12-2015

PERFORMANCE, BODY TEMPERATURE AND BLOOD METABOLITES OF FEEDLOT STEERS AS INFLUENCED BY ENVIRONMENTAL CONDITIONS AND SUPPLEMENTATION OF ZILPATEROL HYDROCHLORIDE

Bradley M. Boyd
University of Nebraska-Lincoln, bboyd4@unl.edu

PERFORMANCE, BODY TEMPERATURE AND BLOOD METABOLITES OF FEEDLOT STEERS AS INFLUENCED BY ENVIRONMENTAL CONDITIONS AND SUPPLEMENTATION OF ZILPATEROL HYDROCHLORIDE

BY

Bradley M. Boyd

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Galen E. Erickson

Lincoln, Nebraska

December, 2015

PERFORMANCE, BODY TEMPERATURE AND BLOOD METABOLITES OF FEEDLOT CATTLE AS INFLUENCED BY ENVIRONMENTAL CONDITIONS AND SUPPLEMENTATION OF ZILPATEROL HYDROCHLORIDE

Bradley M. Boyd, M.S.

University of Nebraska, 2015

Advisor: Galen E. Erickson

Trial one was conducted at the United States Meat Animal Research Center (MARC) near clay center, NE during the summer of 2014. The objective of this trial was to measure the effects of supplementing zilpaterol hydrochloride (ZH) for the final 21 days of the finishing period, and shade, on performance, body temperature, respiration rate, and mobility of finishing beef steers. Feeding ZH increased hot carcass weight, dressing percent, longissimus muscle area, and reduce USDA yield grade. Shade did not affect steer performance and did not reduce body temperature. Zilpaterol hydrochloride increased respiration rate when compared to control cattle and had minimal effect on animal mobility. Zilpaterol hydrochloride and shade had little effect on steer body temperature.

Trial 2 and 3 were conducted during the summer and fall/winter of 2014 at the University of Nebraska-Lincoln (UNL) Agricultural Research and Development Center (ARDC) near mead, NE. The objective of these trials was to assess the effects of environmental conditions on body temperature and blood metabolites across season on finishing steers. Body temperature was correlated to environmental temperature during both trials however the correlations weren't as strong for the winter trial. Many blood metabolites were correlated to environmental and rumen temperatures suggesting that blood metabolites are affected by environmental conditions.

Trial 4 was conducted at UNL ARDC with the objective to determine the effect of feeding Agrimos (Lallemand Animal Nutrition; Montreal, Canada) and 2.5-cm ground wheat straw to finishing steers, during the summer, on body temperature and panting score in addition to performance, and blood metabolites. Hot carcass weight, dressing percent, LM area, and marbling score were not different between treatments. The addition of Agrimos (Lallemand Animal Nutrition; Montreal, Canada) increased steer body temperature with no impact on steer performance. The addition of finely ground wheat straw decreased steer panting score and reduced feed efficiency over both the control and Agrimos fed cattle. Control cattle had greater 12^{th} rib fat depth and as a result USDA yield grade.

Acknowledgements

I would like to thank Galen Erickson for the opportunity provided for me to come to Nebraska and further my education.

I would like to express my gratitude to Terry Mader, Jim MacDonald, Kristin Hales, and Rick Stowell for serving on my committee and being role models for me during my time here.

I appreciate all of the graduate students that have helped me through my studies and provide support throughout my master's program. I wouldn't have been able to complete my research without all of you. I have made lifelong friends and memories I will never forget.

A special thanks to my parents for supporting me throughout my life and for their support and providing me with an opportunity to succeed. I wouldn't be where I am now without your love and support.

Finally, I would like to especially thank my wife Brittany for moving away from her family to Nebraska with me and supporting my decision to attend graduate school.

Thank you to all.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER II.

CHAPTER III.

CHAPTER IV.

LIST OF FIGURES

CHAPTER II.

CHAPTER III.

CHAPTER IV.

INTRODUCTION

The feedlot industry in the United States is extremely competitive with small profit margins; therefore, the addition of any compound to the diet that can increase marketable weight is highly sought. Zilpaterol hydrochloride (ZH; Merck Animal Health; De Soto, KS) is a β-adrenergic agonist that was approved for feeding to beef cattle in the United States in 2006 (FDA, 2006). Performance responses from feeding ZH during the end of the finishing phase are well characterized and clearly show beneficial responses in HCW, dressing %, and yield grade. A 15-kg increase in HCW along with increased dressing percentage and decreased USDA Yield Grade have been consistently observed when ZH was fed at the end of the feeding period (Vasconcelos et al., 2008; Lean et al., 2014; Hilscher et al., 2015). However, recently, there have been concerns of animal welfare issues with the feeding of ZH, which resulted in it being removed from the market by the manufacturer. However, it is unknown whether these concerns are based on scientific fact or simply speculation.

As the feedlot industry continues to be innovative in using both products and management strategies to increase marketable weight the need for a better understanding of how these large animals are affected by environmental conditions is needed. Virtually the entire southern United States can be subject to extended hot periods (West 2003). Animals live in close proximity to the environment and conditions consisting of above normal ambient temperature, relative humidity, and solar radiation coupled with low wind speed can result in reduce performance and even death (Mader et al., 1997). Although some research has been done evaluating blood metabolites as influenced by

environmental conditions, little is known about how animals metabolically change with environmental conditions.

The small intestine is one of the most susceptible tissues to heat damage (Kregel, 2002). Feeding prebiotics in an effort to reduce the negative effects of heat stress has primarily been studied in poultry. Sohail et al. (2011) observed that the feeding of mannan oligosaccharide in the diet of poultry helped reduce some of the detrimental effects of heat stress in terms of reducing oxidative damage to the small intestine. In addition to feeding probiotics, the addition of fiber to a finishing diet may also displace some energy therefore reducing metabolic heat load. Even though environmental stress has been a researched topic for the past few decades little known on how feeding a yeast supplement or fine ground wheat straw will effect feedlot steers from a performance and metabolic standpoint.

Therefore, the objectives of the current studies were to: to further investigate the impact of feeding ZH on heat stress, mobility, and body temperature, in addition to performance and carcass characteristics for steers fed in open or shaded pens (Exp. 1), evaluate the effect of season and ambient temperature on steer blood parameters in addition to measuring rumen temperature (Exp. 2, 3), and finally to determine the effect of feeding a yeast supplement and fine ground wheat straw on steer performance and body temperature, in addition to blood parameters (Exp. 4).

CHAPTER I. Review of Literature

Environmental factors effecting animals

Adverse weather conditions can have a dramatic impact on animal agriculture from both an animal welfare and performance perspective. Virtually the entire southern United States is subject to extended periods of hot weather (West 2003). Animals are dynamic, adaptable, and capable of maintaining life and productive performance in a relatively broad range of environments; however, hot weather can strongly affect animal bioenergetics, with adverse effects on the performance and wellbeing (Hahn, 1997). The external environment animals have to live in can greatly affect an animal's ability to maintain and regulate internal body temperature. As stated by Mader et al. (2002) regulation of body temperature is essential for surviving excessive heat load and involves both physiological and behavioral changes.

Animals have two strategies for coping with adversely hot conditions. First by increasing heat dissipation through evaporative cooling and secondly by reducing feed intake to lower metabolic heat production (Hahn, 1997). Cattle regulate their body temperature by increasing their respiratory rate (Robertshaw, 1985). When the animals' ability to decrease heat load through evaporative cooling is compromised animals begin to show signs of severe heat stress. Evaporative cooling is an effective means of cooling cattle but is compromised by high relative humidity which impedes evaporation (West 2003). With high relative humidity animals fail to cool down as efficiently therefore, the animal's heat load builds up and this is when management strategies must be implemented to cool the animals down. Stressed animals behave in ways to try to mitigate the stress. Animals undergoing heat stress tend to bunch; a behavior influenced

by ambient conditions and is thought to reduce radiant heat absorption as animals provide shade for one another (Mader et al. 2002).

Animals with no way to get out of the ambient weather conditions, such as those in a feedlot setting, are the most susceptible to experiencing the negative effects of adverse weather. Many factors can affect the extent that animals will experience an increased heat load. Summer conditions consisting of above normal ambient temperature, relative humidity, and solar radiation coupled with low wind speed can increase an animal's heat load (Mader et al. 2006). Many environmental conditions interact simultaneously to produce conditions by which an animal can become stressed. While ambient temperature, wind speed, solar radiation, and relative humidity are all important individually they must be considered together in order to determine if conditions will lead to cattle that will become stressed. For example, in times of high temperature and relative humidity when evaporative heat loss is limited, elevated wind speed may actually raise body temperature at a rate faster than it normally would (Mader et al., 2006).

Ambient Temperature

Ambient temperature is the most common climatic indicator used, most likely because of its ease of measure. When the ambient temperature approaches or exceeds the animal's body temperature, the animal must escape, or increase its active cooling by evaporation of water from the respiratory tract or from the skin by sweating (Blackshaw et al. 1994). As the ambient temperature reaches the upper, or lower, limits of the thermal neutral zone the animal must begin expending energy to maintain body temperature.

Effective ambient temperature (EAT) is the actual temperature felt by the animal and may be very different from the air temperature (Aggarwal and Upadhyay, 2013). This has been a useful measurement for assessing the thermal environment effect on animals. Along with EAT comes the thermal neutral zone concept. Christopherson and Young (1986) defined the thermal neutral zone as the range of temperatures in which an animal maintains body temperature in the short term with little or no additional energy expenditure. As the ambient temperature crosses over the thermal neutral barrier the animal must begin to pant, sweat, or increase metabolic rate in order to maintain body temperature. Heat Stress occurs when any combination of environmental conditions cause the effective temperature of the environment to be higher than the animal's thermal neutral temperature (Armstrong, 1994). The greater the gradient between the thermal neutral temperature and the ambient temperature, the more energy the animal must expend in order to maintain body temperature.

Just above and below the thermal neutral zone is an upper critical temperature (UCT) and lower critical temperature (LCT). Yousef (1985) defines the LCT as the ambient temperature below which the rate of heat production of an animal under resting state increases to maintain body heat balance. At temperatures below the LCT the animal's metabolism must increase in order to maintain body temperature. Yousef (1985) also defines the UCT as the air temperature at which the animal increases heat production as a consequence of a raise in core body temperature mainly due to an inadequate evaporative heat loss. As ambient temperature approaches and crosses this upper critical temperature the animal must then find a way to cool itself off to maintain homeothermy. Finch (1986) suggested that the resistance to non-evaporative heat transfer is directly

proportional to the temperature gradients between the animal and the environment. Therefore, ambient temperature has a direct effect on the ability of the animal to transfer body heat to the environment.

Relative Humidity

Evaporative cooling is a contributor in the efficiency by which an animal dissipates heat. As the relative humidity increases, the rate of evaporative cooling that can take place decreases. Relative humidity is defined by Yousef (1985) as the ratio of the mol fraction of water vapor present in a volume of air to the mol fraction present in saturated air, both at the same temperature and pressure. Relative humidity is a key factor in determining how efficiently an animal can dissipate heat. Finch (1986) noted that under conditions of high heat load, about 15% of the animals heat load is lost directly from the core of the animal through the respiratory tract. Therefore, the bulk of heat loss must be transferred to the skin and be lost through conduction, convection, or evaporation off the body surfaces through sweating (Finch, 1986).

With increasing humidity the amount of water that can be evaporated from the skin decreases. Finch (1986) suggested that evaporative resistance to heat transfer is proportional to the absolute humidity gradient between the nasal passage and the air. If this gradient is small, the resistance to evaporation, and thereby heat exchange, will be large. The same is also true with evaporative cooling from the animal's skin. If the air is already saturated, therefore lacking the ability to take up water vapor, evaporative cooling via sweating is minimal (Finch, 1986).

Wind Speed

Summer conditions consisting of above normal ambient temperature, relative humidity, and solar radiation coupled with low wind speed can increase animal heat load and result in reduced performance, decreased animal comfort and death (Mader et al., 1997). Changes in wind speed can alter convective cooling of the animal (Mader et al., 2006). Wind speed is important to consider when determining if animals will experience heat stress or not. Increased air movement over the body surface results in a disruption of the layer of air near the skin surface. This disruption of airspace allows for the removal of warm air as it is replaced by cooler air (Mader et al., 2006). Body heat of the animal is then transferred to the cool air and removed via continuous air movement (Robertshaw, 1985). However, this is only the case when ambient temperature is below animal body temperature. When ambient temperature exceeds body temperature the effects of wind speed are uncertain (Mader et al., 2006).

Solar Radiation

Solar radiation has been considered one of the most important factors that will increase an animal's heat load. Aggarwal and Upadhyay (2013) suggest that the EAT increases 3–5°C in animals exposed to direct sunlight over cattle that have access to shade. In hot weather, solar radiation can have a negative impact on the animal by increasing the heat load but high solar radiation can be beneficial during cold stress by allowing the animal to maintain body temperature.

Solar radiation is of considerable importance as a direct cause of increased body temperatures and respiration rates of dairy cows that are exposed to the sun (Harris et al., 1960). Solar radiation has also been shown to increase sweat gland activity because of the

local heating effects at the neuroglandular junction (Isabirye and Robertshaw, 1972). This phenomenon is important to keep in mind when comparing climate chamber trials as body temperatures of cattle in climate rooms may be markedly different from that measured in a natural environment (Finch, 1986).

The amount of solar radiation that is absorbed by the animal is highly dependent on the animal's hair coat. Absorption of solar radiation due to coat color and hair coat density are both factors that influence evaporative heat loss (Collier et al., 2008). Coat color mediates the impact of solar radiation, and influences the magnitude of heat load on the animal. Animals with dark-colored coats, and hence greater absorptivity to short-wave radiation, acquire greater solar heat loads than animals with lighter colored coats (Walsberg et al., 1978).The inward flow of heat in *Bos Indicus* black steers is 16% larger than for brown steers and 58% larger than for white steers (Finch, 1986). However, *Bos Indicus* cattle evaporate water more efficiently therefore the difference in heat load of different coat colors only has an effect on body temperature when water is limited (Finch, 1986). In contrast, in *Bos Taurus* cattle, dark coat colors have been shown to cause a rise in body temperature even with free access to water as these breeds of cattle cannot evaporate water as efficiently as *Bos Indicus* cattle due to a smaller increasing in sweating rate with increased temperatures (Finch et al., 1984).

Management strategies to reduce environmental stress

In a feedlots located in areas prone to hot summer conditions with little wind and high relative humidity, heat stress can cause substantial economic losses to producers. Although heat stress cannot be completely eliminated, it can be managed. Beede and Collier (1986) suggested shade as management strategies to attenuate the effects of heat

stress. Additionally, sprinkling cattle has long been known to be beneficial in relief of heat stress (Morrison et al., 1973). In contrast, feedlots in areas that experience extremely low ambient temperatures coupled with high relative humidity and wind speed can observe detrimental performance responses to cold stress. The primary mitigation strategy utilized in feedlots is providing cattle with a wind break. Offering wind protection during cold winter conditions has been observed to be beneficial to animal comfort and performance (Tucker et al., 2007; Milligan and Christinson, 1974).

Shade

Shade is a common management practice in areas that experience high relative humidity and temperatures during the summer months. Shade ameliorates heat load of cattle and reduces mortality in extreme weather events (Gaughan et al., 2010). The main roll of shade is to reduce the radiant heat load experienced by the animal. Shade can reduce the radiant heat load on an animal by as much as 30% (Bond et al., 1967).

Mitlöhner et al. (2002) observed an increase in ADG and DMI for shaded cattle when compared to unshaded cattle. Additionally, Mitlöhner et al. (2002) observed a tendency for increased HCW for cattle that were shaded when compared to an unshaded control treatment. Furthermore, Gaughan et al. (2010) observed an increase in final live BW, ADG, DMI, and G:F for cattle fed in shaded pens vs. cattle fed in an open lot system. Conversely, Mader et al., (1999) observed no increase in ADG or DMI for cattle fed in shaded pens when compared to cattle fed in open pens. The lack of shade response observed by Mader et al. (1999) was attributed largely to the mild summer conditions

during the time of the trial suggesting that benefits from shade are only realized during extreme heat events.

Shade has been observed to increase animal performance during extended periods of hot environmental conditions (Gaughan et al., 2010, Mitlöhner et al., 2002). However, Mader et al. (1997) suggested that if shade structures are not adequate in providing solar radiation protection, then any positive production response of shade will be lost. Similarly, Bond and Laster (1975) reported that ADG and G:F during Midwestern summers are unaffected by having access to shade or not. Furthermore, Pusillo et al. (1991) suggested that DMI for cattle in the latter stages of the feeding period exposed to Midwestern climatic conditions are relatively unaffected by presence or absence of overhead shelter.

Shade material plays a major role in how effective shade will be at reducing the heat load experienced by the animals. Eigenberg et al. (2010) evaluated the effectiveness of several different shade materials and concluded that all of the materials tested reduced predicted heat stress experienced by the animals when compared to not having access to the shades; however, shade material did affect the magnitude of response observed. Snow fence provided the least protection and consequently reduced solar radiation reaching the ground only slightly when compared to the non-shaded control. Conversely, the 100% shade cloth blocked almost all of the solar radiation from reaching the ground. However, when choosing a shade material, solar radiation protection is not the only criteria. Shade cloth with 100% protection will not stand up to snow load during the winter, potentially hinders airflow, and would have to be taken down prior to the winter months. Snow fence, however, would hold up to environmental conditions during the winter and could

remain standing year round. Even though snow fence does not provide the best solar radiation protection, it is much more attractive from a management standpoint.

Watering

Applying water to cattle and pens on hot days is a common practice in the feedlot industry. Watering cattle and pen surfaces is relatively cheap and easy for feedlots to implement when compared to other cooling strategies such as installing shades. Sprinkling cattle has long been known to be beneficial in relief of heat stress (Morrison et al., 1973). The objective behind directly wetting cattle is to maximize the amount of heat removed via evaporation (Mader et al., 2003). As water evaporates from around the animal it reduces the air temperature by the amount of heat required to evaporate water, thus increasing the heat gradient between the animal and the atmosphere and allowing for a greater amount of heat flow away from the animal (Mader et al., 2003; Ryan et al., 1992). However, as evaporative cooling relies mainly on the ability for the air to take up moisture, evaporative cooling works better in hot dry climates than hot humid environments. Additionally while sprinkling cattle has been shown to aid in animal cooling, misting cattle hasn't been shown to have as large of a production benefit (Mitlöhner et al., 2001). It has also been suggested that misting cattle failed to increase production due to the fine water droplets clinging to the outer hair of the cattle's coat rather than reaching the skin, resulting in an insulation layer that acts as an evaporation barrier (Mitlöhner et al., 2001).

The wetting of pen surfaces is potentially more beneficial in a feedlot setting than wetting the animal itself. Mader et al., (2003) suggested that application of water to the

pen surface would not only cool the ground and increase the thermal gradient but would also provide for increased thermal conductivity and better heat flow down the gradient. Mader et al., (2007) reported that in sprinkled pens soil temperatures were consistently lower than ambient temperatures and 6 to 15 ℃ lower than non-sprinkled pens.

Numerous trials have noted that dairy cattle given access to sprinklers have increased milk production (Ryan et al., 1992; Chen et al., 1993). However, comparable economic benefits to cooling feedlot cattle is less evident due to compensatory growth following heat stress (Mader et al., 2003). Furthermore, Hahn et al., (1974) reported that heifers stressed at 30.9 ℃ showed compensatory gain 2 weeks after the heat stress was relieved. However, Hahn et al. (1974) noted that compensatory gain and full recovery does not occur in animals subjected to long-lasting severe heat stress. Although performance increases may not be observed for sprinkling cattle, many feedlots utilize the wetting of pens as a method to minimize animal death loss due to heat stress rather than to minimize loss in performance. Therefore, although animal performance may not be increased by watering, preventing animal death provides feedlots with economic incentive to water cattle.

Wind Breaks

In areas where wind is obstructed by buildings, or other objects, the chances of cattle experiencing heat stress on hot summer days is dramatically increased. However, offering wind protection during cold winter conditions has been observed to be beneficial to animal comfort and performance (Tucker et al., 2007; Milligan and Christinson, 1974). Tucker et al. (2007) observed that dairy cattle without provided protection during cold

winter conditions had lower minimum body temperatures and spent more time in postures that minimized surface areas exposed to the wind than cattle that were provided with shelter. Furthermore, 20% - porosity fence has been shown to improve feed utilization and average daily gain of feedlot cattle by 18 and 25% respectively during cold winter conditions (Milligan and Christison, 1974).

However, while providing wind protection during the winter may be beneficial for animal welfare and performance during extreme cold stress conditions, Mader et al. (1997) found that wind breaks had no effect on animal performance during moderate cold stress conditions. Additionally, during the summer months the provided wind protection decreased animal performance when compared to that of the control where no wind protection was provided. These data would suggest that allowing proper air flow during summer months is crucial to maintaining animal performance and although animal comfort is improved by providing wind protection during the winter months observed performance increases are only seen during extreme cold events and minimal performance increases are observed for moderately cold environments.

Animal Response to Environmental Stress

The heat stress response is a highly conserved cascade of protein activation and altered gene expression in response to a variety of stressors (Collier et al., 2008). Like humans, animals can succumb to disease or fail to reproduce or develop properly when exposed to prolonged periods of high heat (Moberg and Mench, 2000). Animals respond to stress in a variety of ways and individual animals can deal with stress better than others. As environmental temperature changes, homotherms must act to maintain body

temperature. In order to maintain a constant body temperature, thermal exchanges between the environment and the animal must be present. These exchanges include both convective and evaporative heat exchanges (Finch, 1986).

Body Temperature

Body temperature follows a diurnal pattern that follows shortly behind that of environmental temperature. Body temperature has been used as a method of assessing the physiological response of an animal to the climatic environment, especially when cattle are exposed to hot conditions (Gaughan et al., 2010). The consistency of an animal's core body temperature is an indication of how well an animal balances heat production and heat losses (Brown-Brandl et al., 2003). Most studies observing body temperatures have only observed body temperatures over short periods usually less than ten days (Gaughan et al., 2010). However, by utilizing rumen temperature boluses, body temperatures can be collected for a much longer period of time. Rumen temperature has been shown to exceed rectal temperature by about 2 ℃; however, rumen temperature has been shown to follow actual core body temperature relatively well (Beatty et al., 2008).

Body temperature has been shown to follow a diurnal pattern tracking in the same pattern as ambient temperature. Rectal temperature has been shown to have a lag time of 4-5 hours after peak environmental temperature (Brown-Brandl et al., 2003). Furthermore, a study conducted by Harris et al. (1960) concluded that solar radiation was of considerable importance as a direct cause of increased animal body temperature.

There are many factors that affect body temperature in cattle. In order to maintain a constant body temperature, the animal must lose the same amount that is gained

through metabolism and the external environment. Finch (1986) have suggested that heat-gain from solar radiation and metabolism usually exceeds heat-loss from radiation, convection and evaporation during the daytime, when temperatures are high, resulting in heat being stored as evidence by increased body temperature. This stored heat must then be lost during the night when heat can be more easily dissipated from the animal. However, when environmental conditions during the night are unfavorable to heat transfer from the animal to the environment the animal fails to lose stored heat and is then more vulnerable to heat stress during the following day. The ability of cattle to lose body heat at night is dependent not only on ambient temperature, but also on atmospheric moisture levels or relative humidity (Mader, 2003).

Physiological response

Measuring an animal's body temperature to access environmental stress is not always feasible specifically in commercial settings. A viable alternative to using body temperature to assess an animal's heat load is to measure the animals observed behavior responses to environmental conditions, such as panting score and respiration rate (Mader et al., 2006). Respiration rate has been shown to be a good indicator of an animal's heat load. However, a lag time between maximum ambient temperature and respiration rate of about 2 hours exists (Gaughan et al., 2000). Therefore, in order to get an estimation of the heat load animals are experiencing, respiration rates must be taken at least 2 to 3 hours after the hottest part of the day as this is when maximum respiration rates will be observed.

The effect of ambient temperature on respiration rate can be affected by numerous things. Respiration rate is influenced by age, sex, genotype, level of performance, nutrition, time of feeding, as well as previous exposure to hot conditions (Gaughan et al., 2000). As environmental conditions place a greater heat load on an animal, the animal must compensate in order to remain in thermal equilibrium. Cattle compensate for increased environmental heat load by increasing respiration rates and panting. Therefore, respiration rates follow the diurnal patterns of ambient temperature (Brown-Brandl et al., 2003).

Metabolic response

Heat shock proteins (HSP) are a family of proteins found in most all living cells. These proteins are present in both prokaryotic and eukaryotic cells, and their high level of conservation suggests they play an important role in fundamental cell processes (Kregel, 2002). Many studies have established that in response to environmental insults cells synthesize HSP (Gutierrez and Guerriero Jr., 1995). Stress-induced accumulation of HSP have been associated with thermotolerance, or the ability of a cell or animal to survive otherwise lethal heat stress (Moseley, 1997). The mechanism by which these proteins grant stress tolerance is not well understood; however, Moseley (1997) suggested that it may relate to the important role of HSPs in the processing of stress-denatured proteins.

Some HSP have been linked to the production of certain metabolites in the blood. Heme oxygenase (HO) is the rate limiting enzyme of heme catabolism and has been shown to have an inducible form (HO-1) which is a member of the HSP32 family (Tomaro et al., 2002; Attuwaybi et al., 2004). The three by-products produced by this

enzyme include carbon monoxide, free iron, and biliverdin, all of which possess free radical savaging properties (Attuwaybi et al., 2004).

Bilirubin is derived from biliverdin which is produced from heme degradation and has previously been thought of as a cytotoxic metabolite because of its role in jaundice in neonates and its possibility of provoking disabling and irreversible brain damage at high concentrations. Recently, it has been discovered that bilirubin may actually play an important physiological role as a powerful anti-oxidant whose activity may surpass that of α-tocopherol (Tomaro et al., 2002; Yamaguchi et al., 1996). Attuwaybi et al. (2004) suggested that the by-products of HO-1 act to mitigate the effects of inflammatory mediators induced by ischemia-reperfusion, which was shown by Kregel et al. (1988) to be simulated during heat stress in rats. Therefore, it can be speculated that the products from this enzyme play an important role in the heat tolerance of the animal's gastrointestinal tract.

