UTILIZING LABORATORY AND FIELD STUDIES TO DETERMINE PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS

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UTILIZING LABORATORY AND FIELD STUDIES TO DETERMINE PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS

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A candidate for the degree of Doctor of Philosophy in Animal Sciences
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LIST OF ABBREVIATIONS

ADG	Average daily gain
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
BUN	Blood urea nitrogen
BW	Body weight
CPK	Creatine phosphokinase
D2	Dopamine-2 receptor
E-	Endophyte-free fescue diet
E-/E-	Animals were on E- pasture and E- seed in the chamber
E-/E+	Animas that were on E- pasture and E+ seed in the chamber
E+	Endophyte-infected fescue diet
E+/E-	Animals that were on E+ pasture and E- seed in the chamber
E+/E+	Animals were on E+ pasture and E+ seed in the chamber
EV	Ergovaline
FI	Feed intake
GGT	Gamma-glutamyl transferase
Hct	Hematocrits
HPLC	High-performance liquid chromatography
HS	Heat stress
NIST	National Institute of Standards and Technology
POST	Post-summer chamber run
PRE	Pre-summer chamber run
PRL	Prolactin
RH	Relative humidity
RR	Respiration rate
T_a	Ambient temperature
$T_{appendage}$	Appendage temperature, combination of ear, and tail
T_{bg}	Black globe temperature
T_c	Core body temperature

TCI	Thermal circulation index
T_{db}	Dry bulb temperature
T_{env}	Environmental temperature
THI	Temperature-humidity index
TN	Thermoneutrality
TNZ	Thermoneutral zone
T_{re}	Rectal temperature
$T_{rum} - T_{re}$	Difference between the 2 locations
T_{rum}	Ruminal temperature
T_{skin}	Skin temperature
T_{trunk}	Trunk temperature, combination of shoulder and rump

UTILIZING LABORATORY AND FIELD STUDIES TO DETERMINE PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS

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ABSTRACT

A series of studies were conducted to investigate the physiological responses of cattle to multiple stressors. These stressors included heat stress, water restriction, and intake of endophyte-infected tall fescue. Many of the symptoms of these stressors are similar including reduced feed intake, reduced growth rate, hyperthermia, and in extreme cases death. However little is known about the interaction between two stressors. In the first experiment, animals were subject to both water restriction and heat stress to determine if dehydration under a controlled heat challenge would compromise thermoregulation. In the remaining experiments, animals placed on either endophyte-infected or endophyte free treatments and given controlled heat challenge to study adaptive changes. Ingestion of endophyte (*Neotyphodium coenophialum*) - infected tall fescue (E+) often impairs animal health and productivity. These effects stem from a collection of symptoms referred to as fescue toxicosis. Clinical signs of fescue toxicosis are dependent on both the ambient temperature and period of exposure making it a good candidate to study the interaction with heat stress.

In the first experiment, Eight Angus steers were maintained for 5 days at thermoneutrality (TN; 19-21°C) in the Brody Environmental Center followed by 14 days of cyclic heat stress (HS; 26-36°C). Water was removed starting on Day 5 of heat stress

(dehydration phase). After 3 days, water was returned starting the rehydration phase. Measurements included rectal temperature (T_{re}) and respiration rate (RR) measured six times daily. Body weight, feed and water intakes, and sweat rate at rump and shoulder were recorded daily during acclimation, dehydration and rehydration. During dehydration, steers lost ~10% of their body weight, which they regained within 36 h of rehydration. As expected, feed intake decreased (~75%) within 24 h of dehydration, but quickly recovered during rehydration. Dehydration reduced RR (~15bpm), which remained low throughout rehydration ($P<0.05$). Similar to RR, T_{re} increased during HS (0.6°C; $P<0.05$). Surprisingly, no increase in T_{re} occurred during dehydration ($P=0.41$), while rehydration caused a 0.8°C drop. Shoulder and rump sweat rates sharply increased with HS, but dropped to TN levels during dehydration ($P<0.05$), before recovering when water was returned. Steers showed no lasting effects of dehydration, with the thermal status of the animal returning to normal after 48 hours of rehydration. Unexpectedly, core body temperature remained relatively unchanged despite dehydration, demonstrating their ability to adapt to changing conditions.

In the second experiment, we utilized both a short-term, controlled exposure and a longer-term field exposure to determine adaptive changes. Each animal was given an initial "stress test" prior to summer to determine a baseline for their response to a controlled heat challenge. This was followed by placement in the summer field environment (South Farm, University of Missouri) for ~3 months, to create the long-term (i.e, real world) scenario. At the end of the summer, animals were split into 4 treatment groups (E-/E-, E+/E+, E+/E-, and E-/E+) and given the same controlled "stress test." Twenty-two Angus steers (365 ± 10kg BW) were housed in the BEC for 7 days at air

temperature (Ta) of 20^{o}C (TN), followed by 7 days of cyclic heat stress (HS; Ta=26^{o}C night; 36^{o}C day). Respiration rate (RR) and rectal temperature (Tre) were measured 6 times daily. Sweat rate was measured at shaved sites (shoulder, rump) on select days. Results from this experiment showed only a few signs of adaptation. With exception of feed intake, animals in the two groups that switched (E-/E+ and E+/E-) treatments responded to the current diet rather than previous exposure, suggesting no adaptation to the toxin. Feed intake was lower for all treatments during the final chamber run which could signify adaptation to heat stress. Sweat rate showed the greatest change between chamber tests, as well as within chamber runs with a reduction after several days in the heat.

In the third experiment, twenty-three Angus steers were subject to both a controlled heat challenge and a field exposure. However, unlike above, animals were placed on pasture prior to a chamber test to adapt to the summer conditions. Steers were given a controlled stress challenge during the peak of summer when they would have the most heat acclimation. In addition to studying adaption of animals to heat and fescue toxicosis, the objective of this experiment was compared the field and chamber exposures to determine how similar the responses under each situation in order to be able to transfer information from one source into another. During the controlled heat challenge, steers were assigned to E+ pasture during the field exposures received a diet containing 40µg ergovaline/kg/d to maintain the fescue toxicosis state. Respiration rate (RR) was measured via flank counting and telemetric temperature transmitters in the rumen of each animal transmitted core temperature ($T_{rum;}$ 20 minute interval). Linear regression fit models for RR, T_{rum}, and air temperature (T_a) was utilized to compare relationships

between field and chamber exposure. Correlation coefficients for respiration rates were similar during both chamber (R =0.69) and field exposures (R = 0.72). Respiration rate showed greater responsiveness to change in T_a under field conditions having twice the slope in the chamber test (4.4 versus 1.75 bpm/°C) and a lower Y-intercept (-42.14 versus +30.97 °C) compared to the chamber run. Ruminal temperature was consistent between exposures showing a similar slope (0.04 °C versus 0.03°C T_{rum}/°C T_a) and Y-intercept (38.4 versus 39.3°C) for its relationship with air temperature. While respiration rate may be the more sensitive indicator of heat stress, ruminal temperature proved to be the more consentient variable between exposures.

In the final experiment, animals were again subjected to both field and chamber exposures. It has been suggested that cattle are more sensitive to endophyte-infected fescue at the end of summer when they have potentially lost some of their heat acclimation. Therefore, this experiment was conducted at the end of summer as temperatures were cooling off. As with the previous experiments, during heat challenges, steers were assigned to either 0 or 40µg ergovaline/kg/d diets to maintain the fescue toxicosis state. Feed intake quickly decreased more than 50% in the E+ animals ($P < 0.05$), while E- animals FI did not change during the challenge. HS resulted in a further decrease in FI for E+ animals ($P < 0.05$). Respiration rate was significantly higher for E+ animals under TN conditions ($P < 0.05$). During HS, E+ animals continued to have the higher rate, however it was short-lived, with E- animals rising to the same level by the end of the chamber exposure. Rectal temperature was also higher for E+ animals at TN ($P < 0.01$). HS resulted in an increase in rectal temperature for both groups with E+ animals showing the greatest increase ($P<0.01$), but like respiration rate both groups came

together and were not different by the end of the trial. Surprisingly, T_{rum} showed no differences between groups during TN or HS. This is likely due to a decline in feed intake and heat production associated with consumption of endophyte infected seed. While E+ animals did show a large response under TN conditions, their response to HS was similar to experiments 2 and 3 suggesting they are not more sensitive to endophyte-infected fescue at the end of summer.

Results of these experiments showed little evidence that repeated exposure to the endophytic toxins gives animals a tolerance to the endophytic toxins. Feed intake, rectal temperature, sweat rate and skin temperature responded similarly for E+ animals regardless of previous exposure suggesting a lack of adaptation. Sweat rate however, did show signs of adaptation in the E- animals being reduced between the start to the end of summer. Sweat rate also showed a decrease after several days in the heat. This reduction occurred even though rectal temperature and respiration rate were still elevated, suggesting that reduction of sweat rate, and possibly water loss, is more important than a reduction of body temperature during heat stress.

CHAPTER ONE

REVIEW OF LITERATURE

1. INTRODUCTION

Over the past two decades, global animal production has increased, especially in developing countries (Renaudeau et al., 2012). Environmental factors such as ambient temperature, solar radiation, and relative humidity are the main limiting factors of production efficiency in regions around the world. In North America, heat stress occurs during the 2 to 3 summer months and during periodic hot spells. Summer heat stress results directly and indirectly in reduced performance, increasing economic losses and animal welfare concerns. Hot spells, on the other hand, can result in mortality along with large changes in performance. According to the National Oceanic and Atmospheric Administration (2011), the summer of 2011 was the second hottest on record for the United States, and the hottest in the last 75 years. While the direction of climate change or variability for the future cannot be predicted with certainty; the U.S. Global Change Research Program has reported that hot summers, similar to recent years, will be more common in the future (Karl et al., 2009). Potential stressors include increased heat wave intensity, frequency, and longevity. Few studies have examined these individual stressors and even less have addressed their interactive impact.

Although acute heat stress at subcritical level may have little effect on an animal, the level of vulnerability for un-acclimated animals, and the combined effect of multiple subcritical stressors could raise the impact to a significant level. Very little research has been conducted looking at multiple environmental stressors. Stressors such as fescue

toxicosis and dehydration exacerbate the symptoms of heat stress, to result in major production losses and even death in extreme cases. The primary and initial production-related effect of heat stress on cattle is a reduction in feed intake (Hahn and Mader, 1997; Mader, 2003; West, 2003). Almost any change in intake or dietary nutrient profile reduces heat production (Mader, 2003; Mader and Davis, 2004) and shifts downward whole body heat content. The acute ability of cattle to withstand a hot environment during exposure to multiple stressors can determined by the animal's immediate reactions to heat stress, as measured by strain on respiration rate, internal body temperature, and by productivity (Dowling, 1956). The goal of this dissertation is to explore animal reaction to such stressors with a focus on the acclimation process.

1.1. Economic Losses

While there have been many breakthroughs in technology and management systems to reduce the impact of heat stress, major productions losses still exist. Annual losses average $897 million, $369 million, $229 million, and $128 million dollars for the dairy, beef, swine, and poultry industries, respectively (St-Pierre et al., 2003). Heat abatement programs have reduced the total economic losses from 2.4 billion to 1.7 billion dollars (St-Pierre et al., 2003). However, as we continue to select for higher producing animals, there will continue to be problems with heat stress conditions.

The magnitude of this loss for bovines is substantial. In July 1995, a heat wave in Iowa resulted in cattle deaths and performance losses of 28 million dollars (Smiley, 1996). During a California heat wave in the summer of 2006, approximately 25,000 cows died (AFP, 2006). This loss was equivalent to $1,500 - 2,500 per head, or between $37.5 and 62.5 million dollars in total. In the last 10 years, economic losses in the feedlot

industry alone have averaged $10 - 20 million dollars per year as a result of adverse climatic conditions (Mader, 2007). For each animal that dies from climatic stress, there is a corresponding economic loss that approaches $5,000, due to mortality and reduced animal performance (Mader, 2007). Across most of the US, loss of body weight gain has been estimated at 10 kg/yr, or an additional 7 days in the feedlot (St-Pierre et al., 2003). In addition, the Canadian Global Coupled Model climate forecast scenarios predict that time to slaughter weight will require 4.8 days more in the future, costing producers an additional $43.9 million annually (Hahn et al., 2002). Another forecast model, the Hadley model, also predicts an additional 2.8 days of production and a loss of $28 million dollars annually. Interestingly, these models also predict that approximately one-half of summer production declines are offset by improvements in productivity during the winter. While some aspects such as warmer temperatures may bring localized benefits, reduced water availability and more frequent extreme weather will still pose a significant risk (Alcamo et al. 2007; Arnell 2004; Easterling et al. 2000; Nijssen et al. 2001). Considering the global importance of US agriculture, any impact is likely to also influence world food security and economic retrocessions.

2. HEAT EXCHANGE AND THE THERMOREGULATORY PROFILE

2.1. Homeothermy

All living organisms exchange heat with their external environment and are in a constant state of dynamic change. Animals are divided into two groups based on thermoregulatory ability: homeotherms or warm-blooded, and poikilotherms or cold-blooded animals. One difference between the two relates to their metabolic rate, with homeotherms having a heat production level that is 7-10 times that of poikilotherms. This

higher metabolic rate in homeotherms provides them with the ability to regulate their internal body temperature independent of ambient temperature within a certain range. Homeotherms maintain a relatively stable internal body temperature range from 33-40°C, relying on the modification of behavior, heat production, and heat loss. Body temperature is an expression of body heat content. The equation for the storage of total body heat or change in body heat content is

$$\text{Equation 1:} \quad S = M+W\pm K \pm C \pm R - E \qquad \text{(IUPS, 2001)}$$

Where S equals the storage of body heat, M equals the metabolic heat production, W is work rate, K is the heat exchanged by conduction, C is the heat exchanged by convection, R is the heat exchanged by radiation, and E is the heat exchanged by evaporation.

2.2. Heat Exchange

Core body temperature is the outcome between the balance between production (i.e., metabolic rate) and heat outflow (i.e., heat loss) (Spiers, 2012). Metabolic rate is the primary source of heat gain for the living homeotherms, as stated previously. Heat exchange into and out of animals occurs by four pathways: conduction, convection, radiant exchange, and evaporation. Under most environmental conditions, temperature represents the driving force for heat exchange between the animal and its environment. However, moisture and heat content of the air, thermal radiation, and airflow also alter total heat exchange. Thus, a combination of environmental variables contributes to the conditions (effective or apparent temperature) to which an animal responds.

Another way of explaining this balance is to separate heat exchange into 3 categories: heat production (metabolism), non-evaporative heat loss (conduction, convection, and radiation), and evaporative heat loss. Non-evaporative is also referred to

as sensible heat transfer because it is associated with the temperature differences of materials (Hansen, 2004). Sensible heat exchange can be negative or positive (i.e., loss or gain) depending on the circumstance. Although heat can be gained through conduction, convection, and radiation, ambient conditions must rise above body temperature to reverse the heat flow into the body, which is not common. As skin temperature increases, the gradient between it and the body core declines, slowing the heat transfer to the surface (McDowell, 1972). As this occurs, evaporative heat loss is the way the animal can lose the excess heat. Evaporative only occurs in one direction, which is away from the body. Evaporative heat loss is referred to as insensible heat exchange because it involves the latent heat of vaporization and change in the kinetic energy of molecular arrangement without a change in material temperature.

2.2.1. Conductive Exchange

Conduction occurs within bodies and between objects in direct contact with each other. Conductive heat transfer (*K*) is defined as the net rate of heat transfer in a solid material or a non-moving gas or fluid (i.e., by conduction) down a thermal gradient, within an organism, or between an organism and its external environment (IUPS Thermal Commission, 2001). Heat exchange by conduction at the skin level is dependent on thermal conductance, surface area, and temperature gradient, and is described using the formula:

Equation 2: $Q_k = (h_k)\,(A_k)\,[(T_1 - T_2) \div (d)]$ (Curtis, 1983)

Where:

h_k is the thermal conductance ($h_k = K \cdot \Delta T^{-1}1$; W/m2/°C), A_k is the surface area (m^2) for heat exchange, $T_1 - T_2$ is the gradient in temperature, associated with skin temperature

and the temperature of air or water, d is the distance between points T_1 and T_2, and K is the thermal conductivity. Heat is only transferred from a higher temperature to lower temperature object. Thus, the amount of heat transferred is proportional to the magnitude of the temperature gradient. Since air has a relatively low thermal conductivity in comparison with water, any air trapped against the skin reduces conductive heat exchange and acts as an effective insulator. The magnitude of heat transfer is also dependent on the nature of the material in contact with the skin in conductive exchange, in particular its thermal conductivity. Thermal conductivity refers to the ability of a material to conduct heat, and is an intrinsic property of that material.

Heat transfer across materials of high thermal conductivity occurs at a higher rate than across materials of low thermal conductivity. In general, metals have the largest values. As a result, a metal surface will feel cooler than a material with a lower thermal conductivity even at the same temperature.

2.2.2. Convective Exchange

Convective heat transfer (C) is defined as the net rate of heat transfer in a moving gas or fluid (i.e., by convection) between different parts of an organism, or between an organism and its external environment (IUPS Thermal Commission, 2001). Convective heat transfer is amplified by thermal gradients and by forces such as wind, fans, pumps or body movement, and usually expressed in terms of unit area of the total body surface (IUPS Thermal Commission, 2001). There are two forms of convective heat exchange: passive convection and forced convection. Passive exchange occurs when air near the skin surface microenvironment is heated to result in a reduction in air density and movement up from the surface (Spiers, 2012). This produces small currents of air that

may result in significant heat loss under resting conditions (Spiers, 2012). Forced convection occurs due to external forces (winds or fans).

Convective heat transfer is described in the following equation as:

Equation 3: $Q_h = (h)\ (A_h)\ (T_s - T_{env})$ (Curtis, 1983)

Where:

h is the coefficient of convective heat transfer ($h_c = C \cdot \Delta T^{-1}$), A_h is the animals surface area that is affected by convection, T_s is the skin or surface temperature, and T_{env} is temperature of the environment or fluid. Convective heat exchange is similar to conduction in that it is driven by the temperature gradient.

2.2.3. Radiative Exchange

Radiant heat exchange (R) is the only means by which heat flows without the aid of a material medium, allowing it to pass through a vacuum. It is defined as the net rate of heat exchange by electromagnetic energy between an organism and its environment, and is usually expressed in terms of unit area of the total body surface, i.e., as a heat flux (IUPS Thermal Commission, 2001). This avenue of heat exchange is described as:

Equation 4: $Qr = Ar\ \sigma\ [(aTe^{-4}) - (eTs^{-4})]$ (Curtis, 1983)

Where:

Ar is the effective radiative surface area of the animal (m^2), A is the absorptivity of the animals surface for thermal radiation, σ is Stefan-Boltzmann constant, Te is the average absolute temperature of animals radiant environment (°K), E is the average emissitivity of environmental surfaces for thermal radiation, and Ts is the average absolute temperature of animals radiant surface (°K). Heat exchange by radiation involves transfer of heat through electromagnetic waves. Any object with a temperature above absolute

zero emits electromagnetic radiation. The electromagnetic radiation emitted carries energy away from the source to surrounding (or distant) objects. This energy is absorbed by objects, causing the average kinetic energy of their particles to increase and causing the temperatures to rise. The hotter the object, the more it radiates. Thermal radiation encompasses a wavelength range of 0.1 to 100 μm containing both the visible light and infrared spectrums. Within the visible spectrum (0.38–0.78 μm wavelengths), the color of an object is an important factor of heat transfer and is derived entirely from the sun or lamps. A "black body" surface has an absorbance of 1 because it completely absorbs all wavelengths of thermal radiation (actual black bodies don't exist in nature). Heat exchange in the infrared spectrum (i.e., 0.78–100 μm wavelengths) is independent of color. Heat transfer in this way occurs by the emission of electromagnetic waves which transmits energy away from the emitting object. Any two objects that "see" each other radiate toward each other with the net direction depending on the surface-temperature gradient (Curtis, 1983). The heat transferred into or out of an object by thermal radiation is a function of several components. These include its surface reflectivity, emissivity, surface area, temperature, and geometric orientation with respect to other thermally participating objects.

2.2.4. Evaporative Exchange

As environmental temperature rises closer to body surface temperature, the animal must invoke evaporative heat loss to dissipate body heat. Evaporative heat loss is relatively independent of temperature, depending mainly on a vapor-pressure gradient. Evaporation of a liquid occurs when liquid molecules have sufficient energy to overcome cohesive forces and escape from the liquid surface into the environment (Curtis, 1983).

Energy is put into a liquid to produce a phase change to a gaseous state. This phase change must happen at the surface of the skin for heat loss to occur. If it does not change to a gaseous state due to high humidity, or if it drips off the animal, there is essentially no heat dissipation. Evaporative heat transfer (*E*) is defined as the rate at which heat energy is transferred by evaporation from or condensation on the skin and the surfaces of the respiratory tract, and usually expressed in terms of unit area of total body surface (IUPS Thermal Commission, 2001). The equation for evaporative heat loss is:

Equation 5: $Qe = (\lambda)\ (Aw)\ (d)\ (Ea - Es)$ (Curtis, 1983)

Where:

λ is the waters latent heat of evaporation at the surface temperature, Aw is the wet area of the animals surface, d is the evaporative diffusion coefficient, Ea is the vapor pressure of air, and Es is the vapor pressure at the evaporation surface. Air speed and relative humidity important are important for maintaining a high vapor pressure gradient in the surface surrounding the animal.

2.3. Thermoregulatory Profile

Environmental conditions that provide maximum comfort and require little or no energy expenditure for body temperature maintenance define the thermoneutral zone (TNZ). The concept of thermoneutral zone can be misleading because it gives the impression that it is a fixed range for a given animal (Hillman, 2009). This is not the case. The TNZ is defined as the range of ambient temperature at which thermoregulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production or evaporative heat loss (IUPS Thermal Commission, 2001). It changes with body size, insulation, posture, resting metabolic rate, and basal metabolic

rate (IUPS Thermal Commission, 2001). Species, breed, age, gender, degree of thermal acclimation, and time of day all alter the limits of the TNZ zone (Yousef, 1985). For instance, the TNZ is narrow for young animals and wider for adult animals of the same species. The TNZ can generally range between 15 to 25°C for most cattle less than a month old and between −10 and 20°C for adult cattle with energy-dense feedlot diets (Gaughan et al., 1999).

As the ambient temperature reaches the limits of the TNZ, it approaches the lower and upper critical temperatures that define the TNZ. Lower critical temperature is the ambient temperature below which the rate of metabolic heat production of a resting animal must be increased by shivering and/or nonshivering thermogenesis in order to maintain thermal balance (IUPS Thermal Commission, 2001). Upper critical temperature is the ambient temperature above which the rate of evaporative heat loss of a resting thermoregulating animal must be increased (e.g., tachypnea or sweating) in order to maintain thermal balance (IUPS Thermal Commission, 2001). Upper critical temperature has also been used to define the ambient temperature above which the mechanisms of heat transfer to the environment of a resting animal are exceeded and the core temperature is forced to rise and, as a consequence, metabolic energy transformation, i.e., the internal heat load, is further increased (IUPS Thermal Commission, 2001).

3. PHYSIOLOGICAL RESPONSES TO HEAT STRESS

3.1. Behavioral Changes

Although many animals have special heat loss mechanisms that enable them to control their core body temperature, such as sweating or panting, these activities involve the use of stored energy and water. Behavioral adjustments are recruited first because

they occur quickly and usually do not require much energy (Hillman, 2009). For instance, increasing body conductance by vasodilation or adjusting the thickness of the hair can be made quickly without large metabolic expenditures (Hillman, 2009). The simplest behavioral modification involves an animal changing their orientation to the direction of the wind or sun (Cabanac, 1975). Cattle will also bunch together to obtain shade from each other as a natural herding response when under stress (Meat & Livestock Australia, 2006). Ansell (1981) found that cattle refused to lie down during heat exposure. Hillman et al. (2005) reported that the core body temperature of cows rises when lying and falls while standing in hot environments. Standing in lakes or streams is also utilized as a behavioral change to increase conductive heat loss to water in order to reduce heat stress. Water has a higher specific heat than air and, as a result, is a superior heat sink. When cattle were exposed to heat they frequently sprinkled drinking water over their bodies (Ragsdale et al., 1951).