Bilirubin is filtered from the blood by the liver where it is then attached to sugars, most commonly, glucuronic acid forming conjugated bilirubin (Kurosaka et al., 1998). Under normal physiological conditions, some of these bilirubin conjugates then escape from the hepatocyte and into the serum where they can be measured in the blood serum as direct bilirubin. This direct bilirubin, when measured accurately, then correspond to changes in conjugated bilirubin concentrations within the liver (Kaplan et al., 2002; Muraca et al., 1987). After conjugation, this conjugated bilirubin is then excreted in bile by the liver via the bile duct and into the small intestine (Harrop and Guzman Barron, 1930). It is suggested by Kaplan et al. (2002) that neonatal jaundice, or high bilirubin levels in the blood, can occur from diminished bilirubin excretion which is primarily the

result of immature conjugative capacity. This would suggest that an increase in conjugated bilirubin concentration in the liver leads to increased excretion of bilirubin though the bile duct further suggesting bilirubin's importance in intestinal integrity.

Olbrich et al. (1972) noted a depression in hematocrit and erythrocytes in cattle subjected to elevated ambient temperatures. This finding was further supported by a study conducted by Lee et al. (1976) where a negative correlation between hematocrit concentration and ambient temperatures was observed. Additionally, in the same trial, hematocrit concentration seemed to drop in dairy cows exposed to hot environments. This finding was in part attributed to a decrease in circulating erythrocytes. Furthermore, Shaffer et al. (1981) observed significant effects of temperature on hemoglobin, A:G ratio, albumin, and blood urea nitrogen concentration in dairy cattle when exposed to cool, intermediate, and hot environments. The observed reduction in red blood cells and hemoglobin was attributed, by Shaffer et al. (1981), to a decrease in cellular oxygen requirements that in turn, reduces the animal's metabolic heat load. Therefore, the oxygen binding capacity of the blood is decreased when the animal is under heat stress conditions (Lee et al., 1976).

Gastrointestinal health

The small intestine is one of the most sensitive tissues to heat damage (Kregel, 2002). During times of severe heat stress blood is transferred away from the small intestine and the rest of the core of the animal's body and transferred to the extremities in order to aid in cooling. This transfer of blood flow away from the gastrointestinal tract (GI) can result in intestinal cellular hypoxia (Yu et al., 2010). Additionally, this

reduction of blood flow can lead to free radical production, ATP depletion, acidosis and cellular dysfunction which can all lead to disruption of the GI barrier (Yu et al., 2010).

Hyperthermia has been shown to increase intestinal permeability in a variety of mammalian species (Lambert et al., 2002). This increased intestinal permeability can then lead to endotoxin, from gut microbial activity, entering into the portal blood flow (Bouchama et al., 1991). Lambert et al. (2002) showed that high temperatures alone didn't appear to induce sufficient oxidative stress to the small intestine. However, Kregel et al. (1988) suggested that with heat stress, blood flow to the intestine initially decreases and then sharply increases with sustained high temperature simulating ischemia-reperfusion, which is tissue injury resulting from a return of blood flow to a tissue after a period of no blood flow and oxygen depletion, of the gut . Ischemia-reperfusion has been well documented to increase intestinal epithelial damage and permeability most likely through increased reactive oxygen species production (Lambert et al., 2002; Attuwaybi et al., 2004).

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and activity of one, or a limited number of bacteria in the colon (Sohail et al., 2012). Feeding prebiotics in an effort to reduce the negative effects of heat stress has been studied mostly in swine and poultry. Sohail et al. (2012) found that the addition of mannan oligosaccharide to the diet of poultry helped in reducing the detrimental effects of chronic heat stress. The increase in reactive oxygen species production in the small intestine due to heat stress observed by Lambert et al. (2002) and Attuwaybi et al. (2004) suggests that damage to the intestinal wall during times of heat stress could potentially be mitigated by increased concentrations of

intestinal antioxidants. Feeding mannan oligosaccharide to poultry was shown by Sohail et al. (2011) to reduce some of the detrimental effects of heat stress in terms of reducing oxidative damage to the small intestine although it is still unclear how the supplemented mannon oligosaccharide acted as an antioxidant.

Dietary fiber has long been known to increase gut health in non-ruminant species. In general, dietary fiber ingestion leads to increased size and length of the digestive organs, including the small intestine, caecum and colon of pigs, chickens and rats (Montagne et al., 2003). Although few data exist, it can be hypothesized that fiber has the same effect in ruminant species lower gut. However, getting soluble fiber to the lower gut of ruminant species can be a challenge.

There are few data on increasing soluble fiber passage from the rumen to the small intestine. However, work on particle size and specific gravity would suggest that reducing particle size and increasing particle specific gravity to between 1.17 and 1.42 allows for particles to pass most rapidly (Welch, 1986). Yansari et al. (2004) suggested that the observed that reduction NDF digestion observed with reduce particle size can be attributed to decreased rumen retention time and digestion. This would suggest that with lower ruminal digestibility that more fiber is potentially reaching the lower intestinal tract to be digested. Increased amounts of fiber reaching the lower intestinal tract has been shown to increase the amount of short chain fatty acids produced in the cecum (Montagne et al., 2003). Short chain fatty acids are potent modulators of growth, function and differentiation of intestinal epithelia and short-chain fatty acids have wound healing and cytoprotective effects (Hongyu et al,. 2001). The presence of short-chain fatty acids, such

as butyrate, has been shown in vitro and in vivo, in the large colon of rats, to induce HSP 25 which aids in providing oxidative protection to intestinal cells (Hongyu et al,. 2001).

Increasing fiber in the diet can also act to decrease metabolizable energy intake and decrease digestive heat production. Mader et al. (1999) observed that feeding roughage at 40% of the diet decreased animal body temperature and respiratory rate over cattle fed 25 and 10% roughage diets. However, while not reported, the high fiber diet fed would have displace enough energy in the diet that a negative production response would have been observed making feeding high levels of fiber illogical from a feedlot production standpoint. Increasing fiber levels during periods of hot weather has been shown to have no beneficial effect on dairy cattle (West et al., 1999). Milk yield decreased when cattle were fed a high fiber diet when compared to cattle fed a low fiber diet in a study conducted by West et al. (1999). However, the observed decrease in milk production was attributed to a displacement of energy rather than an antagonistic effect of feeding fiber in hot climates. Furthermore, in a trial conducted by Magdub et al. (1982) dairy cattle fed a high fiber diet during heat stress had similar intakes to cattle fed a low fiber diet; however, the digestible energy intake of the low fiber cattle was greater than that of the high fiber diet and therefore animal performance was also greater.

Dry Matter Intake

The act of feeding raises the metabolic rate of an animal thus increasing heat production; this is known as the heat increment of feeding (Beatty et al., 2008). During periods of hot weather, DMI of feedlot cattle decreases sharply. Intake begins to decline when mean daily environmental temperatures reach 25 to 27 ℃ (Beede and Collier, 1986). Hahn (1997) observed that initiation of eating events in both cool and hot weather

is associated with peaking or descending portions of the tympanic temperatures. Mader et al. (2002) noted that restricted feeding systems are useful during periods of hot weather and body temperature is reduced when feed is restricted, possibly through reductions in metabolic heat production and a concurrent reduction in metabolic rate.

Numerous studies have noted a decrease in DMI due to heat stress (Mader et al., 1997; Hahn, 1997; Gaughan et al., 2010). Animals on a higher energy diet react more dramatically to hot condition than animals on a lower energy diet (Fuquay, 1981). Although feed intake is decreased with higher temperatures, feed digestibility has been shown to increase (Fuquay, 1981). However, this increase in digestibility is attributed to depressed intakes and therefore a slower rate of passage. Shade has been shown to help offset the depression in DMI experienced in feedlot cattle during periods of hot weather. Gaughan et al., (2010) observed that shaded cattle had significantly greater DMI than that of the control non-shaded cattle. Furthermore, Mitlöhner et al. (2002) also observed that shaded cattle had greater DMI than that of unshaded cattle.

In the northern plains of the United States, performance of feedlot cattle is generally better during the summer than the winter (Mader et al., 1999). Better performance during the summer is due mostly to the fact that cattle fed in cold weather tend to consume more feed but gain less (Young, 1981). Furthermore, Hahn (1995) noted that feed intake during cold weather generally increases; however, mud and ice can hamper movement resulting in decreased feed intake. A cold-adapted animal will have an elevated basal metabolism, increased appetite, and reduced capacity to digest feed (Young, 1983). During times of cold, the maintenance energy requirements for beef cattle have been shown to increase by 30-70% leading to the loss in performance (Young,

1981). This observed increase in maintenance requirements is due mostly to an increased metabolic rate to meet the demand for increased heat production in order for the animal to maintain body temperature.

Beta -adrenergic agonist

Beta-adrenergic agonists (β-agonist) elicit a physiological response when bound to beta-adrenergic receptors located on most mammalian cells (Mersmann, 1998a). Three β-receptors subtypes exist; β_1, β_2, and β_3 (Beermann, 2002). Beta-agonists work by binding to these specific β-receptors on fat and muscle cell surfaces which modify biochemical processes of tissue growth by increasing lipolysis, decreasing lipogenesis, decreasing protein degradation, and increasing protein synthesis (Strydom et al., 2009).

There are two β-agonists commonly used in the beef industry; zilpaterol hydrochloride (ZH; Merck Animal Health; De Soto, KS) and ractopamine hydrochloride (RH; Elanco; Greenfield, IN). Ractopamine was first approved to be fed in the United States in 2003 and ZH was first approved in 2006 (FDA, 2003; FDA, 2006). Both of these β-agonists are labeled to enhance feed efficiency, increase rate of weight gain and increase carcass leanness when orally administered to beef cattle; however, this change can be influenced by dose, duration and type of β-agonist used (Lean et al., 2014).

Mode of Action

Beta-agonists are organic molecules that bind to β-receptors, present on most mammalian cells, and can increase muscle mass and decrease fat through hypertrophy of muscle and reduced fat accretion (Avendaño-Reyes et al., 2006). The exact mechanisms behind how β-agonist work is still not fully understood. As a result of having a variety of

β-receptor subtype structures and distribution across animal tissues and species, the multitude of physiological effects and mechanisms that produce the pharmacological effects observed with oral administration of a β-agonists are complex and very difficult to discern (Mersmann, 1998a).

Almost every mammalian cell type has β-adrenergic receptors embedded in the plasma membrane (Mersmann, 1998a). B-agonists share structural similarities and pharmacological properties with the endogenous catecholamines epinephrine and norepinephrine (Beermann, 2002, Mersmann, 1998a). This structural similarity to epinephrine and norepinephrine then allows for β-agonists to reduce or block the activity of these compounds (Barnes, 1995). When steers were infused with cimaterol via close arterial infusion of the hind limb for 21 d, an increase in the rate of blood flow and extraction of essential amino acids was observed (Beermann, 2002). This increase in uptake reached a maximum at 160% that of the control (Beermann. 2002). Across several studies, a decrease in protein degradation and increase in protein synthesis has been observed (Mersmann, 1998a). However, not every study has observed this effect mainly because it is difficult to measure.

B-agonists and Cattle Performance

Both RH and ZH are approved to be fed in the United States to finishing cattle and are labeled to improve feed efficiency, increase rate of weight gain, and increase carcass leanness (FDA, 2003; FDA, 2006). However this effect is influenced by a number of things. The magnitude of cattle response to β-agonist administration varies greatly among type of β-agonist used and is influenced by age, species, sex, diet, breed, and

duration β-agonist was administered (Beermann, 1998a; Lean et al., 2014; Mersmann, 1998).

A 15 kg increase in HCW along with increased dressing percentage and decreased USDA yield grade have been consistently observed when ZH was fed at the end of the feeding period (Vasconcelos et al., 2008; Lean et al., 2014; Hilscher et al., 2015). Vasconcelos et al. (2008) observed no increase in final live BW between the control group and the average of the other 3 treatments fed ZH over varying lengths of time. While final live BW was not affected by feeding ZH, HCW was increased. Hilscher et al. (2015) also did not observe an increase in final live BW or ADG for cattle fed ZH vs. control. In contrast, Elam et al. (2009) reported an increase in final live BW in addition to increased HCW when cattle were fed ZH.

Montgomery et al. (2009) observed a 0.47 kg/d and 0.056 kg/kg increase in ADG and G:F, respectively, and also a tendency for reduced DMI for steers fed ZH for 20-d duration. Likewise, in a study conducted by Hales et al. (2014), no reduction in DMI was observed; however, there was an increase of 0.8 kg/d of ADG and a 0.016 kg/kg increase in G:F over the entire feeding period when ZH was fed for 21 d. Hilscher et al. (2015) also noted no differences in DMI or ADG for cattle fed ZH, but did note increased G:F over the entire feeding period for cattle fed ZH. Furthermore, Baxa et al. (2010) also reported no difference in DMI for cattle fed ZH. Conversely, in a meta-analysis conducted by Lean et al. (2014), a live BW increase of 8 kg was observed along with a DMI reduction of 0.12 kg/d and an increase of 0.15 kg/d in ADG across numerous studies feeding ZH. However, an observed increase in DMI, ADG, and final live BW has been variable.

Elam et al. (2009) observed a 14 kg increase in HCW when cattle were supplemented with ZH for 20, 30, or 40 d with little change due to increased duration. Similarly, Montgomery et al. (2009) reported a 13 kg increase in HCW as well as a 1.3% increase in dressing percent, and a 7.9 cm^2 increase in LM area. Additionally, Montgomery et al. (2009) reported a 0.38 unit decrease in USDA yield grade. Hilton et al. (2009) also observed an increase in LM area and decreased USDA yield grade when ZH was fed. This is further supported by findings by Hilscher et al. (2015) that reported a 13 kg increase in HCW along with a 7.3 cm^2 increase in LM area and a 0.67 unit decrease in USDA yield grade when ZH was fed.

Zilpaterol hydrochloride and animal welfare

There are limited data evaluating the effects of feeding a β-agonist on animal welfare issues such as mobility and heat stress. However, Hales et al. (2014) observed a positive slope in the regression line for panting score and respiration rates as days fed ZH increased, suggesting that both measures increased as days on ZH increased. While not significant, Hales et al. (2014) reported that cattle fed ZH had greater respiration rates and panting scores. Furthermore, Bernhard et al. (2014) observed increased panting scores in cattle fed ZH over that of the cattle not fed ZH. It is not well understood whether or not this increase is due to a greater amount of heat load on the animal with the increased muscle mass or an unobserved biological effect of increased metabolism due to feeding ZH. Furthermore, Bernhard et al. (2014) observed that the body temperatures of conventionally raised cattle had greater body temperatures than that of conventional fed cattle being supplemented with ZH. There are few studies observing how the feeding of a

β-agonist affects mobility scores. However, Bernhard et al. (2014) noted that ZH had no effect on chute exit velocity or mobility scores.

Conclusions

Feedlot profitability focuses on the ability of a given feedlot to feed cattle with high efficiency and low death loss. Economic losses due to heat stress stem from reduced feed intake, reduced weight gain and in extreme cases death (Mader et al., 1999; Mader et al., 2006; Yousef, 1985). When death from heat stress occurs the animals most affected are the fattest cattle that have been on feed the longest and unfortunately, these are the cattle that the feedlot has the most investment in. When these deaths occur as a result of a severe heat event feedlot profitability can be drastically affected.

With the competitive nature of feeding cattle any exogenous compound that can be administered to elicit an increase in feed efficiency and rate of gain is highly sought. β-adergenic agonists are one of the main substances that have been FDA approved to increase the rate of weight gain and animal performance. ZH is a β-agonist that has been heavily utilized in the feedlot industry since its approval in 2006 (FDA, 2006). ZH has been observed to elicit a dramatic increase in HCW of feedlot cattle (Elam et al., 2009; Montgomery et al., 2009; Hilscher et al., 2015). This observed increase in performance allows feedlots to sell more animal weight from the same amount of inputs driving the economic incentive to utilize these compounds.

With the increase in consumer concern with animal welfare and some raising concerns of ZH causing potentially negative effects on animal welfare it has since been removed from the market by the manufacturer. Additionally, with concerns of animal

welfare, heat stress prevention has begun to be a topic of interest as to improve animal comfort during adverse summer conditions. However, little is known about the mechanism behind how animals respond to heat stress. Therefore the objectives of these studies were to: further investigate the impact of feeding ZH on heat stress, mobility, and body temperature, in addition to performance and carcass characteristics for steers fed in open or shaded pens (Exp. 1). To determine the effect of season and ambient temperature on steer blood parameters in addition to measuring rumen temperature, continuously, throughout the feeding period (Exp. 2, 3). And finally, to determine the effect of feeding a yeast supplement and fine ground wheat straw on steer blood parameters in addition to body temperature and panting score measured continuously throughout the feeding period (Exp. 4).

LITURATURE CITED

Acendaño-Reyes. L., V. Torres-Rodríguez, F. J. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 54:3259-3265.

Aggarwal, A., & Upadhyay, R. 2013. Heat stress and immune function. In Heat Stress and Animal Productivity pp. 113-136

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77:2044-2050

Attuwaybi, B. O., R. A. Kozar, S. D. Moore-Olufemi, N. Sato, H. T. Hassoun, N. W. Weisbrodt, and F. A. Moore. 2004. Heme oxygenase-1 induction by hemin protects against gut ischemia/reperfusion injury. J. Surgical Res. 118:53-57

Barnes, P. J. 1995. Beta-adrenergic receptors and their regulation. Am. J. Respir. Cirt. Care Med, 152:838-860.

Baxa, T. J., J. P. Hutchenson, M. F. Miller, J. C. Brooks, W. T. Nichols, M. N. Streeter, D. A. Yates, and B. J. Johnson. 2010. Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers. J. Anim. Sci. 88:330-337. doi:10.2527/jas.2009-1797

Beatty, D. T., A. Barnes, E. Taylor, and S. K. Maloney. 2008. Do changes in feed intake or ambient temperature cause changes in cattle rumen temperature relative to core temperature. J. Thermal Biology. 33:12-19

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543-554

Beermann, D. H. 2002. Beta-adrenergic receptor agonist modulation of skeletal muscle growth. J. Anim. Sci. 80:E18-E23. doi:10.2134/animalsci2002.0021881200800ES10004x

Bernhard, B. C., C. L. Maxwell, C. F. O'Neill, B. K. Wilson, C. G. Hixon, C. L. Haviland, A. N. Grimes, M. S. Calvo-Lorenzo, C. J. Richards, D. L. Step, B. P. Holland and C. R. Krehbiel. 2014. The effects of technology use in feedlot production systems on the performance, behavior and welfare of steers finishing during late summer. In: Proceedings of the Plains Nutrition Council Spring Conference, San Antonio, TX. p. 142.

Blackshaw, J. K., and A. W. Blackshaw. 1994. Heat stress in cattle and the effect of shade on production and behavior: a review. Aust. J. Exp. Agri. 34:285-295.

Bond, T. E., C. F. Kelly, S. R. Morrison, and N. Pereira. 1967. Solar, atmospheric, and terrestrial radiation received by shaded and unshaded animals. Trans. Am. Soc. Agric. Eng. 10:622-627.

Bond, T. E., and D. B. Laster. 1975. Influence of shading on production of Midwest feedlot cattle. Trans. ASAE 18:957-959.

Bouchama, A., R. S. Parhar, A. El-Yazigi, K. Sheth, and S. Alsedairy. 1991. Endotoxemia and the release of tumor necrosis factor and interleukin 1α in acute heatstroke. J. Appl. Physiol. 70:2640-2644

Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, H. Freetly. 2003. Thermoregulatory responses of feeder cattle. J. Thermal Biology. 28:149-157

Chen, K. H., J. T. Huber, C. B. Theurer, D. V. Armstrong, R. C. Wanderley, J. M. Simas, S. C. Chan, and J. L. Sullivan. 1993. Effect of protein quality and evaporative cooling on locational performance of Holstein cows in hot weather. J. Dairy Sci. 76:819-825

Christopherson, R. J., & Young, B. A. 1986. Effects of cold environments on domestic animals. NATO advanced science institutes series: Series A: Life sciences

Collier, R. J., J. L. Collier, R. P. Rhoads, and L. H. Baumgard. 2008. Genes involved in the bovine heat stress response. J. Dairy Sci. 91:445-454

Eigenberg, R. A., T. M. Brown-Brandl, and J. A. Nienaber. 2010. Shade material evaluation using a cattle response model and meteorological instrumentation. Int. J. Biometeorol. 54:509-515. doi: 10.1007/s00484-010-0343-z.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87:2133-2141. doi:10.2527/jas.2008-1563

FDA. 2003. Freedom of information summary. Original new drug application NADA 141-221. Ractopamine Hydrochloride (Optiflexx). Type A Medicated Article For Beef Cattle. Found at: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm118030.pdf. (Accessed 11 July 2015.)

FDA. 2006. Freedom of information summary. Original new drug application NADA 141-258. Zilpaterol hydrochloride (ZILMAX). Type A medicated article for cattle fed in confinement for slaughter. Found at: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIDrugSummaries/ucm051412.pdf. (Accessed 10 February 2015.)

Finch, V. A., I. L. Bennett, and C. R. Holmes. 1984. Coat colour in cattle: effect on thermal balance, behavior and growth, and relationship with coat type. J. Agric. Sci. 102:141-147

Finch, V. A. 1986. Body temperature in beef cattle: its control and relevance to production in the tropics. J. Anim. Sci. 62:531-542

Fuquay, J. W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 52:164-174

Gaughan, J. B., S. M. Holt, G. L .Hahn, T. L. Mader, and R. Eigenberg. 2000. Respiration rate – is it a good measure of heat stress in cattle. Asian-Aus. J. Anim. Sci. 13:329-332

Gaughan, J. B., S. Bonner, I. Loxton, T. L. Mader, A. Lisle, and R. Lawrence. 2010. Effect of shade on body temperature and performance of feedlot steers. J. Anim. Sci. 88:4056-4067. doi:10.2527/jas.2010-2987

Gutierrez J. A., and Guerriero V. Jr. 1995. Relative abundance of bovine hsp70 mRNA and protein. Biochimica et Biophysica Acta. 1260:239-242

Hahn, L. 1974. Compensatory growth of beef cattle in hot weather and its role in management decisions. In proceedings of the international livestock environment symposium. P 288-295

Hahn, G. L. 1995. Environmental influence on feed intake and performance of feedlot cattle. In proceedings feed intake Oklahoma State University. P207-225. Retrieved from: *beefextension.com/proceedings/feed_intake95/feed_intake95_29.pdf*

Hahn, G. L. 1997. Dynamic responses of cattle to thermal heat loads. J. Anim. Sci. 77:10-20

Hales, K. E., S. D. Shackelford, J. E. Wells, D. A. King, M. D. Hayes, T. M. Brown-Brandl, L. A. Kuehn, H. C. Freetly, and T. L. Wheeler. 2014. Effects of feeding dry-rolled corn-based diets with and without wet distillers grains with soulubles and zilpaterol hydrochloride on performance, carcass characteristics and heat stress in finishing steers. J. Anim. Sci. 92:4023-4033. doi:10.2527/jas.2014-7638

Harris, D. L., R. R. Shrode, I. W. Rupel, and R. E. Leighton. 1960. A study of solar radiation as related to physiological and production responses of lactating Holstein and Jersey cows. J. Dairy Sci. 43:1255-1262

Hilscher, Jr. F. H., E. M. Hussey, B. L. Nuttelman, D. B. Burken, W. A. Griffin, K. J. Vander Pol, J. P. Hutcheson, and G. E. Erickson. 2015. Impact of sorting before feeding zilpaterol hydrochloride on feedlot performance and carcass characteristics of yearling steers**.** J. Anim. Sci. 93:2285-2296. doi:10.2527/jas.2014-8579

Hilton, G. G., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutchenson, W. T. Nichols, M. N. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Effects of feeding zilpaterol hydrochloride with and without monensin and tylosin on carcass cutability and meat palatability of beef steers. J. Anim. Sci. 87:1394-1406. doi:10.2527/jas.2008-1170

Hongyu, R., M. W. Musch, K. Kojima, D. Boone, A. Ma, and E. B. Chang. 2001. Short-chain fatty acids induce intestinal epithelial heat shock protein 25 expression in rats and IEC 18 cells. Gastroenterology. 121:631-639

Isabirye, J. K., and D. Robertshaw. 1972. The sweat gland response to solar radiation in domestic animals. Biometeorology 5:67

Kregel, K. C., P. T, Wall, and C. V. Gisolfi. 1988. Peripheral vascular responses to hyperthermia in the rat. J. Appl. Physiol. 64:2582-2588

Kregel, K. C. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. 92:2177-2186

Kurosaka, K., Senba, S., Tsubota, H., & Kondo, H. 1998. A new enzymatic assay for selectively measuring conjugated bilirubin concentration in serum with use of bilirubin oxidase. Clinica chimica acta, 269(2), 125-136

Lambert, G. P., C. V. Gisolfi, D. J. Berg, P. L. Moseley, L. W. Oberley, and K. C. Kregel. 2002. Molecular biology of thermal regulation selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J. Appl. Physiol. 92:1750-1761

Lean, J. I, J. M. Thompson, and F. R. Dunshea. 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance traits and shear strength of meat in cattle. PLoS One 9(12):e0115904. doi: 10.1371/journal.pone.0115904

Lee, J. A., J. D. Roussel, and J. F. Beatty. 1976. Effect of temperature-season on bovine adrenal cortical function, blood cell profile, and milk production. J. Dairy Sci. 59:104-108

Mader, T. L., J. M. Dahlquist, and J. B. Gaughan. 1997. Wind protection effects and airflow patterns in outside feedlots. J. Anim. Sci. 75:26-36. doi:/1997.75126x

Mader, T. L., J. B. Gaughan, and B. A. Young. 1999. Feedlot diet roughage level for Hereford cattle exposed to excessive heat load. Professional Animal Scientist. 15:53-62

Mader, T. L., J. M. Dahlquist, G. L. Hahn, and J. B. Gaughan. 1999. Shade and wind barrier effects on summertime feedlot cattle performance. J. Anim. Sci. 77:2065-2072. doi:/1999.7782065x

Mader, T. L., S. M. Holt, G. L. Hahn, M. S. Davis, and D. E. Spires. 2002. Feeding strategies for managing heat load in feedlot cattle. J. Anim. Sci. 80:2373-2382

Mader, T. L. 2003. Environmental stress in confined beef cattle. J. Anim. Sci. 80:E110-E119

Mader, T. L., M. S. Davis, and T. Brown-Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. J. Anim. Sci. 84:712-719. doi:/2006.843712x

Mader, T. L., M. S. Davis, and J. B. Gaughan. 2007. Effect of sprinkling on feedlot microclimate and cattle behavior. Int. J. Biometeorol. 57:541-551

Magdub, A., H. D. Johnson, and R. L. Belyea. 1982. Effect of environmental heat and dietary fiber on thyroid physiology of lactating cows. J. Dairy Sci. 65:223-2331

Mersmann, H. J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160-172. doi:/1998.761160x

Milligan, J. D., and G. I. Christison. 1974. Effects of severe winter conditions on performance of feedlot steers. Can. J. Anim. Sci. 54:605-610

Mitlöhner, F. M., J. L. Morrow, J. W. Dailey, S. C. Galyean. M. F. Miller, and J. J. McGlone. 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. J. Anim. Sci. 79:2327-2335. doi:/2001.7992327x

Mitlöhner, F. M., M. L. Galyean, and J. J. McGlone. 2002. Shade effects on performance, carcass traits, physiology, and behavior of heat-stressed feedlot heifers. J. Anim. Sci. 80:2043-2050

Moberg GP, Mench JA, editors. The biology of animal stress. Wallingford, Oxon, UK: CABI Publishing; 2000

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fiber and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. and Tech. 108:95-117

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutchenson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, and T. H. Montgomery. 2009. Dietary zilpaterol hydrochloride. I. feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87:1374-1383. doi:10.2527/jas.2008-1162

Morrison, S. R., R. L. Givens, and G. P. Lofgreen. 1973. Sprinkling cattle for relief from heat stress. J. Anim. Sci. 36:428-431

Moseley, P. L. 1997. Heat shock proteins and heat adaptation of the whole organism. J. Appl. Physiol. 83:1413-1417.

Olbrich, S. E., F. A. Martez, M. E. Tumbleson, H. D. Johnson, and E. S. Hilderbrand. 1972. Effects of constant environmental temperatures of 10 C and 31 C on serum biochemic and hematologic measurements of heat tolerant and cold tolerant cattle. Comp. Biochem. Physiol, 41A:255

Pusillo, G. M., M. P. Hoffman, and H. L. Self. 1991. Effects of placing cattle on feed at two-month intervals and housing on feedlot performance and carcass characteristics. J. Anim. Sci 69:443-450. doi:/1991.692443x

Robertshaw, D. 1985. Heat loss of cattle. Page 55-66 in Stress Physiology in Livestock. Vol. I: Basic Principles. M. K. Yousef, ed. CRC Press Inc., Boca Raton, FL.

Ryan, D. P., M. P. Boland, E. Kopel, D. Armstrong, L. Munyakazi, R. A. Godke, and R. H. Ingraham. 1992. Evaluating two different evaporative cooling management systems for dairy cows in a hot, dry climate. J. Dairy Sci. 75:1052-1059

Shaffer, L., J. D. Roussel, and K. L. Koonce. 1981. Effects of age, temperature-season, and breed on blood characteristics of dairy cattle. J. Dairy. Sci. 64:62-70

Sohail, M. U., Z. U. Rahman, A. Ijaz, M. S. Yousaf, K. Ashraf, T. Yaqub, H. Zaneb, H. Anwar, and H. Rehman. 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. J. Poultry Sci. 90:2573-2577

Sohail, M. U., M. E. Hume, J. A. Byrd, D. J. Nisbet, A. Ijaz, A. Sohail, M. Z. Shabbir, and H. Rehman. 2012. Effect of supplementation of prebiotic mannan-

oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poultry Sci. 91:2235-2240

Strydom, P. E., L. Frylinck, J. L. Montgomery, and M. F. Smith. 2009. The comparison of three β –agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. J. Meat Sci. 81:557-564. doi.org/10.1016/j.meatsci.2008.10.011

Tomaro M. L., and Batlle, A. M. C. 2002. Bilirubin: its role in cytoprotection against oxidative stress. International J. Biochem. & Cell Bio. 34:216-220

Tucker, C. B., A. R. Rogers, G. A. Verkerk, P. E. Kendall, J. R. Webster, and L. R. Matthews. 2007. Effects of shelter and body condition on the behavior and physiology of dairy cattle in winter. Applied Anim. Behaviour Sci. 105:1-13

Vasconcelos, J. T., T. J. Tathmann, R. R. Reuter, J. Leibovitch, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86:2005-2015. doi:10.2527/jas.2008-1032

Walsberg, G. E., G. S. Campbell, and J. R. King. 1978. Animal coat color and radiative heat gain: A re-evaluation. J. Comp. Physiol. 126:211-222

Welch, J. G. 1986. Physical parameters of fiber affecting passage from the rumen. J. Dairy Sci. 69:2750-2754

West, J. W., G. M. Hill, J. M. Fernandez, P. Mandebvu, and B. G. Mullinix. 1999. Effects of Dietary fiber on intake, milk yield, and digestion by lactating dairy cows during cool or hot, humid weather. J. Dairy Sci. 82:2455-2465

West. J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2131-2144

Yamaguchi, T., M. Terakado, F. Horio, K. Aoki, M. Tanaka, and H. Nakajima. 1996. Role of bilirubin as an antioxidant in an ischemia-reperfusion of rat liver and induction of heme oxygenase. Biochem. and biophys. Res. Comm. 223:129-135

Yansari, A. T., R. Valizadeh, A. Naserian, D. A. Christensen, P. Yu, and F. Eftekhari Shahroodi. 2004. Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. J. Dairy Sci. 87:3912-3924

Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. 52:154-163

Young, B. A. 1983. Ruminant cold stress: effect on production. J. Anim. Sci. 57:1601-1607

Yousef, M. K. 1985. Thermoneutral zone. In: Stress Physiology in Livestock. Vol. 1. pp. 67–74. CRC Press, Boca Raton, FL.

Yu, J., P. Yin, F. Liu, G. Cheng, K. Guo, A. Lu, X. Zhu, W. Luan, and J. Xu. 2010. Effect of heat stress on the porcine small intestine: A morphological and gene expression study. Comparative biochem, and Physiol. 156:119-128

CHAPTER II. Effects of shade and feeding zilpaterol hydrochloride to finishing steers on performance, carcass quality, heat stress, mobility, and body temperature

B. M. Boyd[*], S. D. Shackelford[§], K. E. Hales[§1], T. M. Brown-Brandl[§], M. L. Bremer[*], M. L. Spangler[*], T. L. Wheeler[§], D. A. King[§], and G. E. Erickson[*2]

[*] Department of Animal Science; University of Nebraska-Lincoln; Lincoln, NE 68583

[§] USDA, ARS, U.S. Meat Animal Research Center; Clay Center, NE 68933

[1] A contribution of the University of Nebraska Agricultural Research Division, supported in part by funds provided through the Hatch Act. Research partially supported by a grant from the Nebraska Beef Council. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer. The authors have no conflict of interest. The efforts of C. Engle, B. Johnson, L. McPhillips, C. Felber, D. Janssen, A. Menke, C. C. Row, J. Buntyn, R. Oglesbee, Z. Carlson and the entire feedlot crew at U.S. MARC are greatly appreciated.

[2] Corresponding authors: Kristin.Hales@ars.usda.gov and gerickson4@unl.edu

ABSTRACT

Steers (n = 480; 22% with black hides and 78% with red hides) were used to study the effects of shade and feeding zilpaterol hydrochloride (ZH) on performance, carcass quality, heat stress, mobility, and body temperature (BT). A randomized block design with a 2 × 2 factorial treatment arrangement was used with 4 replicates per treatment. Factors included housing type (open or shaded pens) and the feeding of ZH (0 or 8.33 mg/kg DM) the last 21 d on feed with a 3-d withdrawal. Cattle were blocked by BW into a heavy or light block and randomly assigned to pen within each block. Rumen boluses to record BT were inserted prior to ZH feeding. Respiration rate and panting scores were recorded daily during the ZH feeding period. Mobility scores were collected at various time points from before ZH feeding through harvest. Interactions between ZH and housing type were not significant ($P > 0.26$) for animal performance, carcass characteristics, and respiration or panting score. No differences ($P > 0.44$) were observed for DMI, ADG, or G:F on a live basis due to ZH; however, cattle fed in open pens tended ($P = 0.08$) to have a greater ADG than cattle in shaded pens. Cattle fed ZH had 14 kg heavier carcasses with larger LM area ($P < 0.01$) than control cattle. Respiration rates for cattle fed ZH were greater ($P = 0.05$) with no differences ($P = 0.88$) due to housing. Time affected ($P < 0.01$) mobility scores, with observations on the morning of harvest at the abattoir being the worst for all groups of cattle. An interaction ($P < 0.01$) was observed between ZH and housing type for BT. Cattle fed ZH, in both shaded and open pens, had lower ($P < 0.05$) average, maximum, and area under the curve BT than control cattle fed in the same housing type. However, the observed reduction in BT due to ZH was greater

for cattle fed ZH in open pens than for cattle fed ZH in shaded pens. From these results, we conclude that ZH improved HCW with little impact on heat stress or mobility suggesting that animal welfare was not affected by feeding ZH for 21 d at the end of the feeding period.

Keywords: body temperature, mobility, respiration rate, shade, zilpaterol hydrochloride

INTRODUCTION

Zilpaterol hydrochloride (ZH; Merck Animal Health; De Soto, KS) is a β-adrenergic agonist that was approved for feeding to beef cattle in the United States in 2006 (FDA, 2006). Zilpaterol hydrochloride was commonly used in the United States feedlot industry until August 2013. Recently, there have been concerns of animal welfare issues with the feeding of ZH, which resulted in it being removed from the market by the manufacturer. Beta-agonists work by binding to specific β-receptors on fat and muscle cell surfaces which modify biochemical processes of tissue growth by increasing lipolysis, decreasing lipogenesis, decreasing protein degradation, and increasing protein synthesis (Strydom et al., 2009).

Performance responses from feeding ZH during the end of the finishing phase are well characterized and clearly show beneficial responses in final BW, ADG, G:F, and HCW. A 15-kg increase in HCW along with increased dressing percentage and decreased USDA Yield Grade have been consistently observed when ZH was fed at the end of the feeding period (Vasconcelos et al., 2008; Lean et al., 2014; Hilscher et al., 2015).

However, there are few studies evaluating the effect of ZH on animal welfare issues, such as heat stress and mobility of cattle.

Hales et al. (2014) reported an increase in the slope of the regression lines for both panting score and respiration rate as day on ZH increased. However, this increase was not significant, and it is unclear whether this increase was caused by the addition of the β-agonist in the diet or some other variable. Research evaluating animal welfare and heat stress when ZH is supplemented is unavailable; therefore, the objective of this study was to further investigate the impact of feeding ZH on heat stress, mobility, and body temperature, in addition to performance and carcass characteristics for steers fed in open or shaded pens.

MATERIALS AND METHODS

This study was conducted in accordance with, and approved by, the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC, 2014).

Cattle

Four hundred eighty crossbred beef steers were fed at the U.S. Meat Animal Research Center (USMARC) feedlot near Clay Center, Nebraska. The steers were from the MARC II (¼ each Simmental, Gelbvieh, Hereford, and Angus) and MARC III (¼ each Pinzgauer, Red Poll, Hereford, and Angus) composite breed populations. The hide color distribution was 22% black and 78% red. The cattle with black hides were stratified equally across pens. Cattle were started on the experimental diet on January 2, 2014. The diet consisted of 57.35% dry-rolled corn, 30% wet distillers grains with

solubles, 8% alfalfa hay, 4.25% supplement, and 0.40% urea for all pens and treatments. When cattle were to receive ZH, enough type B supplement was added directly to the feed truck to feed all pens on a common treatment. The ZH was fed according to the label at 8.33 mg/kg DM (Merck Animal Health) and the inclusion rate was confirmed by laboratory testing by Merck. During the ZH feeding period, samples of the diet were collected each day. The samples were analyzed 2 times during the ZH feeding period and each time the samples were between 90 and 110% of the 8.33 mg/kg DM feeding recommendation.

Feed bunks were evaluated visually each day of the experiment at approximately 0630 h in order to determine the amount of diet each pen would receive. Feed bunks were managed so that less than 0.10 kg of DM per steer was remaining in the feed bunk at the time of evaluation. Separate trucks were used to feed the cattle to receive ZH and those on the control diet to prevent cross contamination.

Cattle were implanted with Revalor XS (200 mg trenbolone acetate, 40 mg estradiol 17β; Merck Animal Health) and BW was measured on January 28, 2014, using a single-animal scale at the start of the study. At this time, cattle were divided into 2 BW blocks. The blocks were based on differences in BW and were labeled heavy (block 1) or light (block 2); the weight difference between blocks was 53 kg unshrunk BW. Other factors such as sire line, dam line, pre-weaning ADG, and hide color were stratified across pens at this time. Then cattle were assigned to 16 soil-surfaced pens of 30 steers each. The pens were approximately 15.4 m x 61 m with 15.1 m of bunk space and a concrete apron extending 4.7 m from the bunk. This provided the cattle with 31.3 m^2 of

pen space and 0.5 m of bunk space per steer. Shade was provided in 8 of the 16 pens along both side fencelines and shared between adjacent pens. The artificial shade used during the study (Figure 1) was comprised of poles 10 m tall x 15.4 m long (Eigenberg et al., 2013). The north/south structures were equipped with four 15.4 lengths of poly snow-fence and provided an effective 50% shade coverage (Eigenberg et al., 2013). The shade structures tracked the sun during the day and offered 3 m^2 of shade per animal. The other 8 pens were unshaded and unprotected from environmental conditions. All pens were located in the center of the alley so that pens had cattle on either adjacent side and all shaded pens were shaded along both side fencelines.

Cattle in block 1 were fed ZH (21 d) beginning June 19, 2014, and ending July 10, 2014. After a 4-d withdrawal, block 1 cattle were transported to the abattoir on July 14, 2014, and they were harvested the following morning. Steers in block 2 were fed ZH for 21 d beginning July 18, 2014, and ending on August 8, 2014. After a 3-d withdrawal, cattle were transported to the abattoir on August 11, 2014, and they were harvested the following morning. For both blocks, prior to the initiation of the ZH feeding period, cattle were individually weighed and pen mobility scores collected as steers exited their pens to be moved to the processing facility. For pen mobility measurements, an individual would watch a pen of cattle move down the alley and use tick marks to denote the number of animals with a score of 1, 2, 3, or 4. Then, the number of steers with a score of 0 was calculated by difference. These measurements were collected the morning that steers were transported to the abattoir. On the day the cattle were weighed and samples were collected, personnel on horseback moved cattle from pens as a group; cattle were weighed individually, held as a group in a pen, and returned to their respective home pens

as a group. Throughout the experiment, cattle were allowed ad libitum access to water through automatic waters located in the fenceline and shared between 2 adjacent pens.

Sample Collection

On the final sampling day, cattle were removed from the pen and brought to the working facility. After sample collection, they were placed in different pens, but pen treatments were maintained. The new pens were near the cattle shipment facility where the cattle would be loaded onto trucks to curtail any additional stress that may have occurred by returning them to their home pen, and then removing them later that day to be loaded onto trucks. The average distance between the home pens and the working facility was 835 m. In these temporary pens, cattle had ad libitum access to water and were fed 75% of the feed call on the previous day. Later that day, cattle were loaded onto trucks at approximately 1730 h and held overnight at the abattoir for harvest the next morning. Antemortem inspection started at 0600 h and the cattle were harvested soon after. All cattle presented for antemortem inspection at the abattoir were cleared for harvest by a USDA veterinarian.

Experimental Design

The experiment was designed as a randomized block with 2 × 2 factorial arrangement of treatments. Factors consisted of housing type (shaded or open pens), and the inclusion of ZH at 0 or 8.33 mg/kg DM daily for the last 21 d of the finishing period with a 4 d (block 1) and 3-d (block 2) withdrawal prior to harvest. Cattle were blocked by initial BW and the other factors mentioned previously and assigned randomly to pen and pen was then assigned randomly to treatment. Dietary treatments were applied at the end

of the finishing period for both blocks and staggered so that cattle could be harvested in what was predicted to be the warmest weeks of summer (mid-July and early August). Four replications per treatment were used with a total of 16 pens. After block 1 cattle were harvested, block 2 cattle were then shifted into the 8 pens where block 1 cattle had been housed so the receivers for the rumen bolus system did not have to be relocated into new pens.

Heat Stress Measurements

Both blocks of cattle received a SmartStock (SmartStock, LLC, Pawnee, OK) temperature monitoring rumen bolus 5 d prior to the initiation of feeding ZH. The rumen boluses were set to record rumen temperature in 10-min intervals. The rumen temperatures were then transmitted from the boluses to a computer via a receiver located in the steer's home pen; thus, temperature recording stopped when steers left their home pens. Body temperature data were edited such that missing time points and drinking events were imputed using individual animal regressions between the nearest 2 time points on both sides of the missing body temperature (BT) or drinking event. This created a continuous set of data with individual BT in 10-min intervals for the duration of the observation period.

After an adaptation period to humans being near and in the pens prior to initiating ZH feeding, panting scores (0 = no panting; 1 = slight panting, mouth closed, no drool; 2 = fast panting, drool present, no open mouth; 3 = open mouth and excessive drooling, neck extended, head held up; 4 = open mouth with tongue fully extended for prolonged periods plus excessive drooling, neck extended, and head up), and respiration rates were

collected daily by trained individuals during the ZH feeding phase of the study starting at 1300 h and ending by 1530 h. Respiration rates were recorded as the amount of time it took the steer to take 10 breaths, and these data were then used to calculate breaths/min. Panting scores and respiration rates were collected between June 20, 2014, and July 13, 2014, for block 1 and between July 19, 2014, and August 10, 2014, for block 2. Prior to ZH feeding, one-half of the cattle in each pen were selected and identified with a uniquely colored ear tag. One-half of the steers in each pen were evaluated individually on a daily basis such that each one-half of the steers in each pen were evaluated every other day. Panting scores and respiration rates were collected by a team of 2 people, and the first pen observed was rotated daily to minimize time of day effects.

Mobility and Carcass Data

Mobility scores were evaluated 10 times throughout the ZH feeding period. These scores were based on the 0 to 4 Tyson mobility scoring system (Tyson Foods; Springdale, AR). In the mobility system, 0 = normal; 1 = mildly lame; 2 = moderately lame; 3 = severely lame and reluctant to move; and 4 = non-ambulatory/severe distress. The mobility observations were made when cattle were leaving their home pens on weigh/data collection days, as they were loaded onto the truck leaving the feedlot, during unloading at the abattoir, and as they were moved into holding pens at the abattoir. On the day of harvest, mobility scores were evaluated during antemortem inspection, as cattle left the holding pen, and as cattle were moved to the restrainer.

On the day of harvest, HCW and harvest order were recorded. After a 48-h chill, LM area, 12^{th} rib fat thickness, and marbling score were determined by USMARC

personnel using the VBG2000 beef grading camera (Shackelford et al., 2003). Yield grade was calculated (2.5 + (6.35x 12th rib fat) + (0.2 x 2.5[KPH]) + (.0017 x HCW) – (2.06 x LM Area)) for each individual steer and then averaged within pen (USDA, 1997). Dressing percent was calculated for each pen by dividing HCW by final live BW using a 4% shrink.

Statistical Analysis

Performance data (ADG, DMI, G:F, and initial and final live BW) and carcass characteristics (HCW, LM, 12th rib fat, marbling score, and USDA Yield Grade) were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The model included fixed effects of block, dietary treatment (ZH fed or not), housing type (open or shaded pen), and the interaction between dietary treatment and housing type.

Respiration rate was analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with pen as the experimental unit. The model included fixed effects of block, dietary treatment (ZH fed or not), housing type (open or shaded pen), their interaction, and a random residual. Interactions involving time were not significant and thus they were removed from the model. To account for the inherent covariance structure between sequential respiration rate measures, the residual was fitted with a covariance pattern within pen and a covariance of 0 across pens. Multiple covariance patterns were investigated and autoregressive 1 was chosen based on Akaike's information criteria.

Mobility scores were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with pen as the experimental unit. Covariance structure was assessed using

the same methods as the model for respiration rate and the variance components covariance structure was determined to be the best fit. The model included fixed effects of dietary treatment (fed ZH or not), time point of observation, housing type (open or shaded pen), the interaction of dietary treatment and time, and the interaction of dietary treatment and housing type. Interactions for housing type and time were not significant and were therefore removed from the model. Mobility scores were collected from multiple steers within each pen during each time point. The number of steers within each pen with a given mobility score was tallied for each collection point and divided by the number of steers in each pen to create a percentage of the pen with each given mobility score. A percentage was used rather than the number of steers because of death loss (2/480) prior to initiation of ZH feeding. Four time points were then created: before ZH, after ZH, arrival at the abattoir, and time of harvest. One steer was scored as a 3 prior to initiation of ZH feeding, but subsequent scores for this steer improved and consequently this score was removed from the analysis for the earliest time point. We speculate that this steer had foot rot or some other condition that was not relevant to ZH feeding and dissipated by the end of the study. Consequently, frequencies reported are scores of 0, 1, and 2. This scale was used because no steers received a score greater than 2 with the exception of the single steer prior to initiation of the study.

Chute exit speeds were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with pen as the experimental unit. Covariance structure was assessed using the same methods as that of mobility scores and an unstructured covariance structure was determined to best fit the data. The model included the fixed effects of block, dietary treatment (fed ZH or not), time of observation, housing type (shaded or open), the

interaction of dietary treatment and time, and the interaction of dietary treatment and housing type. Interactions for housing type and time were not significant and therefore removed from the model. Prior to analysis, data from steers that stopped walking before crossing the second sensor (the sensors were 7.92 m apart), were removed (n = 75; 7.84%). The number of steers that stopped walking before crossing the second sensor ranged from 5 to 8 per weigh day and were evenly distributed across treatment.

Body temperature was analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with steer as the experimental unit. Covariance structure was assessed using the same methods as the model for respiration and mobility data and the variance components covariance structure was determined to be the best fit for the data. All interactions were analyzed and only those that were significant remained in the model. The model included the fixed effects of day, dietary treatment (fed ZH or not), housing type (open or shade), the interaction of housing type and time, and the interaction of dietary treatment and housing type and the random animal effect and residual. Body temperature measurements were characterized as 4 different phenotypes. The average, maximum, and area under the curve were evaluated. Average and maximum values were calculated on a daily basis. Area under the curve was approximated each day for each individual steer by summing all the temperature points for each day.

Two periods from each block were determined to be relevant heat stress periods using the adjusted temperature humidity index (THI; Mader et al., 2006; Table 1). There was a 2-d and a 3-d period chosen for each block when the daily adjusted THI was the greatest. These 2 hot periods were analyzed separately using the same procedure

described above. Period × ZH and period × housing interactions were not significant (P > 0.40) and thus the effect of period was removed from the model. Steers that lost a bolus and had incomplete data during the observation period or died during the trial (2 steers; 1 open lot control steer, 1 shaded control steer) were removed prior to analysis.

RESULTS

Performance and carcass data are summarized in Table 2. There were no ZH × housing type interactions (P > 0.26) for performance or carcass characteristics (Table 2). Initial BW was not different between dietary treatments or between housing types (P > 0.24). Final live BW was not different between the control and ZH fed cattle (P = 0.43); however, there was a tendency for cattle fed in open lot pens to have a greater final live BW than cattle fed in shaded pens (P = 0.08). Moreover, ADG did not differ between control fed and ZH fed cattle (P = 0.56), but cattle fed in open pens tended to have a greater ADG than cattle fed in shaded pens (P = 0.10). Dry matter intake and G:F was not affected by dietary treatment or housing type (P > 0.39).

Hot carcass weight, dressing percent, and LM area were greater for cattle fed ZH than the control diet (P < 0.01). Nevertheless, there was no difference in HCW, dressing percent, and LM area for cattle fed in shaded vs. open pens (P > 0.17). Twelfth rib fat thickness and marbling scores were not different between dietary treatments or housing types (P > 0.15). Control cattle had a greater USDA Yield Grade compared to cattle fed ZH (P < 0.01), but no differences in USDA Yield Grade were detected between housing types (P = 0.89).

No ZH × housing type interactions were detected ($P > 0.31$) for respiration rates or panting scores (Table 2). Cattle fed ZH had greater respiration rates than cattle fed the control diet ($P < 0.05$), yet respiration rates were not different between housing types ($P = 0.88$). There was a tendency for cattle fed ZH to have a greater panting score than the control cattle ($P = 0.10$), but panting scores were not different between housing types ($P = 0.99$).

There was no ZH × housing or ZH × time interactions observed for mobility score ($P > 0.14$). Consequently, only the main-effect means of dietary treatment and time for mobility are presented in Tables 3 and 4, respectively. There was no difference in mobility between the control cattle and ZH fed cattle for the percentage of steers scoring 0 ($P = 0.91$) or 0 and 1 ($P = 0.21$; Table 3). No steers during the study received a mobility score of 4 or 5 at any time, and only the one steer previously discussed received a mobility score of 3. There were no ZH × housing or ZH × time interactions for chute exit speed ($P > 0.48$; data not shown), and cattle fed the control diet vs. cattle fed the diet containing ZH did not differ in chute exit speed ($P = 0.68$; Table 3).