One of the most common behaviors observed in heat stressed cattle is to seek shade to reduce solar radiation (Hillman, 2009). Cattle will begin to seek shade as ambient temperature exceeds 20˚C, although heat adapted animals may not utilize shade 28˚C (Meat & Livestock Australia, 2006). Natural shade, such as trees, are their first preference, however artificial shade structures are beneficial (Meat & Livestock Australia, 2006; Rovira and Velazco, 2010). During periods of extreme heat stress, cattle are known to graze for longer periods at night than during the day (Payne et al., 1951).

3.2. Metabolism

The main source of body heat accumulation in cattle is metabolic heat (Spiers, 2012). Living animals use oxygen to burn carbohydrates, fats, and protein in a process

called metabolism (DeShazer and Yen, 2009). The chemical activity is used for work, growth, and animal products, with a large portion going to waste heat (DeShazer and Yen, 2009). Metabolism generates one third of the heat load of a cow standing in a hot environment (Finch, 1986). In the resting animal, a large portion of this metabolic heat load is maintained in the core and must be transported by the blood to the skin and extremities where it is transferred to the animal's surroundings (Finch, 1986).

Cattle adapt to hot environments by decreasing heat production or increasing heat dissipation (McDowell, 1972). Increasing heat dissipation is sufficient when heat loads are short (i.e., 1–2 days) (Meat & Livestock Australia, 2006). However, if these conditions continue, cattle must reduce metabolic heat to control their core body temperature (Finch, 1986). This is achieved by a reduction in feed intake. It is widely accepted that feed intake "drive" is somehow related to the energy/oxygen metabolism of the animal (Gill and Romney, 1994). However, it is not known whether a reduction in feed intake comes before or after a reduction in metabolism.

3.3. Feed Intake

Food intake is directly related to all aspects of energy metabolism with the release of heat for maintenance, activity, and production (Finch, 1986; Purwanto et al. 1990). Higher than normal feed intake increases metabolic rate and possibly heat accumulation which gives rise to a greater requirement for heat loss. When environmental temperature is elevated above the thermoneutral zone, the metabolic rate also increases as a result of the increased body temperature and Q_{10} effect (Whittow, 1971). This gives rise for increased heat loss effort and a need to reduce feed intake. This reduction in food intake is followed by a reduction in metabolic rate, helping balance heat production with loss

(Turner and Taylor, 1983). However, this connection between reduced feed intake of cattle and increased environmental temperature is only partly understood.

The extent to which feed intake is reduced due to changes in body or ambient temperature varies depending on the type of feed, air temperature, thermal radiation, and body temperature (Yousef, 1968). Feed intake reductions between 3 – 25% have been reported in the literature (Bianca et al., 1965; Johnson et al., 1966; Conrad, 1985). Cattle on high energy grain diets (consistent with a higher heat of digestion) can reduce dry matter intake by more than 25% and may never return to previous levels of intake despite lower temperatures (Meat & Livestock Australia, 2006). Cattle on low quality roughage (low energy) diets experience variable intake reductions, often around 10%, and are more likely to return to full feed when conditions return to normal (Meat & Livestock Australia, 2006).

3.4. Mechanisms of Heat Exchange

To maintain internal body temperature within narrow limits requires sensitive and quick-acting mechanisms which balance most changes in heat production. The rate of heat transfer from an animal to the surrounding environment is dependent on both the thermal and vapor pressure gradients. The influence of the thermal environment on an animal is primarily exerted through energy exchanges which involve convection, conduction, radiation, and evaporation.

3.4.1. Conduction

Conduction has two primary roles within cattle. It is partially responsible for the movement of heat from the core to the skin surface and the flow of heat from the periphery to surrounding objects in contact with the skin surface. Therefore, conductive

heat flow to the surrounding in cattle mainly applies to ground or water contact. When standing, the heat transfer with the ground has to occur through the animal's feet; which constitutes only 2% of the body surface area (Meat & Livestock Australia, 2006). If an animal is lying on a cool or wet surface when as much 20–30% of the body surface may be in contact with the ground, conductive heat transfer will be much greater and depend on several variables including thermal conductance, thermal gradient, and area of contact (Meat & Livestock Australia, 2006). While conduction is probably the least important mode of heat dissipation in cattle during heat stress (McDowell, 1972), it is important for other livestock animals such as swine. Conduction and the thermal conductivity of objects also play an important role in thermal perception (Spiers, 2012).

3.4.2. Convection

Since cattle are often considered to be furred animals, the fur entraps a layer of air close to the skin and this tends to resist passive or natural convection (Robertshaw, 1985). In most conditions, passive convection is of little importance to cattle. However, forced convection breaks up the layer of air retained by the fur (i.e., microclimate) and greatly increases sensible heat loss through the fur (Gebremedhin, 1987). Convection also plays a role in respiratory heat loss apart from evaporative cooling. The enhanced air flow through the nasal passages and upper respiratory tract can carry a large amount of body heat (Robertshaw, 1985). The inspired air is not only humidified, but adjusted to core body temperature by the time the air reaches the trachea (Robertshaw, 1985). On expiration, the animal is able to dissipate a significant amount of body heat.

Convective heat transfer by circulation is a main mode of heat transfer to the periphery in animals (Spiers, 2012) that requires very little energy expenditure.

Increasing blood flow to the skin by vasodilation increases the thermal gradient between the skin surface and air prompting heat loss to the surroundings (McDowell, 1972; Hillman, 2009). The adjustment of skin temperature to meet the needs of temperature regulation occurs primarily in the extremities; whereas variation in the trunk region is very small (Ames, 1970).

3.4.3. Radiation

Studies on animal heat exchange in the long-wave portion of the electromagnetic spectrum indicate that the net transfer is away from the animal (Robertshaw and Finch, 1976). The magnitude of radiant heat transfer is complex. Radiation can be a significant source of incoming radiant heat for animals during the middle of the day. At the same time, the night sky acts as an important heat sink, cooling the animal following days of excessive heat loads. Exposure is a function of direct sunlight to the surface of the animal, as well as the amount reflected from the ground. For the most part, solar radiation in cattle is a function of surface area exposed to the radiation, and the color and structure of their coat (Robertshaw 1985). The intensity of solar radiation that reaches the earth's surface varies, but can be as high as 1000 W/m^2 (Walsberg, 1983). The primary site of absorption of radiant heat is the surface of the hair in cattle.

Many studies have looked at the effect of solar radiation on heat gain in cattle. Da Silva et al. (2003) found that light colored hair coats have reflectance values much greater than those of dark colored hair coats. A black coat has an absorbance of nearly 1.00, whereas white fur has an absorbance of 0.37 and red fur of 0.65 (Cena and Monteith, 1975). However, Nelore cattle, which have a mixture of white and dark hairs, had a higher reflectance value than white cattle. Skin color also plays a role. Non-

pigmented skin has a reflectance level that is lower than light grey skin (Da Silva et al, 2003). Red and brown skins have reflectance levels close to those of black and gray skin in the same range (Da Silva et al, 2003). Another issue with solar radiation becomes coat structure. Cena and Monteith (1975) have shown that number of hairs, length, diameter, and the angles relative to incident of radiation are all key factors that are unaffected by coat color, making the impact of solar radiation on animals even more complex..

3.4.4. Evaporation

Under hot conditions, the amount of heat that can be lost via conduction, convection, and radiation is limited. When the ambient temperature is above or equal to skin temperature, evaporation is the only avenue available for cattle to lose heat (Collier and Zimbelman, 2007). The vaporization of water from the body takes place from the respiratory tract and through the skin via sweating. It is important to note that all animals experience passive evaporative heat loss from both skin and respiratory surfaces (Hillman, 2009). However, both panting and sweating undergo tremendous change during heat stress.

3.4.4.1. Respiration

In every animal, there is increased frequency of breathing above the upper critical ambient temperature to increase heat dissipation (Spiers, 2012). In cattle, this increase in respiratory rate (panting) involves an increased ventilation of the dead space (Robertshaw, 1985). Heat is carried by the blood supply to the nasal mucosa and the cool blood drains into the venous sinuses at the base of the skull (Robertshaw, 1985). Cattle pant with their mouth closed causing heat exchange to take place at the mucosa of the

upper respiratory tract. At higher rectal temperatures, the respiratory rate declines while tidal and minute volumes increase. If sustained, this ultimately leads to low blood levels of carbon dioxide and increased blood pH (Whittow, 1971). A transition from rapid, shallow breathing to a slower, deeper type of respiration allows for maximal respiratory evaporative cooling with minimal disruption of the blood gases (Whittow, 1971). The last phase of respiratory frequency is open-mouthed panting, with the tongue protruding, and coincides with the peak respiratory rate and may be dictated by airway resistance (Hahn, 1999).

Respiratory rate has long served as an indicator of heat load and heat strain on the animal during hot weather (Hahn, 1999). The normal range of respiration rate for cattle at thermoneutrality is 30 to 60 bpm (Smith, 1996). A respiration rate of 90 to 110 bpm is indicative of cattle under moderate to high thermal stress, whereas above 130 bpm is considered to be an excessive heat load (Eigenberg et al., 2005). Respiratory rate is influenced by ambient temperature, solar radiation, relative humidity, and wind speed. Of these, ambient temperature is the most important (Hahn, 1999). Hahn et al. (1997) showed that respiration rate is strongly correlation with ambient temperature above 21°C, increasing at a rate of 4.3 bpm per °C. Respiratory rate eventually hits a ceiling characterized by a shift from rapid shallow breathing to slower open mouth panting (Hahn et al., 1997). While most studies have looked at respiration rate, the panting score as defined by Meat & Livestock Australia (2006) book may, in fact, be a better indicator of thermal strain. Although evaporative cooling is enhanced by panting, it is not as effective as sweating for heat dissipation.

3.4.4.2. Sweating

During heat stress, sweating accounts for about 85% and panting for about 15% of the total evaporative heat loss in cattle (Maia et al., 2005). The amount of sweat produced by each sweat gland in cattle is much less than that of humans (Robertshaw, 1985), with the total\ produced per unit of surface area also being less. Droplets of water on the skin are only visible under a stereo microscope and not to the naked eye (Ferguson and Dowling, 1955). This led many researchers to state that cattle are not a sweating species (Worstell and Brody, 1953). Many environmental factors affect sweating rates in cattle. An increase in relative humidity from 30 to 90% can lower the sweating rate from 500 to 60 g/m^2h (Maia et al., 2005). Changing air velocity over the hair coat surface from 0.2 to 0.9 m/s raises sweating rate from about 75 to 350 g/m^2h (Hillman et al., 2001).

There are two types of sweat glands: eccrine and apocrine. Eccrine glands (also called atrichial) are open to the surface of the skin and arranged in a spiral fashion through the epidermis. Apocrine glands (also called epitrichial) are larger, associated with hair follicles, and have ducts that open into the hair follicle (Ingram and Mount, 1975). Apocrine glands release fluid with adrenergic stimulation, while the eccrine glands rely on cholinergic pathways (Robertshaw, 1977). Humans possess both eccrine and apocrine glands; however cattle only possess apocrine (Findlay et al., 1950; Dowling, 1955; Nay and Hayman, 1956).

The total number of sweat glands is determined at birth (Folk and Semken, 1991). The distribution of these glands in cattle varies greatly with region, with greater numbers in the trunk regions and fewer in the legs and ventral region (Hillman, 2009). These differences in numbers result in differences in sweat rates during heat exposure (Volcani and Schindler, 1954; Berman, 1957; Scharf et al., 2008). Findlay and Yang (1950) were

one of the first to show that the ventral region of the neck and trunk had the greatest number of sweat glands, while the forehead and legs slowed the lowest numbers in Ayrshire dairy cows. They determined the average number of sweat glands per square cm to be 1,871. Though not extensively studied, it generally accepted that the regional distribution of sweat glands is similar across a variety of breeds (McLean, 1963).

4. FESCUE TOXICOSIS

4.1. Introduction

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. –*Schedonorus arundinaceus* [Schreb.] Dumort) is one of the most important cultivated pasture grass in the USA, occupying over 35 million acres (Hoveland, 2003). It is the predominant cool-season perennial forage that supports approximately 20% of beef cattle in the United States (West and Waller, 2007). Tall fescue was introduced from Europe sometime in the 1800s. It quickly became known for its tolerance to drought, insects, and extreme soil pH (Cowan, 1956). This led to it (primarily the Kentucky-31 variety) being planted across the U.S. throughout the 1940s and 1950s (Browning, 2003). Its low demand on labor and grazing control make tall fescue a highly favorable cultivar for livestock producers. In general, the nutritive value of tall fescue is similar to that of other perennial, cool-season grasses. The nutritive value of tall fescue (15 to 18% crude protein) depends on plant maturity and soil fertility. However, unlike other cool-season grasses, the relationship between the nutritive value of tall fescue and animal daily response is variable.

After widespread adoption of fescue in the 1940s, problems arose with the well-being and performance of their cattle (Stuedemann and Hoveland, 1988). The cause of these problems was discovered in the late 1970s to be from alkaloids produced by

endophytic fungi (*Neotyphodium coenophialum*). Unfortunately, removal of the fungi from the plant eliminated the toxicity problem but resulted in a weakened plants tolerance. It has been estimated that over 90% of tall fescue pastures in the U.S. are infected with the fungal endophyte (Bacon and Siegel, 1988; Glenn et al., 1996). Fescue has since become known for its three general problems: fescue foot, fat necrosis, and fescue toxicosis.

4.2. Economic Impact Associated with Endophyte-Infected Fescue

Fescue toxicosis is one of the most notorious grass-related livestock problems in the United States, affecting more than 8.5 million beef cows and 700,000 horses (Stuedemann and Seman, 2005). Annual economic losses to the beef industry from decreased conception and weaning weights associated with fescue toxicosis have been estimated to be more than $600 million (Hoveland, 1993). Reduced weaning weight alone costs the cow/calf sector $338 million. In Missouri, it is estimated that fescue toxicosis is responsible for $160 million loss for the beef industry. Further economic impacts occur for the horse and sheep industry (Thompson et al., 2001). Altered thermoregulatory ability, decreased feed intake, and reduced average daily gain further increase the adverse economic effect of fescue toxicosis (Spiers et al., 2005). All in all, this severe livestock disorder costs U.S. livestock producers up to $1 billion each year. Considering inflation and losses to other livestock, the economic cost associates with this problem will continue to rise (Panaccione et al., 2001).

4.3. Symbiotic Relationship of Fescue with Endophyte

Neotyphodium coenophialum grows in the intercellular space in tall fescue plants, and has a mutualistic, symbiotic relationship (Bacon and Siegel, 1988). The fungus lives

its entire life cycle within the plant, thus called an endophyte. Unlike most fungi, this one is not visible externally on the tall fescue plant. The endophyte grows within the cell walls of the tall fescue, with mycelia growing throughout the intracellular spaces (Kimbrough et al., 1993). It does not invade the plant cells nor is it pathogenic to the plant (Thompson et al., 2001). The endophyte infects the developing seed and its life cycle follows that of the fescue grass. The plant supplies both a home and nutrition for the fungus and, in return, the host plant receives benefits that include: improved drought tolerance, root development (deeper in the soil), water conservation, tolerance of pests, utilization of nitrogen, and seedling vigor and growth potential (Hill et al., 1990; Hill, 1994; Sleper and West, 1996; Latch, 1997). Tall fescue benefits from the endophyte-grass association, in part, because of a group of ergot alkaloids produced by both the endophyte and the plant (Roberts and Andrae, 2004). These ergot alkaloids can include compounds such as clavine alkaloids, lysergic acid amides, and ergopeptines (Roberts and Andrae, 2004). In addition to the drought and insect tolerance, the endophyte is also leads to a series of health disorders in grazing animals (Roberts and Andrae, 2004). As stated previously, removal of the fungi from the plant eliminated the toxicity problem, but resulted in a weakened plant to stressful environmental conditions and less competitive advantage with other plant species (Hoveland, 2003).

4.4. Toxins Associated with Fescue Toxicosis

The search for the causative agents of fescue toxicosis has been ongoing since animal disorders were first discovered. Ergot alkaloids have emerged as the generally accepted toxic agents of the tall fescue endophyte. However, no definitive experiment has been reported to demonstrate this (Bush and Fannin, 2009). Some of the ergot

alkaloids associated with endophyte infected tall fescue include ergovaline, ergotamine, ergocryptine, ergocornine, ergosine, lysergic acid amide, and ergonovine (Porter, 1995; Bush and Fannin, 2009). Of the ergot alkaloids, ergopeptides and lysergic acid amides have received the most attention (Browning, 2003). Ergopeptine alkaloids, including peramine and loline alkaloids, are readily found and inhibit the activities of root eating nematodes and other insects. They are believed to have minimal toxicity in mammals (Porter, 1995); however, they may reduce reproduction and growth in large animals in high enough quantities (Thompson et al., 2001). Ergovaline, on the other hand, is the most frequently researched of these alkaloids as it comprises more than 85% of total ergopeptine alkaloids found in the infected fescue and is thought to be the major toxin responsible for fescue toxicosis (McCollough et al., 1994; Porter, 1995; Oliver, 1997). Using isolated bovine tissues, ergovaline has been shown to cause vasoconstriction (Dyer, 1993; Klotz et al., 2007). Vasoconstriction results in reduced blood flow to the skin and lower peripheral skin leading to overheating and fescue foot. In an in vivo experiment, McLeay et al. (2002) using purified ergovaline (administered intravenously), found altered cardiovascular function, reduced skin temperature, and induced heat strain in sheep. Because of this, fescue samples are often tested for ergovaline concentration to indicate the toxic potential of tall fescue pasture or hay (Roberts and Andrae, 2004). However, there is evidence that ergovaline may not be the only contributor to fescue toxicosis and may work in concert with other ergot alkaloids to produce the toxicological syndrome (Gadberry et al., 2003).

Ergovaline contains D-lysergic acid, L alanine, L-valine and L-proline slightly differing from another alkaloid, ergotamine, (the most common ergopeptine) containing

L-valine in place of L-phenylalanine (Panaccione et al., 2001). Though ergotamine is found in endophyte-infected tall fescue at lower levels than ergovaline (Yates et al., 1985), it is has still been heavily researched do to its similar structure and pharmacodynamic properties (Porter, 1995). Osborn et al. (1992) demonstrated that consumption of ergotamine by steers induced decreased feed intake and peripheral skin temperature, increased rectal temperature and respiration rate, and reduced weight gains which are all consistent signs of fescue toxicsosis. Similarly, McLeay et al. (2002) found that ergotamine and ergovaline had similar effects on cardiovascular and thermoregulatory function in sheep. Ergovaline and ergotamine together consistently show the performance problems observed in cattle grazing endophyte-infected tall fescue making them the primary toxins believed to be associated with fescue toxicosis.

Other research by Hill et al. (2001) has reported that ergovaline and ergotamine have a difficult time crossing the gastrointestinal tract suggesting they are not the cause of the symptoms. In the study, researchers suggest that the primary ergot alkaloids transported across gastrointestinal tissue are lysergic acid and lysergic acid amides (Hill et al., 2001). Lysergic acid amides have been shown to elicit similar physiological responses as ergopeptides in terms of vasoconstriction in isolated bovine tissue (Oliver et al., 1993) and altered hormone profiles in cattle (Browning et al., 1998). Although candidates have been proposed as possible toxicants, the primary agent or set of agents is still unknown. Additional research is required to confirm which of these or other compounds are of primary concern for each of the affected physiological systems in grazing animals.

4.5. Toxicological Conditions Associated with Fescue Toxicosis in Cattle

Over the last several decades, tall fescue has gained a reputation for a variety of livestock health problems that result in poor performance (Strickland et al., 1993). Three separate syndromes associated with tall fescue (Ball et al., 2002) include: fescue foot, bovine fat necrosis, and summer slump. The type of thermal strain (i.e., cold versus hot) experienced by livestock following intake of infected fescue is an important determinant of the specific clinical outcome (Hemken et al., 1981). Extreme heat for example exacerbates toxicosis-related health problems and cold increases the incidence of fescue foot.

4.5.1. Fescue Foot

Symptoms of fescue toxicosis are generally less severe in cooler temperatures (Chestnut et al., 1991), however during the winter, sloughing of the tips of the ears, tails, and hooves can result. This condition, known as fescue foot, is characterized by tissue necrosis and dry gangrene of the distal extremities (Yates, 1983). Clinical signs start with swelling and reddening at coronary band, knuckling of the pastern joint, and arching of the back in extreme cases. These signs are due to the effects ergot alkaloids on blood vessels, and result in damage to vessel-lining cells, enhanced blood clotting, vasoconstriction of the vessel lumens (Tor-Agbidye et al., 2001), and ultimately regional reduction or lack of blood flow (Strickland et al., 1993; Oliver, 2005). Fescue foot in cattle develops during long-term intake of endophyte-infected tall fescue (E+) hay or pasture (Spiers et al., 2005). Jensen et al. (1956) reported that cattle became lame as early as 18 d after grazing E+, whereas Jacobson et al. (1970) reported gangrene as early as 25 d after the start of E+ grazing. In the early stages of fescue foot, red or flushed color is observed at the coronary band of the hoof. If cattle remain on E+ tall fescue, gangrene can develop with

eventual necrosis and sloughing of the hooves (Bush et al., 1979; Waller, 2009). Although fescue foot is an acute and costly problem when it occurs, tall fescue toxicosis causes more financial losses to cattle producers because of its often unobserved widespread incidence (Waller, 2009).

4.5.2. Fat Necrosis

During post-mortem examinations cattle grazing E+ have been diagnosed with a disorder known as liptomatosis, or bovine fat necrosis (Wilkinson et al., 1983). Typically there are no external signs of fat necrosis. Fat necrosis results in hard fat deposits in the abdomen that can interfere with digestion or parturition (Waller, 2009). It is now known that ergot alkaloids can be retained in fat tissue (Realini et al., 2003). This suggests that fat deposits may serve as a reservoir for toxic alkaloids. The retention of alkaloids by fat tissues may serve as a clue to the cause of fat necrosis (Roberts and Andrae, 2004). The occurrence of fat necrosis in cattle has been associated with cattle grazing E+ pastures that have received high levels of nitrogen fertilization (Stuedemann et al., 1985) mainly from poultry litter or other manure. Even though fat necrosis is not a widespread problem, it can be costly to individual beef cattle producers (Williams et al., 1969).

4.5.3. Summer Slump

In beef cattle, the term "summer slump" has been used to refer to visual symptoms that occur during most summers (Strickland et al., 1993). Symptoms can include failure to shed winter hair coats, intolerance to heat (i.e., ambient elevated temperature), and reduction in weight gain, milk production, and pregnancy rate (Strickland et al., 1993). In horses, mares have serious reproduction problems with prolonged gestation, dystocia, agalactia, and abortions. Behaviorally, animals often seek

shade, form wallows around water troughs and in shaded areas, and spend less time grazing than their unaffected counterparts (Strickland et al., 1993; Oliver, 2005). Despite the term “summer slump”, research has shown that animal performance is reduced throughout the year, with a large decrease in weight gain during the spring.

4.6. Effects of Endophyte-Infected Fescue on Livestock Performance

4.6.1. Feed Intake

Decreased weight gain and feed intake are common responses by most species (e.g., cattle, rats, and horses) to consuming an E+ diet (Strickland et al., 2009). Feed intake, in particular, is an extremely sensitive indicator of fescue toxicosis (Spiers, 2012). The depression in feed intake associated with fescue toxicosis can be turned on and off with addition and removal, respectively, of the E+ diet (Spiers, 2012). In addition, a drop in feed intake can take place with no additional clinical signs of fescue toxicosis (Spiers et al., 2012). Causes of reduced feed intake are not known, but could be due to a number of possibilities including endophyte-produced toxicants acting on the feeding and satiety centers, gastrointestinal tract motility, blood flow patterns, and/or a sensation of sickness are all possibilities (Strickland et al., 2009). It is believed that the majority of decline in weigh gains are due to the reduced feed intake. However, there is evidence that digestibility may also be altered in sheep, cattle, and horses (Redmond et al., 1991; McCann et al., 1992).

Average daily gain for steers grazing tall fescue has been used as a reliable indicator of the severity of tall fescue toxicosis. Schmidt et al. (1982) fed endophyte-E+ and endophyte-free (E-) tall fescue seed to beef steers during a 53 day trial. Steers fed the E+ diet gained 0.76 kg/d less than those fed E- seed. Jackson et al. (1984) compared

performance of steers consuming either E+ fescue or orchard grass. Steers on E+ fescue lost weight; weighting 14 kg less than steers consuming orchard grass. Paterson et al. (1995) in a review summarized 11 trials. He reported that steer gains were 30 to 100% lower when steers consumed E+ fescue versus E- fescue. During a controlled study, Aldrich et al. (1993) fed E+ and E- fescue to steers housed at either 22 or 32°C. At 32°C, cattle consumed 22% less feed and 62% more water than animals housed at the lower temperature showing that air temperature further exacerbates the feed intake response.

In addition to the decline in feed intake when given diets, E+ fescue also influences behaviors such as grazing times. Cattle grazing E+ pastures tend to shift their grazing time to cooler periods of the day and often will graze more at night than during daylight (Bond et al., 1984; Stuedemann et al., 1985). Howard et al. (1992) reported that cattle assigned to E+ pastures spent more time idling and standing, and had fewer prehensile bites than cattle grazing endophyte free tall fescue suggesting they spend less time foraging.

In addition to the decline in feed intake, Hannah et al. (1990) suggested that inhibition of ruminal fiber digestion in animals consuming infected seed as a possible explanation for the reduced intake observed. Several controlled studies have been conducted looking at digestibility in sheep. Matthews et al. (2005) and Westendorf et al. (1993) both reported that dry matter, neutral detergent fiber, and acid detergent fiber digestibilities were depressed by intake of E+ fescue. Osborn et al. (1992) observed that even though some E+ fed steers consumed feed more than the calculated maintenance level (NRC, 1984) they lost weight, further suggesting that a reduction in efficiency is occurring. Currently, it is still unknown how the alkaloids affect nutrient absorption, feed conversion, and feed intake. Theories include a lack of sufficient nutrient absorption time

or the use energy for other functions such as detoxification (Oliver et al. (2000; Strickland et al., 2009).

4.6.2. Respiration

As stated above, the level of thermal stress experienced during E+ fescue intake is an important determinant of the clinical signs (Spiers et al., 2005). Under cold or TN conditions, consumption of E+ fescue has little impact on respiration rate. However, intake of an E+ diet during HS results in a marked increase in respiration rate under both field (Jacobson et al. 1970; Waller et al., 2009) and chamber conditions (Spiers et al., 2012; Scharf et al., 2012). This increase is likely due to the inability to transfer heat to peripheral tissues via blood flow (Rhodes et al., 1991). In order to maintain thermal balance, the animal switches to evaporative heat loss. There is also evidence that the alkaloids have direct effects on receptors in the lung tissue and blood platelets that may cause hypoxemia, resulting in a subsequent reflex increase in respiration rate (Strickland et al., 2009). Respiration rate, reported in the literature, are highly variable depending on the level of stress and level of the alkaloid intake (Oliver, 1997).

4.6.3. Core body temperature

Hyperthermia is a primary characteristic of fescue toxicosis that often is used to define the magnitude of this condition (Strickland et al., 2009). However, during cold or TN conditions, consumption of the endophyte results in either lower or unaltered core body temperature. Osborn et al. (1992) observed no change in core body temperature or respiration rate, in steers consuming an E+ diet at 21ºC. Intake of the same diet during HS increased both core body temperature and respiration rate. Schmidt et al. (1982)

reported increased rectal temperature in steers grazing E+ fescue as well as E+ tall fescue hay. This increased core body temperature is not always evident. Aldrich et al. (1993) placed cattle on an E+ diet during cycling HS (22 to 32°C) and reported lower rectal temperature for some cattle compared to controls. This is contrary to most studies (Bond et al., 1984; Hemken et al, 1981; Rhodes et al., 1991; Osborn et al. 1992).

In recent years, studies have looked at the core body temperature response in different breeds. McMurphy et al. (1990) and Cole et al. (2001) placed Angus steers on E+ fescue diets and reported an elevated rectal temperature, whereas rectal temperature of Brahman steers were unaffected by this diet. McMurphy et al. (1990) also noted that rectal temperature in steers with lower Brahman influence showed an elevated respiration rate on E+ fescue with no rectal temperature difference. Rectal temperatures were also found to be similar for both breeds during cooler measurement periods (McMurphy et al., 1990). However, these studies may be confounded due to the level of stress. It is well understood that the level of thermal strain experienced by livestock alters the response. Since Brahman cattle are already heat tolerant, the level of strain the animals are under is lower than that of Angus animals. Much more research is necessary to determine if the core body temperature response is different between breeds. Along with an increase in body temperature due to reduced heat loss, several studies have showed changes in the circadian rhythm of animals. Al-Haidary et al. (2001) reported that the poor performance in beef cattle during heat stress is strongly associated with the disruption of the normal patterns of diurnal body temperature, not just the hyperthermia. During this study, core temperature increased above control animals near midnight. However, none of the other variables (i.e., skin temperature, skin or respiratory vaporization) were affected. This is

likely due a steady accumulation of metabolic heat following digestion of the daily meals and the inability to dissipate the accumulated heat.

Carr and Jacobson (1969) showed that injection of an endophyte extract (intramuscular) a skin temperature drop and rectal temperature increase. They suggested that elevated core body temperature was the result of the inability to transfer core heat to peripheral tissues resulting in increased body heat content. This is now understood to be true. Aldrich et al. (1993) reported no effect on metabolic heat production or respiratory vaporization in cattle fed an E+ diet during heat stress. However, skin vaporization was reduced by 50% resulting in hyperthermia. Al-Haidary et al. (1995) injected (intraperitoneal) cattle with ergovaline for 3 days of heat stress and noted that it resulted in increased core body temperature and respiration rate. At the same time, skin temperatures around the hip and back were lowered, suggesting reduced blood flow and heat loss. Rhodes et al. (1991), using radiolabeled microspheres to measure vascular flow rates, found that flow rate to the skin covering the ribs was reduced in steers fed a diet containing E+ fescue seed. It is now known that this reduced skin temperature is due to peripheral vasoconstriction and it results from the agonistic properties of E+ toxicants with α-2 adrenergic and serotonin receptors (Oliver, 2005).

4.6.4. Vasomotor Activity

The effect of fescue toxicosis on vasomotor activity has been well documented (Strickland et al., 1993; Strickland et al., 2009; Strickland et al., 2011). Blood vessel issues are caused by a series of alkaloids produced by the endophyte, including lysergic acid, ergotamine, and ergovaline (Klotz et al., 2008). These ergot alkaloids, act as an agonist and stimulate alpha-1 adrenergic receptors, resulting in vasoconstriction of

peripheral blood vessels (Oliver, 2005). This impairs heat transfer from core to peripheral tissues, which produces hyperthermia. In addition, some ergot alkaloids are agonists for serotonin receptors that reduce vascular lumen size through activation of vascular smooth muscle cells (Oliver, 2005). By inducing vasoconstriction as well as vascular smooth muscle cell hyperplasia (Oliver and Schultze, 1997) and endothelial cell damage, blood flow to the skin is substantially reduced. Recently vesicular glutamate transporters have been shown to be inhibited by ergot alkaloids causing a deleterious effect on the normal physiological function of tissues that express vesicular glutamate transporters (Strickland et al., 2011). These tissues include the brain and numerous organs. However, it is currently unclear which ergot alkaloids, metabolites, and/or alkaloid combinations found in endophyte infected tall fescue are the primary toxicants or the combination of mechanisms by which these alkaloids may affect vasomotor activity (Strickland et al., 2009).

4.6.5. Blood parameters

Most blood cellular parameters are not affected by E+ fescue intake. However, an increased number of erythrocytes and decrease in size (mean corpuscular volume) has been reported (Oliver et al., 2000). Several enzyme activities, hepatic enzyme activity in particular, are decreased by intake of E+ fescue (Schultze et al., 1999; Oliver et al., 2000). The reduced enzymes include serum alkaline phosphatase, aspartate aminotransferase, and aniline transaminase (Piper et al., 1991; Thompson and Steudeman, 1993). A reduction in serum alkaline phosphatase and an increased serum alanine transaminase are both prominent signs of fescue toxicosis that suggest cellular injury (Schultze et al., 1999). Piper et al. (1991) proposed that the depression in

transaminases in animals is likely due to depression of overall hepatic detoxifying efficiency. It is unclear whether the alkaloids specifically affect the enzymatic activity, but decreased feed intake is known to be the contributing factor (Oliver et al., 2000), resulting in reduced growth and tissue metabolism and body mass.

Several serum parameters are known to change with fescue toxicosis. Serum sodium and potassium levels are decreased, and blood urea nitrogen levels are increased in cattle grazing on E+ pastures (Oliver et al., 1997). Blood urea nitrogen (BUN) levels are reduced in animals consuming E+ fescue. These levels can indicate many problems, but in general indicate the level of urea nitrogen and protein metabolism (Srikandakumar et al., 2003), as well as dehydration (Schmidt-Nielsen and Schmidt-Nielsen, 1952). Other conditions resulting in a reduction in blood urea levels include liver damage (Satirapoj et al., 2007), malnutrition, and impaired nutrient absorption (Preston et al., 1965).

Serum creatinine has been reported to be elevated in animals consuming E+ fescue (Schultze et al., 1999; Oliver et al., 2000). Creatinine is influenced by the glomerular filtration rate; therefore an increase suggests a reduction in renal filtration rate. Serum globulin and triglyceride on the other hand are reported to be lowered in animals consuming E+ fescue (Oliver et al., 2000). Some of the reductions found in triglyceride could be due to a reduction that occurs during heat stress (Abeni et al. 2007). Globulin represents a portion of the amino acid pool of the body and is believed to be indicative of the nutritional status of the animal which is probably due to the reduced feed intake response.

Serum cholesterol is also reduced by feeding an E+ diet to cattle (Stuedemann et al., 1985. It is unknown if this reduction is due to increased cholesterol uptake by the

tissues or decreased hepatic secretion (Thompson et al., 2001). Stuedemann et al. (1985) reported an association between high-nitrogen fertilization of tall fescue and reduced serum cholesterol.

Very few mineral levels are affected by intake of E+ fescue (Oliver et al., 2000). However, serum copper levels are decreased (Oliver et al., 2000). Dennis et al. (1998) reported lower copper levels in animals consuming E+ fescue. Reduced copper levels have been suggested to contribute to the decreased mean corpuscular volume and have decreased hemoglobin values leading to the inhibition of hemoglobin synthesis (Strickland et al., 2009). Copper deficiency also has an impact on immune function, suggesting cattle consuming E+ fescue may have a lowered immune response (Saker et al., 1998).

4.6.5.1. Prolactin

Prolactin is a hormone normally associated with milk secretion in cattle, however, decreased serum prolactin levels has been reported in nearly all animals consuming E+ fescue (Waller et al., 2009). The effect of fescue toxicosis on prolactin level is independent of air temperature. Aldrich et al. (1993) showed a reduction in prolactin levels in cattle consuming an E+ diet during exposure to heat (32ºC) and thermoneutral conditions (22ºC). Similarly, Gadberry et al. (2003) reported a 93.6% reduction in serum prolactin level of lambs fed an E+ diet compared to lambs on an E- diet. This reduction occurred regardless of the ambient temperature, leading the authors to suggest that change in prolactin level is a more sensitive to fescue toxicosis than feed intake, skin temperature, or core body temperature. Serum prolactin concentration increases in response to an increase in air temperature (Schams, 1972; Head et al., 1976; Johnson,

1985; Wetteman and Tucker, 1974), therefore a major reduction represents a good indicator of fescue toxicosis in that it must go against the heat-induced increase.

Prolactin has more known functions than all other pituitary hormones combined (Freeman et al., 2000), therefore suppression could have multiple impacts. It is known that depression of prolactin in animals consuming E+ diets indicates an impact on both pituitary and neural functions. Ergot alkaloids are potent dopaminergic agonists and pituitary secretion of prolactin is inhibited by dopamine stimulation (Lamberts and Macleod, 1990). Mizinga et al. (1993) studied the dopamine D2 receptor density and binding affinity in rats fed E+ fescue. They observed that endophyte treatment did not change D2 receptor density, but rather increased D2 receptor affinity for ergot alkaloids. Turkington and Frantz (1972) reported that prolactin receptors are found not only in mammary glands but also in liver, kidney, cerebral cortex, and seminal vesicles.

Dull and shaggy hair coat changes associated with fescue toxicosis may also be a result of depressed serum PRL (Thompson et al., 2001). Lipham et al. (1989) treated steers with metaclopramide (dopamine antagonist) and observed increased serum PRL, improved hair coat quality, and BW gain. From these results, it can be speculated that retention of a rough hair coat (Aiken et al., 2006) often seen in cattle on E+ pastures could partially be associated with reduced prolactin. There is also evidence that malnutrition affects hair coat or the shedding of a hair coat, which could be partly responsible (Reference). Other effects of prolactin include the impact on gonad function and various tissues such as liver, kidney, cerebral cortex, and seminal vesicle (Strickland et al., 1993).

5. ADAPTATION, ACCLIMATION, AND ACCLIMATIZATION

Animals have developed coping mechanisms to minimize the impact of various environmental stressors on their biological systems (Roy and Collier, 2012). These responses are broadly described as adaptation, acclimation, and acclimatization. Adaptation is defined as a change which reduces the physiological stain produced by a stressful component of the total environment (Yousef, 1985). The magnitude of environmental stress can only be measured indirectly through the response of the animal. Adaptation may involve physiological shifts in the animal within its lifetime to the environment or genetic adaptation that involves forces of selection across generations. It may be confined to a small area of tissues or it may affect the entire animal (Yousef, 1985). Adaptation can be broken down into two subcategories: acclimation and acclimatization. Acclimation is a physiological change occurring within the lifetime of an organism, which reduces the strain caused by experimentally induced stressful changes, in particular climate factors (Yousef, 1985). Acclimatization, which is also a physiological change that occurs within a lifetime, reduces the strain caused by stressful changes in natural climates, such as seasonal changes. Acclimation and acclimatization both refer to phenotypic, and not genotypic, changes (Roy and Collier, 2012). In both cases, the overall impact is to improve the fitness of the animal in the environment; however, the responses will decay if the stress is removed. Throughout this dissertation, the term acclimation will refer to both acclimation and acclimatization.

5.1. Adaptation to Heat stress

5.1.1. Physical

As discussed in detail for short-term heat stress, coat color and consistency (i.e. length and thickness) has a major impact on radiative heat flow. Brody (1956) showed that cattle tended to have light-colored fur in the tropics that would better reflect solar radiation, thus keeping the animal from becoming overheated. He also reported that cattle undergo summer lightening and winter darkening of their hair that would alter radiative heat flow for a reduction and increase, respectively, in heat load. It has also been observed that cattle increase sebum (oily/waxy matter) secretion with heat stress, which gives the hair a reflective and protective sheen against solar radiation (Brody, 1956). There is evidence of the existence of a major gene, designated as the slick hair gene, which is responsible for producing a very short, sleek hair coat (Olson et al., 2003). Cattle with slick hair were observed to maintain a lower rectal temperature compared to animals whose coats are more wooly (Olson et al., 2003). Cattle also go through seasonal variations in coat characteristics. Seasonal changes in hair cover are influenced by daily photoperiod, as well as by ambient temperature. As cattle shed their winter hair coat, it allows for an increase in heat exchange. In addition, the rate of shedding is associated with the thermal status of the animal (Webster, 1974). Dowling (1956) found that differences in winter and summer coats were correlated with the core temperature of shorthorn cattle, although the difference was not substantial. Limiting nutrition has also been shown to induce the formation of a winter-type hair coat (Yeates, 1956).

5.1.2. Physiological

Body size also has an impact on adaptation to hot environments. Extra-large skin folds result in a larger surface area to mass ratio and consequently more heat loss per unit of weight. The ears, dewlap, navel flap, and vulva are much larger and more corrugated

in the loosely built, heat-tolerant and cold-sensitive Indian cattle than in the compactly built, cold-tolerant and heat-sensitive European cattle. Although there are differences in the surface area and subcutaneous fat layers in heat tolerant and intolerant cattle, other factors are very similar. For example, there are no differences in skin thickness (independent of subcutaneous fat) between Indian and European cattle (Dowling, 1955). However, these adaptations are genetic and do not change during the lifetime of the animal. The known modes of acclimation to its thermal environment through physiological means includes metabolism, redirection of blood flow, and changes in evaporation.

5.1.2.1. Metabolism

One of the most researched avenues of acclimation is the reduction in body heat production or metabolism. Cattle experience reductions in feed intake (Kibler and Brody, 1951), growth rate (Cartwright, 1955), and milk production (Johnson, 1965) in response to heat stress. When these reductions occur it is termed metabolic acclimation, implying adaptation to a variable, such as environmental temperature. (Mount, 1979). By its nature, metabolic acclimation is a form of adaptation that is dependent on a food supply and level of nutrition. However, other forms of metabolic adaptation exist. For instance, it is generally accepted that the metabolic rate of heat-adapted animals is lower than temperate species (Robertshaw, 1985). For example, as environmental temperature rises, feed consumption by tolerant cattle may begin to decline around 32-35°C, whereas intolerant cattle may decline closer to 26-29°C (Ingram and Mount, 1975). The heat tolerant cattle have a lower metabolic rate and make more efficient use of ingested food (Ingram and Mount, 1975). Their rates of weight gain under hot conditions exceed those

of heat intolerant cattle, but are much lower at thermoneutrality. This lower metabolism is a huge advantage under hot conditions. In fact, some breeds of tolerant cattle are able to maintain a lower rectal temperature while evaporating 40% less than heat intolerant cattle.

5.1.2.2. Blood flow

A major adaptation to thermal stress is peripheral vasodilation and increased blood flow to accommodate evaporative and convective heat losses (Beede and Collier, 1986), concomitantly reducing blood flow to internal organ systems such as the reproductive tract and ruminant stomach. The vasomotor response is the cheapest energetic response of all thermoregulatory defense reactions, with the major energy expenditure being from cardiac work. Engelhardt and Hales (1977) quantified distribution of capillary blood flow to the muscular and mucosal layers of the rumen, reticulum, and omasum of sheep experiencing various thermoregulatory demands. They reported that 7% of cardiac output was to the stomach regions and 95% of blood flow was in the mucosa, with only 5% to the muscle layers at thermoneutrality. During exposure to heat stress (40°C) blood flow decreased in mucosa of the dorsal rumen by 32%, and was reduced 31% in the reticulum, compared with the thermoneutral environment (18°C). A reduction in blood flow to the digestive tract during heat stress could potentially be a trigger for the reduced feed intake.

Thermal insulation is decreased during heat stress, particularly with vasodilation and increased blood flow to the skin of the extremities and ears, which have relatively little hair cover and a high surface area to volume ratio. These factors increase heat transfer between animal and environment. Vasodilation under a heavy coat contributes

little to heat exchange because the consequent decrease in internal insulation is only a small portion of the total insulation. Blood flow to the periphery increases so that heat loss via conduction and convection is enhanced (Choshniak et al., 1982).

5.1.2.3. Sweating rate

In an extensive study looking at changes in sweating activity over multiple seasons, Schleger and Turner (1965) found striking differences in sweating rates between summer and autumn-winter periods. Interestingly, respiration rates and rectal temperatures were similar during the same periods. McLean (1963) found during a 7 month experiment that respiration rate, skin and rectal temperatures declined over time while sweat rate increased. He attributed this to both age and seasonal effects. Kibler and Yeck (1959) found no difference in sweating of calves raised at 10.0 and 26.6°C when subsequently exposed to a range of environmental conditions. This lack of acclimatization evidence in cattle demonstrates that seasonal difference in sweating function are more likely associated with seasonal variations in hair growth (Turner and Schledger, 1960) and level of skin activity (Dowling and Nay, 1960).

As cattle adapt to chronic thermal stress, their energy metabolism or basal metabolic rate decreases, while water and electrolyte metabolism increase (Johnson et al., 1967; McDowell et al., 1968; Collier et al., 1982). These adaptations are reflected in lower concentrations of hormones such as thyroxine (Bianca, 1965; Collier et al., 1982) and growth hormone (Mitra et al., 1972). Although aldosterone concentrations also are lower in chronically heat-stressed cattle it is a reflection of need to increase urinary sodium loss to conserve potassium (Beede and Collier, 1986). Collectively, these results indicate that lowered energy metabolism is a major adaptation to chronic thermal stress.

Likewise, increased water and electrolyte metabolism are associated with adaptation to thermal stress as evaporative cooling requirements increase with heat stress (Beede and Collier, 1986).

5.1.3. Heat tolerance

Heat tolerance, in its simplest form, is the ability to tolerate ambient temperatures above the upper critical temperature (UCT) (Berman, 2012). For homeotherms, they are often characterized as heat tolerant if they remain comfortable or are able to balance heat production and heat loss at particularly high ambient temperatures (IUPS Thermal Commission, 2001). The ability to describe heat tolerant cattle is very subjective and lacks a true definition. Heat tolerance, in this sense, does not involve reduction of ambient heat stress or increasing heat loss by human intervention (Berman, 2012). While production is the end result and probably the best overall indicator of heat tolerance, it is also takes the longest to record. Therefore, most researchers use a measure of body temperature (rectal, abdominal, or ruminal). It would appear, therefore, to be a physiologically reasonable and desirable way of assessing an animal's innate overall capacity to cope successively with a hot environment versus an acquired tolerance (Bianca, 1961).

5.2. Adaptation to Fescue Toxicosis

There is almost no information regarding adaptation to fescue toxicosis over an extended period in either laboratory or field environments. This is a complicated problem, as one is dealing with both adaptation to the endophyte, and possible adaption to the animal's thermal environment. The combination of altered thermoregulatory ability and reduced caloric intake over an extended period of time has major negative impacts on

general animal health and performance. Therefore, any adaptation would be of significant importance and reflect the "real-world" situation. A few studies have looked at core temperature, feed intake, and body weight gain in rats with differing results. Spiers et al. (2005) and Settivari et al. (2008) reported that feed intake gradually improves overtime when animals are fed an E+ diet under both TN and HS conditions. Kishore et al. (2012), however, found that feed intake and growth rate showed no signs of adaptation under TN or HS conditions. They also reported a reduction in hyperthermia during heat exposure with repeat E+ treatment. The authors suggested that conditioning animals to fescue toxicosis and heat stress prior to exposure may be beneficial (Kishore et al., 2012). Settivari et al. (2008) also reported an adaptation in growth rate of rats on an E+ diet. However, despite the adaptation, the growth rate was still only 70% of the control rats. The authors suggested that activation of genes associated with gluconeogenesis, lipid metabolism and protein catabolism, and hepatic detoxification mechanisms may partially contribute to the adaptation observed in E+ rats (Settivari et al., 2006). In a study conducted with lambs, fecal recovery of ergovaline and lysergic acid were measured over a 21 day study (Zinner, 2011). The authors reported results that lambs adapt to E+ seed consumption through increased nutrient digestibility and increased ergovaline and lysergic acid excretion (Zinner, 2011). This could result in the recovery of the decrease in feed intake reported in other studies. There is some evidence that sharp changes in ambient temperature exposes major sensitivities to fescue toxicosis. If this is the case, than any adaptation to heat alone or fescue toxicosis would be ineffective against rapid temperature chances.