The effect of time was significant ($P < 0.01$) on overall cattle mobility, in that cattle were more mobile early in the feeding period, but mobility decreased over time until harvest (Table 4). Additionally, time affected chute exit velocities with cattle taking more time to travel 7.93 m at the end of the ZH feeding period than before ($P < 0.01$). Even though there was not a significant time × ZH interaction ($P > 0.14$), the effects of ZH feeding across time are presented in Table 4. At the beginning of the trial, cattle assigned to the ZH treatment had a tendency ($P = 0.07$) to have a greater proportion of

steers that scored 0 (more mobile) than cattle in the control treatment. Likewise, there was a tendency ($P = 0.06$) for cattle fed ZH to have a lesser proportion of cattle that scored 0 and 1 (less mobile) than the cattle in the control group at the point where cattle were going to the restrainer. For the remainder of the time points, there were no differences between the ZH and control treatments ($P > 0.16$) in the number of steers scoring 0 or 0 and 1. Furthermore, housing did not have an effect on mobility ($P > 0.70$; data not presented).

Zilpaterol hydrochloride × housing type interactions were observed for BT (Table 5; $P < 0.01$). Feeding ZH in open and shaded pens decreased BT relative to the control group ($P < 0.01$). Cattle fed ZH in open pens had the lowest average BT followed by cattle fed ZH in shaded pens, control cattle in shaded pens, and control cattle in open pens ($P < 0.05$). Maximum BT followed this same pattern ($P < 0.05$). Area under the curve and the average magnitude of BT each day, also followed the same pattern as average and maximum BT. A housing × time interaction was observed for all body temperature measures ($P < 0.03$; data not reported). There was no difference ($P > 0.05$) between housing type for maximum, average, and area under the curve for most days; however, there was a difference ($P < 0.05$) for a few days (n = 4, 6, and 6 d, respectively) during the feeding period where cattle fed in open pens had lower values than cattle fed in shaded pens leading to the interaction.

The hot periods were defined by the greatest adjusted THI for a period of 2 or 3-d (Table 1). During these hot periods, an interaction between ZH and housing type (Table 6; $P < 0.05$) was observed for average BT and area under the curve BT; cattle fed ZH in

open pens had the lowest BT followed by cattle in shaded pens, both ZH and control, and control cattle in open pens having the greatest BT ($P < 0.05$). For maximum BT, cattle fed ZH in open pens and in shaded pens were not different ($P = 0.52$) and had the lowest maximum BT, followed by control cattle fed in shaded pens, and control cattle in open pens with the greatest values ($P < 0.05$).

DISCUSSION

The effect of ZH on performance and carcass characteristics has been well documented. Vasconcelos et al. (2008) observed no increase in final live BW between the control group and the average of the other 3 treatments fed ZH over varying lengths of time. Although final live BW was not affected by feeding ZH, HCW was increased, which is consistent with results from the present study. Furthermore, Hilscher et al. (2015) did not observe an increase in final live BW or ADG for cattle fed ZH vs. control. In contrast, Elam et al. (2009) reported an increase in final live BW in addition to increased HCW when cattle were fed ZH. In our study, cattle fed ZH had numerically greater final live BW which was similar to other literature reports (Vasconcelos et al., 2008; Hilscher et al., 2015).

Montgomery et al. (2009) observed a 0.47 kg/d increase in ADG, and a 0.056 kg/kg increase in G:F, and a tendency for decreased DMI for steers fed ZH for 20-d. Likewise, Hales et al. (2014) observed no reduction in DMI in response to ZH, but there was an increase of 0.80 kg/d of ADG and a 0.016 kg/kg increase in G:F over the entire feeding period when ZH was fed for 21 d. In the present study, no effect on DMI, ADG, or G:F were noted. Similarly, Hilscher et al. (2015) noted no differences in DMI or ADG

for cattle fed ZH, but did note increased G:F over the entire feeding period for cattle fed ZH. Furthermore, Baxa et al. (2010) reported no difference in DMI for cattle fed ZH. Conversely, in a meta-analysis conducted by Lean et al. (2014), a live BW increase of 8 kg was observed along with a reduction in DMI of 0.12 kg/d and an increase of 0.15 kg/d in ADG across numerous studies feeding ZH. However, responses in DMI, ADG, and final live BW has been variable in available literature.

The 14-kg increase in HCW noted in the present study is consistent with the findings of other literature. Elam et al. (2009) observed a 14-kg increase in HCW when cattle were supplemented with ZH for 20, 30, or 40 d. In addition, Montgomery et al. (2009) reported a 13-kg increase in HCW as well as a 1.3% increase in dressing percent, and a 7.9 cm^2 increase in LM area. Additionally, Montgomery et al. (2009) reported a 0.38 unit decrease in USDA Yield Grade which is consistent with the results from the present study. Hilton et al. (2009) observed an increase in LM area and decreased USDA Yield Grade when ZH was fed. This is further supported by findings of Hilscher et al. (2015) that reported a 13-kg increase in HCW along with a 7.3 cm^2 increase in LM area and a 0.67 unit decrease in USDA Yield Grade when ZH was fed. Additionally, a numerical decrease in marbling score and 12^{th} rib fat was observed in the present study for ZH fed cattle, which is consistent with other research (Vasconcelos et al., 2008; Hilton et al., 2009; Montgomery et al., 2009). The HCW and dressing percentage results in the present study are consistent with that of Lean et al. (2014) which reported a 15-kg increase in HCW and 1.7% increase in dressing percentage across a minimum of 27 studies.

Summer conditions consisting of above normal ambient temperature, relative humidity, and solar radiation can increase an animal's heat load resulting in decreased performance, decreased animal comfort, and eventually death of the animal (Mader et al., 2006). One of the objectives of the present study was to evaluate the effects of feeding ZH during summer heat stress conditions and determine if the severity of heat stress worsened. Increased respiration rates were observed in the present study with a tendency for increased panting scores in cattle fed ZH compared to the control. These data are consistent with the ZH feed label (Merck Animal Health) that states increased respiration rates may be observed in conjunction with ZH feeding. Hales et al. (2014) observed a positive slope in the regression line for panting score and respiration rates as days fed ZH increased suggesting that both measures increased as days on ZH increased. Although not significant, Hales et al. (2014) reported that cattle fed ZH had a numerically greater respiration rate and panting scores consistent with the findings in the present study. Whether or not this increase is due to a greater amount of heat load on the animal with the increased muscle mass or an unobserved biological effect of increased metabolism due to feeding ZH is not well understood.

Although respiration rates and panting scores were increased in cattle fed ZH, the average and maximum BT were lower for cattle fed ZH than for the control group, for both open and shaded housing, which contradicts the theory that feeding a β-agonist increases the heat load on the animal. For cattle not fed ZH, shaded cattle had lower average and maximum BT than cattle in open lots, which is consistent with data reported by Gaughan et al. (2010) suggesting that shade decreases BT. Conversely, in the present study when ZH was fed in open lots, cattle had lower average and maximum BT than

cattle in shaded pens. Many studies have reported decreases in DMI associated with feeding ZH. Potentially, the decreased BT could be associated with the heat of fermentation in the rumen because BT was measured via rumen bolus. Even though no differences in DMI were observed with feeding ZH, DMI was numerically lower over the entire feeding period. However, there are very few studies evaluating the effect of β-agonists on heat stress potential of cattle and the summer conditions during this trial were relatively mild. It is possible, that different responses to ZH would be observed under conditions of harsher heat stress.

With the lower BT observed for cattle fed ZH vs. that of the control group, it can be speculated that the increase in respiratory rate is a side effect of feeding ZH rather than a response to increased heat load. Although data does not exist in the literature to directly support this, Finch (1986) suggested that 15% of heat loss in cattle under high heat loads is lost directly through the respiratory tract. The mechanism by which respiration rate is increased in cattle fed ZH is not well understood. However, the fact that lower BT were observed in cattle fed ZH would suggest that the observed increase in respiration rate is not a correlated biological response of cattle attempting to moderate BT. A review by Mersmann (1998), suggested that the addition of an orally administered β-adergenic agonist could increase blood flow to skeletal muscle and adipose tissues. Finch (1986) noted that under conditions of high heat load, only about 15% of the animals heat load is lost directly from the core of the animal through the respiratory tract. Therefore, the bulk of heat loss must be transferred to the skin and be lost through conduction, convection, or evaporation off the body surfaces through sweating (Finch, 1986). Blackshaw et al. (1994) noted that transfer of heat from the body core depends on blood flow to the skin.

If feeding a β-agonist increases blood flow to the skin as observed by Mersmann (1998), it can be speculated that with more blood being transferred away from the core of the body to muscle and fat, this could aid in cooling the animal and lead to decreased body temperatures through conductive heat loss although there is no direct evidence of this in literature.

There is very little published data on cattle mobility as impacted by ZH feeding. However, Bernhard et al. (2014) noted that ZH had no effect on chute exit speed or mobility score, which is consistent with the findings of the present study. As time progressed from starting ZH to the day of harvest, when cattle were going up to the restrainer at the abattoir, mobility decreased across all groups of cattle. After arrival at the abattoir, the number of 0 mobility scores decreased by 2.6% when mobility was measured near the holding pens. Furthermore, between arrival at the abattoir and harvest the next morning, the number of animals with a mobility score of 0 decreased by an additional 3.2%. Combined, these data suggest that cattle mobility decreases as cattle gain weight and that transport and standing on concrete at the abattoir further exacerbates this issue. However, further research may help better explain the mechanism by which mobility is decreased. It is important to note that mobility score measurements are subjective and that scores taken at the feedlot were on soil surfaces, whereas at the packing plant these scores were taken on concrete which can affect the way an animal appears to walk. Also, all cattle passed antemortem inspection by a USDA veterinarian; no welfare or health concerns were noted for any of the cattle.

The effects of heat stress on BT, panting score, respiration rate, and animal performance of cattle has been well documented in literature. Mader et al. (1999) suggested an effective means of helping animals maintain temperature regulation in hot environments is to reduce incoming thermal radiation by providing shade. Gaughan et al. (2010) evaluated the effects of shade on BT and reported that during a severe heat event, shade decreased cattle BT by 2.3%. In the present study, for cattle not fed ZH there was a slight decrease in average and maximum BT for shaded cattle when compared to the open lot cattle but only by approximately 0.1%. This could be due in part to the mild summer conditions experienced during this study with the majority of day adjusted THI (Mader et al., 2006) falling in or below the alert category of 75 to 78 (Table 1). Gaughan et al. (2010) reported no difference in BT for shaded and unshaded cattle for the first period before the heat wave, which suggests that BT is well regulated and shade is only beneficial in reducing BT during severe heat episodes. Panting scores and respiration rates were not different for shaded and unshaded cattle in the present study further suggesting the shade used was not effective at mitigating heat stress in the absence of a heat wave. Mitlöhner et al. (2001) observed a 29% decrease in respiration rates for shaded cattle over that of unshaded cattle which is consistent with other research (Gaughan et al., 2004; Brown-Brandl et al., 2005).

The shades used in the present study were made of layered snow fence. A study conducted by Eigenberg et al. (2010) concluded that using snow fence as a shade material may not be the most effective means of providing shade, but it is one of the most cost effective shade materials and does reduce respiration rates compared to cattle in open pens without shade. In the present study, minimal production response to shade was

observed. Mader et al. (1997) suggested that if shade structures are not adequate, then any positive production response of shade will be lost. Although, the type of shade used has been shown by Eigenberg et al. (2010) to reduce respiration rates when compared to unshaded cattle, it was the least effective of all the materials observed potentially resulting in the lack of shade response noted in the present study.

The effect of shade on animal performance has been well documented. Gaughan et al. (2010) observed an increase in final live BW, ADG, DMI, and G:F for cattle fed in shade vs. cattle fed in an open lot system. In the present study, there were no differences observed for any animal performance or carcass characteristics between shaded and unshaded cattle, further suggesting that the shade provided was inadequate or heat stress was insufficient to hinder performance. However, Pusillo et al. (1991) suggested that DMI for cattle in the latter stages of the feeding period exposed to Midwestern climatic conditions are relatively unaffected by presence or absence of overhead shelter. Similarly, Bond and Laster (1975) reported that cattle ADG and G:F during Midwestern summers are unaffected by having access to shade or not. This could suggest that the cattle were simply not heat stressed enough to benefit from having access to shaded pens. Additionally, as G:F and DMI for the present study was calculated for the entire feeding period starting in January, and the effect of heat stress and shade may have been masked by the winter and spring months. As no increase in performance was observed in the present study, this could suggest that with mild environmental conditions the shade constructed of snow fence that was used in the present study may have little effect on cattle performance.

In the present study, the use of ZH for 21 d at the end of the feeding period increased HCW, dressing percent, LM area, and improved yield grade with little effect on live animal performance. Shade used in the present study had little effect on cattle performance or carcass characteristics. Although respiration rates and panting scores were, or had a tendency, to be greater for cattle fed ZH, average and maximum BT for cattle fed ZH were lower than that of the control. This suggests that the inclusion of ZH had little impact on the heat load experienced by the animal. Overall no impact was observed for feeding ZH on cattle mobility; however with time, mobility decreased for all cattle up until harvest. Based on the observations in this study, it can be concluded that the use of ZH improved carcass characteristics with little impact on heat stress or mobility suggesting that animal welfare was not affected by feeding ZH during the last 21 d of the feeding period.

LITERATURE CITED

Baxa, T. J., J. P. Hutchenson, M. F. Miller, J. C. Brooks, W. T. Nichols, M. N. Streeter, D. A. Yates, and B. J. Johnson. 2010. Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers. J. Anim. Sci. 88:330-337. doi:10.2527/jas.2009-1797

Bernhard, B. C., C. L. Maxwell, C. F. O'Neill, B. K. Wilson, C. G. Hixon, C. L. Haviland, A. N. Grimes, M. S. Calvo-Lorenzo, C. J. Richards, D. L. Step, B. P. Holland and C. R. Krehbiel. 2014. The effects of technology use in feedlot production systems on the performance, behavior and welfare of steers finishing during late summer. In: Proceedings of the Plains Nutrition Council Spring Conference, San Antonio, TX. p. 142.

Blackshaw, J. K., and A. W. Blackshaw. 1994. Heat stress in cattle and the effect of shade on production and behavior: a review. Aust. J. Exp. Agri. 34:285-295.

Bond, T. E., and D. B. Laster. 1975. Influence of shading on production of Midwest feedlot cattle. Trans. ASAE 18:957-959.

Brown-Brandl, T. M., R. A. Eigenberg, J. A. Nienaber, and G. L. Hahn. 2005. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, part 1 analyses of indicators. Biosys. Eng. 90:451-462. doi:10.1016/j.biosystemseng.2005.02.001

Bruneel, A., M. Dehoux, A. Barnier, A. Boutten. 2013. External evaluation of the dimension vista 1500 intelligent lab system. J. of Clinical Laboratory Analysis. 26:384-397

Eigenberg, R. A., T. M. Brown-Brandl, and J. A. Nienaber. 2010. Shade material evaluation using a cattle response model and meteorological instrumentation. Int. J. Biometeorol. 54:509-515. doi: 10.1007/s00484-010-0343-z.

Eigenberg, R. A., T. M. Brown-Brandl, and M. D. Hayes. 2013. Shade structure design and evaluation. ASABE Meeting Presentation no 131595866. Am. Soc. Agric. Biol. Eng. Kansas City, MO.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87:2133-2141. doi:10.2527/jas.2008-1563

FDA. 2006. Freedom of information summary. Original new drug application NADA 141-258. Zilpaterol hydrochloride (ZILMAX). Type A medicated article for cattle fed in confinement for slaughter. Found at: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIDrugSummaries/ucm051412.pdf. (Accessed 10 February 2015.)

Finch, V. A. 1986. Body temperature in beef cattle: its control and relevance to production in the tropics. J. Anim. Sci. 62:531-542. doi:10.2134/jas1986.622531x

Gaughan, J. B., L. A. Tait, R. Eigenberg, and W. L. Bryden. 2004. Effect of shade on respiration rate and rectal temperature of Angus heifers. Aust. Soc. Anim. Prod. 25:69-72.

Gaughan, J. B., S. Bonner, I. Loxton, T. L. Mader, A. Lisle, and R. Lawrence. 2010. Effect of shade on body temperature and performance of feedlot steers. J. Anim. Sci. 88:4056-4067. doi:10.2527/jas.2010-2987

Hales, K. E., S. D. Shackelford, J. E. Wells, D. A. King, M. D. Hayes, T. M. Brown-Brandl, L. A. Kuehn, H. C. Freetly, and T. L. Wheeler. 2014. Effects of feeding dry-rolled corn-based diets with and without wet distillers grains with soulubles and zilpaterol hydrochloride on performance, carcass characteristics and heat stress in finishing steers. J. Anim. Sci. 92:4023-4033. doi:10.2527/jas.2014-7638

Hilscher, Jr. F. H., E. M. Hussey, B. L. Nuttelman, D. B. Burken, W. A. Griffin, K. J. Vander Pol, J. P. Hutcheson, and G. E. Erickson. 2015**.** Impact of sorting before feeding zilpaterol hydrochloride on feedlot performance and carcass characteristics of yearling steers. J. Anim. Sci. 93:2285-2296. doi:10.2527/jas.2014-8579

Hilton, G. G., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutchenson, W. T. Nichols, M. N. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Effects of feeding zilpaterol hydrochloride with and without monensin and tylosin on carcass cutability and meat palatability of beef steers. J. Anim. Sci. 87:1394-1406. doi:10.2527/jas.2008-1170

IACUC. 2014. Policies and Procedures Manual for the Use of Animals at the University of Nebraska-Lincoln. Number 988.

Lean, J. I, J. M. Thompson, and F. R. Dunshea. 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance traits and shear strength of meat in cattle. PLoS One 9(12):e0115904. doi: 10.1371/journal.pone.0115904

Mader, T. L., J. M. Dahlquist, and J. B. Gaughan. 1997. Wind protection effects and airflow patterns in outside feedlots. J. Anim. Sci. 75:26-36. doi:/1997.75126x

Mader, T. L., J. M. Dahlquist, G. L. Hahn, and J. B. Gaughan. 1999. Shade and wind barrier effects on summertime feedlot cattle performance. J. Anim. Sci. 77:2065-2072. doi:/1999.7782065x

Mader, T. L., M. S. Davis, and T. Brown-Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. J. Anim. Sci. 84:712-719. doi:/2006.843712x

Mersmann, H. J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160-172.

Mitlöhner, F. M., J. L. Morrow, J. W. Dailey, S. C. Galyean. M. F. Miller, and J. J. McGlone. 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. J. Anim. Sci. 79:2327-2335. doi:/2001.7992327x

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutchenson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, and T. H. Montgomery. 2009. Dietary zilpaterol hydrochloride. I. feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87:1374-1383. doi:10.2527/jas.2008-1162

Pusillo, G. M., M. P. Hoffman, and H. L. Self. 1991. Effects of placing cattle on feed at two-month intervals and housing on feedlot performance and carcass characteristics. J. Anim. Sci 69:443-450. doi:/1991.692443x

Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 2003. Online prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade, and marbling score using the MARC beef carcass image analysis system. J. Anim. Sci. 81:150-155. doi:/2003.811150x

Strydom, P. E., L. Frylinck, J. L. Montgomery, and M. F. Smith. 2009. The comparison of three β –agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. J. Meat Sci. 81:557-564. doi.org/10.1016/j.meatsci.2008.10.011

USDA, 1997. Official United States standards for grades of carcass. Beef. Agric. Marketing Serv., USDA, Washington, DC.

Vasconcelos, J. T., T. J. Tathmann, R. R. Reuter, J. Leibovitch, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing

diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86:2005-2015. doi:10.2527/jas.2008-1032

Table 1. Adjusted temperature humidity index (THI), temperature, and humidity during the zilpaterol hydrochloride (ZH) treatment phase of both blocks of cattle

Day[1]	Block 1			Block 2		
	Adjusted THI[2]	Temperature (◦C)	Humidity (%)	Adjusted THI[2]	Temperature (◦C)	Humidity (%)
1	69.9	22.2	69.2	61.3	17.5	86.0
2	70.2	24.4	69.0	65.6	19.9	84.9
3	69.6	22.7	78.6	70.9	24.8	78.5
4[3]	71.4	22.4	79.1	77.8	29.4	67.4
5[3]	69.2	20.6	79.9	76.5	26.1	73.5
6[3]	69.1	19.9	86.1	71.3	22.0	75.8
7	71.9	22.0	78.6	68.2	23.4	75.5
8[3]	68.9	22.0	83.7	77.2	27.7	70.1
9[3]	65.2	21.7	90.9	78.9	26.6	83.4
10	67.0	20.8	84.3	70.1	22.5	68.9
11	72.1	23.8	85.6	66.7	18.9	75.7
12	70.9	23.5	79.2	68.6	19.8	74.8
13	62.7	19.0	70.9	69.1	20.2	68.7
14	60.4	16.5	71.0	68.4	21.0	70.2
15	63.0	18.6	73.5	68.4	20.7	75.6
16	63.9	21.6	71.0	70.3	21.8	75.3
17[4]	73.9	25.0	77.7	72.9	23.1	72.1
18[4]	78.0	26.2	75.4	74.0	23.6	79.8
19[4]	72.7	23.4	73.9	73.7	24.5	77.5
20	67.3	20.5	74.0	72.1	22.9	85.7
21	66.4	20.0	78.9	72.4	22.2	86.1
22	68.1	22.3	81.0	71.7	22.2	84.2
23[4]	73.5	24.5	80.1	68.4	20.9	88.4
24[4]	74.3	24.1	78.8	71.4	21.9	81.8
25	75.1	23.9	77.5	N/A	N/A	N/A

[1]Day since cattle started on the ZH treatment. Day 21 to 25 is withdrawal period.

[2]Adjusted Temperature Humidity Index calculated as (0.8 x ambient temperature + [(% relative humidity /100) x (ambient temperature – 14.4)] +46.4) + (4.51 – (1.992 x wind speed) + (0.0068 x solar radiation)] Where > 84 is emergency, 79 to 84 is danger, 74 to 79 is alert, and < 74 is normal (Mader et al., 2006).

[3]Days used as hot period for block 2.

[4]Days used as hot period for both blocks 1.

Table 2. Main-effect means of zilpaterol hydrochloride (ZH) feeding and housing type on performance and carcass characteristics of finishing beef steers

	Open		Shade		*P* - Value			
Item	Control	ZH	Control	ZH	Diet[1]	Housing[2]	Interaction	SEM[3]
Performance								
Initial BW, kg	360	362	358	359	0.37	0.24	0.72	3.1
Final BW, kg	645	649	635	640	0.43	0.08	0.90	7.6
DMI, kg/d	9.9	9.6	9.6	9.7	0.61	0.55	0.26	0.21
ADG, kg	1.58	1.58	1.52	1.55	0.56	0.10	0.68	0.034
G:F, kg/kg	0.160	0.164	0.159	0.160	0.44	0.39	0.53	0.0020
Carcass characteristic								
HCW, kg	410	425	406	418	<0.01	0.17	0.61	8.1
Dressing %	63.5	65.6	63.9	65.3	<0.01	0.78	0.29	0.30
LM Area, cm^2	89.0	96.12	88.3	93.9	<0.01	0.27	0.59	0.20
12^{th} Rib Fat, cm	1.64	1.59	1.63	1.52	0.15	0.39	0.54	0.020
Marbling score[4]	473	470	478	466	0.50	0.92	0.67	10.0
USDA Yield Grade[5]	3.6	3.2	3.5	3.2	<0.01	0.89	0.68	0.09
Non-performance characteristics								
Respiration (breaths/min)	92.9	99.7	91.8	101.9	0.05	0.88	0.69	5.82
Panting score[6]	0.59	0.64	0.52	0.72	0.10	0.99	0.31	0.107

[1]Main effect of ZH inclusion.

[2]Main effect of housing type.

[3]Pooled standard error of simple effects means, n = 4 pens/mean.

[4]300 = slight, 400 = Small, 500 = Modest.

[5]Calculated as 2.5 + (6.35 x 12^{th} rib fat) + (0.2 x 2.5[KPH]) + (.0017 x HCW) – (2.06 x LM Area) (USDA, 1997).

[6]Panting scores based on 0 to 4 scale with 0 = no panting and 4 = severe distress.

Table 3. Main effect of zilpaterol hydrochloride (ZH) on mobility score calculated as the proportion of animals in a treatment that received the score[1]

Item	Control	ZH	SEM[2]	*P*-value
0 score	90.49	90.63	0.808	0.91
0 and 1 score [3]	99.00	98.44	0.344	0.21
CES[4]	4.94	5.02	0.145	0.68

[1]Mobility scores are based on the Tyson mobility scoring system where 0 is no lameness and 4 is non-ambulatory.

[2]Pooled standard error of main-effect means, n = 8 pens/mean.

[3]The percentage of animals receiving a score of 0 or 1 added together. The percentage of animals that scored a 2 can be calculated as 100% - the percent of 0 and 1 scores together.

[4]CES = Chute exit speed reported as seconds to travel 7.93 m.

Table 4. Zilpaterol hydrochloride (ZH) effect on percentage of animals in a treatment with a given mobility score at different time points[1]

Score	Control				Zilpaterol Hydrochloride				SEM[2]	*P* -value				
	Before ZH	After ZH	Unloading at plant	Up to restrainer	Before ZH	After ZH	Unloading at plant	Up to restrainer		Before ZH[3]	After ZH[3]	Unloading at plant[3]	Up to restrainer[3]	Int[4]
0	93.3	91.1	89.4	87.3	96.3	90.5	87.4	83.6	1.88	0.07	0.79	0.39	0.16	0.14
0 and 1[5]	98.8	99.6	99.0	98.1	99.2	99.2	98.0	95.7	1.01	0.54	0.48	0.26	0.06	0.49
CES[6]	4.60	5.28	-	-	4.70	5.35	-	-	0.15	0.66	0.75	-	-	0.84

[1]Mobility scores are based on the Tyson mobility scoring system where 0 is no lameness and 4 is non-ambulatory.