CHAPTER TWO

IMPACT OF DEHYDRATION ON PRODUCION AND THERMOREGULATION OF ANGUS STEERS UNDER DIFFERENING AMBIENT CONDITIONS

1. ABSTRACT

Evaporative cooling via panting or sweating is the most effective means of maintaining core temperature of cattle exposed to heat for an extended period. Water restriction during heat stress alters this ability. Therefore, a study was conducted to determine if dehydration under a controlled heat challenge would compromise thermoregulation. Eight Angus steers were maintained for 5 days at thermoneutrality (TN; 19-21°C) in the Brody Environmental Center (University of Missouri). This was followed by 14 days of cyclic heat stress (HS; 26-36°C). Water was removed starting on Day 5 of heat stress. After 3 days, water was returned starting the rehydration phase. Measurements included rectal temperature (T_{re}) and respiration rate (RR) measured six times daily. Body weight, feed and water intakes, and sweat rate at rump and shoulder were recorded daily during acclimation, dehydration and rehydration. During dehydration, steers lost ~10% of their body weight, which they regained within 36 h of rehydration. As expected, feed intake decreased (~75%) within 24 h of dehydration, but quickly recovered during rehydration. Transition from TN to HS caused RR to double (40-80bpm; $P<0.05$). Dehydration reduced RR (~15bpm), which remained low throughout rehydration ($P<0.05$). Similar to RR, T_{re} increased during HS (0.6°C; $P<0.05$). However, no increase in T_{re} occurred during dehydration ($P=0.41$), while rehydration caused a 0.8°C drop before recovery. Shoulder and rump sweat rates sharply increased with HS, but dropped to TN levels

during dehydration ($P<0.05$), before recovering when water was returned. Haematocrit did not provide a reliable indication of dehydration as it only slightly increased during dehydration, and was not different from TN level ($P=0.08$). Steers in the present study showed no lasting effects of dehydration, with the thermal status of the animal returning to normal after 48 hours of rehydration. Unexpectedly, core body temperature remained relatively unchanged despite dehydration, demonstrating their ability to adapt to changing conditions.

2. INTRODUCTION

Ruminants have evolved numerous behavioral and physiological strategies that enable them to survive long periods of water scarcity. One of the most important is the ability to maintain water balance through effective use of food moisture, drinking water, and metabolic water. The importance of maintaining balance for cattle has been known as early as 1557 when Tusser stated:

"In summer-time daily, in winter in frost,
If cattle lack drink, they be utterly lost." (From "A hundredth good pointes of husbandrie")

During water restriction there are competing demands between temperature-regulatory mechanisms, that are affected by the loss of water, and cell solute and volume-regulatory mechanisms that require water and solute retention (Olsson, 2005). In response, ruminants reduce fecal and renal water losses to protect plasma volume during dehydration (Silanikove, 1994). In addition, cattle will reduce their feed intake and decrease metabolism. The lower metabolic rate allows for conservation of water, due to a reduction in heat generation which, in part, is dissipated through evaporation.

Although the impact of dehydration has been previously studied, there is limited knowledge of the physiological changes associated with water restriction in *Bos taurus* cattle. The majority of the research on dehydration has focused on East African ruminants and Zebu cattle which are known for their ability to withstand harsh environments (Taylor, 1970; Maloiy, 1973; Kay, 1997; Silanikove, 2000). This is for good reason, as desert regions of the world have limited rainfall which limits production. Under good animal management practices in the United States, availability of water should not be a major issue of concern; however, it can become an issue during seasonal drought and transport.

A recent study by Scharf et al. (2010) showed a decline in sweat rate and a rise in core body temperature during prolonged heat stress, despite *ad libitum* access to water. This would suggest that body water retention is more important to the animal than evaporative heat loss for the control of core body temperature. While it has been known for more than 50 years that dehydration causes an animal to conserve water by reducing evaporative water loss (Schmidt-Nielsen and Schmidt-Nielsen, 1952), little is known about dehydration-induced reductions in sweat rate under controlled conditions. Bianca (1965) withheld water from steers for 4 days at a constant air temperature (T_a) of 40°C, and reported a 1.5 h delay in the onset of sweating. However, he did not examine the associated changes in blood parameters. In addition, most research has concentrated on either the acute thermoregulatory or endocrine changes associated with heat stress and dehydration (Silanikove, 1994). This prompted a study to determine the physiological and biochemical responses to dehydration under less severe thermoneutral conditions and during a controlled cyclic heat challenge. The purpose of this study was to determine if 1)

3 days without water would compromise thermoregulation of the steers during both a neutral and heat stress challenge, and 2) study changes in sweat rate associated dehydration.

3. MATERIALS AND METHODS

3.1. Animals

Eight Angus steers (400 ± 7 Kg BW) obtained from the University of Missouri Beef Research Farm in April 2007 and maintained at that location in feedlots prior Trial 1 (conducted in May), and between Trials1 and 2 (conducted in September). While at the feedlots, steers were placed on a typical feedlot corn-soy receiving diet. They were transported to the Brody Environmental Center at the University of Missouri one day prior to starting each trial. The Brody Environmental Center consists of four 6.1 × 9.1 meter chambers, two of which were used in the present study. Each chamber was divided into four stanchions, with each animal loosely restrained to the stanchion by a chain. Air movement in the chamber was held at 15 room changes per hour (126 cubic meters of volume). Light cycle during the chamber study was a 12-h light: dark (0600:2000 h) schedule. The experimental protocol and procedures for this use of animals were approved by the University of Missouri Animal Care and Use Committee.

3.2. General Procedure

Steers in Trial 1 were housed at a thermoneutrality (TN; 19 - 21°C). Chambers at TN had a set-point of 20°C, with only slight fluctuations across days (Figure 2.1). Percent relative humidity (RH) was maintained under 50% during the entire study so as to minimize the effect of this environmental variable on heat loss and thermoregulatory ability. Chamber environmental conditions were controlled using a Fisher-Porter

Controller (698B179U01) and a Sensycon I/P Converter (Controller Type 27/06–65). Steers were acclimated to chambers and the environmental conditions under the stated conditions for 7 days to provide a reliable baseline reading. This was followed by 3 days each of water restriction and rehydration for a total of 13 days. Three days of water restriction was chosen as it was known to be a safe interval, but still elicit the effects of dehydration (Bianca, 1965). Steers were provided water in 5 gallon buckets to record water intake prior to dehydration. During dehydration periods, buckets were removed. Rehydration was accomplished by gradual return of water (~2 gallons every 1hr) to avoid water toxicity (Silanikove, 1994). At the end of Trial 1, animals were moved to University of Missouri Beef Farm for a 4 month recovery period (May through September).

A second study was conducted to determine how responses found in Trial 1 would differ under heat stress conditions. Steers in Trial 2 were housed for 5 days at the same T_a as in Trial 1 prior to initiation of HS, which consisted of daily cyclic T_a (26°C night: 36°C day) for 14 days (Figure 5.1). During the daily HS cycle, T_a increased as a step-up function with 3 set points throughout the rise phase, followed by a 4-hour stable period (36°C; 1200 to 1600 hours). The decline phase consisted of 2 set points to reach the stable low temperature (26°C; 0000 to 0600 hours). As in Trial 1, RH was maintained under 50% during the entire study (TN: 40 to 50%; HS: 35 to 45%). This procedure has been used in a number of previous studies (Spiers et al., 2001; Scharf et al., 2008). Water was removed starting on Day 5 of HS, and after 3 days was returned gradually to start the rehydration period and avoid water toxicity. Animals during both trials were individually maintained in stanchions, with water and feed available *ad libitum* prior to dehydration.

Data loggers (Hobo H8 Pro; Onset Computer, Bourne, MA; accuracy: ± 0.2°C and ± 3% RH) were used to record T_a and RH every 10 minutes. Body weight, feed (FI) and water intakes were recorded during acclimation, with daily measurements during dehydration and rehydration periods. Animal measurements, including respiration rate (RR), skin temperature (T_{skin}), and rectal temperature (T_{re}), were taken 6 times daily (0600, 1100, 1300, 1600, 1900, and 2100 hours). Determinations of RR were made by counting flank movement over a 1-minute interval. Skin temperatures, at 5 different shaved sites (ear, shoulder, rump, tail head, and lower tail), were measured using a calibrated infrared thermometer (Model C-1600M, Linear Laboratories, Fremont, CA). Readings were taken less than 30 cm away from each skin site with a thermometer target ratio of 3:1. Rectal temperature was measured using a traceable thermistor thermometer (Model 8110–20, Cole-Parmer Instruments, Chicago, IL). This was accomplished by inserting a YSI probe (model 400, YSI Inc., Yellow Springs, OH; accuracy: 0.1°C) approximately 15 cm into the rectum for 2 minutes.

Sweat rate was measured at 0900, 1400, and 1900 hours on specific days throughout the study at shaved shoulder and rump sites. Days selected targeted Pre-dehydration days (Both TN and HS) and daily dehydration and rehydration periods. Sweat rates were taken using a factory calibrated, digital moisture sensor (Vapometer; Delfin Technologies Ltd, Finland) that determines transepidermal water loss, and has recently been verified using rodents and humans (Fluhr et al., 2006). All calibrations were certified and performed at the company laboratory using 3 different relative humidities. The Vapometer uses a closed system approach, free of ambient airflow, to

measure ambient relative humidity and temperature. The device is then held on the skin for 10 to 20 s before the evaporation rate is displayed in g/m^2h (accuracy: ± 10%).

Blood samples were collected at approximately 0900 hours during baseline readings prior to dehydration and daily at the same time during dehydration and rehydration periods via jugular venous puncture. Samples were collected into 15-mL tubes and allowed to clot before centrifugation. Serum was separated by centrifugation (2,300 x *g* for 25 minutes; 4°C) before being removed and stored at −20°C for later analysis. Blood serum parameters included hematocrit, osmolarity and biochemical profiles. Most serum measurements were components of a biochemical profile produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an auto-analyzer (Olympus AV400; Olympus America, Inc., Melville, NY). These include albumin, chloride, cholesterol, creatinine phosphokinase, creatinine, globulin, glucose, magnesium, potassium, sodium, total protein, triglyceride, and urea nitrogen. Serum concentrations of prolactin was determined by RIA procedures previously validated at the University of Missouri (Lutz et al. 1991). Minimum detectable concentration of prolactin in serum was 1.2 ng/tube. Intra-assay CV was 9.2%. Serum osmolarity was determined by freeze point analysis using an Osmette II (Precision Systems, Inc., Natick, MA; Special thanks to Luis Polo-Parada for use of this machine). Hematocrit was measured using a centrifuge (Adams Autocrit Centrifuge CT-2905; Block Scientific, Inc., New York, NY).

Urine was manually collected for baseline levels prior to dehydration and daily during dehydration and rehydration using a cup beneath the animal. Subsamples were obtained after collection of urine for osmolarity and specific gravity. Urine osmolarity

was determined by freeze point analysis using the same device as for blood, and urine specific gravity was measured using a standard urinometer.

3.3. Statistical Analyses

Data was analyzed using a repeated measures ANOVA procedure in JMP statistical software (SAS Institute; Cary, NC). All evaluations were conducted using periods TN (thermoneutral), HS (heat stress), dehydration or rehydration. The analysis included either RR, Tskin, sweat rate, blood parameters or Tre as the dependent variable. Skin temperatures were included as an average of the shoulder and rump sites (Ttrunk), or an average of ear, tail head and lower tail (Tappendage). Period, time, and period by time were set as fixed effects, with animal nested within breed as a random effect. For ANOVA analyses present in text, experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD adjustment procedures for multiple mean comparisons. Regression coefficients for slope and model *r*, as well as P-values for the hypothesis test that the regression coefficients are significantly different from zero, are reported.

4. RESULTS

4.1. Trial 1 – Thermoneutral

Prior to dehydration, FI increased from Days 1 to 5 (6.87 vs. 9.00 kgs; $P \leq 0.05$) before stabilizing at 8.55± 0.45 kgs on the day prior to water restriction (Figure 2.2). Water intake was consistent across baseline readings with an average daily intake of 38 liters per animal. There was approximately a 60% reduction in FI within 24 hours of dehydration for seven of the steers (8.55 to 3.11 Kgs), with an overall 70% reduction by the third day of dehydration ($P < 0.001$; Figure 2.2). Surprisingly, one animal showed no

reduction during the first 24 hours of dehydration, but the same 70% reduction by the third day of water restriction. This FI reduction resulted in an average of approximately 4% body weight loss over the entire dehydration period. During rehydration, FI increased from 3.11 to 6.76 ± 0.45 kgs per animal during the first 24 hours and further increased to baseline level by the end on the 2nd day of rehydration ($P < 0.05$; Figure 2.2). As with FI recovery, body weight quickly returned to pretreatment level within 24 hours of rehydration, suggesting that weight loss was mainly water loss.

Respiration rate showed no differences from Days 1 through 6, with an average maximum rate of 47 ± 1.32 bpm ($P = 0.10$; Figure 2.3). Unlike FI, there was no response to the first 24 hours of dehydration (Figure 2.3). It was not until the second day of dehydration that RR was different from baseline ($P< 0.05$; 45 versus 33 ± 1.32 bpm) with a further decrease to 29 ± 1.32 bpm on the third day. Respiration rate showed a rapid recovery, returning to baseline level within 3 hours of water availability (41 ± 1.32 bpm; $P < 0.05$; Figure 2.3).

In the absence of thermal stress, sweat rate was low for baseline readings (approximately 25 g/m^{2}h; Figure 2.4a). Both shoulder and rump sweat rates displayed a slow decreased during dehydration, only reaching a significant reduction of 40 percent on the final day of dehydration ($P < 0.05$). Unlike RR, sweat rate remained low for the first two days of rehydration (Figure 2.4a).

Regional skin temperature differences existed, with trunk temperatures (Shoulder and Rump) being approximately 0.5°C higher than extremity skin temperature (Ear and Tail) throughout Trial 1 ($P < 0.05$; not shown). Skin temperatures across all sites showed no changes during the 3 days of dehydration ($P = 0.32$). Rehydration caused an

immediate decline in all skin temperature sites with extremity skin temperatures showing the largest drop ($P < 0.01$; approximately 3.5°C; not shown). The decrease was transient, returning to baseline levels by 1600 hours ($P < 0.05$; i.e., 6 hours into rehydration period). The Thermal Circulation Index (TCI) was calculated [$TCI = (T_{skin} - T_a) / (T_{re} - T_{skin})$; Burton and Edholm, 1955] as an indicator of steady-state blood flow and heat transfer to the skin. During early rehydration, TCI values decreased ($P < 0.05$) suggesting that reductions in blood and heat flows to the periphery may be responsible for the noted decrease in skin temperatures.

Rectal temperature showed a minimal reduction during dehydration (0.3°C), being significantly different from baseline levels only on the third day of dehydration ($P < 0.05$; Figure 2.5). However, the magnitude of this reduction had no physiological significance, and quickly returned to baseline level during the first 3 hours of rehydration. During both baseline and rehydration periods, steers displayed a circadian rhythm with rectal temperature being lowest and highest at 0600 and 1700 hours, respectively ($P < 0.05$; Figure 2.5). All other times of the day were similar. However, this circadian rhythm disappeared during all days of dehydration, with no discernible rhythm over the 6 daily readings.

Hematocrit (Hct), which is a common measure of dehydration, was highly variable across individuals, with some steers having a 20% higher baseline level than other steers ($P<0.05$) during baseline readings (Figure 2.6a). Furthermore, Hct actually decreased during the first 24 hours of water restriction ($P < 0.05$), followed by an increase above baseline level during the second and third days of dehydration ($P < 0.10$; Figure 2.6a). There was a rapid response to rehydration, with Hct rapidly returning to

baseline levels within 24 hours after return of water. Serum osmolarity, unlike Hct, increased to above baseline levels within 24 hours and remained high throughout dehydration (>300 mOsm; $P < 0.05$; Figure 2.7a). It then quickly returned to baseline levels (< 285 mOsm) within 24 hours of rehydration ($P<0.05$; Figure 2.7a).

Certain blood parameters did not change with dehydration or rehydration, including Gamma-glutamyl transferase, creatine phosphokinase, aspartate aminotransferase, glucose, and prolactin ($P \geq 0.45$). While not significantly different, there was a tendency for prolactin concentrations to decease with dehydration ($P < 0.10$), being lowest on the third day of water restriction (25.5 vs. 43.1 ± 4.0; Table 2.1). Urea nitrogen, creatinine, total protein, sodium, albumin, and chloride, in contrast, exhibited significant increases with dehydration ($P < 0.05$; Table 2.1). It is interesting to note that while these blood parameters increased with dehydration, the time table for change was quite different. Urea nitrogen, sodium, and chloride all showed sharp increases being significantly ($P < 0.05$) different from baseline after 24 hours of dehydration (Table 2.1). Creatinine took 48 hours, however, and total protein did not change until 3 days without water ($P < 0.05$; Table 2.1). Breaking down total protein into its components of albumin and globulin showed that each required 3 days of dehydration before there was a significant shift above baseline levels ($P < 0.05$). All blood variables that increased with dehydration quickly returned to baseline levels within 24 hours after return of water ($P < 0.05$; Table 2.1).

Others variables, including potassium, calcium, phosphorous, and magnesium, did not change with dehydration ($P = 0.24$), but showed a response during rehydration ($P < 0.05$; Table 2.1). Phosphorous and magnesium all decreased with rehydration, and

maintained at low levels even after 3 days of access to water ($P < 0.05$; not shown). Calcium, however, only showed a decrease for the first day of rehydration, with a return to baseline level after 48 hours ($P < 0.05$; not shown). Potassium gave an unusual result by not changing with dehydration or the first day of rehydration, but increased above baseline for the second and third days of rehydration ($P < 0.05$; Table 2.1).

Along with serum levels of sodium and potassium, we also measured urinary levels. Urinary potassium was stable, exhibiting no change throughout the study ($P = 0.79$; not shown). However, urinary sodium showed a rapid increase during dehydration. While serum sodium only increased 8.6% after 3 days of dehydration, urinary sodium increased more than 670% before returning to baseline within 24 hours of rehydration ($P < 0.001$). This increase with dehydration and decrease with rehydration coincided with both urine osmolarity and urine specific gravity following the same trend ($P < 0.001$). Urine osmolarity and specific gravity was highly correlated ($P < 0.05$; $R = 0.89$; linear).

4.2. Trial 2 – Heat stress

Similarly to Trial 1, FI increased during the 3 days of TN before stabilizing to approximately 8.18 kgs/day prior to imitation of HS (Figure 2.2). Heat stress had no effect of feed intake which was 9.00 kgs/day the day before dehydration (Figure 2.2). As expected, FI dramatically decreased within 24 hours of dehydration being lowest on Day 2 of water restriction (~75% of baseline levels; Figure 2.2). However, unlike Trial 1 where one animal had a delayed feed intake response, all animals showed the decrease during the first day (Figure 2.2). Steers maintained a steady weight gain until the dehydration phase, whereby they lost ~10% of their body weight, which they quickly regained within 36 hours of rehydration. During rehydration, FI increased from 3.89 to

8.06 ± 0.58 kgs during the first 24 hours and stabilized at baseline level by the end on the second day of rehydration (Figure 2.2).

Respiration rate was stable throughout TN ($P = 0.31$; 36.1 ± 1.8 bpm; Figure 2.3). Transition from TN to cyclic HS caused RR to double (41.3 versus 70.8 ± 1.8 bpm; $P<0.05$; Figure 2.3). It continued to increase during HS before stabilizing at approximately 84.7 ± 1.8 bpm before the start of dehydration. Dehydration caused a small drop in RR (~15bpm) which did not become significantly different from baseline until Day 3 of dehydration ($P < 0.05$; Figure 2.3). Likewise, recovery of RR to baseline levels during rehydration required 4 days ($P < 0.05$).

Like during Trial 1 at TN, sweat rate was low for baseline readings for both Shoulder and Rump sites (~25 g/m^2h; Figure 2.4b). Transition to HS resulted in a sharp increase in sweat rates of both sites, being more than triple that of TN values ($P < 0.01$; 93 versus 25 g/m^2h; Figure 2.4b). Both Shoulder and Rump sweat rates showed a large time of day differences ($P < 0.01$) during HS, being lowest in the morning at 0900 and highest at 1400 h. Despite showing no TN differences between sites, Shoulder sweat rate at 1400 was significantly higher ($P < 0.01$) than Rump sweat rates. Dehydration had little impact on either Shoulder or Rump sweat rates during the first 24 hours (Figure 2.4b). During Day 2 and 3 of dehydration, Shoulder and Rump sweat rates showed no difference at the 0800 h readings ($P = 0.21$). However, there was significant difference ($P < 0.05$) in the 1400 reading. Unlike during HS, the 1400 h reading showed no increase above the 0800 h reading for both sites ($P < 0.05$). The reduction in sweating rate only occurred when ambient temperature was high, and never decreased to TN levels (Figure 2.4b). Similarly to RR, rehydration did not cause immediate recovery of Shoulder sweat

rate ($P < 0.05$). In fact, sweat rates took nearly 4 days to recover to pre-dehydration peaks at 1400 h for Shoulder sweat rate and 3 days recovery for Rump sweat rate ($P < 0.01$; Figure 2.4b).

As during Trial 1, regional skin temperature existed with Trunk temperatures (Shoulder and Rump) being approximately 0.5°C higher than extremity skin temperature (Ear and Tail) at TN (not shown). These skin site differences were reduced after initiation of HS (~0.30°C). Skin temperature at both sites showed a linear increase ($R = 0.97$) with air temperature increasing during the day being lowest at 0600 h and highest at 1600 h ($P < 0.05$; 4°C increase throughout the day; not shown). Though not significantly different, $T_{appendage}$ tended to have a lower minimum and maximum daily temperature than T_{trunk}, although the range was similar regardless of water access. During dehydration the skin site differences increased to approximately 0.5°C ($P < 0.05$). Rehydration resulted in an immediate drop in skin temperature at both sites. This drop increased to before dehydration levels by the end of the first day of rehydration.

Rectal temperature increased from Day 1 through Day 3 ($P < 0.05$) before stabilizing to approximately at 38.6°C before initiation of HS (Figure 2.5). Similarly to RR, T_{re} increased during HS (TN: 38.6 to HS: 39.0°C; $P<0.05$) with a maximum daily T_{re} occurring the day before dehydration being significantly different from all other HS days (39.3 vs. 39.0°C; $P<0.05$; Figure 2.5). During both TN and HS readings, steers displayed a circadian rhythm with T_{re} being lowest at 0600 h and highest at 1700 h ($P < 0.05$). A stable daily T_{re} was maintained during the first 2 days of dehydration ($P = 0.21$; 39.3°C; Figure 2.5). It was not until the third day of dehydration that daily T_{re} increased above HS levels ($P < 0.05$). As prior to dehydration, steers showed a similar rhythm with T_{re} being

lowest at 0600 h and highest at 1700 h ($P < 0.05$). Rehydration caused a drop (39.4-38.8°C) before recovering to pre-dehydration level ($P < 0.05$; Figure 2.5). The circadian rhythm that was pronounced during previous periods was not found during the rehydration period.

Hematocrits as during Trial 1 proved to be an ineffective measure of dehydration. Transition from TN to HS showed no effect of hematocrit ($P = 0.33$; Figure 2.6b). Likewise, dehydration resulted in little change during the first 2 Days ($P = 0.14$; 32.8 versus 34.0 ± 1.5; Figure 2.6b). In fact, it was not till the final measure on Day 3 and the first measure during rehydration that any significance was found with hematocrit being higher than baseline levels ($P < 0.01$; 37.7 versus 32.8 ± 1.5; $P < 0.05$; Figure 2.6b). Rehydration resulted in an eventual decline to baseline levels on Day 3 of rehydration ($P < 0.01$). Serum osmolarity showed no change ($P = 0.28$) during transition from TN to HS (273 versus 280 ± 12 mOsm; Figure 2.7b). Dehydration caused an immediate increase ($P < 0.01$) in serum osmolarity, reaching 320 ± 12 mOsm during the first 24 hrs of water restriction. Serum osmolarity continued to increase reaching a maximum of 350 ± 12 mOsm during the final day of water restriction ($P < 0.01$; Figure 2.7b). Rehydration resulted in a decrease in osmolarity ($P < 0.05$). However, it was not until after 4 days of rehydration that osmolarity dropped below 300 mOsm or baseline level ($P < 0.05$; Figure 2.7b).

Blood parameters in Trial 2 were similar to Trial 1 responses during dehydration and rehydration, despite the addition of HS. Similarly to results found in Trial 1, some blood parameters did not change with dehydration or rehydration ($P \geq 0.21$; gamma-glutamyl transferase, creatine phosphokinase, aspartate aminotransferase, glucose, and

prolactin). Gamma-glutamyl transferase, creatine phosphokinase, aspartate aminotransferase, and glucose also showed no response during transition from TN to HS ($P \geq 0.13$). Prolactin, however, showed a significant increase during transition from TN to HS ($P < 0.05$). There was also a tendency for prolactin concentration to decease with dehydration ($P < 0.10$), being lowest on the 3rd day of water restriction (35.2 vs. 23.3 ± 3.7; Table 2.2).

Urea nitrogen, creatinine, total protein, sodium, albumin, and chloride all exhibited significant increases during dehydration ($P < 0.05$; Table 2.2). Only total protein and creatinine exhibited change during transition from TN to HS, with total protein being significantly higher than TN ($P<0.05$; Table 2.2), while creatinine showed a trend to increase ($P < 0.10$; Table 2.2). Breaking down total protein into its components of albumin and globulin showed that neither changed during dehydration. However, both increased ($P < 0.05$), resulting in a significant increase in total protein ($P < 0.05$; Table 2.2).

All blood variables that increased with dehydration quickly returned to baseline levels within 24 hours after return of water (Table 2.2). Others variables, including potassium, calcium, phosphorous, and magnesium, did not change with dehydration, but showed a response during rehydration ($P < 0.05$). However, all responses were transient and returned to baseline within 3 days of rehydration.

Similarly to Trial 1, urinary sodium and potassium levels were measured. Urinary potassium was stable, exhibiting no change throughout HS, dehydration, and rehydration ($P = 0.41$). However, urinary sodium showed a trend to increase during HS ($P < 0.01$), with a large increase during dehydration ($P < 0.01$). As during Trial 1, urinary sodium

increased approximately 450% (Trial 1 – 670%) before returning to baseline within 24 hours of rehydration (not shown). This increase with dehydration and decrease with rehydration coincided with both urine osmolarity and urine specific gravity following the same trend.

5. DISCUSSION

In all animals, the body water pool must remain relatively constant over the long-term, although livestock in particular are able to tolerate large short-term fluctuations. This is due to the large fore-stomach which can maintain a water content ranging from 15 to 40% of their body weight (Shkolnik et al. 1980; Shkolnik and Silanikove, 1981). Adolph (1982) divided mammals into two categories of drinkers: those that gradually replenish lost water (including humans) and those that can do so rapidly (including ruminants). In either case, animals drink primarily to replace lost fluid, giving little regard for anticipation of future needs.

In the present experiment prior to dehydration, steers were consuming 38.33 L which is consistent with Bianca et al. (1965) reported in their dehydration study. It is also consistent with the water requirements of 3 to 5kg per kg dry matter intake at 15 to 25°C (Gaughan et al., 2002) showing that the animals were in balance prior to dehydration. Silakinove (1994) divides the stages of dehydration in ruminants into two phases with Phase 1 gradually turning into Phase 2. During Phase 1, food intake and salivation are only marginally reduced still allowing for near-normal fermentation in the rumen. During Phase 2, food intake, salivation, and digesta content in the rumen fall severely (Silakinove, 1994). In the British breeds of cattle, if water was withheld for a single day, appetite can be depressed more than 20% (Bianca, 1963). In the present study, feed

intake quickly dropped more than 60% over a 24 hour period. While we did not measure rumen motility or salivation, it is clear that the steers quickly moved from Phase 1 to Phase 2 over the 24 hours. An interesting note is the change in feed intake decrease between Trial 1 and Trial 2. When the effects of heat load were added to that of dehydration, the results are mixed. The gazelle and Oryx show no decrease, whereas sheep and zebu cattle have been reported to have a further drop in feed intake (Maloiy et al., 2008). In the present study, feed intake was only approximately 5% different during dehydration between thermoneutral and heat stress trials ,suggesting that dehydration-induced reductions in feed intake is independent of ambient temperature in Angus cattle. This rapid decrease in feed intake does pose a problem with dehydration studies as water restriction can be confounded with reduced feed intake (Li et al., 1999).

The major reduction in feed intake resulted in ~4% loss in body weight during Trial 1 at thermoneutrality and a ~9% loss during heat stress (Trial 2). While these losses are significant, they are lower than Bianca et al. (1965), who reported a 12% loss in body weight over 4 days of dehydration at 15°C suggesting animals in the present may not have been severely dehydrated. This is much lower than the reported 15-18% loss of body weight that cattle are known to tolerate (Bianca, 1963; Silanikove, 1994). In European cattle, the rumen can contribute up to 49% of the total body fluid loss during dehydration (Silanikove, 1989). This is one of the reasons that during rehydration body weight is quickly regained within 48 hours as found in the current study. The role of the rumen as a water reservoir also explains ruminant's capacity to withstand a greater level of weight loss during dehydration than most monogastric mammals. Despite having this

reservoir, they still must utilize water available through ingested food and metabolic water, and restrict water loss below normal levels to control their water deficit.

Respiratory rate in hydrated animals increases at ambient temperature above 21°C, resulting in increased respiratory evaporation (Hahn et al., 1997). In the current study, transition from thermoneutrality to heat stress resulted in a doubling of respiration rate from 40 to 80 bpm. Dehydrated animals however tend to have lower respiratory rate, and initiate panting at higher ambient temperatures than do normally hydrated animals (Cain et al., 2006). During both Trial 1 and 2 of the present study, respiration rate decreased by approximately 15 bpm during dehydration. This represents approximately a 33% (Trial 1) and 18% (Trial 2) drop in respiration rate, and is less than 47% reported by Rumsey and Bond (1976) for cattle after 4 days of dehydration. This is consistent with other ruminant species (Taylor, 1970; Maloiy et al., 1978; Lowe et al., 2002). In the present study, no difference was found in ambient temperature at which respiration rate was initiated. It makes sense that there would be only a marginal drop in respiration rate, as respiratory evaporation only accounts for 35% of total evaporation with sweating rate accounting for the remaining 65% (Jenkinson, 1972). Salt and electrolytes are also not lost during panting, as in sweating. However, panting possess a risk of respiratory alkalosis and the increase in work and associated heat production by the respiratory muscles (Jenkinson, 1972).

Evaporative cooling can account for more than 30% of the total dissipation of heat load in ruminants, as well as 80% of the water loss (Taylor, 1972). In the current study, dehydration caused reduction in both respiratory and sweating rate, which suggests a decrease evaporative heat loss. In particular, the small reduction in sweating rate at

thermoneutrality during Trial 1 was somewhat surprising. It is possible that the water content of the skin may be decreasing as blood is being shifted away from the periphery. When dehydrated, several ruminants under heat strain have been reported to reduce sweating and the rate of cutaneous evaporation by 12–89% (Schmidt-Nielsen, 1964, Schoen 1968, Maloiy 1970). In the current study, shoulder sweat rates were reduced 37% below pre-dehydration level during Trial 1 (thermoneutral) and 62% during Trial 2 (heat stress). The core body temperature at which dehydrated animals begins to sweat, as noted for respiration rate, is often higher than that of normally hydrated animals (Schmidt-Nielsen, 1964; Taylor 1969, 1970). In the current study, dehydration during heat stress produced a small reduction in sweating rate during the cool hours of the day, but no change during the peak hours of the day. It may be that if the animals were pushed to a higher level of heat strain than used in the present study, we would see an increase in the sweat rate even during water restriction.

Many studies have reported that after dehydrated animals drink water, sweating and panting resume rapidly before water has been absorbed from the gut (Baker and Turlejska, 1989; Baker, 1989; McKinley et al., 2008). This is surprising as the act of drinking would have to override the inhibition on evaporative mechanisms (McKinley et al., 2008). In the current study, we did not encounter this effect. The fact that we slowly gave water back to the steers may have influenced this effect. However, we did not see full recovery of panting or sweating for more than 48 hours after water was returned. This suggest that Angus cattle may have to replenish the blood volume in order to regain sweating.

One of the objectives of our trials was to determine the priority of body temperature regulation over regulation of water balance during dehydration. It has been suggested that it is important for an animal to maintain core body temperature within a narrow limit (Cabanac, 1975). However, studies (Schmidt-Nielsen et al., 1970; Scharf et al., 2010) that include the present one have provided evidence that suppression of evaporative cooling mechanisms for maintenance of water regardless of hyperthermia may take precedence over preventing increases in body temperature. In the current study when steers became dehydrated, they suppressed sweat rate and respiration rate reducing further fluid loss at the expense of a higher core temperature. This would suggest that water balance may be more important at least up to a certain temperature threshold. Dehydration-induced hyperthermia has been suggested to be adaptive in conserving water as it increases the temperature at which the animals switch from thermoregulation via convection and radiation to evaporative cooling. This increase in body temperature also reduces the temperature gradient which reduces heat gain from the environment.

Another mechanism for conserving body water utilized by ruminants is known as adaptive heterothermy. The best known example of this increased diurnal core temperature fluctuation (i.e. adaptive heterothermy) in response to dehydration is in the camel (Schmidt-Nielsen, 1964). The camel can save approximately 1.3% of its body water pool by allowing its core body temperature to rise from 34 to 41°C during the day and disposing of the stored heat at night (Schmidt-Nielsen, 1964). The concept of adaptive heterothermy is still controversial (Mitchell et al., 2002). During Trial 1 at TN, a circadian rhythm was present before and after dehydration, but absent during water restriction. In Trial 2 of the present study, there was only a small increase in the diurnal

temperature fluctuations with dehydration suggesting no adaptive heterothermy. If animals in the present study were using this mechanism than large fluctuations in body temperature would have been found.

Salivary flow rate and food intake have been reported to be linearly and inversely related to the increase in plasma osmolarity (Silanikove, 1994). It has been suggested that the basis for the reduction in food intake and saliva secretion upon dehydration is related to the increase in Na concentration (Silanikove, 1994). In the present study, the reduction in plasma volume resulted in an overall increase in serum proteins, Na, Cl, albumin, total protein and urea nitrogen to ultimately result in an increase in osmolarity. Sodium, chloride, creatinine, and urea are consistently increased across species during dehydration increasing serum osmolarity (Burgos et al., 2000; Cain et al., 2006). The increase in serum Na results from increased sodium retention in the kidney which helps to conserve water (El-Nouty et al., 1980). Eventually the renal sodium excretion must increase to stabilize the serum sodium level resulting in the high urinary sodium (Michell and Moss, 1995; Burgos et al., 2000) found in the present study.

Similarly to sodium, recycling urea helps to reduce urine volume and conserve water (Maltz et al., 1981; Burgos et al., 2000). The increase in serum urea level found in the present study is possibly related to increase in secretion of vasopressin. An increase in serum vasopressin is known to promote urea reabsorption in the kidney. The rise in urea level can also be associated with catabolism of body proteins during dehydration (Abdelatif et al., 2010).

Serum creatinine in the current study was significantly increased with dehydration. This increase in serum creatinine can be a consequence of general reduction

in urinary excretion rate, or it could be related to changes in clearance. The plasma creatinine can be used as an indicator of glomerular filtration rate (GFR) in animals (Perrone et al., 1992). It has been reported in sheep that GFR is reduced during dehydration resulting in a decrease in creatinine clearance (Nawaz and Shah, 1984). This would be consistent with the results found in this study.

Many of the various blood parameters measured did not respond uniformly to dehydration, indicating that many are regulated independently of each other. Variables, including potassium, calcium, phosphorous, and magnesium failed to rise above their normal level. This is consistent with results reported by Bianca et al., (1965) who found that phosphorous and potassium did not change with dehydration until Day 4 when potassium began to fall. They attributed this to the lack of dietary intake of potassium.

Other blood parameters showed no change in response to water restriction. These included Gamma-glutamyl transferase, creatine phosphokinase, aspartate aminotransferase, glucose, and prolactin. Of particular interest were the responses of serum glucose and prolactin.

Studies looking at serum glucose during dehydration have reported either no difference or a reduction in levels. It is somewhat surprising that serum glucose did not change in the current study considering the large decrease in feed intake. From the results in the current study it appears that changes in various hormones might have aided in the maintenance of normal glucose level despite the change in food intake.

Prolactin is also of interest as it has more actions than all other pituitary hormones combined including being a stress hormone (Bole-Feysot et al., 1998). Prolactin has also been implicated in water balance in lower vertebrates such as fish (Loretz and Bern,

1982; Bole-Feysot et al., 1998). In the present study, prolactin showed only a numerical decrease while it has been reported to decrease with dehydration by others (Schams and Himmler, 1978). The difference in the results of this study may be related to changes in body fluid volume. In a study conducted by Doris and Bell (1984), they determined that the initial reduction in prolactin during dehydration is closely related to changes in packed cell volume levels. In the present study, packed cell volume or hematocrit was highly variable during the first days of dehydration which may have resulted in a lack of prolactin response found in the current study.

Hematocrits which are used as a diagnostic indicator of dehydration proved not to be a reliable indicator of dehydration. While hematocrits did increase with dehydration which is in agreement with others (Bianca et al., 1965), it was not consistent between animals. Animals at baseline levels were very different with hematocrits ranging from 16 to 40% pre-dehydration. Unlike Bianca et al. (1965), in the present study hematocrit values actually decreased in the first 24 hours during Trial 1 and showed no change during Trial 2 possibly suggesting a release of water from the rumen. It has been shown in sheep that under moderate dehydration, the water balance in the body is kept virtually unaltered by fluid drawn from the rumen during the first two days of water depravation (Hecker et al., 1964). They also reported that 54% of total body fluid loss was from the rumen, while Silanikove (1994) found that 49% was from the rumen in European type cattle. One explanation for the decrease found during Trial 1 is a dumping of water from the rumen to maintain water balance when water loss across the skin was low. During Trial 2 when water restriction occurred under heat stress, hematocrit was maintained since water loss across the skin was high.

Water is conserved when dehydrated ungulates reduce total urine volume and increase concentration (Taylor and Lyman, 1967). Reductions of this type in urine volume have been shown to range from 0-76% and increases in urine osmolarity of 3-239% have been documented (Cain et al. 2006). The normal quantities of sodium and potassium excreted in the urine are consistent with that of Bianca et al. (1965) for baseline levels. They reported very similar results with marked increases in sodium, but found a reduction in urinary potassium. During both trials in the present study, urinary potassium did not change while urinary sodium showed more than 600% increase which possibly caused the increase in specific gravity and osmolarity. Urine specific gravity and osmolarity were highly correlated with each other ($R^2 = 0.80$).

Homeothermy was maintained during both trials and never resulted in runaway hyperthermia, although changes did occur in evaporative heat loss mechanisms. Steers in both trials of the present study showed no lasting effects of 3 days water restriction with all parameters returning to normal after 48 hours rehydration. Dehydration appeared more complex than rehydration, resulting in increases in some blood parameters and no change in others. In contrast, rehydration decreased in all blood parameters to suggest blood dilution. While haematocrits may be used currently as an indicator of dehydration, other variables may ultimately be a better indicator. Some of the first and most important clinical signs of dehydration are those that are easily measurable. Dehydration induces dryness and wrinkling of the skin which subsides slowly after being picked up or pinched into a fold. The body and face have a shrunken appearance and the eyeballs recede into the sockets. However, the most obvious sign of dehydration in the present study was the loud vocalization that occurred as soon as someone was in sight of the animals. Serum

osmolarity and urinary sodium along with serum urea nitrogen and creatinine all show promise as possible future indicators of dehydration.

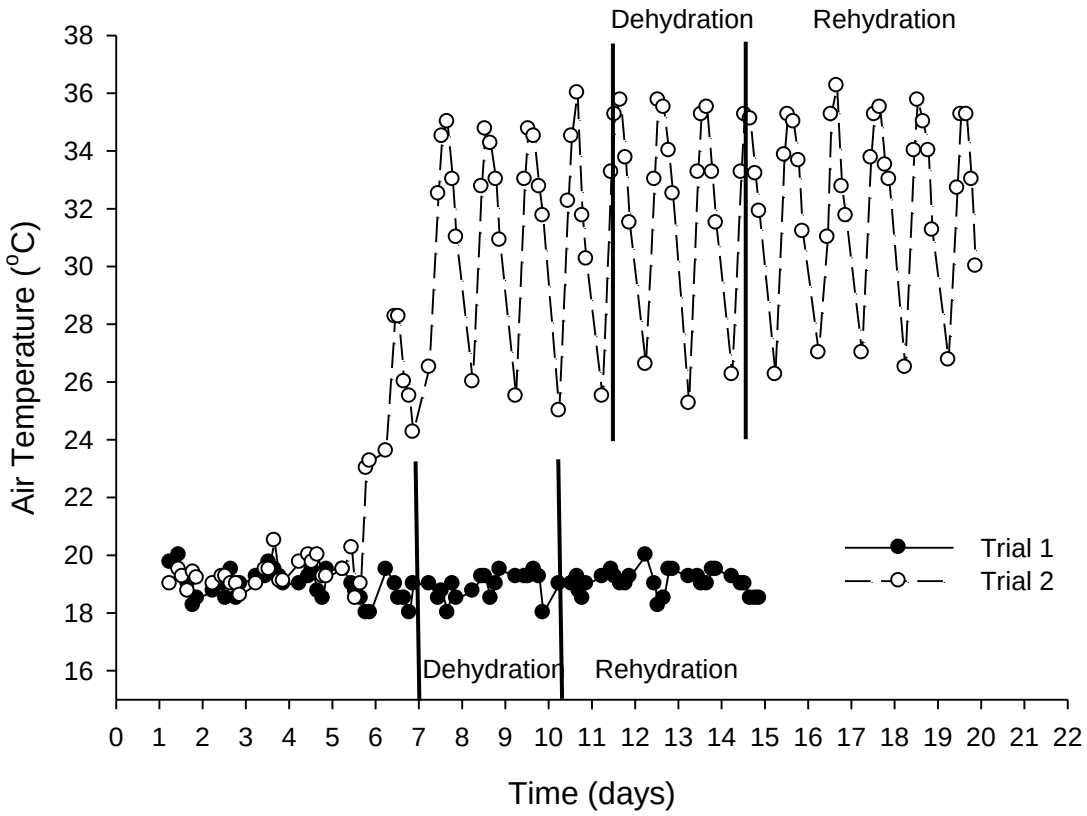

Figure 2.1 – Air temperature shown as a function of time in days for Trial 1 and Trial 2. The solid black line represents Trial 1 under TN conditions (Dehydration on days 7 through 10). The black dashed line represents Trial 2 under HS conditions (Dehydration on days 11 through 14).

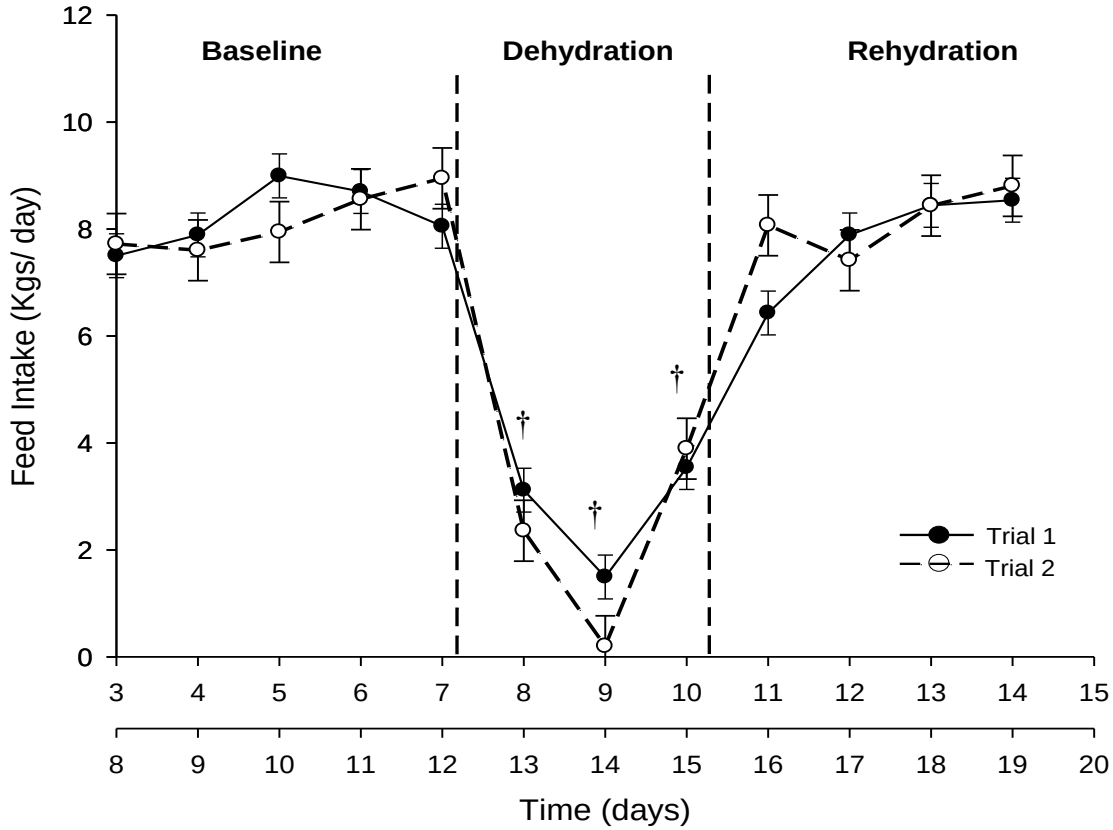

Figure 2.2 - Daily feed intake is shown as a function of time in days for Trial 1 and Trial 2. The solid black line represents Days 3 to 15 of Trial 1 under TN conditions. The black dashed line represents Days 8 to 20 of Trial 2 under HS conditions. The dashed vertical line separates designate the start and end of the dehydration period. The vertical line on top of each variable represents +1 SEM. Dagger (†) signifies values are significantly ($P < 0.05$) different from baseline.

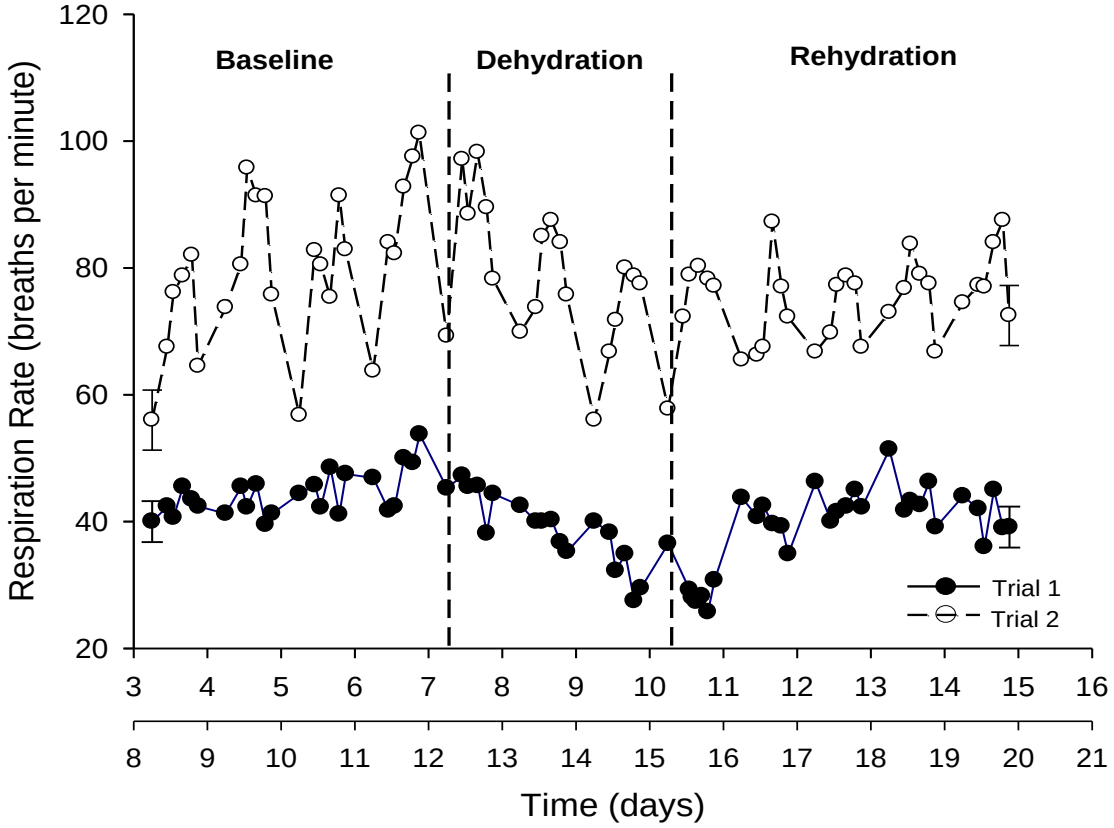

Figure 2.3 – Mean respiration rate (± 1 SEM) as a function of time in days for Trial 1 and Trial 2. All six sample times during a day are shown. The solid black line represents Days 3 to 15 of Trial 1 under TN conditions. The black dashed line represents Days 8 to 20 of Trial 2 under HS conditions. The dashed vertical line separates designate the start and end of the dehydration period.