[2]Pooled standard error of the simple effect means, n = 8 pens/mean.

[3]Main effect of ZH.

[4]Time × ZH interaction.

[5]The number of animals receiving a mobility score of 0 or 1 added together. The percentage of animals that scored a 2 can be calculated as 100% - the percent of 0 and 1 scores together.

[6]CES = Chute exit speed; reported as seconds to travel 7.93 m.

Table 5. Simple-effect means for cattle body temperature (BT) observed during the presence of a zilpaterol hydrochloride (ZH) × housing interaction

	Open		Shade			*P* - values		
Measurement	Control	ZH	Control	ZH	SEM[1]	Diet[2]	Housing[3]	Interaction
Average BT, ˚C	39.13-d	38.98[a]	39.10[c]	39.08[b]	0.011	<0.01	<0.01	<0.01
Maximum BT, ˚C	40.31[d]	40.12[a]	40.26[c]	40.17[b]	0.016	<0.01	0.99	<0.01
AUC BT[4]	14,752[d]	14,711[a]	14,743[c]	14,738[b]	1.6	<0.01	<0.01	<0.01

[a,b,c]Values within rows with unique superscripts differ $P < 0.05$.
[1]Pooled SEM, n = 4 pens/mean.
[2]Main effect of ZH.
[3]Main effect of housing type.
[4]AUC = Area under the curve

Table 6: Simple-effect means for cattle body temperature (BT) in the presence of a zilpaterol hydrochloride (ZH) × housing type interaction for 2 selected hot periods[1]

	Open		Shade			*P* - values		
Measurement	Control	ZH	Control	ZH	SEM[2]	Diet	Housing	Interaction
Average BT, ˚C	39.17[c]	39.04 [a]	39.12[b]	39.11[b]	0.035	<0.01	0.57	<0.01
Maximum BT, ˚C	40.50	40.32	40.44	40.33	0.050	<0.01	0.31	0.05
AUC BT[3]	14760[c]	14728[a]	14749[b]	14744[b]	5.1	<0.01	0.57	<0.01

[a,b,c]Values within rows with unique superscripts differ $P < 0.05$.

[1]Hot periods were based on highest adjusted THI index values. The hot periods are defined in Table 1.

[2]Pooled SEM, n = 4 pens/mean.

[3]AUC = Area under the curve.

Figure 1. Photograph of the shade structures used during the experiment. The shades were incorporated into the fence line of each pens and the pens were North/South oriented. Each pen contained 2 shade structures, one on the West fence line and one on the East fence line.

CHAPTER III. Metabolic and body temperature responses to environmental conditions across seasons in finishing steers[2]

B. M. Boyd*, Terry L. Mader*, Curt Bittner*, Dirk Burken*, Henry Hilscher*, Gene Wijffels†, John B. Gaughan§, Megan Sullivan§, Judy Cawdell-Smith§, Galen E. Erickson*[3]

* Department of Animal Science; University of Nebraska-Lincoln; Lincoln, NE 68583; † Animal, Food and Health Sciences, Commonwealth Scientific and Industrial Research Organisation; Queensland, AUS 4067; § School of Agriculture and Food Sciences, University of Queensland, Queensland, AUS 4072

[2] A contribution of the University of Nebraska-Lincoln funded in part by the Commonwealth Scientific and Industrial Research Organisation (CSIRO; Qld, Aus) and the University of Queensland (Qld, Aus).
[3] Corresponding author; gerickson4@unl.edu

ABSTRACT

Two finishing trials were conducted utilizing 160 crossbred beef steers across two seasons (summer: n=80, and winter: n=80; initial BW = 489 ± 20.4, 387 ± 15.9 kg, respectively) at the University of Nebraska-Lincoln Agricultural Research and Development Center near Mead, Neb. Continuous rumen temperature was collected throughout the duration of both trials. Blood samples were taken via jugular venous puncture every 2 weeks until 4 weeks prior to harvest. This resulted in a total of 6 collections during the summer and 8 collections during the winter. Two vacutainer tubes were used for analysis, one blood plasma tube and one blood serum tube. Cattle in both trials were fed the same diet consisting of 51% HMC, 40% Sweet Bran, 5% wheat straw and 4% supplement. Individual steer rumen temperature was placed in the statistical model as a covariate. Only blood measures where rumen temperature was significant as a covariate were reported. These selected blood measures were correlated using the CORR procedure of SAS (SAS Institute, Inc. Carry, N. C.) to the three day, prior to blood collection, average environmental temperature and the comprehensive climate index (CCI). Correlations between the change in blood measures and the respective change in rumen temperature across collection points were also analyzed. Additionally, environmental conditions were correlated to DMI and rumen temperature. Direct bilirubin, red blood cell count, hematocrit, and hemoglobin concentration were all correlated ($P > 0.22$), during at least one season, to both the 3-d average environmental conditions and 3-d rumen temperature. Rumen temperature was positively correlated to CCI and environmental temperature (r =0.65, r =0.63; respectively) during the summer. During the winter, rumen temperature was negatively correlated to CCI and

environmental temperature (r =-0.27 and r =-0.19). Intake was negatively correlated to both CCI and environmental temperature (r =-0.32; r =-0.30) in the summer trial, however, during the winter trial DMI was positively correlated to CCI and environmental temperature (r =0.22 and r =0.24). Certain blood metabolites change in response to environmental conditions and may be important during times of environmental stress. Additionally, environmental conditions can affect both DMI and rumen temperature.

Keywords: blood, body temperature, CCI, environment, heat stress, rumen temperature

INTRODUCTION

Environmental stress can cause substantial economic losses for producers through both losses in animal performance and mortality. Summer conditions consisting of above normal ambient temperature, relative humidity, and solar radiation coupled with low wind speed can increase an animal's heat load resulting in reduced performance, decreased animal comfort, and death (Mader et al., 1997). In addition, cold stress can result in sustained performance losses, particularly when coupled with wet and/or windy conditions. Steers housed outside in adverse winter conditions consume more feed, but grow slower, because less energy is available for productive purposes (Young, 1981).

Even though environmental stress has been a researched topic and some blood metabolites have been studied, little is known about how hot and cold environmental temperatures affect blood metabolites in feedlot cattle. Lee et al. (1976) observed a negative correlation between hematocrit concentration and ambient temperature in dairy cattle and suggested the oxygen binding capacity of blood is decreased under hot conditions. Furthermore, it has been noted that environmental temperature can effect

hemoglobin, albumin/globulin ratio, blood urea nitrogen, and erythrocyte concentration in dairy cattle (Olbirch et al., 1972; Shaffer et al., 1981). Conversely, Bide et al. (1973) noted that blood urea nitrogen, creatinine, lactate dehydrogenase and glucose change during the last half of the feeding period as muscle growth slows and fat deposition increases, with little impact attributed to environmental conditions. Although some research has observed the influence of environmental conditions on blood metabolites it remains unclear how environmental conditions directly influences the blood metabolites of feedlot cattle

Therefore, the objective of the current trials were to determine the effect of season and ambient temperature on steer blood parameters in addition to measuring rumen temperature continuously throughout the feeding period.

MATERIAL AND METHODS

These studies were conducted in accordance with, and approved by, the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC, 2014).

Experimental procedure

Two finishing studies were conducted at the University of Nebraska-Lincoln (UNL) Agricultural Research and Development Center (ARDC) research feedlot near Mead, Nebraska. Both studies utilized 80 crossbred beef steers housed in the same 8 pens during 2 seasons. Cattle were received at the UNL ARDC research feedlot between October, 11, 2013 and November 22, 2013. These cattle were sourced from ranch and livestock sale barns. Upon receiving, initial processing of the cattle included: vaccination

for Bovine Rhinotracheitis Virus, Bovine Viral Diarrhea types 1 and 2, Bovine Respiratory Syncytial Virus, Parainfluenza 3 Virus, and *Mannheimia Haemolytica* (Bovishield Gold One Shot; Zoetis Inc., Florham Park, NJ); vaccination for the prevention of *Histophilus Somni* (Somubac; Zoetis Inc.); and administration of an anthelmintic for the prevention of harmful species of gastrointestinal roundworms, lungworms, eyeworms, grubs, sucking lice and mange mites (Dectomax; Zoetis Inc.) in addition to administration of a panel tag for identification and an electronic identification. Two weeks following initial processing, cattle were revaccinated for prevention of infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea caused by bovine virus diarrhea (BVD) virus Types 1 and 2, and disease caused by parainfluenza3 (PI3) virus and bovine respiratory syncytial virus (BRSV; Bovi-sheild Gold 5; Zoetis Inc.). Additionally, cattle received vaccination for the prevention of *Clostridium chauvoei*, *septicum*, *novyi*, *sordellii*, *perfringens* Types B, C and D, and *Histophilus somni* (Ultrabac 7; Zoetis Inc.).

The first trial (summer) was conducted during the summer of 2014 utilizing summer yearlings (initial BW = 489 ± 20.4 kg). Cattle were started on trial May 22, 2014 and harvested on September 10, 2014 at Greater Omaha Packing Co., (Omaha, NE). The second trial (winter) was conducted during the fall and winter of 2014 utilizing fall yearlings (initial BW = 387 ± 15.9 kg). Cattle, for the second trial, were started on trial on September 11, 2014 and harvested on January 14, 2015 at Greater Omaha Packing Co., (Omaha, NE).

Cattle in both experiments were limit fed a diet consisting of 50% Sweet Bran (Cargill, Blair, Neb) and 50% alfalfa hay at an estimated 2% of BW for five days prior to

an initial BW being collected. This method of collecting an initial body weight was used so that cattle weight variation due to gut fill was minimized (Watson, 2013). Initial BW were collected over a 2 d period, on day 0 and 1 of the experiment, and then averaged to create a single initial BW. Cattle were then sorted into 8 pens of 10 steers each providing 56 m^2 of pen space per steer. Steers were stratified by hide color and initial BW, and assigned randomly to pen within strata. Cattle in both trials were implanted with Revalor 200 (200 mg trenbolone acetate, 20 mg estradiol; Merck Animal Health, De Soto, KS) on day 14 of both trials.

Cattle were adapted to the finishing diet over a 21-d period by reducing alfalfa inclusion in the diet and increasing concentration of high moisture corn (HMC). Cattle in both trials were fed the same finishing diet consisting of 40% Sweet Bran (Cargill, Blair, Neb), 51% HMC, 5% wheat straw, and 4% supplement. Bunk readings were performed once daily at 0600 h to determine if any adjustments were necessary based on the quantity of feed estimated to be remaining in the bunk at time of feeding. Steers were fed once daily, in the morning, in concrete fence-line feed bunks providing 91 linear cm of bunk space per steer. A Roto-Mix (Roto-Mix®, Dodge City, KS) mixer box mounted to a truck was used to deliver feed to the pens. Feed refusals were collected when accumulated for more than 48 h at the discretion of the unit manager, sampled, frozen, and dried in a forced air oven at 60ºC for 48 h described by Buckner et al. (2011) to calculate DMI. There were no treatments implemented across pens during these studies because the objective was to determine the effect of season and ambient temperature on steer blood parameters in addition to measuring rumen temperature continuously throughout the feeding period.

Blood Samples

On the first day of both trials, blood samples were collected via jugular venous puncture from each steer to obtain a baseline measure. Subsequent blood samples were collected from every steer in two week intervals until 4 weeks prior to harvest. However, one steer during the winter trial was unable to go through the alley way due after the second blood collection, therefore, blood samples were no longer collected from this steer for the remainder of the trial. This blood collection schedule resulted in 6 blood collections for the summer trial and 8 blood collections for the winter trial. For the summer trial, blood collections took place on: 5/22/14, 6/5/14, 6/19/14, 7/3/14, 7/17/14, and 7/31/14. For the winter trial, blood collections took place on: 9/11/14, 9/25/14, 10/9/14, 10/23/14, 11/6/14, 11/20/14, 12/4/14, and 12/18/14. Blood samples were collected prior to feeding and pen order remained constant for all blood collections. Three 10-mL vacutainer tubes and one 5-mL vacutainer tube was collected from each steer. Two of the 10-mL vacutainer tubes contained EDTA. Samples were placed on ice after collection and transported to the ruminant nutrition lab at UNL and centrifuged at 1200*g* for 10 min at 4 ℃. Plasma was removed and placed into four 2 mL screw capped tubes and frozen at – 80 ℃ until shipment to the Commonwealth Scientific and Industrial Research Organisation (CSIRO; Queensland, Aus) for subsequent analysis. The third, 10 mL vacutainer tube contained a clot activator and serum separation gel. This tube was placed on ice after collection and transported to the UNL ruminant nutrition lab where it was centrifuged at 1200*g* for 10 min at 4 ℃ and kept on ice until the sample was picked up by Nebraska Lab-Link (Lincoln, Neb) for analysis. Finally, the 5 mL vacutainer tube containing EDTA was placed on ice after collection and transported to the UNL ruminant

nutrition lab were it was kept on ice until it was picked up by Physicians Lab (Omaha, Neb) for analysis.

Blood plasma was analyzed for: white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils using the ADVIA 2120 hematology analyzer (Siemens Medical Solutions, Erlangen, Germany) with veterinary software as described by Harris et al., (2005). The blood serum was analyzed for: alkaline phosphatase alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, bilirubin, calcium, glucose, lactate dehydrogenase, phosphorus, and uric acid using a Dimension Vista 1500 analyzer (Siemens Medical Solutions, Erlangen, Germany) as described by Bruneel et al. (2012).

Environmental Stress Measurements

Environmental temperature, humidity, solar radiation, wind speed and barometric pressure data were collected and saved to a central computer every thirty minutes throughout the duration of both trials using a Davis Vantage Pro 2 (Davis Instruments Vernon, IL) weather station located between the drovers alley and the settling basin behind the center pen of the pens used in the current trial. The comprehensive climate index (CCI; Mader et al., 2010) was calculated and averaged to create 3-d, 7-d, and 14-d averages prior to each blood collection for both trials. Additionally, the 3-d, 7-d, and 14-d average environmental temperatures were calculated in the same manner (Table 7). A CCI of 21.1 at 0600 and 27.8 at 1200 was utilized as the cutoff to water cattle. This

threshold resulted in cattle being sprinkled a total of 13 times throughout the duration of the summer trial and 0 times during the winter trial.

Cattle in both trials received a SmartStock (SmartStock; LLC. Pawnee, OK) temperature monitoring rumen bolus at the initiation of both trials. Boluses transmitted each individual steer's body temperature, in a ten minute interval (summer) or a twenty minute interval (winter), to a receiver located near the animals home pen and then transmitted to a central computer where data were stored. Therefore, when cattle were removed from their home pen, rumen temperature readings were no longer recorded. Any drinking events were removed using a cutoff reading of below 35 ℃ to identify drinking events. In addition to removing drinking events, any missing data points were removed and replaced with a dot in the SAS (SAS Institute, Inc. Carry, N. C.) system and an average body temperature was then calculated for each individual steer for every hour. This method created a final data set with individual steer body temperatures every hour throughout the duration of the finishing period.

Statistical analysis

Due to an inadequate number of rumen temperature readings for numerous animals, the first blood collection was not used for the summer trial analysis and the first two collections were not used for the winter trial analysis resulting in an analysis of 5 collection points for the summer trial and 6 collection points for the winter trial.

The GLIMMIX procedure of SAS (SAS Institute, Inc. Carry, N. C.) was used to determine whether or not blood metabolites were affected by steer rumen temperature. The model included the fixed effects of collection and the three days prior to collection

rumen temperature placed in the model as a covariate. Only the blood measures where the covariate of rumen temperature was significant were chosen to be used for further analysis. Rumen temperature was then removed as a covariate for all other analysis.

The change in blood measure between one collection to the next, within study (summer and winter), was then correlated to the respective change in the 3-d average rumen temperature using the CORR procedure of SAS with animal as the experimental unit. Blood data were also correlated to the 3-d, prior to blood collection, average environmental temperature (3-d temperature) and CCI (3-d CCI) using the CORR procedure of SAS with animal as the experimental unit. Pen intakes (DM offered) were correlated to the previous day environmental temperature and CCI using the CORR procedure of SAS with pen as the experimental unit. Animal body temperature was also correlated with the previous day environmental temperature and CCI using the CORR procedure of SAS with animal as the experimental unit.

RESULTS AND DISSCUSSION

Only blood measures where the 3-d rumen temperature prior to blood collection was significant, for at least one season (summer or winter), as a covariate were determined to be of relevance and are reported (Table 8).Additionally, a correlation coefficient of ±0.20 was used as the cutoff to determine if the correlations between the blood metabolites to body temperature were biologically meaningful.

Environmental conditions during the first summer trial were relatively mild with no average CCI being greater than 27.4 (Table 7). Blood collection 3 had the warmest 3-d average and collection 5 had the warmest 7-d and 14-d average. Although mild overall,

warm periods were observed during the summer with 20 d having a CCI > 29.44. Unfortunately 9 of the 20 d occurred consecutively between August 18 and August 27, 2014 and there were no blood collections during this time point. Winter trial conditions started out mild for the first two collections, however, the final three collections had average 3-d, 7-d and 14-d CCI < -2.58.

Rumen temperature has been shown to be an accurate measure of animal body temperature although it may be slightly higher than actual body temperature (Beatty et al., 2008). Rumen temperature was positively correlated to the CCI and environmental temperature (r = 0.65 and r = 0.63 respectively; Table 11) during the summer. This finding is supported by Brown-Brandl et al., (2003) where they observed that rectal temperature has a lag time of 4-5 hours after peak environmental temperature for feedlot cattle. Correlations to previous day environmental conditions and rumen temperature were also evaluated. If lag time is only 4-5 hours (Brown-Brandl et al., 2003) this may suggest that previous day average environmental temperature may, in fact, be too far in the past and have little effect on the animal's body temperature. For the summer trial, rumen temperature was less correlated to previous day CCI and environmental temperature (R= 0.32, and r =0.40, respectively; data not presented) than for the same day environmental conditions (r =0.65, and r =0.63, respectively).

During the winter trial, rumen temperature was negatively correlated to the CCI and environmental temperature (r =-0.27 and r =-0.19 respectively; Table 11). The negative correlations observed suggests that as environmental temperature decreased rumen temperature increased. However, while these correlations exist, rumen temperature

stayed constant as temperatures got colder leading to the observed correlations. This is supported by other research that has shown that rumen temperatures of sheep are not significantly decreased by exposure to cold conditions (Bailey *et al.*, 1962). Therefore, the negative correlations observed in the current study are attributed to environmental temperatures decreasing while rumen temperatures were relatively constant. Rumen temperature for the winter trial were not correlated to previous day environmental conditions ($r < 0.04$; data not presented) similar to that of rumen temperatures during the summer trial.

During the summer, dry matter offered (DMO) was negatively correlated to both the same day CCI and environmental temperature ($r = -0.38$). This correlation is supported by findings from numerous studies that have noted a decrease in intake due to heat stress (Mader et al., 1997; Hahn, 1997; Gaughan et al., 2010). However, during the winter trial, DMO was positively correlated to the previous day CCI and environmental temperature ($r = 0.19$ and $r = 0.21$ respectively). This is contradictory to findings by Hahn (1995) who noted that feed intake during cold weather generally increases, however, it was noted that mud and ice can hamper movement resulting in decreased feed intake. Although mud and ice were not an issue during the current trial, the positive correlation observed in the current study would suggest that as temperature decreases DMO also decreases. However, it is important to note that DMO for pens are being analyzed rather than true DMI as orts were not collected every day, which may affect results.

Blood urea nitrogen: creatinine ratio (BC), blood urea nitrogen (BUN), calcium (Ca), white blood cell count, platelet count, monocytes, and both absolute and percent

basophil count were not correlated (0.19 < r >-0.18; Table 10) to 3-d CCI, 3-d environmental temperature or the 3-d rumen temperature for both the summer and the winter trials. The change in lactate dehydrogenase (LDH) was not correlated (r >-0.06; Table 9) to the 3-d rumen temperature during either the summer or winter trials. However, LDH was negatively correlated to environmental conditions during the summer (r >-0.25) and also during the winter (r >0.62) trial. Glucose (Glu) was negatively correlated (r <-0.37) to both the 3-d CCI and 3-d environmental temperature for the winter trial but were not correlated (r >-0.16) to the 3-d CCI or 3-d environmental temperature for the summer trial. Additionally, Glu was not correlated to the 3-d rumen temperature for either the summer or winter trials (r >- 0.11).

Blood urea nitrogen, creatinine, LDH and Glu have been shown to change during the last half of the feeding period as protein deposition is slowing and fat deposition is increasing (Bide et al., 1973). This may offer some insight to some of the observed correlations to environmental conditions across the feeding period to these blood measures. A strong correlation (r >0.62) between lactate dehydrogenase and environmental conditions was observed during the winter trial and a moderate correlation during the summer trial. The changes in lactate dehydrogenase during the latter portion of the feeding period may help explain these correlations. This observed correlation may be due to lactate dehydrogenase changing as days on feed increase, rather than environmental conditions affecting lactate dehydrogenase. Furthermore, this phenomenon may also help explain some of the correlations observed between environmental conditions and Glu, BUN, and BC.

Uric acid was positively correlated (r >0.51) to the 3-d CCI and 3-d environmental temperature for the winter trial, however, it was not correlated (r <0.17) for either the 3-d CCI or the 3-d environmental temperature for the summer trial. Additionally, uric acid concentration were not correlated (r =0.08) to the 3-d rumen temperature for either the summer or winter trials. This is supported by findings from Lin et al. (2006) who observed plasma uric acid concentration in broiler chickens was not increased due to acute heat stress. The change in alkaline phosphatase (ALKP) was negatively correlated (r =-0.21) to the 3-d rumen temperature during the winter but was not correlated (r =0.11) during the summer trial. Additionally, ALKP was not correlated to environmental measures for either the summer or winter trials (r <0.17, r >-0.16; respectively).

Mean corpuscular hemoglobin concentration (MHCH) was positively correlated (r >0.22) to 3-d environmental temperature for the winter trial but was not correlated (0.05 < r >-0.12) to the 3-d CCI during the winter trial or both the 3-d CCI and 3-d environmental temperature during the summer trial. Additionally, MHCH was not correlated (r <0.11) to the 3-d rumen temperature for either the summer or winter trial. Phosphorus was negatively correlated to 3-d rumen temperature for both the summer and winter trials (r =-0.21, r =-0.20; respectively). Additionally, phosphorus was positively correlated (r =0.33) to environmental temperature for the winter trial; however, was not correlated (0.03 > r <-0.12) to CCI for the winter trial, or either environmental condition for the summer trial.

The change in aspartate aminotransferase (AST) was negatively correlated (r <-0.20) to the change in 3-d rumen temperature for both the summer and winter trial. AST was also observed to be negatively correlated to environmental temperature during the summer trial (r =-0.26) and both environmental conditions during the winter trial (r <-0.40). However, AST was not correlated to CCI during the summer trial (r =- 0.16). The negative correlations observed in the current study for AST are in agreement with findings by Srikandakumar and Johnson (2004) who observed a decrease in AST in dairy cattle when exposed to hot conditions. However, Srikandakumar and Johnson (2004) suggested the important finding was that the concentration of these enzymes were not increased as a decrease in AST is not of physiological concern. Furthermore, while Yokus and Cakir (2006) observed no statistical differences in AST between seasons, numerically, cattle had the lowest concentration of AST in August when compared to October, February and May in Holstein cows suggesting that concentration of AST were lowest during the warmest month.

The change in red blood cell count (RBC), hemoglobin, hematocrit, and eosinophils were negatively correlated (r <-0.21; Figure 2; Figure 3) to the 3-d rumen temperature during the summer trial but were not correlated (r =0.01) for the winter trial. However, hemoglobin, and hematocrit were negatively correlated (r <-0.22) for both 3-d CCI and 3-d environmental temperature for both the summer and winter trials. Red blood cell count was negatively correlated (r <-0.23) to 3-d environmental conditions during the winter trial; however, was not correlated (r >-0.10) to environmental conditions during the summer trial. Finally, eosinophils were negatively correlated (r =- 0.29) to

environmental temperature during the summer trial but was not correlated (r >-0.13) to CCI during the summer trial or either environmental measure during the winter trial.

These findings are supported by previous findings from Lee et al. (1976) where a negative correlation was observed between hematocrit concentration and ambient temperature. Furthermore, Olbrich et al. (1972) observed a depression in hematocrit concentration in cattle subjected to elevated ambient temperatures. Additionally, in the current study, a negative correlation was observed between hemoglobin and both environmental temperature and CCI for the summer and winter trials. This is supported by findings from Shaffer et al. (1981) who observed significant effects in hemoglobin concentration due to environmental temperature. Furthermore, Olbrich et al (1972) observed a depression in red blood cells in cattle subjected to elevated ambient temperatures. This depression on RBC was also observed in the current trial with negative correlations to environmental temperature and CCI during the winter and a slight negative correlation to CCI during the summer trial. Shaffer et al., (1981) observed similar findings and attributed this association to a decrease in cellular oxygen requirements, therefore, resulting in reduced metabolic heat load. Furthermore, it was suggested by Lee et al. (1972) that the oxygen binding capacity of blood decreases when animals are under heat stress conditions.