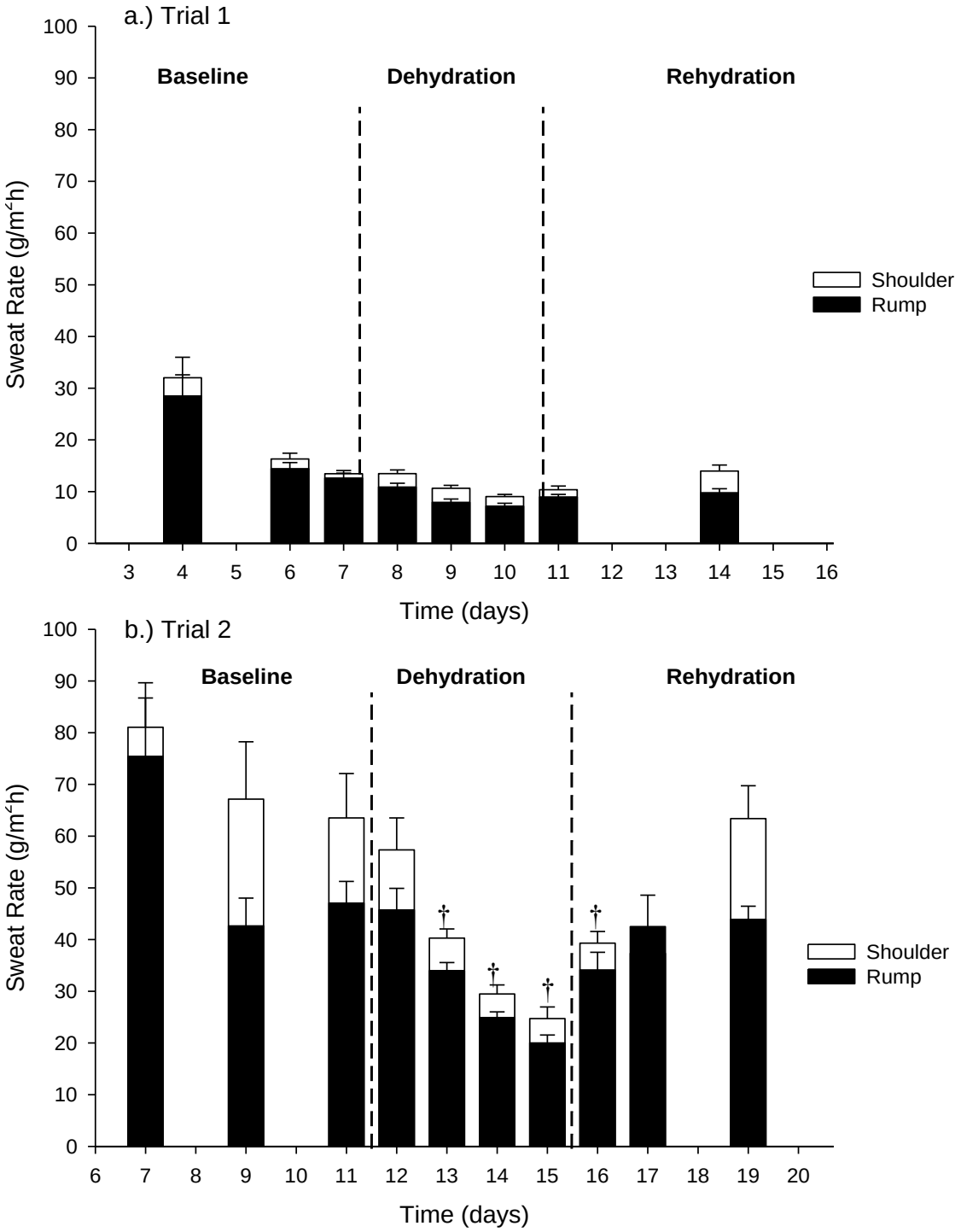

Figure 2.4 – (a) Mean shoulder and rump sweat rates (± 1 SEM) as a function of time in days for Trial 1. (b) Mean shoulder and rump sweat rates (± 1 SEM) as a function of time in days for Trial 2. Baseline reading in trial 2 represents a HS reading. Open boxes represent shoulder rates and black boxes represent rump sweat rates. Only PM reading is represented in this figure. Dagger (†) signifies values are significantly ($P < 0.05$) different from baseline.

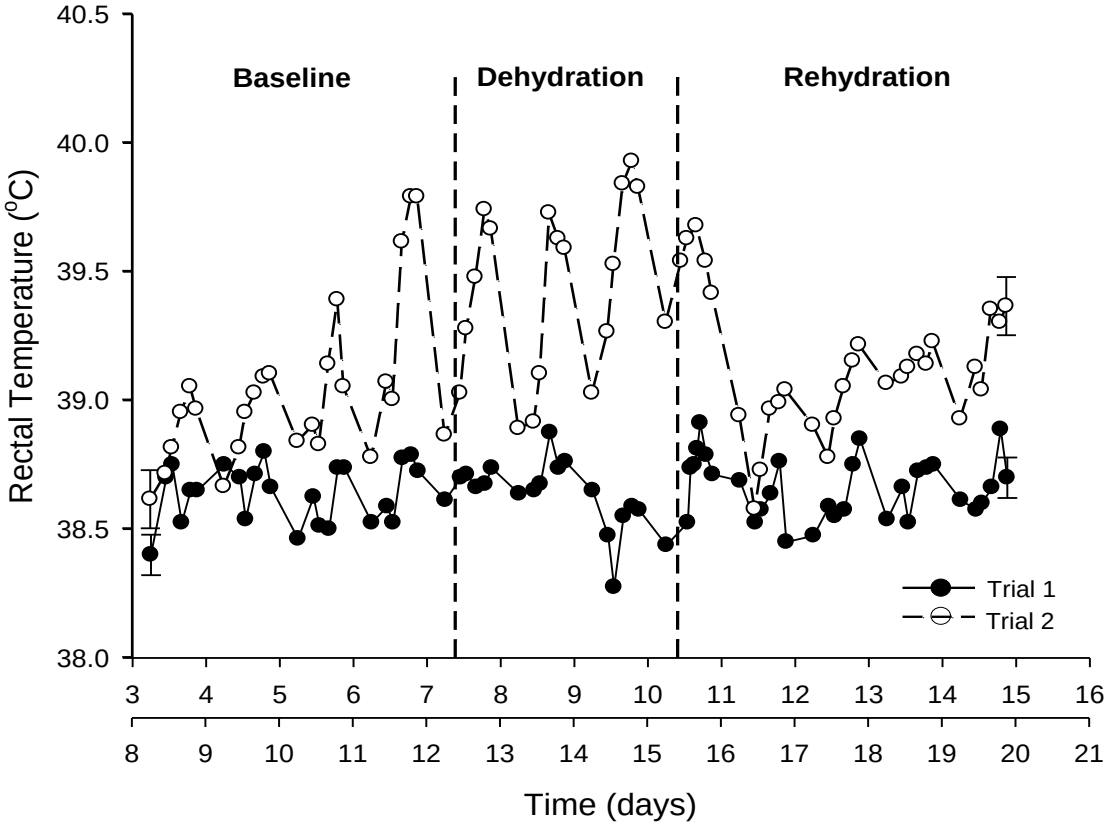

Figure 2.5 - Mean rectal rate (± 1 SEM) as a function of time in days for Trial 1 and Trial 2. All six sample times during a day are shown. The solid black line represents Days 3 to 15 of Trial 1 under TN conditions. The black dashed line represents Days 8 to 20 of Trial 2 under HS conditions. The dashed vertical line separates designate the start and end of the dehydration period.

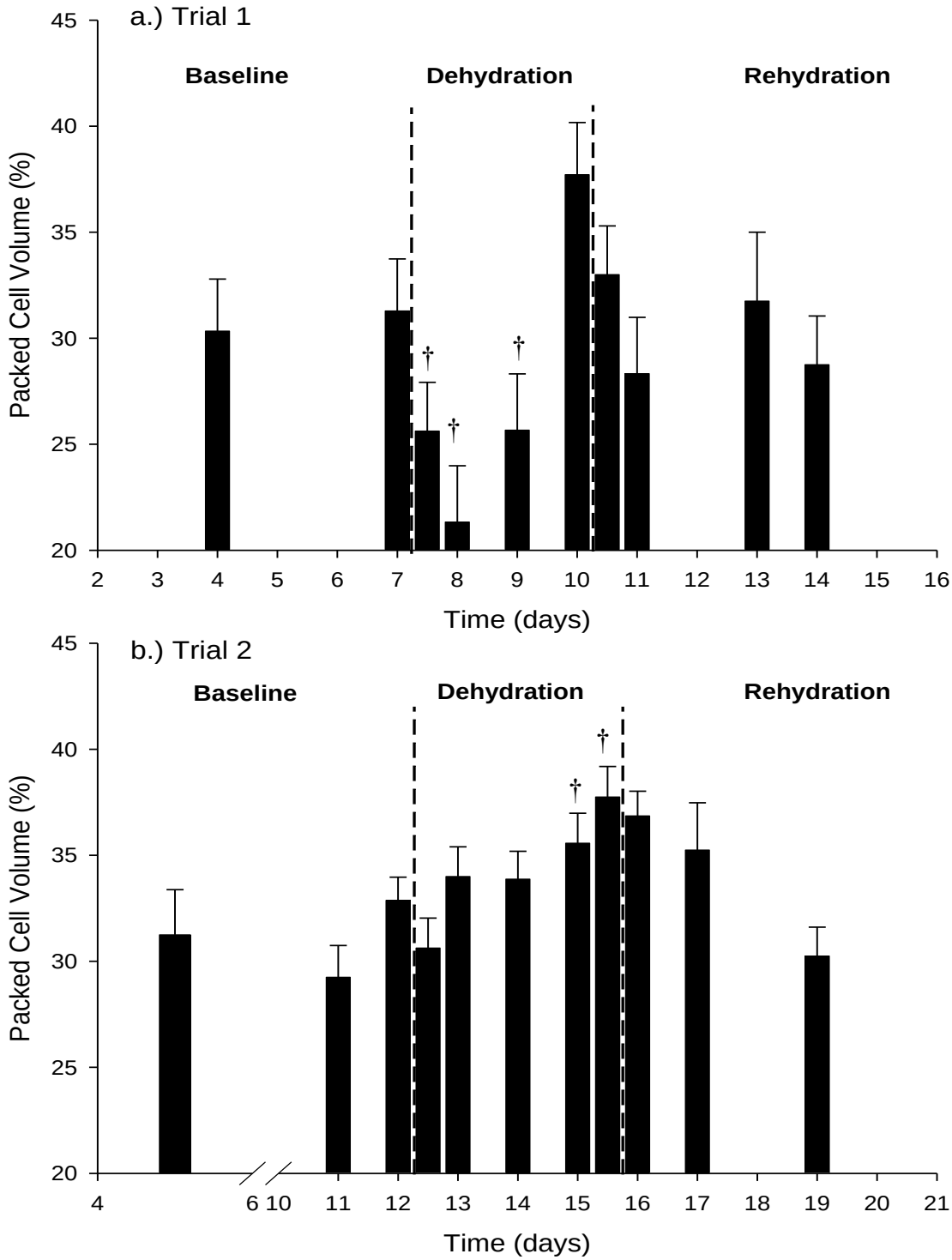

Figure 2.6 – (a) Mean packed cell volumes (± 1 SEM) as a function of time in days for Trial 1. (b) Mean packed cell volumes (± 1 SEM) as a function of time in days for Trial 2. Line break showed represents transition from TN to HS. Dagger (†) signifies values are significantly ($P < 0.05$) different from baseline.

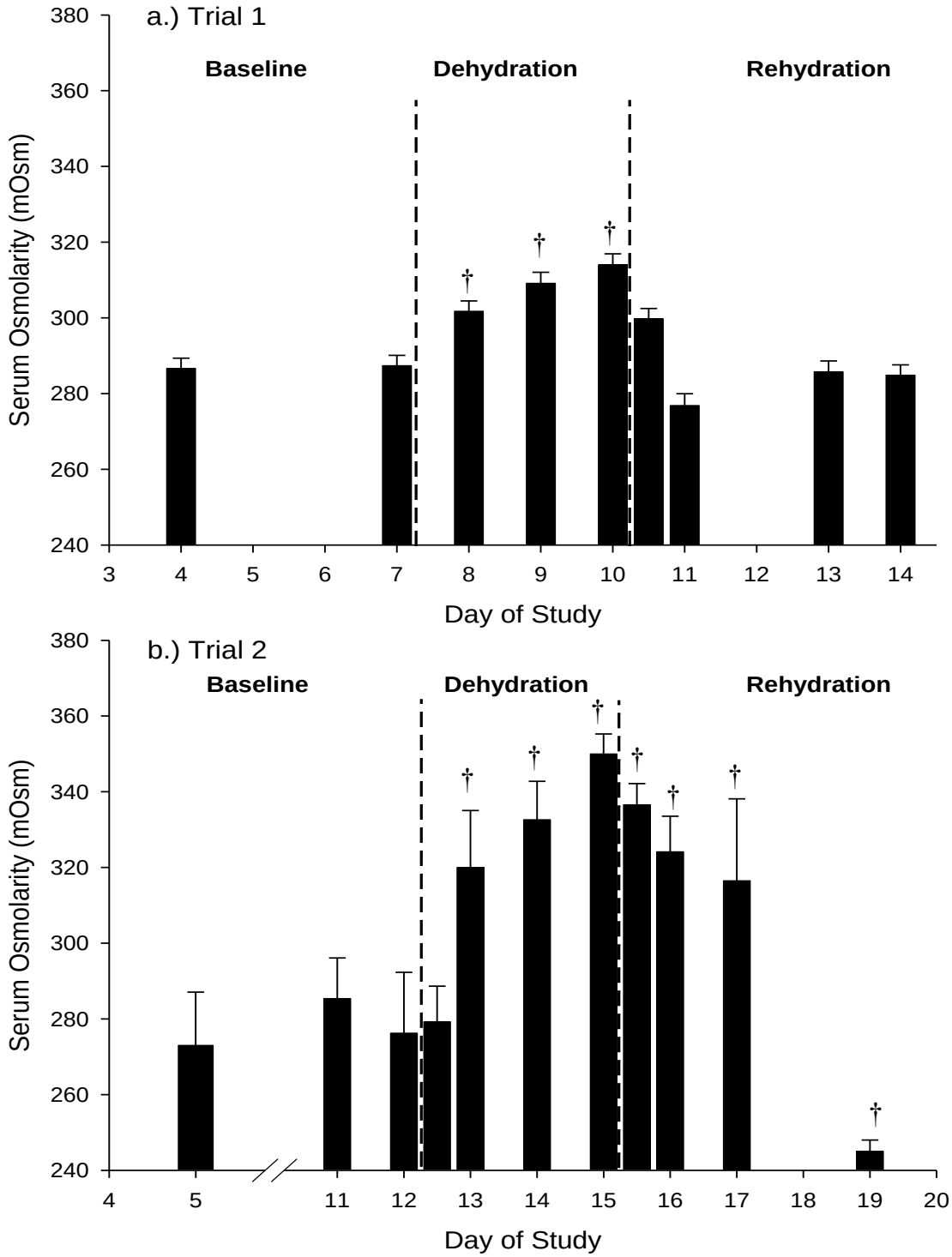

Figure 2.7 – (a) Serum Osmolarity (± 1 SEM) as a function of time in days for Trial 1. (b) Serum Osmolarity (± 1 SEM) as a function of time in days for Trial 2. Line break showed represents transition from TN to HS. Dagger (†) signifies values are significantly ($P < 0.05$) different from baseline.

Table 2.1 – Group mean serum biochemistry values for cattle during Trial 1under thermoneutral conditions

	Baseline		Water restriction			Rehydration			Std Error
Measurement	1	2	1	2	3	1	2	3	
Albumin	3.3	3.27	3.41	3.5	3.57	3.16	3.18	3.18	0.08
Chloride	98.87	101	107	109.42	111	98.2	100.57	100.25	1.56
Creatinine	1.35	1.32	1.5	1.67	1.78	1.56	1.24	1.28	0.08
Globulin	3.2	3.18	3.4	3.52	3.72	3.18	3.15	3.25	0.13
Potassium	4.12	4.21	4.3	4.41	4.3	3.92	5.32	4.73	0.13
Prolactin	47.98	43.1	43.21	37.26	25.5	38.38	41.04	36.16	4.35
Sodium	139.37	140.62	148.37	151.57	152.57	137	137.71	138.75	2.35
Total Protein	6.5	6.46	6.81	7.02	7.3	6.34	6.34	6.43	0.16
Urea N	5.87	6.37	9.62	13.85	16.57	11	6.42	6.25	0.88

Table 2.2 – Group mean serum biochemistry values for cattle during Trial 2 under heat stress (26°C night, 36°C day) conditions.

	Baseline		Water restriction			Rehydration			Std Error
Measurement	1	2	1	2	3	1	2	3	
Albumin	3.19	3.35	3.48	3.38	3.51	3.16	3.34	3.24	0.08
Chloride	94.63	100.00	108.38	107.00	108.00	94.16	91.38	99.50	2.66
Creatinine	1.49	1.63	1.74	1.68	1.88	1.53	1.41	1.31	0.08
Globulin	3.19	3.09	3.71	3.61	3.60	3.36	2.95	3.31	0.12
Potassium	4.01	4.35	4.36	4.20	4.06	3.68	3.69	4.15	0.15
Prolactin	22.85	35.20	34.88	28.50	23.34	32.64	33.36	37.95	3.70
Sodium	135.63	139.13	147.50	151.25	147.75	132.88	136.38	140.00	1.79
Total Protein	6.38	6.44	7.19	6.99	7.11	6.54	6.34	6.51	0.15
Urea N	9.63	10.00	11.88	15.63	14.50	12.00	7.13	8.13	0.93

CHAPTER THREE

ACCLIMATION OF ANGUS STEERS TO LONG-TERM HEAT STRESS AND FESCUE TOXICOSIS IN THE FIELD USING A CONTROLLED HEAT CHALLENGE

1. ABSTRACT

Most studies of heat stress are conducted using short-term, controlled exposure (i.e., 1-2 weeks) in environmental chambers or long-term summer exposure to variable field environments. This study combines both situations by utilizing the controlled conditions in the Brody Environmental Center (BEC) at the University of Missouri to effectively provide each animal with an initial baseline "stress test." This was followed by placement in the summer field environment (South Farm, University of Missouri) for ~3 months, to create the long-term (i.e, real world) scenario. After this period, animals were split into 4 treatment groups (E-/E-, E+/E+, E+/E-, and E-/E+) and given the controlled "stress test" to determine the adaptive change. During the second controlled heat challenges, steers were assigned to diets of either 0 or 40µg ergovaline/kg/d. The 4 groups were used to determine if: 1) there would be improved overall performance to heat at study end, 2) placement on E+ pasture would improve responses to E+ under controlled conditions, 3) placement of E+ pasture would alter the animals response to heat stress alone, and 4) summer heat acclimation would alter the animal's response to an E+ challenge. Twenty-two Angus steers (365 ± 10kg BW) were housed in the BEC for 7 days at air temperature (Ta) of 20^{o}C (TN), followed by 7 days of cyclic heat stress (HS; Ta=26^{o}C night; 36^{o}C day). Respiration rate (RR) and rectal temperature (Tre) were measured 6 times daily. Sweat rate was measured at shaved sites (shoulder, rump) on

select days. Following the initial test, steers were placed on fescue pasture from May to September, 2006, when Ta and THI ranges were 7.0 - 38.1^{o}C and 49.8 - 86.9, respectively. Results from this experiment showed only a few signs of adaptation. With exception of feed intake, animals in the two groups that switched (E-/E+ and E+/E-) treatments responded to the current diet rather than previous exposure, suggesting no adaptation to the toxin. Feed intake was lower for all treatments during the final chamber run which could signify acclimation to heat stress. Sweat rate showed the greatest change between chamber tests, as well as within chamber runs with a reduction after several days in the heat. This reduction occurred even though rectal temperature and respiration rate were still elevated, suggesting that reduction of sweat rate, and possibly water loss, is more important than reduction of body temperature during heat stress.

2. INTRODUCTION

One of the greatest challenges facing beef producers in many regions of the world is heat stress and the strain it has on livestock health and productivity. Reduced feed intake, growth or production, efficiency, and reproduction are recognized results of heat stress (Hahn, 1999). Although brief heat stress may have little effect, vulnerability is a concern for un-acclimated animals and feedlot cattle, particularly during sustained hot weather, or acute heat loads imposed by heat waves (Hahn and Mader, 1997). In addition, heat stress effects are exacerbated by consumption of an endophytic toxin (*Neotyphodium coenophialum)* resulting in a condition known as fescue toxicosis. Fescue toxicosis is common condition occurring during the summer months in southeast and Midwest regions of the United States due to consumption of endophyte infected tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. –*Schedonorus arundinaceus* [Schreb.] Dumort).

Across the United States, heat stress results in an estimated annual economic loss of $370 million for the beef industry (St-Pierre et al., 2003), while losses due to fescue toxicosis result in more than $600 million (Hoveland, 1993). With the possibilities of climate change resulting in increased heat stress in the future, there is a need to understand how cattle adapt to heat and fescue toxicosis.

Most studies of heat stress and/or fescue toxicosis are conducted under short-term, controlled (i.e., 1-2 weeks) or long-term, field situations. Each approach contains unique problems that reduce the ability to derive meaningful results needed to develop realistic solutions. Studies in environmentally-controlled chambers have been valuable in establishing the thermoregulatory changes that occur during heat stress. However, application of such information to field situations is often difficult due to diurnal variation in ambient temperature, relative humidity, and difficulty in controlling other aspects of the animal's environment. Studies done in chambers or under field conditions usually become a measure of the time taken by the animal to lose control of temperature regulation, and are not necessarily a measure of its adaptability (Lee, 1953). The goal of this study was to perform a baseline "stress test" before placing cattle on pasture to create a long-term real world scenario, after which animals were placed under carefully monitored field conditions and then given another "stress test" to determine adaptive change to heat stress and fescue toxicosis.

3. MATERIALS AND METHODS

Twenty-two Angus steers (365 ± 10kg BW at start) were obtained from the University of Missouri Beef Research Farm. These animals have been raised on tall fescue for many generations and represent animals that possibly that they have acquired

some tolerance to the fescue endophyte indirectly through selection. Steers were housed in covered feedlots at the Beef Farm prior to the beginning of the study. The experiment consisted of 2 chamber runs (i.e., pre- and post-field exposure) and a field exposure (Figure 3.1). Chamber runs were conducted in the Brody Environmental Center which consists of four 6.1 × 9.1 m chambers, two of which were used in the present study. The chambers are divided into six stanchions, with each animal loosely restrained to the stanchion by a chain. Due to space requirements, the 24 animals were split into 2 groups (11 animals per group) and run 2 weeks apart. Air movement in the chamber was held at 15 room changes per hour (124 cubic meters of volume). Light cycle during the chamber study was a 12-h light: dark (0600:2000) schedule. During chamber exposures, steers were housed for 7 d at a thermoneutral Ta (TN; 20^{o}C) prior to initiation of heat stress (HS; Figure 3.2). Heat stress consisted of daily cyclic air temperature (26°C night: 36°C day) for 7 d (Figure 3.2). Chambers at TN had a set-point of 20°C showing slight fluctuations across days. Chambers during HS cycle increased daily as a step-up function with 3 set points throughout the rise phase, followed by a 4-h stable period (36°C; 1200 to 1600 h). The decline phase consisted of 2 set points to reach the stable low temperature (26°C; 0000 to 0600 h). Relative humidity was maintained under 55% during the entire study (TN: 40 to 55%; HS: 35 to 45%). Chamber environmental conditions were controlled using a Fisher-Porter Controller (698B179U01) and a Sensycon I/P Converter (Controller Type 27/06–65). This procedure has been used in a number of previous studies (Spiers et al., 2001; Scharf et al., 2008) .Environmental conditions were measured as during field exposure with Hobo H8 data loggers (Onset, Bourne, MA) to record Ta and RH every 10 minutes.