Direct bilirubin was positively correlated to both 3-d CCI and 3-d environmental temperature (r =0.24, r =0.26) for the summer trial but was not correlated (r =0.03) to 3-d environmental measures for the winter trial. Additionally, the change in direct bilirubin was positively correlated (r =0.25; Figure 4) to the 3-d rumen temperature for the summer

trial but was not correlated (r =-0.06) during the winter trial. This may suggest that bilirubin may be of importance during warmer conditions but not as much when temperatures are cooler and are at or below the animal's thermoneutral zone. The lack of correlation observed during the winter trial is confirmed by Bide et al., (1973) where they observed that bilirubin concentration in cattle, fed in Canada, were unchanged through the feeding period when no unusually warm weather was experienced across two trials conducted in two different seasons. Although, there have been few studies evaluating bilirubin concentration in farm animals, there have been many studies conducted in human and rat subjects observing the effects of bilirubin on the body due to bilirubin's importance in neonatal jaundice. A study conducted by Ceran et al. (2001) found that bilirubin has a protective effect in the rat small intestine towards ischemia/reperfusion injury. Thus, it can be speculated that if heat stress simulates ischemia/reperfusion injury (Kregel et al., 1988) that increased bilirubin concentration in the small intestine can be beneficial during times of heat stress. Bilirubin is a powerful antioxidant that is secreted from the liver directly into the small intestine where oxidative stress has been shown to occur during times of heat stress. This combined with the correlations observed in the current study suggests the possibility that bilirubin is of high importance in the animals biological mechanism for maintaining intestinal integrity and homeostasis during times of heat stress.

Absolute lymphocyte count, and ALT were not correlated ($0.15 > r < -0.15$) to the change in 3-d rumen temperatures for either the summer or winter trials. However, absolute lymphocyte count, and ALT were positively correlated ($r = 0.20$) to 3-d environmental temperature for the summer trial; however, was not correlated ($r < 0.12$) to

3-d CCI for the summer trial or 3-d environmental temperature and 3-d CCI during the winter trial. This finding is contradictory to findings by Boyd and Ford (1967) who observed no obvious correlation between environmental conditions and ALT in dairy cows. Furthermore, Yokus and Cakir (2006) observed no change in ALT concentration between seasons in cows. It is unclear why these correlations were observed during the current trial; however, in the findings by Boyd and Ford (1967) significant fluctuations in ALT across months were observed suggesting that ALT concentration naturally fluctuate independently of other variables. Therefore, the correlations observed in the current study may be simple variation being detected across days on feed.

Numerous studies have shown that animal body temperature is related to environmental temperature and follows diurnal patterns, and is directly affected by solar radiation (Brown-Brandl et al., 2003; Harris et al. 1960; Finch 1986; Mader et al., 2006). In the current study the observed correlations were significant and positive during the summer trial which is supported by other research suggesting that as environmental temperature increases, rumen temperature also tends to increase within a day. Using the covariate method of analysis to determine if blood measures were of importance it allowed for the effect of time on the measurement to be accounted for in determining blood measures that are affected by rumen temperature. However, the effect of days on feed may still be influencing some of the observed correlations. With any metabolic process, blood metabolites are closely regulated which makes changes difficult to detect and it is important to note that all blood measures observed in this study were within normal physiological concentration for healthy cattle. There were several blood measures where body temperature was significant as a covariate but once correlated to either the 3-

d rumen temperature or 3-d environmental conditions, the correlation coefficients weren't significant. This may be due in part to the complexity of animal chemistry and the integration between animal systems in the heat stress response. However, while there are some metabolites that were not well correlated, there were some that were correlated to both rumen temperature and environmental conditions for either the summer or winter trials or both. These blood measures included direct bilirubin, red blood cell count, hemoglobin, and hematocrit for the summer trial and red blood cell count, hemoglobin, and hematocrit for the winter trial. The fact that these measures are correlated to both rumen temperature and environmental conditions may suggest that these are of particular importance in both the heat and cold stress response of a finishing steer. It is clear that environmental conditions can change metabolic conditions within a steer however, it is important to keep in mind that many factors are all acting on these blood parameters at once making interpretation of these changes difficult.

LITERATURE CITED

Bailey, C. B., R. Hironoka and S. B. Slen. 1962. Effect of the temperature of the environment and the drinking water on the body temperature and water consumption of sheep. Can. J. Anita. Sci. 42:1.

Beatty, D. T., A. Barnes, E. Taylor, and S. K. Maloney. 2008. Do changes in feed intake or ambient temperature cause changes in cattle rumen temperature relative to core temperature. J. Thermal Biology. 33:12-19

Bide, R. W., W. J. Dorward, and M. E. Tumbleson. 1973. Clinical chemistry of grain-fed cattle. I. A preliminary study of a basic biomedical profile. Can. J. Anim. Sci. 53:697-707

Boyd, J. W., and E. J. Ford. 1967. Normal variation in alanine aminotransferase activity in sheep and cattle.J. Acric. Sci. Camb. 68:358-389

Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, H. Freetly. 2003. Thermoregulatory responses of feeder cattle. J. Thermal Biology. 28:149-157

Buckner, C. D., M. F. Wilken, J. R. Benton, S. J. Vanness, V. R. Bremer, T. J. Klopfenstein, P. J. Kononoff and G. E. Erickson, 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol byproducts. Prof. Anim. Sci. 27:57-64.

Ceran, C., k. Sönmez, Z. Türkyılmaz, B. Demiroğulları, A. Dursun, E. Düzgün, and N. Kale. 2001. Effect of bilirubin in ischemia/reperfusion injury on rat small intestine. Journal of pediatric surgery, 36:1764-1767

Gaughan, J. B., S. Bonner, I. Loxton, T. L. Mader, A. Lisle, and R. Lawrence. 2010. Effect of shade on body temperature and performance of feedlot steers. J. Anim. Sci. 88:4056-4067. doi:10.2527/jas.2010-2987

Hahn, G. L. 1995. Environmental influence on feed intake and performance of feedlot cattle. In procedings feed intake Oklahoma State University. P207-225. Retreived from: beefextension.com/proceedings/feed_intake95/feed_intake95_29.pdf

Hahn, G. L. 1997. Dynamic responses of cattle to thermal heat loads. J. Anim. Sci. 77:10-20

Harris, N., J. Kunicka, and A. Kratz. 2005. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. Lab Hematol. 11:47-61

IACUC. 2014. Policies and Procedures Manual for the Use of Animals at the University of Nebraska-Lincoln. Number 902

Kregel, K. C., P. T, Wall, and C. V. Gisolfi. 1988. Peripheral vascular responses to hyperthermia in the rat. J. Appl. Physiol. 64:2582-2588

Kregel, K. C. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. 92:2177-2186

Lee, J. A., J. D. Roussel, and J. F. Beatty. 1976. Effect of temperature-season on bovine adrenal cortical function, blood cell profile, and milk production. J. Dairy Sci. 59:104-108

Lin, H., E. Decuypere, J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparitive Biochemistry and Physiology. 144:11-17

Mader, T. L., J. M. Dahlquist, and J. B. Gaughan. 1997. Wind protection effects and airflow patterns in outside feedlots. J. Anim. Sci. 75:26-36. doi:/1997.75126x

Mader, T. L., L. J. Johnson, and J. B. Gaughan. 2010. A comprehe nsive index for assessing environmental stress in animals. J. Anim. Sci. 88:2153-2165

Olbrich, S. E., F. A. Martez, M. E. Tumbleson, H. D. Johnson, and E. S. Hilderbrand. 1972. Effects of constant environmental temperatures of 10 C and 31 C on serum biochemic and hematologic measurements of heat tolerant and cold tolerant cattle. Comp. Biochem. Physiol, 41A:255

Shaffer, L., J. D. Roussel, and K. L. Koonce. 1981. Effects of age, temperature-season, and breed on blood characteristics of dairy cattle. J. Dairy. Sci. 64:62-70

Srikandakumar, A., and E. H. Johnson. 2004. Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian milking Zebu cows. Tropical Animal Health and Production. 36:685-692

Watson, A. K., B. L. Nuttelman, T. J. Klopfenstein, L. W. Lomas, and G. E. Erickson. 2013. Impacts of a limit-feeding procedure on variation and accuracy of cattle weights. J. Anim. Sci. 91:5507-5517

Yokus, B. and U. D. Cakir. 2006. Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biological Trace Element Research. 109: 255-266

Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. 52:154-163

Table 7. Environmental temperature (Temp) and comprehensive climate index (CCI)

		Three Day Avg[3]		Seven Day Avg[4]		Fourteen Day Avg[5]	
Collection[1]	Season[2]	CCI[6]	Temp[7]	CCI[6]	Temp[7]	CCI[6]	Temp[7]
1	S	22.42	22.32	15.76	16.09	13.93	15.19
	W	19.18	19.43	19.77	18.78	22.84	20.42
2	S	24.14	21.24	25.53	22.92	25.09	22.19
	W	19.24	18.18	20.31	19.00	17.92	16.83
3	S	27.36	27.28	23.50	23.58	22.74	21.92
	W	15.29	15.36	10.84	12.43	16.19	15.96
4	S	20.82	19.52	23.29	21.55	25.59	22.63
	W	14.90	15.16	12.84	13.54	10.89	12.25
5	S	21.34	18.31	25.75	21.90	26.58	22.55
	W	6.94	10.48	3.93	8.35	8.58	10.76
6	S	23.82	19.92	26.57	22.86	26.78	23.54
	W	-9.71	-4.88	-12.76	-6.62	-9.32	-3.11
7	S	-	-	-	-	-	-
	W	-8.34	-3.92	-7.23	-1.69	-6.28	0.01
8	S	-	-	-	-	-	-
	W	- 10.88	-3.03	-2.58	3.57	-3.74	1.97

averages in relation to blood collections

[1] Summer collections taken every other week beginning on 5/22/2014 and ending 7/31/2014. Winter collections taken every other week beginning on 9/11/2014 and ending on 12/18/2014

[2] Season either defined as S = Summer or W = Winter

[3] Three days prior to blood collection weather averages correlated to blood measures

[4] Seven days prior to blood collection weather averages correlated to blood measures

[5] Fourteen days prior to blood collection weather averages correlated to blood measures

[6] CCI = Ambient Temperature (Ta; ℃)+($e^{(0.00182\times\text{relative humidity(RH)}+1.8\times10\text{^-}5\times Ta\times RH)}$)×(0.000054×$Ta^2$+0.00192×Ta–0.0246)×(RH - 30))+(-6.56/$e^{(1/(2.26\times\text{wind speed (WS)}+0.23)\text{^}(0.45\times(2.9+1.14\times10\text{^-}6\times ws\text{^}2.5-\log0.3(2.26\times WS+0.33)\text{^}-2))))}$-0.00566×$WS^2$+3.33) + (0.0076×Solar Radiation (RAD)-0.00002×RAD×Ta+0.00005×Ta^2×√(RAD) +0.01×Ta-2) Mader et al., (2010)

[7] Environmental Temperature (℃)

Table 8. The effect of the 3-d average body temperature prior to blood collection as a covariate on blood measures

Blood Measure	*P*-Value Summer[1]	Winter[2]
Alkaline phosphatase, U/L	0.03	<0.01
Alanine aminotransferase, U/L	0.97	<0.01
Aspartate aminotransferase, U/L	0.29	<0.01
Blood Urea Nitrogen : Creatinine, Ratio	<0.01	<0.01
Direct bilirubin, mg/dL	0.03	0.80
Blood urea nitrogen, mg/dL	<0.01	<0.01
Calcium, mg/dL	0.02	0.31
Glucose, mg/dL	0.04	0.72
Lactate dehydrogenase, U/L	0.04	0.01
Phosphorus, mg/dL	<0.01	0.76
Uric acid, mg/dL	<0.01	<0.01
White blood cell, count	<0.01	0.92
Red blood cell, count	<0.01	0.02
Hemoglobin, g/dL	<0.01	0.03
Hematocrit, %	<0.01	0.02
Mean corpuscular hemoglobin conc.	0.05	0.63
Platelet, count	0.04	0.26
Lymphocytes, absolute	<0.01	0.21
Monocytes, absolute	0.01	0.32
Eosinophils, %	0.77	0.05
Eosinophils, absolute	0.30	0.03
Basophils, %	<0.01	0.02
Basophils, absolute	<0.01	0.01

[1] Summer collections taken every other week beginning on 5/22/2014 and ending 7/31/2014

[2] Winter collections taken every other week beginning on 9/11/2014 and ending on 12/18/2014

Table 9. Correlation between the change in blood measures and the respective change in three day, prior to blood collection, average rumen temperature between consecutive collection points

	Summer[1]		Winter[2]	
Blood Measure	R - Value	*P* - Value	R - Value	*P* - Value
Alkaline phosphatase, U/L	0.11	0.04	-0.21	<0.01
Alanine aminotransferase, U/L	0.15	<0.01	-0.15	<0.01
Aspartate aminotransferase, U/L	-0.21	<0.01	-0.20	<0.01
Blood Urea Nitrogen : Creatinine, Ratio	-0.18	<0.01	0.18	<0.01
Direct bilirubin, mg/dL	0.25	<0.01	-0.06	0.22
Blood urea nitrogen, mg/dL	-0.03	0.57	0.13	0.01
Calcium, mg/dL	-0.13	0.02	-0.13	0.01
Glucose, mg/dL	-0.11	0.04	-0.03	0.53
Lactate dehydrogenase, U/L	-0.01	0.83	-0.06	0.25
Phosphorus, mg/dL	-0.21	<0.01	-0.20	<0.01
Uric acid, mg/dL	0.08	0.16	0.08	0.11
White blood cell, count	-0.02	0.74	0.03	0.51
Red blood cell, count	-0.29	<0.01	0.01	0.83
Hemoglobin, g/dL	-0.29	<0.01	0.01	0.70
Hematocrit, %	-0.30	<0.01	0.01	0.80
Mean corpuscular hemoglobin conc.	0.11	0.06	0.01	0.91
Platelet, count	-0.13	0.02	-0.08	0.14
Lymphocytes, absolute	-0.02	0.76	-0.04	0.38
Monocytes, absolute	0.11	0.04	0.02	0.74
Eosinophils, %	-0.22	<0.01	-0.11	0.02
Eosinophils, absolute	-0.22	<0.01	-0.09	0.07
Basophils, %	-0.08	0.16	0.02	0.74
Basophils, absolute	-0.11	0.05	0.05	0.34

[1] Summer collections taken every other week beginning on 5/22/2014 and ending 7/31/2014

[2] Winter collections taken every other week beginning on 9/11/2014 and ending on 12/18/2014

Table 10. Correlation between blood measure and 3-d average comprehensive climate index (CCI) or 3-d average environmental temperature (Temp)

	Summer[1]				Winter[2]			
	CCI		Temp[3]		CCI		Temp[3]	
Blood Measure	R-Value	P-Value	R-Value	P-Value	R-Value	P-Value	R-Value	P-Value
Alkaline phosphatase, U/L	0.06	0.19	0.17	<0.01	-0.15	<0.01	-0.16	<0.01
Alanine aminotransferase, U/L	0.09	0.06	0.20	<0.01	-0.02	0.54	-0.03	0.44
Aspartate aminotransferase, U/L	-0.16	<0.01	-0.26	<0.01	-0.40	<0.01	-0.41	<0.01
Blood Urea Nitrogen : Creatinine, Ratio	-0.14	<0.01	-0.01	0.89	0.07	0.08	0.08	0.04
Direct bilirubin, mg/dL	0.24	<0.01	0.26	<0.01	0.03	0.42	0.03	0.40
Blood urea nitrogen, mg/dL	-0.11	0.02	0.00	0.96	0.09	0.02	0.11	0.01
Calcium, mg/dL	0.00	0.99	-0.07	0.16	-0.13	<0.01	-0.12	<0.01
Glucose, mg/dL	-0.09	0.05	-0.16	<0.01	-0.37	<0.01	-0.38	<0.01
Lactate dehydrogenase, U/L	-0.25	<0.01	-0.28	<0.01	-0.63	<0.01	-0.62	<0.01
Phosphorus, mg/dL	-0.04	0.35	-0.12	<0.01	0.03	<0.01	0.33	<0.01
Uric acid, mg/dL	0.16	<0.01	0.17	<0.01	0.52	<0.01	0.51	<0.01
White blood cell, count	0.05	0.27	0.08	0.07	0.08	0.03	0.08	0.04
Red blood cell, count	-0.10	0.03	0.03	0.54	-0.25	<0.01	-0.23	<0.01
Hemoglobin, g/dL	-0.30	<0.01	-0.25	<0.01	-0.57	<0.01	-0.54	<0.01
Hematocrit, %	-0.29	<0.01	-0.22	<0.01	-0.59	<0.01	-0.57	<0.01
Mean corpuscular hemoglobin conc.	0.05	0.27	-0.08	0.10	0.19	<0.01	0.22	<0.01
Platelet, count	0.01	0.83	-0.06	0.19	0.10	0.01	0.10	0.02
Lymphocytes, absolute	0.12	0.01	0.22	<0.01	0.11	0.01	0.10	0.01
Monocytes, absolute	0.01	0.81	0.05	0.29	0.19	<0.01	0.18	<0.01
Eosinophils, %	-0.13	<0.01	-0.29	<0.01	-0.06	0.11	-0.06	0.14
Eosinophils, absolute	-0.11	0.02	-0.24	<0.01	-0.05	0.22	-0.04	0.27
Basophils, %	0.04	0.42	0.01	0.81	0.18	<0.01	0.18	<0.01
Basophils, absolute	0.06	0.20	0.05	0.25	0.16	<0.01	0.15	<0.01

[1] Summer collections taken every other week beginning on 5/22/2014 and ending 7/31/2014

[2] Winter collections taken every other week beginning on 9/11/2014 and ending on 12/18/2014

[3] Environmental temperature

Measure	Season[1]	Avg. value[2]	CCI	*P* – value	Temp[3]	*P* - value
Ruminal Temperature	S	39.37	0.65	<0.001	0.63	<0.001
	W	39.08	-0.27	<0.001	-0.19	<0.001
DMI	S	13.42	-0.32	<0.001	-0.30	<0.001
	W	13.56	0.22	<0.001	0.24	<0.001

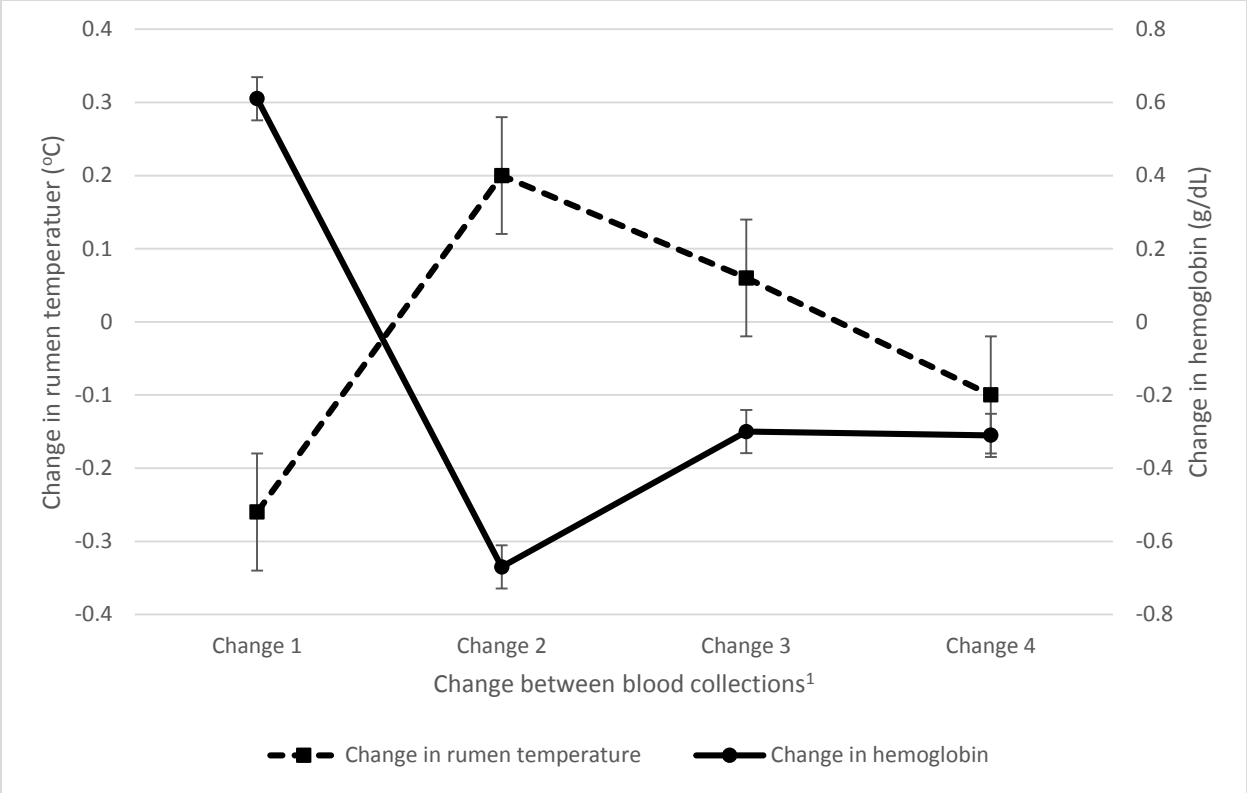
Table 11. Correlation between environmental temperature and comprehensive climate index (CCI) to animal body temperature and DMI

[1] Season either defined as S = summer or W = winter

[2] Overall average value for DMI (kg) and rumen temperature (℃) of cattle for summer and winter trials

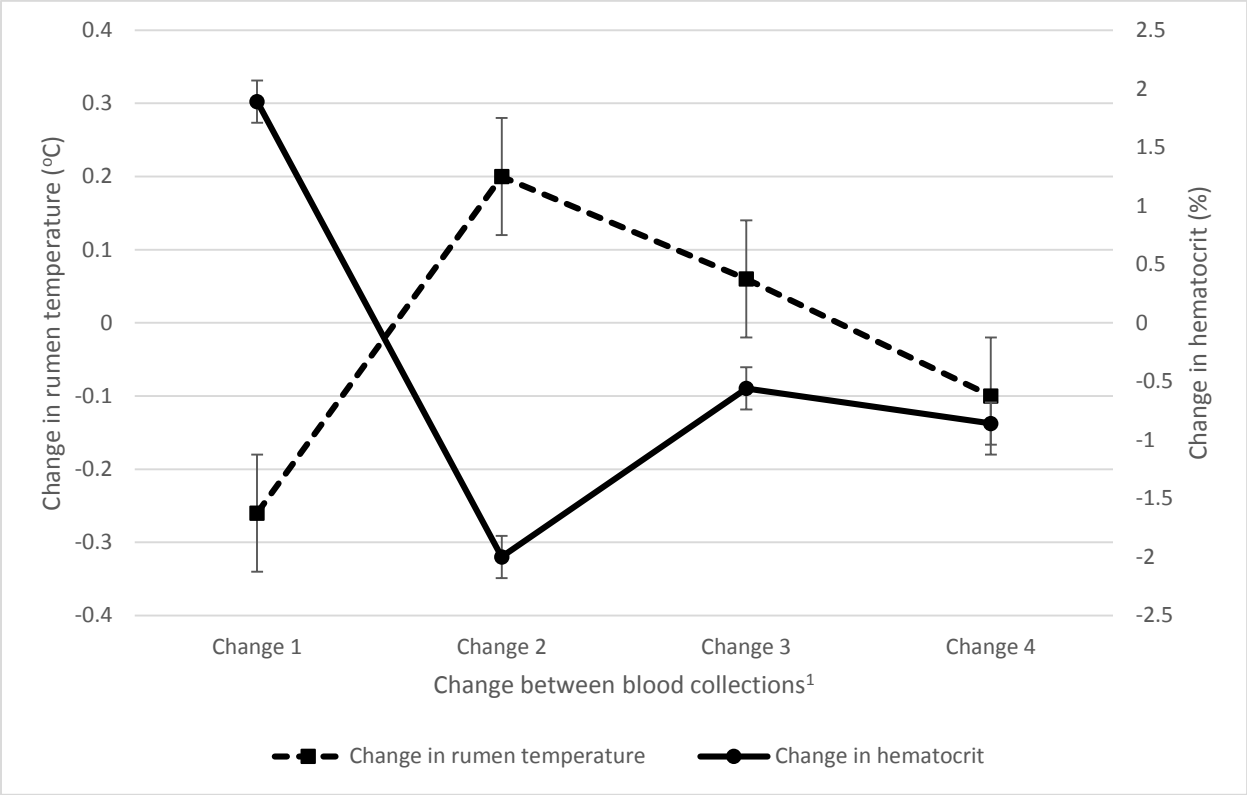
[3] Correlation to daily average environmental temperature

Figure 2. Change in rumen temperature and the change in hemoglobin concentration between summer blood collections



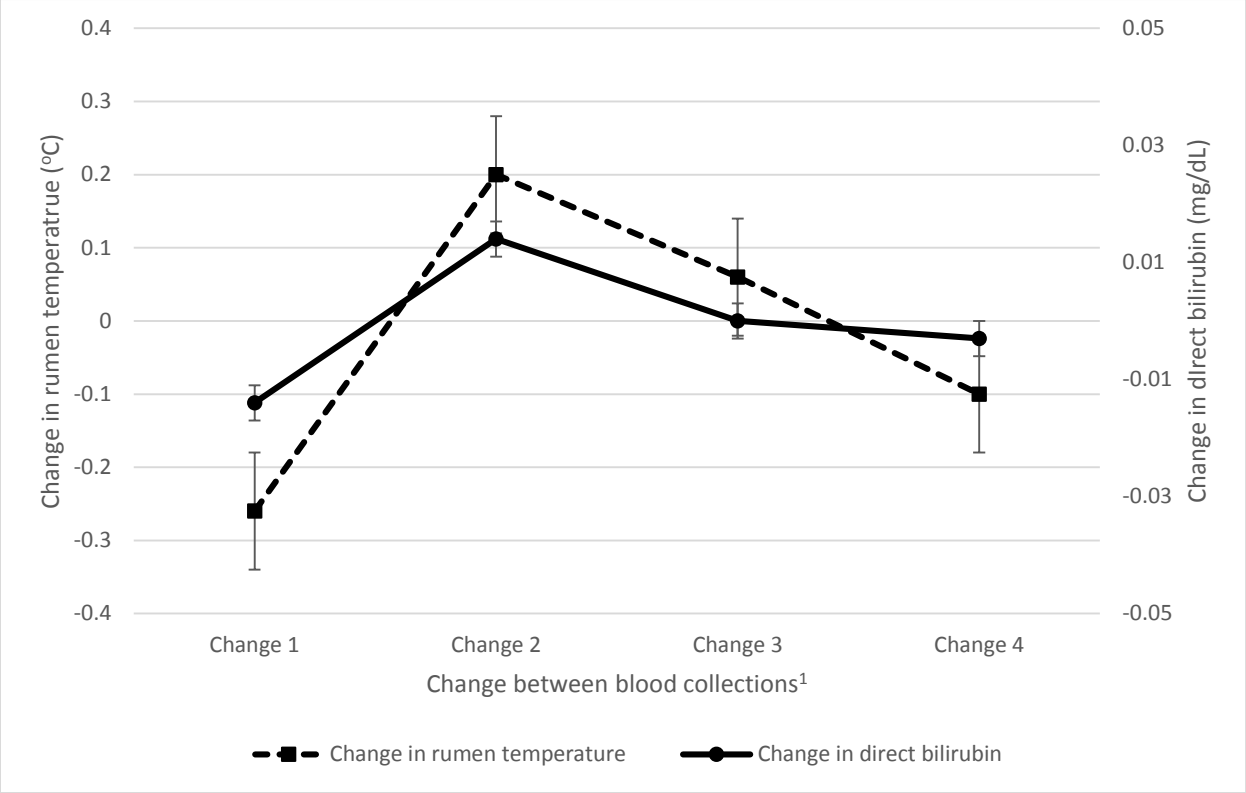
[1] Change 1 = change between summer blood collection 2 and collection 3; Change 2 = change between summer blood collection 3 and collection 4; Change 3 = change between summer blood collection 4 and collection 5; Change 4 = change between summer blood collection 5 and collection 6