During the first chamber run, animals were placed on an endophyte-free (E-) tall fescue seed diet to perform a baseline stress test prior to the field exposure (Figure 3.1). The E- seed was top-dressed over a supplement concentrate diet (39% each of corn and soybean hulls, 20% dried corn distiller's grains, and 2% mineral supplement). The field exposure consisted of a 3 month exposure during the summer (Figure3). Animals were assigned to an endophyte-infected (E+; n=12) or E- (n=12) tall fescue pasture. For ergovaline analysis, pasture samplings were collected by walk in a zigzag pattern through the 2 pastures. Approximately 30 samplings were collected for both E+ and E- pasture. Samplings included the entire plant above ground including some root material. Samples were freeze dried and ground for analysis. Ergovaline content was measured by HPLC (detection limit = 50 ppb and CV = 7%; Rottinghaus et al., 1993). Ergovaline concentrations for the E+ pastures ranged from 335 ppb in June to 1225 ppb when seed heads were present in July. Ergovaline concentrations were lower through August (~165 ppb) prior to transport to the chambers. Ergovaline content for the E- pasture was almost negligible ranging from 15 to 60 ppb throughout the summer.

This field exposure was followed by a second chamber run identical to the first, except animals were split again giving 4 treatment groups (Figure 3.1). The first group (E-/E-) remained on E- for the entire experiment and were considered to be the controls. The second group (E+/E+) was on E+ (40 µg ergovaline/Kg BW/day) treatment for the whole experiment and thought to be the most stressed treatment group. The third group (E+/E-) was switched from E+ pasture to E- seed under control conditions. The final group (E-/E+) was moved from E- pasture to E+ seed (40 µg ergovaline/Kg BW/day) in the chambers. These groups were used to determine if consuming E+ over summer would

alter their response to the heat stress challenge or to test if summer heat alters the animal's response to an E+ challenge.

Animal measurements, including respiration rate (RR), skin temperature (Tskin), and rectal temperature (Tre), were taken 6 times daily (0600, 1100, 1300, 1600, 1900, and 2100 h). Daily measurements of feed intake were made during the entire trial period by measuring refusal. Shoulder and rump sweat rates at shaved skin sites were measured twice daily on selected days. Days chosen represent TN, early and late heat stress periods. Sweat rate was determined using a calibrated, digital moisture sensor (Vapometer; Delfin Technologies Ltd, Finland) that determined transepidermal water loss. Other recent studies have used the same type of device to measure moisture loss in a range of situations and environments (Nuutinen et al. 2003). Determination of respiration rate (RR) was made by counting flank movement over a 1-minute interval. Skin temperatures, at 5 different shaved sites (outer ear, shoulder, rump, tail head, and lower tail), were measured using a calibrated infrared thermometer (Model RAYST80XB, Raytek Corporation, Santa Cruz, CA; accuracy ± 1%). Readings were taken less than 35 cm away from each skin site with a thermometer target ratio of 50:1. Rectal temperature was measured using a calibrated thermistor thermometer (Model 8110–20, Cole-Parmer Instruments, Chicago, IL). This was accomplished by inserting a YSI probe (model 400, YSI Inc., Yellow Springs, OH; accuracy: 0.1°C) approximately 15 cm into the rectum for 2 minutes.

Blood were sampled at thermoneutrality prior to heat stress, and again at the beginning and end of the heat stress sessions via a jugular venous puncture. Samples were collected into a 15 ml tubes and allowed to clot prior to centrifugation. Serum was

separated by centrifugation (2,300 x *g* for 25 minute; 4°C) before being removed and stored at −20◦C for later analysis. Serum analyses used standard procedures. Most serum measurements were components of a larger biochemical profile produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an auto-analyzer (Olympus AV400; Olympus America, Inc., Melville, NY). These include albumin, alkaline phosphatase, calcium, chloride, cholesterol, creatinine phosphokinase, creatinine, globulin, glucose, potassium, sodium, total protein, triglyceride, and urea nitrogen. Serum leptin concentrations were determined by a sensitive ovine leptin radioimmunoassay validated for bovine serum (Delavaud et al., 2000). Standards were assayed in quadruplicate and samples in triplicate 200 μl volumes. Assay sensitivity and intra-assay coefficient of variation were 0.03 nmol/L and 3.1% respectively. Serum concentrations of prolactin were determined by radioimmunoassay procedures previously validated at the University of Missouri (Lutz et al. 1991). Minimum detectable concentrations of prolactin in serum were 1.19 ng/tube. Intra-assay coefficient of variation was 9.2%.

As during the chamber exposures, data loggers (Hobo H8 Pro; Onset Computer, Bourne, MA; accuracy: ± 0.2°C and ± 3% RH) were used to record air temperature (Ta) and percent relative humidity (RH), as well as black globe temperature (BG; hollow copper sphere; 15.24 cm diameter; flat black exterior; Bond and Kelly 1955; located between animal pastures) for assessment of radiant heat load under field conditions. Determinations of respiration rate (RR) were made by counting flank movement over a 1-minute interval twice a day (i.e., 0900 and 1500 h). These points were selected as they represent both low and high points of the daily core temperature cycle.

To record core body temperature under field conditions, a calibrated, telemetric, temperature transmitting bolus (SmartStock LLC, Pawnee, OK) was placed into the rumen of each animal (oral administration using a bolus gun) prior to the study to record ruminal temperature (Trum). The telemetric system is composed of a telemetric bolus (3 cm x 8.25 cm, 115 g), an antenna, a receiver unit, a base receiver unit, and a personal computer equipped with a software program for data logging. The boluses were designed to transmit every 20 minutes. Boluses were calibrated to a NIST (National Institute of Standards and Technology) thermometer prior to ingestion by the animal. The data was filtered for maximum hourly value which was used for all analyses. This avoided the incorporation of thermal artifacts associated with water intake.

The experimental protocol and procedures for this use of animals were approved by the University of Missouri Animal Care and Use Committee.

3.1. Statistical Analysis

3.1.1. Chamber Exposure

All evaluations at TN were performed using the last 6 d (i.e., Days 2 through 6) before the increase in T_a. Transition to HS analyses was performed using days 6 through 8 and HS analysis used days 8 through 15. The analysis included RR, skin temperatures, sweat rate, or T_{re} as the dependent variables. Skin temperatures were included as an average of the shoulder and rump sites (T_{trunk}), or an average of ear, tail head and lower tail ($T_{appendage}$). Treatment, time, and treatment by time effects were set as fixed, with animal nested within breed as a random effect. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD adjustment procedures for multiple mean comparisons. Simple linear regression procedures of JMP® were utilized

to establish relationships between animal variables (RR, T_{trunk}, $T_{appendage}$, T_{re}) and T_a. Regression coefficients for slope and model R, as well as P-values for the hypothesis test that the regression coefficients were significantly different from zero, are reported. Blood analyses were performed using the repeated measures ANOVA procedure in JMP® with fixed and random effects as described above.

3.1.2. Field exposure

The effects of period on ambient variables (T_a, T_{bg}, and Temperature Humidity Index (THI) were modeled using the repeated measures ANOVA procedures of JMP® (SAS Institute; Cary, NC) with the ambient variable modeled as the dependent variable, with period, time of day, and period by time of day interaction as independent variables that were modeled as fixed effects. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD (Steele and Torrie, 1980) adjustment procedures for multiple mean comparisons.

A repeated measure ANOVA (Steele and Torrie, 1980), constructed using JMP, was also used to test the effects of period on T_{rum}. The model included T_{rum} as the dependent variable, with the independent variables of treatment, hour (0700 to 1800 h), and period fit as fixed effects, and animal within period, treatment by period, treatment by hour, and period by hour interaction as random effects. Respiration rate, T_{rum}, and T_a observations were averaged for each of the two days within period observed at 0800 and 1500 h. These means were tested by way of ANOVA, using JMP to determine if any statistical differences existed.

4. RESULTS

4.1. Pre-Summer Chamber Exposure

During the first chamber run, all animals received the same E- diet and exposed for 7 days to thermoneutral (TN) conditions followed by 7 days of heat stress (HS; Figure 3.2). This baseline test was prior to assignment to treatment and was designed to be a pre-summer challenge for comparison to the post-summer chamber run. If animals were divided into POST chamber run treatments, no differences were found in feed intake ($P = 0.81$; Figure 3.4), respiration rate ($P = 0.97$; Figure 3.5), skin temperature (both trunk and appendage; $P = 0.73$; not shown), rectal temperature ($P = 0.44$; Figure 3.6) or sweat rate ($P = 0.76$; Figure 3.7).

Feed intakes were consistent throughout both TN and HS periods (Figure 3.4). Feed intake at TN was lowest on Day 2 of the study (10.9 ± 1.2 kgs), but quickly stabilized by the end of TN being highest on Day 8 (13.8 ± 1.2 kgs; Figure 3.4). Respiration rates were increased across the 7 days of TN, being lowest on day 2 and highest on day 5 ($P < 0.05$; 43 vs. 53 ± 2 bpm). Respiration rate was lowest at 1900 hrs (45 bpm) and highest at 0600 (52 bpm) throughout TN ($P < 0.05$). Transition to HS (day 8) resulted in an increase in RR from 51 to 72 bpm by the end of the day ($P < 0.05$; Figure 3.5). Respiration rate increased during the first 3 days of HS ($P < 0.05$) before stabilizing on day 12 ($P > 0.05$; Figure 3.5). As during TN, RR showed time of day differences being lowest at 0600 and highest at 1600 ($P < 0.05$).

Skin temperatures at the trunk (shoulder and rump) and appendages (ear and tail) showed similar trends under TN conditions, both being highest on day 5 of TN ($P < 0.05$) and not different on other days ($P > 0.05$). Time of day differences were also similar being highest from 0600 to 2100 and lowest from 1100 to 1600 ($P < 0.05$). Trunk

temperature was approximately 0.5°C greater than appendage temperature under TN conditions. Transition to HS resulted in an immediate increase in trunk temperature (30.1 vs. 36.4°C) and appendage temperature (29.6 vs. 36.1°C; $P < 0.01$). Skin temperatures (both trunk and appendage) continued to increase during the first 3 days of HS ($P < 0.05$), stabilizing on Day 12 and not being different after ($P = 0.14$). Skin temperatures (both trunk and appendage) showed a rhythm similar to air temperature being highest at 0600 and lowest at 1600 ($P < 0.05$). Trunk, appendage and air temperatures were highly correlated (Trunk: $R = 0.86$; appendage $R = 0.87$). Similarly, the correlation between trunk and appendage temperature was also very high ($R = 0.98$). The difference between trunk and appendage temperature was reduced during HS (~0.2°C vs. ~0.5°C) compared to TN.

Sweat rate at both the shoulder and rump regions were not different at TN, ranging from 36 to 63 g/m^2h ($P = 0.42$; Figure 3.7a & 3.7b). Transition to HS resulted in a rapid increase in sweat rate at both regions (54 to 251 g/m^2h; $P < 0.001$) being significantly different from TN on day 9 at the 0900 reading ($P < 0.05$). During HS, there was a time of day effect with the 0900 reading being significantly lower than the 1500 reading ($P < 0.01$). Shoulder sweat rate showed the greatest response reaching levels in excess of 300 g/m^2h ($P < 0.05$; Figure 3.7a) by 1500 on day 9 while rump sweat rates only reached 250 g/m^2h ($P < 0.05$; Figure 3.7b). After 6 days of HS, sweat rates at both regions decreased; however, levels were still more than double the rates under TN conditions (~135 g/m^2h; $P < 0.05$; Figure 3.7a & 3.7b).

Rectal temperature showed a similar response, with no difference at TN ranging from 38.8 on day 1 to 39.0°C on day 7 ($P = 0.09$; Figure 3.6). Rectal temperature showed

a time of day effect with 1100 and 1300 (~38.7°C) having the lowest temperature and 1900 to 2100 having the highest temperature (~39.0°C; $P < 0.05$). Transition from TN to HS resulted in an increase in Tre ($P < 0.05$; 39.0 to 39.3°C; Figure 3.6). However, this increase above TN levels did not occur until day 9 (2nd day of HS). As during TN, Tre showed a daily rhythm having a high temperature from 1600 to 1900 hours and a low temperature at 0600 (~40.0°C vs. 39.3°C; $P < 0.05$). As with RR, Tre continued to increase from days 9 to 12 (~39.2 vs. 39.6°C; $P < 0.05$) reaching 40.2 on the final day ($P < 0.05$; Figure 3.6). Despite the increase in daily Tre, the daily rhythm remained the same.

4.2. Field Exposure

Following the chamber runs, steers were randomly split into E+ and E- pastures for 3 months. While on pasture, RR and Trum were measured to monitor the thermal status of the animal. Due to difficulty receiving transmitter values at night, only the rise of the day (0600 to 2000 h) was used in the analysis.

Respiration rate showed no treatment, day x treatment, time x treatment, or day x time x treatment effects ($P \geq 0.18$; Figure 3.8). There was a significant time of day effect with 1500 h being significantly higher than the 0900 h reading on every day with exception of July 10th ($P < 0.05$; Figure 3.8). On July 7th, ambient temperature only achieved a maximum air temperature of 23.7°C, whereas, the rest of the day's maximum air temperature was above 28°C. Respiration rates showed the greatest response during the 2nd and 3rd week of July (Figure 3.8) when ambient temperatures were above 34°C ($P < 0.05$; Figure 3.3). During this time period, RR at both 0900 and 1500 were significantly higher than the rest of the field exposure ($P < 0.05$). Interestingly, a similar heat exposure

also occurred during early August (Figure 3.3), where temperatures were above 34°C, however RR did not increase to the same level ($P < 0.05$). Linear and polynomial regressions (between RR, Ta, BG, and THI) showed that both treatments had similar responses to a change in Ta (Figure 3.9). There was a tendency for E- animals to have a higher correlation coefficient for Ta ($R = 0.74$ vs. 0.71) and BG (0.73 vs. 0.68), however THI which had the highest correlation ($R = 0.79$) was the same for both treatments (Figure 3.9).

Similarly to RR, Trum showed no significant treatment differences ($P = 0.51$). As stated above, only the rise of the day for Trum (0600 to 2000 h) is reported. Similarly to RR, the 2nd and 3rd week of July showed the greatest response in Trum being significantly higher than the rest of the field exposure ($P < 0.05$). Because, the analysis only included the rise of the day, a circadian rhythm could not be determined. However, hours 0600 through 1100 were significantly lower than hours 1200 through 2000 ($P < 0.05$). Surprisingly, there was a trend for E- animals to maintain a higher Trum than E+ animals (39.5 vs. 39.3 ± .12°C) during the middle of the day (1000 to 1400; $P < 0.11$; Figure 3.10), however this is likely of no physiological significance. There was also a tendency for the E- animals to have a lower Trum during the morning (38.4 vs. 38.6 ± 0.19°C; Figure 3.10). Linear and polynomial regressions showed a much lower correlation with Ta, BG, and THI than RR ($R \leq 0.35$; not shown).

4.3. Post-Summer Chamber Exposure

After the field exposure, animals were once again split up into the final 4 groups. Animals began treatment as soon as they arrived in the chambers for POST run. Feed intake during the first 3 d in the chamber fluctuated at TN, decreasing from day 2 to 3

(8.3 vs. 6.7 kg) before stabilizing on days 4 and 5 at approximately 10 kg ($P < 0.05$). Days 5, 6, and 7 were not different from each other suggesting that feed intake stabilized prior to initiation of HS ($P = 0.14$; Figure 3.11). While no treatment differences were found during TN, E+/ E+ showed a significantly lower feed intake than E-/E- animals ($P < 0.05$; 7.3 vs. 10.6 ± 0.87 kg per day) during HS. Feed intake was numerically lower throughout the trial, however it was not significantly different until day 10 ($P < 0.05$; Figure 3.11). Though not significantly different, the 2 groups that switched treatment at the start of the second chamber trial (i.e., E+/E- and E-/E+) tended to responded to their current treatment on rather than the pasture they had been on during the field exposure (Figure 3.11). The E-/E+ group was not different from any groups throughout the study ($P = 0.23$). Similarly the E+/E- group was not different until days 13, 14, and 15 where it was significantly higher than the E+/E+ group ($P < 0.05$; Figure 3.11).

Respiration rate was also not different between treatment groups under TN conditions ($P = 0.24$; Figure 3.12). Similarly to feed intake, RR was low for all groups (~35 bpm) during the first 3 days of TN in comparison to PRE chamber run (Figure 3.12). However, RR increased over the TN trial not being different on days 4, 5, 6, and 7 (~55 bpm) suggesting the animals were stable prior to HS ($P < 0.05$; Figure 3.12). While not significant, E+ animals showed a numerically higher RR (~54 bpm) compared to steers receiving the E- seed (~44 bpm; $P < 0.14$). During TN, 0600 represented the highest RR being significantly than all other times of the day ($P < 0.05$). This time of day effect was found throughout the TN period regardless of treatment ($P = 0.81$). Transition to HS resulted in a rapid increase in RR, which was significantly different from TN on day 9 at 0600 ($P < 0.05$; Figure 3.12). Respiration rate continued to increase until day 10

at approximately 95 bpm and was not different there after ($P < 0.05$; Figure 3.12). There was a time of day effect for RR, with 1300 and 1600 having the highest values (~84 bpm), and 0600 and 2100 the lowest (~99 bpm; $P < 0.05$). This time of day effect was consistent despite the increase in RR during days 8, 9, and 10 ($P < 0.05$).

Skin temperatures at both the trunk and appendage regions showed no treatment differences across day, time, or day x time ($P \geq 0.19$) during TN or HS. Under TN conditions, skin temperature showed a time of day effect with 0600 and 2100 being the highest temperature of the day (~33.4°C) and 1100 to 1900 having the lowest temperature (~32.5°C; $P < 0.05$). During TN, trunk skin temperature was approximately 1°C higher than appendage temperature (~33.0°C vs. 32.0°C). As expected, transition to HS resulted in a large increase in skin temperature at both regions ($P < 0.01$) being significantly different from TN by 1300 on day 8 ($P < 0.05$). The skin temperature difference between regions was reduced during HS (HS: ~0.3°C vs. TN: ~1.0°C) compared to TN. Skin temperatures followed Ta, being lowest from 2100 to 0600 and highest from 1300 to 1600 ($P < 0.05$).

Unlike RR and Tskin, rectal temperature during the second chamber run displayed treatment differences (Figure 3.13). During TN, E+/E+ animals were significantly higher than all other groups ($P < 0.05$). As with many of the parameters, Tre was low during the first 3 days of TN (~38.6°C) being significantly different from days 4 through 7 of HS (~38.9°C; $P < 0.05$). These changes were consistent across treatments. A rhythm was found at TN with 1100 to 1600 having the lowest temperature of the day (~38.7°C) and 2100 to 0600 having the highest temperature (38.9°C; $P < 0.05$). Transition to HS resulted in an increase in Tre for all treatments, with E+/E+ being significantly higher (P

< 0.05) than E-/E- and E+/E- groups during the first 3 days of HS. While animals receiving the E+ seed (i.e., E+/E+ and E-/E+ groups) showed a significantly higher Tre than animals receiving E- seed (i.e., E-/E- and E+/E-; $P < 0.01$); there were no differences between the E+/E+ and E-/E+ or between the E-/E- and E+/E- ($P = 0.18$). All treatment groups showed a continual increase until Day 10 ($P < 0.05$; Figure 3.13). After Day 10, no treatment differences were found between groups ($P = 0.13$). Both the E-/E- and E+/E- animals showed a trend toward an increase from Day 11 to 14 ($P < 0.10$). During HS, Tre showed a pronounced circadian rhythm with 0600 and 1100 having the lowest Tre (~39.8°C), while 1600 and 1900 showed the highest Tre (~40.2°C). This rhythm was independent of treatment ($P = 0.90$).

No treatment, day, or time of day differences were found for shoulder and rump sweat rates under TN conditions ($P = 0.89$) during Trial 2. Transition to HS increased sweat rate at both the shoulder (~100 vs. 40 g/m^2h) and rump sites (~70 vs. 40 g/m^2h; $P < 0.05$); with different responses between treatments ($P < 0.05$; Figures 14a & 14b). Both E- seed groups (E-/E- and E+/E-) increased sweat rate above that of the E+ groups by Day 10 ($P < 0.05$; Figures 14a & 14b). After a 6 d in the HS environment, sweat rate at both regions showed a decrease; but remained above TN levels ($P < 0.05$; Figures 14a & 14b). Animals in the E+ seed groups (E+/E+ and E-/E+) did show a numerical increase in shoulder sweat rate; however, it was not significantly different from TN levels (Figure 3.14a). Likewise, rump sweat rate was not different from TN levels for the E+ seed groups (Figure 3.14b).

4.4. Comparison between Chamber Runs

Figure 3.15 shows a comparison between the PRE and POST feed intakes. The PRE feed intakes were numerically greater than POST feed intakes across all treatments. While E-/E- and E+/E- animals showed no significant differences, E+/E+ and E-/E+ were significantly lower than PRE levels ($P < 0.05$). This difference was only found during the HS conditions. No differences were found under TN conditions for treatment or chamber run. Respiration rate and skin temperatures showed a lack of adaptation with no differences between PRE and POST chamber runs (not shown). Rectal temperature showed no treatment differences at TN between PRE and POST runs (Figure 3.16). Transition to HS resulted in an increase in Tre during both chamber runs ($P < 0.01$). However, E+/E+ animals showed a large increase in Tre during the first 4 d of HS being significantly different from the PRE chamber run ($P < 0.05$; Figure 3.16). The E-/E+ group also tended to have a higher Tre during the first 4 d of HS ($P < 0.09$; Figure 3.16). Though all treatments tended to have a higher Tre during the first 3 d of HS, by the end of each trial the animals were all at similar levels. In fact, POST animals stabilized 1 d (day 11 vs. 12) faster than the PRE animals (Figure 3.16). Sweat rate, specifically shoulder sweat rate, showed the greatest change between PRE and POST (Figure 3.17). Sweat rate for both regions (shoulder and rump) rapidly increased during initiation of HS prior to Tre and RR ($P < 0.01$) during PRE testing; rising above all values in the POST chamber run ($P < 0.001$). Sweat rate for E-/E- and E+/E- groups increased well above the E+ groups ($P <0.05$) during the POST runs (Figure 3.17). However, levels only reached ~150 g/m^2h in comparison to more than ~350 g/m^2h during the PRE run (Figure 3.17). E+/E+ and E+/E- groups showed very little increase in sweating only tending to be higher than TN levels during the POST chamber run(~80 g/m^2h; Figure 3.17). Some

characteristics of sweat rate responded the same between the 2 chamber runs. Sweat rate decreased after approximately 5 days in the heat despite the fact that Tre and RR were still elevated.