Figure 3. Change in rumen temperature and the change in hematocrit concentration between summer blood collections



[1] Change 1 = change between summer blood collection 2 and collection 3; Change 2 = change between summer blood collection 3 and collection 4; Change 3 = change between summer blood collection 4 and collection 5; Change 4 = change between summer blood collection 5 and collection 6

Figure 4. The change in rumen temperature and change in direct bilirubin concentration between summer blood collections



[1] Change 1 = change between summer blood collection 2 and collection 3; Change 2 = change between summer blood collection 3 and collection 4; Change 3 = change between summer blood collection 4 and collection 5; Change 4 = change between summer blood collection 5 and collection 6

CHAPTER IV. The effect of supplementing mannan oligosaccharide or finely ground fiber, during the summer, on body temperature, performance, and blood metabolites of finishing steers[4]

B. M. Boyd*, Terry L. Mader*, Curt Bittner*, Henry Hilscher*, Gene Wijffels†, John B. Gaughan§, Megan Sullivan§, Judy Cawdell-Smith§, Galen E. Erickson*[5]

* Department of Animal Science; University of Nebraska-Lincoln; Lincoln, NE 68583; † Animal, Food and Health Sciences, Commonwealth Scientific and Industrial Research Organisation; Queensland, AUS 4067; § School of Agriculture and Food Sciences, University of Queensland, Queensland, AUS 4072

[4] A contribution of the University of Nebraska-Lincoln funded in part by the Commonwealth Scientific and Industrial Research Organisation (CSIRO; Qld, Aus) and the University of Queensland (Qld, Aus).
[5] Corresponding author; gerickson4@unl.edu

ABSTRACT

Crossbred beef steers (n=96) were utilized in a study conducted at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot near Mead, NE to determine the effect of feeding Agrimos (Lallemand Animal Nutrition; Montreal, Canada) and 2.5-cm ground wheat straw to finishing steers during the summer on body temperature and panting score in addition to performance, and blood metabolites. Three treatments with four replications per treatment were set up in a completely randomized design. Treatments consisted of a basal control diet (CON), consisting of 68.5% corn, 20% modified distillers grains plus solubles, 7.5% sorghum silage, and 4% supplement; the inclusion of Agrimos (MOS; 30g/steer daily), and 2.5-cm ground wheat straw replacing 5% corn (WHT). Cattle were stratified by initial BW between pens and pen was assigned randomly to treatment. Rumen boluses to collect body temperature were inserted on d 21 of the trial after cattle were adapted to finishing diets. Blood was collected in July and August (7 collection weeks) of the trial via jugular venous puncture. There were no differences ($P > 0.19$) observed for final BW, ADG, and DMI among treatments. Additionally, no difference ($P > 0.24$) was observed for carcass-adjusted final BW or ADG. Feed efficiency was decreased ($P < 0.02$) on both a live- and carcass-adjusted basis for cattle fed WHT when compared to CON and MOS. Hot carcass weight, dressing %, LM area, and marbling score were not different ($P > 0.36$) among treatments. Cattle fed the CON had greater 12th rib fat depth and USDA yield grade ($P < 0.02$) than cattle fed WHT and MOS. Both average and maximum body temperatures were greater ($P < 0.01$) for cattle fed MOS than for cattle fed CON or WHT. There was

no difference ($P = 0.18$) for area under the curve body temperature between treatments. Panting scores were least ($P < 0.01$) for cattle fed the WHT when compared to CON and MOS. Time and treatment interactions ($P < 0.05$) were observed for bilirubin, blood urea nitrogen, calcium, chloride, carbon dioxide, creatinine, potassium, lactate dehydrogenase, phosphorus, total protein, triglyceride, uric acid, red blood cell count, hemoglobin, and hematocrit concentration. No effect on animal performance was realized from the addition of MOS to the diet, however, body temperature was increased slightly. Additionally, the WHT treatment decreased G:F and reduced panting score but did not affect body temperature.

Key words: Blood Metabolites, Body Temperature, Fiber, Finishing Cattle, Heat Stress

INTRODUCTION

The small intestine is one of the most susceptible tissues to heat damage (Kregel, 2002). Blood flow to the intestine initially decreases during heat stress; however, after sustained high temperatures blood flow to the small intestine increases sharply (Kregel et al., 1998). This phenomenon simulates ischemia-reperfusion of the gut which has been documented to increase intestinal epithelial damage through an increase in reactive oxygen species (Lambert et al., 2002; Attuwaybi et al., 2004). Feeding prebiotics in an effort to reduce the negative effects of heat stress has primarily been studied in poultry. Sohail et al. (2011) observed that the feeding of mannan oligosaccharide in poultry diets helped reduce some of the detrimental effects of heat stress in terms of reducing oxidative damage to the small intestine. In addition to feeding probiotics, feeding increased levels

fiber has been shown to increase the amount of short chain fatty acids present in the cecum of the animal. Some short chain fatty acids, such as butyrate, have been shown in rats to induce heat shock protein 25 which aids in providing oxidative protection to the intestinal cells (Hongyu et al., 2001). The addition of fiber to a finishing diet may also displace some energy, thereby reducing metabolic heat load. Mader et al. (1999) observed a decrease in steer body temperature when steers were fed a diet consisting of 28% alfalfa hay and barley straw when compared to steers fed a traditional 6% alfalfa hay finishing diet.

Even though environmental stress has been a researched topic for the past few decades, little is known on how feeding a yeast supplement or fine ground wheat straw will affect feedlot steers from a performance and metabolic standpoint. Therefore, the objective of the current study was to determine the effect of feeding a yeast supplement and fine ground wheat straw on steer performance and body temperature, measured continuously throughout the feeding period, in addition to blood parameters.

MATERIAL AND METHODS

This study was conducted in accordance with, and approved by, the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC, 2015; #902).

Experimental procedure

A finishing study was conducted utilizing crossbred beef steers (n=96, initial BW = 480 kg ± 25 kg) to study the effects of feeding mannan oligosaccharide (Agrimos; Lallemand Animal Nutrition; Montreal, Canada) and finely ground wheat straw on steer

performance, body temperature, panting score, and blood metabolites during summer conditions. Steers were fed at the University of Nebraska-Lincoln (UNL) Agricultural Research and Development Center (ARDC) research feedlot near Mead, Nebraska. Cattle were received at the UNL ARDC research feedlot between October, 3, 2014 and November 07, 2014 and sourced from ranches and livestock markets. Upon receiving, initial processing of the cattle included: vaccination for Bovine Rhinotracheitis Virus, Bovine Viral Diarrhea types 1 and 2, Bovine Respiratory Syncytial Virus, Parainfluenza 3 Virus, and *Mannheimia Haemolytica* (Bovishield Gold One Shot; Zoetis Inc., Florham Park, NJ); vaccination for the prevention of *Histophilus Somni* (Somubac; Zoetis Inc.); and administration of an anthelmintic for the prevention of harmful species of gastrointestinal roundworms, lungworms, eyeworms, grubs, sucking lice and mange mites (Dectomax; Zoetis Inc.). Steers were administered a panel tag, metal ear clip, and an electronic ID for identification. Two weeks following initial processing, cattle were revaccinated for prevention of infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea caused by bovine virus diarrhea (BVD) virus Types 1 and 2, and disease caused by parainfluenza3 (PI3) virus and bovine respiratory syncytial virus (BRSV; Bovishield Gold 5; Zoetis Inc.) Additionally, cattle received vaccination for the prevention of *Clostridium chauvoei*, *septicum*, *novyi*, *sordellii*, *perfringens* Types B, C and D, *and Histophilus somni* (Ultrabac 7; Zoetis Inc.). After arrival steers were backgrounded on corn stalks and supplemented with wet corn gluten feed (SweetBran, Cargill Inc., Blair, NE), followed by being placed on an 84-d growing period until initiation of the current trial in May 2015.

Cattle were limit fed a diet consisting of 50% Sweet Bran (Cargill, Blair, Neb) and 50% alfalfa hay at an estimated 2% of BW for five days prior to an initial BW being collected. This method of collecting an initial BW was used to minimize gut fill variation (Watson, 2013). Initial BW were collected over a 2-d period on day 0 and 1 of the experiment and averaged to establish initial BW. Cattle were sorted into 12 pens of 8 steers each providing 70 m^2 of pen space per steer. Steers were stratified by initial BW and assigned randomly to pen within strata and treatment was assigned randomly to pen. Cattle were delay implanted with Revalor 200 (Merck Animal Health, De Soto, KS) on day 21 of the trial. The trial was conducted during the summer of 2015 utilizing summer yearlings (initial BW = 479 ± 11.5 kg). Cattle were started on trial May 26, 2015 and harvested on September 23, 2015 at Greater Omaha Packing Co., (Omaha, NE).

The study was set up as a completely randomized design with three treatments and four replications per treatment. The basal diet consisted of 34.25% high moisture corn (HMC), 34.25% dry rolled corn (DRC), 20% modified distillers grains plus solubles, 7.5% sorghum silage, and 4% supplement (Table 12). Cattle were adapted to finishing diets over a 21-d period by reducing alfalfa inclusion in the diet and increasing levels of HMC/DRC blend. The first treatment was a control (CON) where cattle were fed the basal diet. The second treatment consisted of feeding cattle Agrimos (MOS; Lallemand Animal Nutrition, Montreal, Canada) at an inclusion rate of 30g/steer daily added into the supplement. The third treatment consisted of feeding 2.5-cm ground wheat straw (WHT) at an inclusion of 5% of the diet DM replacing 5% of the DRC/HMC blend.

Blood Samples

Blood samples were collected via jugular venous puncture from each steer to obtain a baseline measure on the first day cattle were fully stepped onto the finishing diet (day 21). Blood samples were then collected two weeks following the baseline measure on the first Thursday in July. Blood was sampled from every steer during each week throughout the month of July with a final blood collection in August. This blood collection schedule resulted in a total of 7 blood collections throughout the duration of the trial. Therefore, blood was collected on 6/18/2015, 7/2/201, 7/9/2015, 7/16/2015, 7/23/2015, 7/30/2015, and 8/13/2015. Three 10 mL vacutainer tubes and one 5 mL vacutainer tube was collected form each steer. Two of the 10 mL vacutainer tubes contained ethylenediaminetetraacetic acid (EDTA). Samples were placed on ice after collection and transported back to the ruminant nutrition lab at UNL and centrifuged at 1200*g* for 10 min at 4 ℃. Plasma was removed and placed in four 2 mL screw capped tubes and frozen at – 80 ℃ until shipment to the Commonwealth Scientific and Industrial Research Organisation (CSIRO; Queensland, Aus.) for subsequent analysis. The third 10 mL vacutainer tube contained a clot activator and serum separation gel. This tube was placed on ice after collection and transported to the UNL ruminant nutrition lab where it was centrifuged at 1200*g* for 10 min at 4 ℃ and kept on ice until the sample was delivered to Nebraska Lab-Link (Lincoln, Neb) for analysis. Finally, the 5 mL vacutainer tube also contained EDTA. This sample was placed on ice after collection and brought back to the UNL ruminant nutrition lab where it was kept on ice until delivered to Physicians Lab (Omaha, Neb) for analysis within 2 h.

Blood plasma was analyzed for: white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils using the ADVIA 2120 hematology analyzer (Siemens Medical Solutions, Erlangen, Germany) with veterinary software as described by Harris et al., (2005). The blood serum was analyzed for: alkaline phosphatase alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, bilirubin, calcium, glucose, lactate dehydrogenase, phosphorus, and uric acid using a Dimension Vista 1500 analyzer (Siemens Medical Solutions, Erlangen, Germany) as described by Bruneel et al. (2012).

Environmental Stress Measurements

Environmental temperature, humidity, solar radiation, wind speed and barometer were collected and automatically saved to a central computer every thirty minutes throughout the duration of the trial using a Davis Vantage Pro 2 (Davis Instruments Vernon, IL) weather station located between the drovers alley and the settling basin behind the center pen, of the pen group, used in the current trial. The comprehensive climate index (CCI; Mader et al., 2010) was calculated and averaged to create a 3-d, 7-d, and 14-d, average prior to each blood collection; additionally, the 3-d, 7-d, and 14-d average heat load index (HLI; Gaughan et al., 2008) were calculated in the same manner (Table 13). A CCI of 22.2 at 0600 and 27.8 at 1200 was utilized as the cutoff to water cattle. This threshold resulted in cattle being sprinkled a total of 12 times throughout the duration of the trial.

All cattle received a SmartStock (SmartStock; LLC. Pawnee, OK) temperature monitoring rumen bolus during the first blood collection on d 21 of the trial. Boluses were programmed to transmit each individual body temperature in twenty minute intervals to a receiver located near the steer's home pen which then transmitted the reading to a central computer where data were stored. Therefore, when cattle were removed from their home pen, rumen temperature readings were no longer recorded.

Any readings below 35 ℃ were presumed to be drinking events and were removed. In addition to removing drinking events, temperatures were averaged by hour and by steer and any missing data points were then regressed in Microsoft Excel using the trend function with the previous 15 body temperature readings being used to create the trend. This method created a final data set with individual steer body temperatures every hour throughout the duration of the finishing period. However, due to a large number of missing bolus readings for the second replication (Table 14) only three animals within each pen of this replication with the least missed readings for the entire trial were chosen to be used for the analysis of body temperatures.

In addition to body temperature, panting scores were collected by a trained individual 5 times weekly at 1300 h. Scores were taken from outside of the pen in the feed alley as to not disturb the animals while taking the scores. The identification numbers of animals with a panting score of 3 or above were recorded in order to ensure that there wasn't a single animal within a pen always scoring greater than a 3. Panting scores were then compiled and averaged by pen for each collection day.

A hot period between June 25, 2015 and July 27, 2015 was chosen as a warm period and body temperature, panting score, and DMI were calculated separately for analysis during this time period. This time period was selected because feed refusals were collected on these days so actual DMI could be calculated. Additionally the month of July was the warmest period during the trial (Table 13).

On the final day of the trial, all pens were weighed prior to loading for transport to the abattoir to determine final live weight. Cattle were loaded at 17:00 and shipped to Greater Omaha Packing Co., (Omaha, NE) the evening prior to the scheduled harvest date. Cattle were held overnight and HCW and harvest order were recorded at time of harvest. After a 44-h chill, LM area, 12^{th} rib fat thickness, and marbling score were determined using the USDA grading camera. Yield grade was calculated (2.5 + (6.35x 12^{th} rib fat) + (0.2 x 2.5[KPH]) + (.0017 x HCW) – (2.06 x LM Area)) for each individual steer and then averaged within pen (USDA, 1997). Dressing percent was calculated for each pen by dividing HCW by final live BW using a 4% shrink.

Statistical analysis

Performance and carcass data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc. Carry, N. C.) with pen as the experimental unit. The model consisted of the fixed effects of dietary treatment (WHT, MOS, and CON). Blood parameters were analyzed as a repeated measure using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with pen as the experimental unit. The model consisted of the fixed effects of dietary treatment, time and the interaction between dietary treatment (WHT, MOS, and CON) and time along with a random residual. To account for the inherent covariance

structure between sequential blood measures, the residual was fitted with a covariance pattern by steer and a covariance of 0 across steers. Multiple covariance patterns were investigated and unstructured was chosen based on Akaike's information criteria.

Body temperatures were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc.) with pen as the experimental unit. Covariance structure was assessed in the same manner as that of blood measure and autoregressive 1 covariance structure was determined to best fit the data. The model consisted of the fixed effects of dietary treatment and day along with a random residual. Body temperature was characterized as 3-different phenotypes. Average, maximum and area under the curve (AUC) was evaluated. The average and maximum were calculated in excel using the average and max functions respectively on a per animal basis and then averaged by pen for analysis. Area under the curve was also calculated in excel using the sum function where all body temperatures were summed by animal by day and then averaged by pen for analysis. Panting scores were also analyzed using the GLIMMIX procedure of SAS. Covariance structure was assessed the same as that of blood measure and autoregressive 1 was determined to be the best fit. The model consisted of the fixed effects of dietary treatment and day along with a random residual.

A warm period was chosen between June 25, 2015 and July 27, 2015 and body temperatures and panting score were analyzed separately for this time point. The warm period body temperatures and panting scores were analyzed using the GLIMMIX procedure of SAS. The model included the fixed effects of dietary treatment and day along with a random residual. Simple covariate structure determined to be the best fit for

panting scores and autoregressive 1 covariance structure was determined the best fit for the body temperatures. Body temperature was analyzed as AUC, maximum, and average body temperatures and were calculated in the same manner as those for the overall trial.

RESULTS AND DISCUSSION

There was no difference ($P > 0.19$; Table 15) between treatments for initial BW, final BW, ADG, DMI, warm period DMI, NEg intake, carcass adjusted final BW, or carcass adjusted ADG. However, G:F was lower ($P < 0.05$) for WHT when compared to CON or MOS cattle due to numerical differences in DMI and ADG. These findings are supported by West et al., (1999) who observed no positive production effect of feeding increased fiber levels to dairy cattle during times of hot weather. West et al. (1999) observed a decrease in milk production in dairy cattle fed increased fiber levels which was attributed to displaced energy from the increased fiber content, which would also explain the reduced G:F observed in the current trial for cattle on the wheat diet. Mader et al. (1999) observed a decrease in ME intake for steers fed a high roughage diet when compared to a steers fed a traditional 6% roughage diet. This is contradictory to the current study where no difference was observed in NEg intake between the WHT and the CON, however, total roughage inclusion in the WHT treatment of the current trial totaled 12.5% whereas a 28% roughage diet was fed by Mader et al. (1999). Although not significant, in the current trial cattle fed WHT had, numerically, lower energy intake, possibly explaining the observed reduction in G:F as corn was displaced by the added fiber.

Mader et al. (1999) also observed no difference in DMI between a traditional 6% roughage finishing diet and the 28% roughage diet agreeing with the results in the current trial. However, it is important to note that in the current trial, cattle fed WHT had numerically greater intake for the overall feeding period and during the warm period when compared to MOS and CON. Likewise ADG was also numerically lower for WHT cattle. These numerical differences then contributed to the significant decrease in G:F observed for WHT cattle when compared to the CON and MOS. Additionally, Bagheri et al. (2009) observed no change in DMI or feed efficiency in dairy cattle when supplemented MOS, which is in agreement with the results of the current trial.

Hot carcass weight, dressing percentage, LM area, and marbling scores were not different ($P > 0.36$) between treatments. However, a difference ($P < 0.02$) in 12th rib fat depth and USDA yield grade was observed. Cattle fed CON had the greatest 12th rib fat depth and USDA yield grade when compared to MOS and WHT cattle which were not different ($P > 0.05$). This result is most likely due to the numerically greater energy intakes for the control cattle which allowed for the animals to accumulate more fat prior to slaughter.

Average and maximum body temperature was greatest ($P < 0.05$; Table 16) for cattle fed MOS when compared to CON and WHT treatments which were not different ($P > 0.05$) from one another. This finding is contradictory to findings reported by Mader et al. (1999) who observed that increased fiber level in the diet decreased body temperature in Hereford steers; however, a much greater level of fiber was supplemented than in the current trial. Cattle fed WHT in the current trial had reduced panting scores (*P*

< 0.05) when compared to the CON and MOS cattle, which were not different from one another ($P > 0.05$). This finding is in agreement with the findings by Mader et al. (1999) who observed a decrease in respiratory rate for cattle fed the high roughage diet when compared to cattle fed the high energy or conventional diet. However, these findings are contradictory to data presented in a review article by Fuquay (1981) where feeding increased fiber was observed to increase rectal temperatures and respiration rates in dairy cattle when compared to a low fiber diet, however, the level of fiber in either diet was not disclosed and dairy cattle are already fed greater levels of fiber when compared to finishing cattle.

There are few data evaluating the effect of supplementing MOS on body temperature, therefore, the reason an increase in body temperature was observed for cattle fed MOS is unknown. Energy intake was similar between all treatments and the addition of the Agrimos to the supplement was the only difference between CON and MOS diets. As body temperature was greater for MOS cattle, metabolic heat load was presumably increased by the addition of MOS and since energy intake was similar across treatments the increase in body temperature must be due to an effect on energy utilization due to supplementation of MOS. However, although average and maximum body temperatures were different between treatments, area under the curve body temperature was similar ($P = 0.18$) among treatments suggesting that, overall, the total magnitude of body temperatures was unaffected by treatment. Therefore, as maximum temperature was greater for MOS cattle, minimum temperature must then have also been lower to make up for the difference. Cattle with higher heat loads accumulated during the day attempt to

dissipate body temperature rapidly at night, which leads to overcompensation in anticipation for a higher heat load again the following day leading to observed decreases in body temperature (Gaughan and Mader, 2014; Mader and Kreikemeier, 2006). This supports the findings in the current study where MOS cattle had greater maximum and average body temperature then CON and WHT cattle, but total magnitude of body temperatures was not different.

Average and maximum body temperatures were also greatest ($P < 0.01$; Table 17) for MOS cattle during the selected warm period. Panting scores also followed a similar pattern and were the greatest ($P < 0.05$) for MOS cattle, least for WHT, and CON cattle were intermediate, agreeing with the findings by Mader et al. (1999) that increased fiber can reduce heat load on a finishing steer. There was no observed difference in area under the curve body temperature ($P > 0.05$) between treatments; however, cattle fed MOS had greater area under the curve body temperature numerically. This would suggest that the addition of MOS to the diet has little impact on reducing physical heat stress experienced by the animal. However, because no decrease in performance was observed, this may suggest that the observed slight increase in body temperature is of little impact on the animal during the conditions present in the current study.

There was a treatment × time interaction ($P < 0.05$; Table 18) observed for red blood cell count, hemoglobin, and hematocrit concentration. These interactions would suggest that over time and, as environmental conditions changed, there were differences in how these dietary treatments affected steers metabolically. The observed interaction in red blood cell count, hemoglobin, and hematocrit concentration could be of importance as

other research would suggest these metabolites are correlated with environmental temperature. Lee et al. (1976) observed a negative correlation between hematocrit concentration and ambient temperature. Furthermore, Olbrich et al. (1972) observed a depression in hematocrit concentration and red blood cell count in cattle subjected to elevated ambient temperatures.

Shaffer et al. (1981) observed significant effects in hemoglobin concentration due to environmental temperature. These findings were attributed to a decrease in cellular oxygen requirements, thereby resulting in reduced metabolic heat load. Furthermore, it was suggested by Lee et al. (1972) that the oxygen binding capacity of blood decreases when animals are under heat stress conditions. The addition of both WHT and MOS to the diet appeared to decrease both hemoglobin and hematocrit concentration during most of the trial (Fig. 1; Fig. 2, respectively) and seemed to prevent the spike from occurring during collection 5 where the 14-d average CCI and HLI was the greatest (Table 13). This may suggest that the addition of WHT and MOS to the diet may prevent dramatic metabolic changes from occurring during periods of hot weather; however, there are no data to further support this.

Bilirubin has also been found to change in response to environmental conditions. In the current study, an interaction ($P = 0.02$) was observed between treatment and time for bilirubin concentration. This interaction may be of importance as bilirubin has been observed by Ceran et al. (2001) to have a protective effect in the rat small intestine by preventing oxidative injury, which can be a result of heat stress (Tomaro et al., 2002; Yamaguchi et al., 1996). Thus, increased bilirubin concentration may be beneficial

during times of heat stress relative to the small intestine. The addition of MOS in the diet appeared to maintain serum bilirubin concentration during blood collection 5 when the greatest 14-d average CCI and HLI was observed (Fig. 3), which suggests that the addition of MOS may aid in lower gut health during times of warm weather. However, this assumes bilirubin is actually beneficial in mitigating lower gut inflammation and oxidation in finishing cattle as Attuwaybi et al. (2004) suggested it does in mice.

Time × treatment interactions ($P < 0.05$) between blood urea nitrogen, creatinine, blood urea nitrogen:creatinine ratio, and lactate dehydrogenase (LDH) were also observed. Blood urea nitrogen, creatinine, and LDH have all been observed to change during the last half of the feeding period as muscle growth is slowing and fat deposition is increasing (Bide et al., 1973). This along with the CON cattle having increased fat depth ($P < 0.05$) may help to explain the observed interaction between these values with treatment and time. There were also interactions ($P < 0.05$) observed between time and treatment for calcium, chloride, carbon dioxide, potassium, phosphorous, total protein, triglyceride, uric acid. However, there are few data observing these blood metabolites in relation to environmental conditions. Therefore, their significance is unknown and may be related to growth, BW change, or age.

Albumin:globulin ratio differed ($P = 0.01$) across treatments with WHT cattle being the lowest, MOS cattle being the greatest and CON cattle intermediate. There was also a difference observed for red blood cell distribution width ($P = 0.03$) following the same trend as that of Albumin:globulin ratio. Differences between treatments ($P < 0.05$) were also observed for phosphorus, absolute lymphocyte count, absolute monocyte count,

and absolute basophil count with CON cattle having the lowest concentration and WHT having the greatest with MOS being intermediate. Data presented by Schley and Field (2002) suggested the dietary fiber reaching the lower intestine aids in enhancing the immune system and may increase lymphocytes and other immune related cells such as monocytes and basophils. This may help explain the observed increase in these cell types in the WHT diet. Additionally, if this is the case, this may suggest that the reduction in particle size to 2.5-cm, as in the current trial, was sufficient to increase fiber flow to the lower gut and elicit the observed response in monocyte and basophil concentration. This is further supported by the total white blood cell count being greater ($P < 0.05$) in the current study for cattle fed the WHT diet than for cattle fed the CON or MOS diets.

There was also a difference ($P < 0.05$) observed between treatments for mean corpuscular volume and mean corpuscular hemoglobin observed in the current trial. Cattle fed the WHT diet had the lowest mean corpuscular volume and mean corpuscular hemoglobin when compared to MOS and CON cattle. However, these differences are most likely due to the slightly higher red blood cell count compared to hemoglobin and hematocrit concentration in the WHT diet. Even though these were not significant, there was a tendency ($P < 0.10$) for cattle fed MOS and WHT to have lower concentration of hemoglobin and hematocrit. Additionally there was a tendency ($P = 0.15$) for differences among treatments for red blood cell count with cattle fed WHT having the greatest red blood cell count. These together may be causing the observed difference in mean corpuscular volume and mean corpuscular hemoglobin as these values are calculated based on the concentration of red blood cells, hemoglobin, and hematocrit. Triglyceride

concentration was also observed to be different ($P = 0.02$) with cattle fed the CON diet having the lowest concentration compared with WHT and MOS treatments which were similar.

In the present study there was no observed performance benefits for cattle supplemented with MOS or WHT. The addition of MOS to the diet slightly increased body temperature both overall and during the selected warm period. The addition of WHT decreased G:F, however, no other performance traits were affected. CON cattle had greater 12th rib fat depth and USDA yield grade than both the WHT and MOS cattle although no other differences were observed for carcass characteristics. While body temperature remained unchanged by the addition of WHT to the diet, panting scores were decreased when compared to both the CON and MOS diets possibly suggesting that the addition of finely ground wheat straw to the diet may alleviate some of the environmental stress experienced by the steers and improve animal comfort. Many blood metabolites reacted differently across time between treatments however both WHT and MOS appeared to decrease hemoglobin and hematocrit concentration, which have been shown to be positively correlated to environmental conditions suggesting that these supplements may aid the steer's metabolic reaction to environmental conditions. Even though some benefits to animal comfort may be provided by the addition of MOS and WHT to the diet, the lack of performance response suggests these strategies do not alleviate environmental stress.

LITURATURE CITED

Attuwaybi, B. O., R. A. Kozar, S. D. Moore-Olufemi, N. Sato, H. T. Hassoun, N. W. Weisbrodt, and F. A. Moore. 2004. Heme oxygenase-1 induction by hemin protects against gut ischemia/reperfusion injury. J. Surgical Res. 118:53-57

Bagheri, M., G. R. Ghorbani, H. R. Rahmani, M. Khorvash, N. Nili, and K. H. Südekum. 2009. Effect of live yeast and mannan-oligosaccharides on performance of early lactation Holstein dairy cows. Asian-Aust. J. Anim. Sci. 22:812-818

Bide, R. W., W. J. Dorward, and M. E. Tumbleson. 1973. Clinical chemistry of grain-fed cattle. I. A preliminary study of a basic biomedical profile. Can. J. Anim. Sci. 53:697-707

Bruneel, A., M. Dehoux, A. Barnier, A. Boutten. 2013. External evaluation of the dimension vista 1500 intelligent lab system. J. of Clinical Laboratory Analysis. 26:384-397

Ceran, C., k. Sönmez, Z. Türkyılmaz, B. Demiroğulları, A. Dursun, E. Düzgün, and N. Kale. 2001. Effect of bilirubin in ischemia/reperfusion injury on rat small intestine. Journal of pediatric surgery, 36:1764-1767

Fuqyay J. W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 52:164-174

Gaughan, J. B., T. L. Mader, S. M. Holt, A. Lisle, 2008. A new heat load index for feedlot cattle. J. Anim. Sci. 86:226-234

Gaughan, J. B., and T. L. Mader. 2014. Body temperature and respiratory dynamics in unshaded beef cattle. Int. J. Biometeorol. 58:1443-1450

Harris, N., J. Kunicka, and A. Kratz. 2005. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. Lab Hematol. 11:47-61

Hongyu, R., M. W. Musch, K. Kojima, D. Boone, A. Ma, and E. B. Chang. 2001. Short-chain fatty acids induce intestinal epithelial heat shock protein 25 expression in rats and IEC 18 cells. Gastroenterology. 121:631-639

IACUC. 2015. Policies and Procedures Manual for the Use of Animals at the University of Nebraska-Lincoln. Univ. Neb. Lincoln, NE

Kregel, K. C., P. T, Wall, and C. V. Gisolfi. 1988. Peripheral vascular responses to hyperthermia in the rat. J. Appl. Physiol. 64:2582-2588

Kregel, K. C. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. 92:2177-2186

Lambert, G. P., C. V. Gisolfi, D. J. Berg, P. L. Moseley, L. W. Oberley, and K. C. Kregel. 2002. Molecular biology of thermal regulation selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J. Appl. Physiol. 92:1750-1761

Mader, T. L., J. B. Gaughan, and B. A. Young. 1999. Feedlot diet roughage level for Hereford cattle exposed to excessive heat load. Professional Anim. Sci. 15:53-62

Mader, T. L., and W. M. Kreikemeier. 2006. Effects of growth-promoting agents and season on blood metabolites and body temperature in heifers. J. Anim. Sci. 84:1030-1037

Schley, P. D., and C. J. Field. 2002. The immune-enhancing effects of dietary fibers and prebiotics. British J. Nutrition. 87:221-230

Sohail, M. U., Z. U. Rahman, A. Ijaz, M. S. Yousaf, K. Ashraf, T. Yaqub, H. Zaneb, H. Anwar, and H. Rehman. 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. J. Poultry Sci. 90:2573-2577

Tomaro M. L., and Batlle, A. M. C. 2002. Bilirubin: its role in cytoprotection against oxidative stress. International J. Biochem. & Cell Bio. 34:216-220

Watson, A. K., B. L. Nuttelman, T. J. Klopfenstein, L. W. Lomas, and G. E. Erickson. 2013. Impacts of a limit-feeding procedure on variation and accuracy of cattle weights. J. Anim. Sci. 91:5507-5517

West, J. W., G. M. Hill, J. M. Fernandez, P. Mandebvu, and B. G. Mullinix. 1999. Effects of Dietary fiber on intake, milk yield, and digestion by lactating dairy cows during cool or hot, humid weather. J. Dairy Sci. 82:2455-2465

Yamaguchi, T., M. Terakado, F. Horio, K. Aoki, M. Tanaka, and H. Nakajima. 1996. Role of bilirubin as an antioxidant in an ischemia-reperfusion of rat liver and induction of heme oxygenase. Biochem. and biophys. Res. Comm. 223:129-135

Ingredient (%)	CON	WHT	MOS
High moisture corn	34.25	31.75	34.25
Dry rolled corn	34.25	31.75	34.25
Modified distillers grains plus soulubles	20.0	20.0	20.0
Sorghum Silage	7.5	7.5	7.5
Wheat Straw (2.5 cm)	-	5.0	-
Supplement			
Fine ground corn	1.4018	1.4018	1.1476
Limestone	1.7080	1.7080	1.7080
Salt	0.3	0.3	0.3
Urea	0.4	0.4	0.4
Tallow	0.1	0.1	0.1
Beef trace mineral	0.05	0.05	0.05
Rumensin	0.0165	0.0165	0.0165
Vitamin A-D-E	0.015	0.015	0.015
Tylan	0.0087	0.0087	0.0087
Agrimos (30g/steer daily)	-	-	0.2542

Table 12. Composition of diets between control (CON), wheat straw (WHT), and Agrimos (MOS) fed cattle

	Three day average[1]		Seven day average[2]		Fourteen day average[3]	
Collection	CCI[4]	HLI[5]	CCI[4]	HLI[5]	CCI[4]	HLI[5]
1	25.21	15.34	23.83	15.43	25.68	18.13
2	27.45	23.30	27.07	22.71	26.82	21.17
3	19.20	11.33	21.43	13.61	24.25	18.16
4	33.38	27.89	31.93	27.30	26.68	20.46
5	27.59	22.69	30.52	25.51	31.22	26.41
6	25.41	17.17	28.79	22.61	29.66	24.06
7	28.04	23.22	29.69	24.73	28.16	21.61

Table 13. Comprehensive climate index (CCI) and heat load index (HLI) averages in relation to blood collections

[1]Three days prior to blood collection weather averages correlated to blood measures

[2] Seven days prior to blood collection weather averages correlated to blood measures

[3] Fourteen days prior to blood collection weather averages correlated to blood measures

[4] Comprehensive climate index = Ambient Temperature (Ta; ℃)+($e^{(0.00182\times\text{relative humidity(RH)}+1.8\times10\text{^-}5\times Ta\times RH)}$×(0.000054×$Ta^2$+0.00192×Ta–0.0246)×(RH - 30))+(-6.56/$e^{(1/(2.26\times\text{wind speed (WS) }+0.23)\text{^}(0.45\times(2.9+1.14\times10\text{^-}6\times ws\text{^}2.5-\log0.3(2.26\times WS+0.33)\text{^-}2))))}$-0.00566×$WS^2$+3.33) + (0.0076×Solar Radiation (RAD)-0.00002×RAD×Ta+0.00005×Ta^2×√(RAD) +0.01×Ta-2) Mader et al., (2010)

[5] Heat load index (HLI; black globe temp (BG) > 25 ℃) = 8.62 + (0.38 × RH) + (1.55 × BG) − (0.5 × WS) + e2.4 − WS; HLI BG < 25 = 10.66 + (0.28 × RH) + (1.3 × BG) – WS (Gaughan et al., 2008)

Table 14. Missing rumen bolus temperature readings by treatment.

	Treatment	Total Missed (%)[1]	Corrected Missed (%)[2]
Replication 1	Control	27.77	-
	Wheat	16.57	-
	Agrimos	16.70	-
Replication 2	Control	47.06	31.00
	Wheat	36.25	17.67
	Agrimos	66.46	44.00
Replication 3	Control	36.19	-
	Wheat	35.44	-
	Agrimos	26.76	-
Replication 4	Control	15.13	-
	Wheat	39.62	-
	Agrimos	20.75	-

[1] Total missing bolus readings with all animals in each replication left in the analysis

[2] Total missing bolus readings after only using the three animals with the most readings from the second replication.

	Control	Wheat	Agrimos	SEM	P-Value
Live Performance					
Initial BW, kg	479	480	479	2.1	0.98
Final BW, kg	701	696	701	5.9	0.80
ADG, kg	1.87	1.83	1.88	0.044	0.66
DMI, kg/d	12.4	12.7	12.3	0.21	0.43
Hot Period DMI[1], kg/d	11.9	12.6	11.9	0.28	0.19
NEg Intake, Mcal/d	14.50	14.02	14.38	0.245	0.39
G:F	0.151[b]	0.144[a]	0.154[b]	0.0019	0.01
Carcass Adjusted Performance					
Final BW, kg	692	681	690	5.9	0.38
ADG, kg	1.80	1.71	1.78	0.044	0.24
G:F, kg/kg	0.145[b]	0.134[a]	0.146[b]	0.0029	0.02
Carcass Characteristics					
HCW, kg	436	429	435	3.7	0.38
Dressing %	62.3	61.7	62.0	0.28	0.36
LM Area, cm^2	87.1	88.4	89.0	1.29	0.45
12^{th} Rib Fat, cm	1.52[b]	1.30 [a]	1.35 [a]	0.056	0.01
Marbling[2]	474	471	476	13.0	0.97
USDA Yield Grade[3]	3.9[b]	3.5[a]	3.5[a]	0.13	0.02

Table 15. Main effect of Agrimos and wheat straw supplementation on performance and carcass traits.

Values within rows with unique superscripts are different ($P < 0.05$)

[1]Period between 6/25/2015 and 7/27/2015

[2]300 = slight, 400 = Small, 500 = Modest.

[3]Calculated as 2.5 + (6.35x 12^{th} rib fat) + (0.2 x 2.5[KPH]) + (.0017 x HCW) – (2.06 x LM Area) USDA, 1997.

Table 16. Main effect of treatment on body temperature and panting measurements

					P-Value	
	Control	Wheat	Agrimos	SEM	Trt	Interaction[1]
Average	39.06[a]	39.06[a]	39.22[b]	0.034	<0.01	<0.01
Maximum	40.56[a]	40.50[a]	40.83[b]	0.064	<0.01	<0.01
AUC[2]	2456	2455	2462	1.3	0.18	0.95
Panting Score[2]	1.75[b]	1.72[a]	1.76[b]	0.010	<0.01	<0.01

Values within rows with unique superscripts are different ($P < 0.05$)

[1] Interaction between time and treatment

[2] Area under the curve = Total magnitude of individual animal body temperature within each treatment

[3] Panting scores based on 0 to 4 scale with 0 = no panting and 4 = severe distress

Table 17. Main effect of treatment on body temperature and panting measurements during the selected warm period[1]

					P-Value	
	Control	Wheat	Agrimos	SEM	Trt	Interaction[2]
Average	39.16[a]	39.16[a]	39.33[b]	0.053	<0.01	<0.01
Maximum	40.86[a]	40.78[a]	41.07[b]	0.072	<0.01	<0.01
AUC[3]	2460	2460	2467	2.6	0.18	0.95
Panting Score[4]	1.87[ab]	1.84[a]	1.90[b]	0.010	0.05	<0.01

Values within rows with unique superscripts are different ($P < 0.05$)

[1] Warm period between 6/25/2015 and 7/27/2015

[2] Interaction between time and treatment

[3] Area under the curve = Total magnitude of individual animal body temperature within each treatment

[4] Panting scores based on 0 to 4 scale with 0 = no panting and 4 = severe distress

Table 18. Main effect of control (CON), wheat straw (WHT) and Agrimos (MOS) diets on blood measures

					P-Value	
	CON	WHT	MOS	SEM	Trt	Int[1]
Albumin:Globulin, ratio	0.87[ab]	0.83[a]	0.90[b]	0.016	0.01	0.98
Albumin, mg/dL	3.49	3.46	3.53	0.026	0.18	0.55
Alkaline phosphatase, U/L	132.4	123.0	113.4	6.21	0.10	0.85
Alanine transferase, U/L	30.5	32.5	29.5	0.96	0.09	0.08
Aspartate transferase, U/L	108.0	98.0	103.3	4.05	0.22	0.23
B:C[2], ratio	9.30	8.87	9.16	2.1	0.33	0.05
Direct Bilirubin, mg/dL	0.00	0.00	0.00	0.063	0.99	0.71
Bilirubin, mg/dL	0.18	0.17	0.19	0.005	0.26	0.02
Total Bilirubin, mg/dL	0.19	0.18	0.19	0.005	0.35	0.04
Blood Urea Nitrogen, mg/dL	11.4	10.9	11.5	0.27	0.23	0.01
Calcium, mg/dL	9.99	9.86	9.92	0.042	0.11	0.03
Cholesterol, mg/dL	95.5	97.9	88.9	3.08	0.10	0.14
Creatinine Kinase, U/L	156.7	180.9	177.5	12.79	0.35	0.89
Chloride, mEq/L	102.2	101.9	102.3	0.20	0.29	<0.01
Carbon Dioxide, mM/L	27.9	27.5	27.5	0.25	0.46	0.05
Creatinine, mg/dL	1.23	1.24	1.27	0.024	0.51	0.02
GGT[3], U/L	30.1	28.0	29.6	1.07	0.34	0.80
Globulin, mg/dL	4.08	4.16	3.98	0.058	0.09	0.69
Glucose, mg/dL	74.1	76.5	75.0	1.61	0.58	0.12
Potassium, mEq/L	4.84	4.78	4.82	0.029	0.27	0.01
Lactate Dehydrogenase, U/L	1454	1425	1386	23.4	0.12	0.04
Sodium, mEq/L	142.0	141.7	142.0	0.20	0.39	0.56
Phosphorus, mg/dL	7.39[a]	7.61[b]	7.57[ab]	0.071	0.05	0.04
Total Protein, mg/dL	7.57	7.61	7.50	0.060	0.43	0.05
Triglyceride, mg/dL	15.7[a]	17.8[b]	18.4[b]	0.72	0.02	0.03
Uric Acid, mg/dL	0.58	0.59	0.61	0.019	0.68	0.03
White blood cell, cells/L	9.13[a]	10.05[b]	9.50[a]	0.212	<0.01	0.64

Red blood cell, cells/L	8.20	8.29	7.94	0.132	0.15	<0.01
Hemoglobin, g/dL	13.6	13.3	13.2	0.142	0.07	<0.01
Hematocrit, %	37.9	37.0	36.9	0.38	0.10	<0.01
MCV[4], fL/cell	46.5[b]	45.0[a]	46.9[b]	0.54	0.03	0.35
MCH[5], pg/cell	16.7[b]	16.1[a]	16.7[b]	0.20	0.05	0.54
MCHC[6], g/dL	35.8	35.8	35.7	0.07	0.21	0.96
RDW[7], μm	20.9[ab]	20.6[a]	21.5[b]	0.23	0.03	0.18
Platelet, cell/L	439.7	465.9	463.5	15.23	0.40	0.63
Neutrophils, %	27.4	26.9	27.8	0.77	0.73	0.45
Absolute Neutrophils, cell/μL	2.52	2.72	2.64	0.089	0.27	0.64
Lymphocytes, %	61.0	60.8	59.8	0.87	0.57	0.65
Absolute Lymphocytes, cell/μL	5.56[a]	6.10[b]	5.69[ab]	0.159	0.04	0.38
Monocytes, %	5.81	5.82	5.69	0.152	0.80	0.87
Absolute Monocytes, cell/μL	0.53[a]	0.58[b]	0.54[ab]	0.018	0.05	0.80
Eosinophils, %	4.77	5.37	5.71	0.351	0.16	0.11
Absolute Eosinophils, cell/μL	0.45	0.56	0.55	0.040	0.10	0.07
Basophils, %	0.99	1.06	1.01	0.027	0.20	0.76
Absolute Basophils, cell/μL	0.10[a]	0.11[b]	0.11[ab]	0.003	0.02	0.32

Values within rows with unique superscripts are different ($P < 0.05$)

[1]Interaction between time and treatment

[2] Blood urea nitrogen: creatinine ratio

[3] Gamma-glutamyl transpeptidase

[4] Mean corpuscular volume

[5] Mean corpuscular hemoglobin

[6] Mean corpuscular hemoglobin concentration

[7] Red blood cell distribution width

Figure 5. The effect of treatment on hemoglobin concentration across blood collections

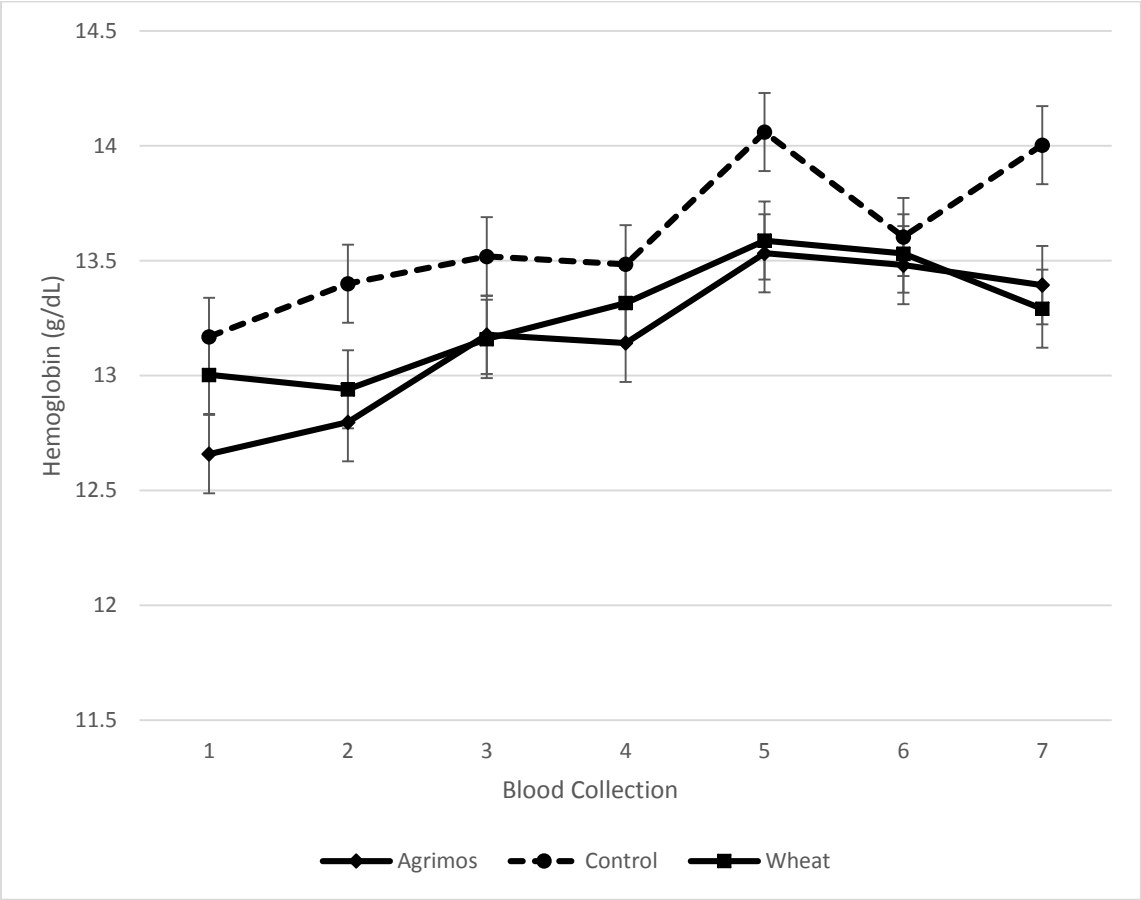


Figure 6. The effect of treatment on hematocrit concentration across blood collections

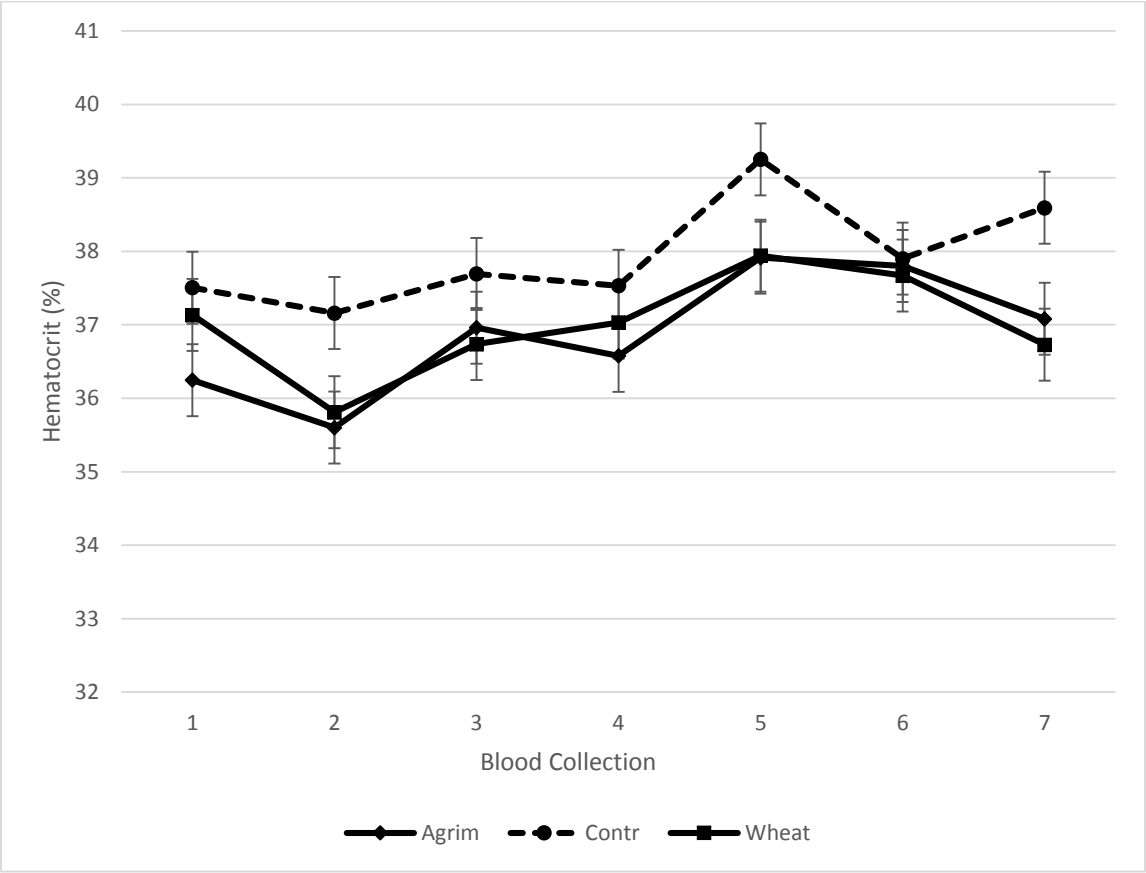


Figure 7. The effect of treatment on bilirubin concentration across blood collections