4.5. Blood Parameters

A number of blood parameters were measured to look at both adaptions to heat, as well as classic signs of fescue toxicosis. Calcium was the only parameter to show a difference between trials being significantly higher in PRE than POST runs ($P < 0.05$; not shown). During both runs, several blood parameters showed changes during initiation of HS that were independent of treatment. Sodium, chloride, total protein, albumin, and triglyceride all increased during HS ($P < 0.05$; Table 3.1). Cholesterol, on the other hand, showed a decrease during HS ($P < 0.05$). Only 4 parameters (albumin: globulin ratio, alkaline phosphatase, prolactin, and leptin) showed significant treatment differences ($P < 0.05$; Table 3.1). Decreased prolactin is a classic symptom of fescue toxicosis. During the present study, E+/E+ and E-/E+ steers (15.4 and 13.6 ng/ml respectively) showed decreased prolactin levels compared to E- seed groups (E-/E-: 32.1 ng/ml and E+/E-: 32.2 ng/ml; Table 3.1). Similarly, E+/E+ and E+/E- steers had a larger albumin: globulin ratio than did the E- groups ($P < 0.05$; 1.11 vs. 0.90 ± 0.03). Alkaline phosphatase, which known to decrease with E+ seed, showed a significant treatment difference only between the E-/E+ and E-/E- group (94.4 vs. 48 ± 8.8 U/L; Table 3.1). No differences were found between the rest of the treatments; however, there was a trend for the E+/E+ group to have a lower alkaline phosphatase level than the E-/E- group ($P < 0.06$; 69.2 vs. 94.4 ± 8.8 U/L; Table 3.1). Leptin results were similar to alkaline phosphatase, with the groups that switched (i.e., E-/E+ and E+/E- Table 3.1) not being different from the E+/E+ and E-

/E-. However, unlike alkaline phosphatase, the E+/E+ groups showed higher serum levels of leptin than the E-/E- animals (8.43 vs. 6.2 ± 0.59 ng/ml; $P < 0.05$; Table 3.1). There was also a trend for the E-/E+ group to being higher than the E-/E- group (6.87 vs. 6.2 ± 0.59 ng/ml; $P < 0.10$; Table 3.1).

5. DISCUSSION

One of the goals of this experiment was to see if exposure to the summer environment would result in acclimation to heat stress and/or fescue toxicosis (e.g., lower core temperature, respiration rate, and sweat rate) at the end of the summer (i.e. September). To accomplish this task, an experiment was created resulting in 4 treatment groups. The first group (E-/E-) remained on E- pasture and seed for the whole experiment and acted as the controls. This E-/E- was used to see if heat stress through the summer would improve their response to heat stress in a chamber run at the end of summer (i.e. POST). A second group (E+/E+) was placed on E+ pasture and E+ seed during the second chamber trial. This group was used to determine if there was a combined adaptation and impact of heat stress and fescue toxicosis (e.g., reduced feed intake, reduced prolactin, hyperthermia, etc.; Strickland et al., 2009). This group might also have shown an improved response to E+ seed under controlled conditions in the case of positive adaptation. The third group (E+/E-) was switched from E+ pasture to E- seed during the final chamber run. This group was used to determine if consuming E+ over summer would alter their response to the heat stress challenge during the POST test. Finally, the fourth group was placed on E- pasture and changed to E+ seed during the final chamber run (i.e., POST). This group was to test if summer heat alters the animal's response to an E+ challenge.

The first chamber exposure (PRE) was developed as an initial challenge to determine if there were major differences in responsiveness of the animals prior to being placed on treatments. During this exposure, while receiving E- seed, none of the parameters measured were different (i.e. RR, Tre, skin temperature, feed intake, sweat rate). Respiration rate, Tre, skin temperature, and sweat rate all increased with HS exposure, which is consistent with previous literature (Hahn, 1999, Scharf et al., 2008). Feed intake increased throughout TN and showed little change under HS conditions. Typically, feed intake is reduced after 3 days in the heat (Hahn, 1999). Animals in the present study had a relatively low feed intake in comparison to feedlot animals shown in Hahn (1999), which could lead to the differences found in this chamber run. While RR is considered to be one of the first indicators of an increased thermal load and Tre is a primary indicator of thermal status, sweat rate showed the largest response during the PRE challenge. Sweat rate at both the shoulder and rump regions showed little differences under TN conditions, but rapidly increased more than 5 times that amount during HS. It is known that there are regional differences in the number of sweat glands (Findlay and Yang, 1950; McLean, 1963) with the shoulder region possessing a greater number than the rump region. Therefore in this experiment, those regions were selected and showed that indeed, the shoulder region during HS increased to a greater level than the rump region (~350 vs. 250 g/m^2h).

During the field exposure, RR and Trum showed no treatment differences. Typically, animals grazing E+ pasture are known to have an increased respiration rate compared to cattle grazing E- pastures (Jacobson et al. 1970; Waller et al., 2009). However, many of these studies measured respiration rates after moving the animals to a

more accessible location. In the present study, animals were not moved or restrained in any way during the field exposure. Many studies have also reported higher rectal temperatures for steers grazing E+ tall fescue compared to E- and novel endophyte (Jesup MaxQ and ArkPlus) pastures (Schmidt et al., 1982; Nihsen et al., 2004; Beck et al. 2008). Again, in order to obtain rectal temperatures for these studies, the animals must be restrained or wear an uncomfortable data logger. In the current study, a temperature transmitter was placed into the rumen of the animal allowing the animal to remain undisturbed. To my knowledge, no one has looked at the effects of endophyte on ruminal temperature. It is known that different sub-dermal sites respond differently to environmental stimuli (Bligh, 1957; Guidry and McDowell, 1966; Seawright et al., 1984; Hahn et al., 1990). It is possible that ruminal temperature is not as sensitive as other core body regions (i.e., rectal, tympanic, intraperitoneal). Behaviorally, E+ animals are known to often seek shade, form wallows around water troughs and in shaded areas, and spend less time grazing than E- animals (Schmidt and Osborn, 1993; Strickland et al., 1993; Oliver, 2005). Behavioral changes were not recorded during this experiment; however, these changes in time spent under shade or grazing might result in the lack of differences found for Trum and RR.

Since measurements during the field exposure showed none of the typical signs of fescue toxicosis (increased RR and hyperthermia; Strickland et al., 1993); we were hopeful that animals on E+ pasture had adapted to the toxins. During the chamber study, animals were given a larger dosage of the toxin that they would have received on pasture which resulted in increased signs and symptoms. Feed intake, which is a sensitive indicator of fescue toxicosis was reduced in both the E+/E+ and E-/E+ groups during HS.

However, only the E+/E+ group was significantly different from the E-/E- group. The E-/E+ group was only numerically lower suggesting that summer HS may have helped them respond better to the E+ seed during POST. Also, the E+/E+ group might be experiencing the long-term effects of E+ exposure. Aldrich et al. (1993) reported that to steers on E+ housed at 32ºC had a 22% reduction in feed and compared to animals housed at 22ºC temperature, which is consistent with the results of the present study. However, other studies have shown a reduction in feed intake irrespective of temperature (Strickland et al., 2009; Spiers et al., 2012). In the present study, feed intake during the first 3 days was low as they adjusted to the new diets, which may have masked the E+ feed intake reduced noted by other researchers.

As during the field exposure, RR showed no treatment differences when tested in the chambers. All groups increased RR during transition to HS. This result could suggest that the E+/E+ group and E-/E+ groups have adapted to the heat and toxins, resulting in no differences. However, the E+/E- group was also not different. As stated above, RR is one of the first outward signs of an increased thermal load and is known to precede a change in core body temperature to effectively maintain normal heat balance (McDowell 1972). Hahn et al. (1997) reported that the T_a threshold for increases in RR varies across individuals, ranging from 17 to 23°C; which is well below the 26°C low point for the second chamber run. It is possible that the heat strain for all the animals was large enough to increase RR to the same level regardless of the diet. Skin temperatures showed no treatment differences during TN or during HS. It is well known that consumption of endophyte infected fescue results in peripheral vasoconstriction and that this leads to an increase in heat retention (Rhodes et al., 1991; Oliver, 2005). In the present study,

transition to HS resulted in an increase in skin temperature which followed the air temperature in the chamber. Since skin temperature is known to be highly correlated with air temperature (Scharf et al., 2012), it is likely that during HS, the E+ effect was masked. However, any retention in heat due to vasoconstriction results in an increased heat load and an increase in body temperature.

Rectal temperature showed no treatment differences under TN conditions in the second chamber trial. Despite vasoconstriction that occurs during consumption of E+ seed (Strickland et al., 2009; Klotz et al., 2010); during TN conditions the animals are able to easily dissipate heat resulting in the absence of a Tre change. Transition to HS resulted in both E+ groups (E+/E+ and E-/E+) having higher Tre than the E- groups (E-/E- and E+/E-). No differences were found between the animals receiving the E+ seed in the POST run or between animals receiving the E- seed. Since the animals responded only to their current diet and not their pasture exposure, it suggests that Tre did not adapt through the summer. By the end of the trial (~day12), all treatments came together resulting in no differences in Tre. This is consistent with others, as animals typically need 3 days in the heat to adjust intakes and increase heat loss (Hahn, 1999; Scharf et al., 2012).

Of the parameters measured, sweat rate at both the shoulder and rump regions showed the greatest change between chamber runs. Similarly to rectal temperature, there were no treatment differences during TN conditions. This is not surprising as under TN conditions we are measuring diffusion of water across the skin rather than active sweating (Cain et al., 2006). Transition to HS resulted in a large increase for both shoulder and rump regions only for the E-/E- and E+/E- groups. The groups receiving E+

seed (E+/E+ and E-/E+) did not increase above TN levels which could result in the Tre responses shown in the present study. Similarly to Tre, animals responded similarly to the seed they were currently receiving rather than their previous pasture exposure suggesting a lack of adaptation to the toxins. While the driving mechanism or controller driving sweat rate is poorly understood, some researchers have found that skin temperature or reduced blood flow are good candidates (Berman, 1971; Whittow, 1962; McLean, 1963). Since E+ seed is known to cause vasoconstriction and reduce blood flow (Rhoads et al., 1991; Strickland et al., 2009), it is feasible that the animals sweat rate is driven down due to this. It has been shown in humans that a reduction in peripheral blood flow lowers sweat rate (Kimura et al., 2007). With the E+ animals not having the ability to increase sweat rate, the animals must rely on other heat loss mechanisms such as RR or accept a higher core body temperature which are all classic signs of fescue toxicosis (Strickland et al., 2009). As shown during the PRE chamber run, shoulder and rump sweat rates responded similarly to HS with the rump having a reduced rate. E+ seed animals similarly animals receiving E+ seed showed no increase at the rump site, while the E- seed animals increased well above TN levels.

Only one blood parameter showed a change between PRE and POST chamber runs. Calcium was higher during early summer versus late summer. Calcium is known to be reduced during heat stress (Shaffer et al., 1981), which could be responsible for some of the differences that were found. Values for several parameters increased during HS. Sodium and chloride increased during HS which is somewhat unexpected. A reduction in serum sodium concentration during heat stress is expected and has been described by El-Nouty et al. (1980). Normally, an increase in serum sodium is the result of an animal

becoming dehydrated. However, animals had ad lib access to water at all times. It is more likely that serum sodium was increased due to the feedlot diet that they were on. An increase in serum chloride would also increase due to the increase in sodium. Total protein constitutes a portion of the amino acid pool of the body and is believed to be indicative of the nutritional status of the animal. Total protein is made up mostly of albumin and globulin. In the present study, total protein increased with heat stress. This has been shown by others (Shaffer et al., 1978). However, globulin did not change with heat stress, showing that albumin is the factor changing total protein concentration. Like many blood parameters, albumin is not well studied with regard to heat stress (Parker et al., 2003). Cholesterol was reduced during HS for all treatments which is consistent with previous literature (Abeni et al., 2007). Prolactin, leptin, and alkaline phosphatase also showed treatment differences. Reductions in prolactin and alkaline phosphatase are usual symptoms of fescue toxicosis (Schultze et al., 1999; Waller et al., 2009, Strickland et al., 1993; Strickland et al., 2009). Very little is known about the effects of fescue on serum leptin concentrations. In the present study, E+ seed animals showed a higher leptin concentration than E- animals. It has been shown that plasma leptin is strongly related to adipose cell size and number in cattle (Delavaud et al., 2002). It is also known that treatment of animals with leptin causes a dose-dependent decrease in food intake, loss of body weight, loss of fat depots, and an increase in energy metabolism **(**Houseknecht et al., 1998). Since E+ animals have a reduced feed intake, it could be that leptin is playing a role in the feedback mechanisms. The results from this study for leptin should be studied in greater detail in a future study.

Comparing PRE and POST chamber runs showed only a few signs of adaptation. Both RR and skin temperature showed no signs of adaption responding the same between trials and on treatments. Feed intake showed large treatment differences and was numerically lower for all groups than PRE, however this was not significant. Interestingly, the E-/E+ group showed a similar feed intake PRE and POST suggesting that the E- pasture during the summer may have helped alleviate the response to the E+ seed given in the chamber. This is an extremely important finding since fescue toxicosis-induced reduction in feed intake is probably the most significant deleterious effect of this condition. Rectal temperature showed treatment differences during the POST period, but no differences in the magnitude of responses. This temperature did stabilized one day faster during the POST than PRE chamber runs; however, POST increased to a higher level at a faster rate than under the PRE condition. However, this does not indicate adaptation. Sweat rate showed a significant change between PRE and POST chamber runs.

Sweat rate for the shoulder and rump regions was reduced during the POST run, despite the fact that Tre was similar between the two chamber runs. In an extensive study looking at changes in sweating activity over multiple seasons, Schleger and Turner (1965) found striking differences in sweating rates between summer and autumn-winter periods for what animal?. These differences occurred in spite of the fact that respiration rates and rectal temperatures were similar during all seasons. It is believed that the seasonal difference in sweating function is more likely associated with the marked seasonal variation in hair growth (Turner and Schledger, 1960) and level of skin activity (Dowling and Nay, 1960) which is under photoperiodic control (Yeates, 1955). Another

surprising result of the present study was a reduction in sweat rate of animals after five days of HS. It is unclear why sweat rate would decrease if they have the ability to sweat more and this could result in a reduced core temperature. A reduction in sweat rate after several hours of heat strain has been reported in sheep, goats, and humans (Jenkinson and Robertshaw, 1971; Collins and Weiner, 1962). However, this reduction occurs when sweat rate is continuously monitored for secretion rates over minutes. This reduction after several days warrants further study.

This experiment was designed as a hybrid study utilizing both a controlled chamber test and a longer-term field exposure. The expectation was that performing a "stress test" prior to any treatment provided a good baseline for comparison to the final heat challenge to reveal adaptive changes. Results from this experiment showed only a few signs of adaptation. With exception of feed intake, animals in the two groups that switched (E-/E+ and E+/E-) treatments responded to the current diet rather than previous exposure suggesting no adaptation to the toxin. As a result, these groups are eliminated from future studies of this nature. Feed intake was lower for all treatments during the final chamber run which could signify acclimation to heat stress. Sweat rate showed the greatest change between chamber tests, as well as within chamber runs with a reduction after several days in the heat. This reduction occurred even though rectal temperature and respiration rate were still elevated, suggesting that reduction of sweat rate, and possibly water loss, is more important than reduction of body temperature during heat stress. Further research is necessary to study the short-term heat response and adaptation of sweat rate overtime in regard to heat stress and fescue toxicosis.

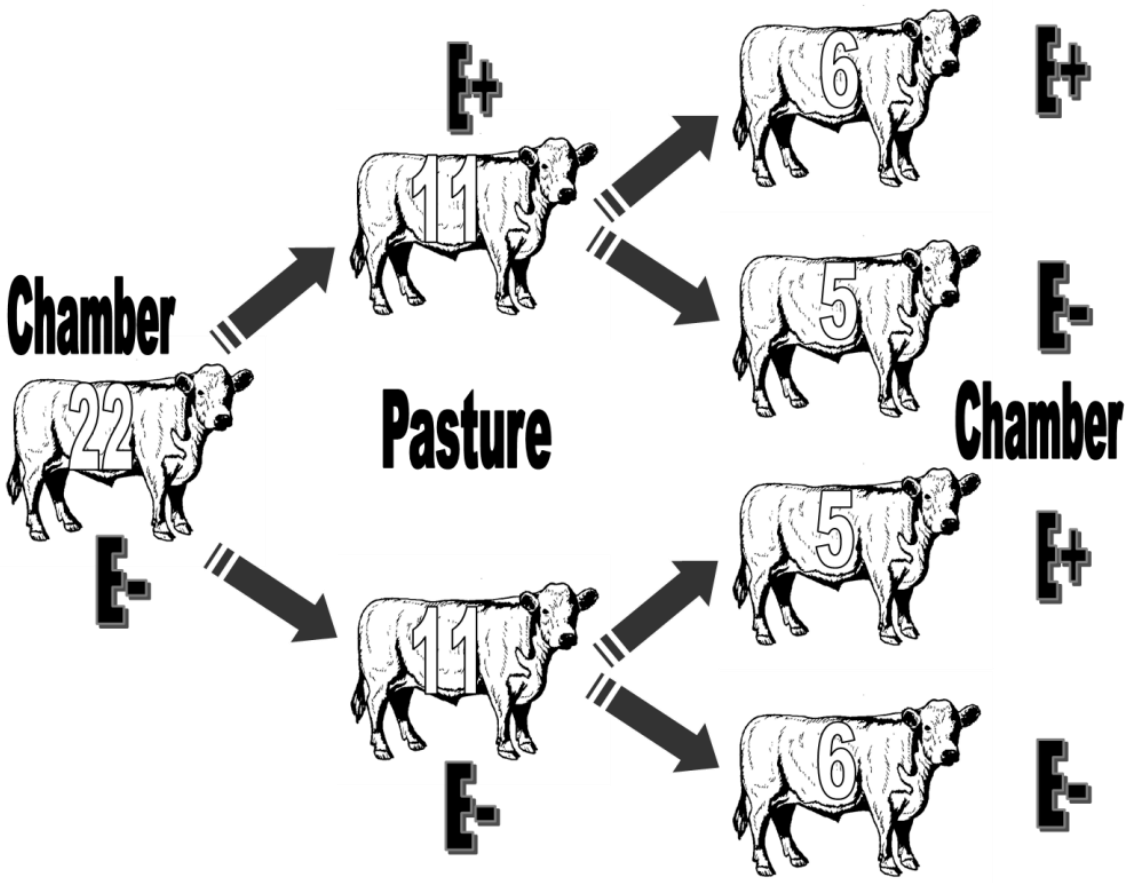

Figure 3.1 – Diagram of the experimental design. Experiment consisted of a baseline chamber run followed by a pasture or field exposure. Following the pasture exposure, a second chamber run was conducted.

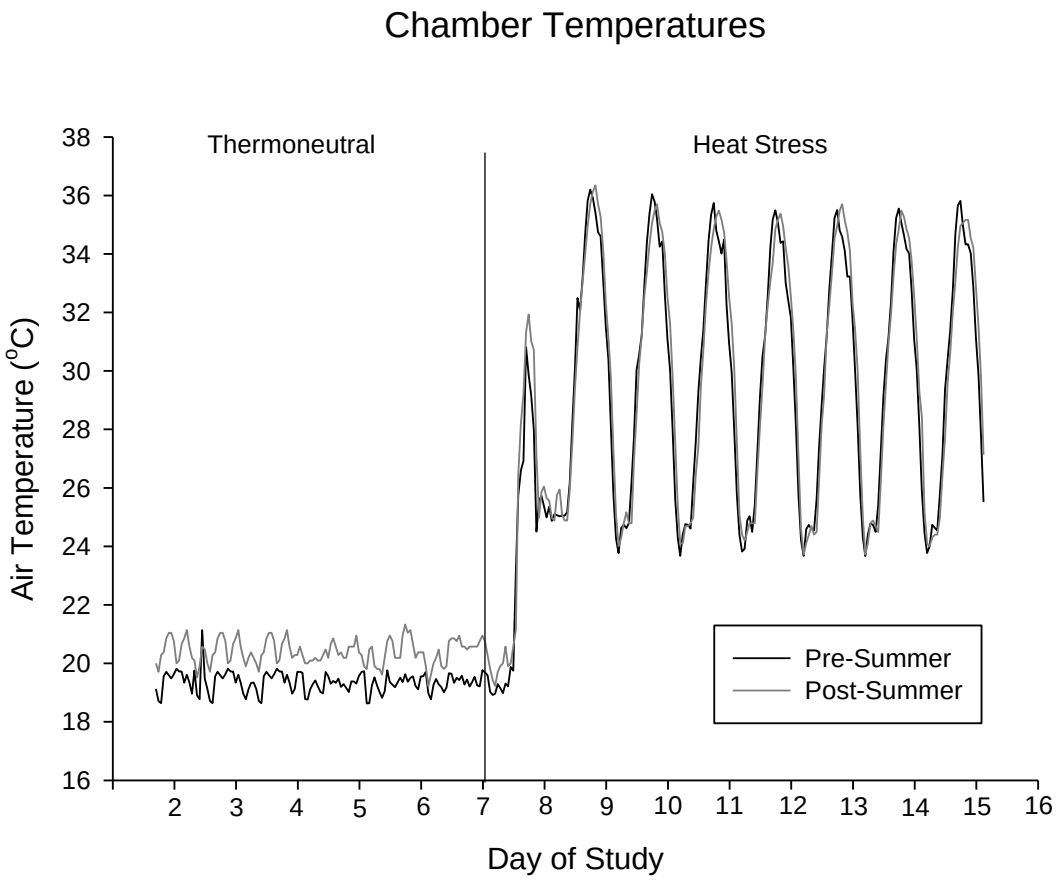

Figure 3.2 - Mean room air temperature beginning on Day 2 at thermoneutrality (19-21°C) and continuing through Day 15 which was the last day of heat stress (Night: 26°C; Day: 36°C). Values were collected hourly for each day. Pre-summer chamber run shown in black. Post-summer chamber run shown in gray.

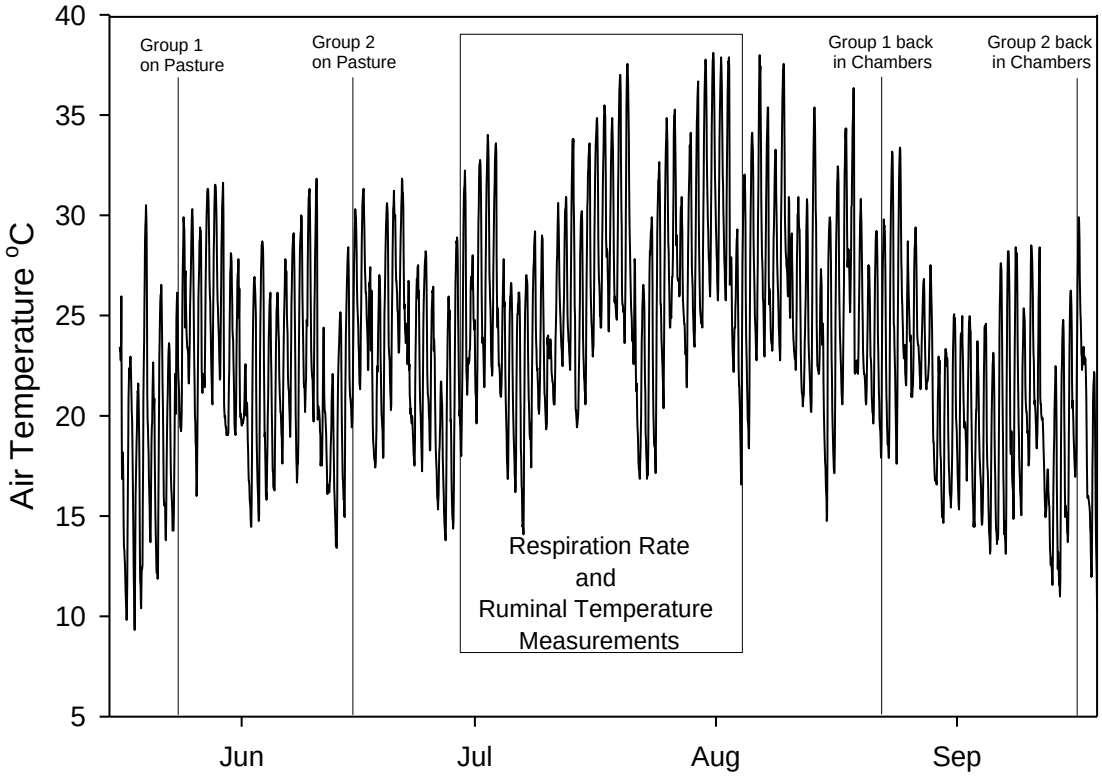

Figure 3.3 – Hourly air temperature (T_a) of the field environment during the experiment. Vertical lines designate when animals entered the chambers. Days within the box represent days where respiration rate and ruminal temperature were collected. Values were collected hourly for each day.

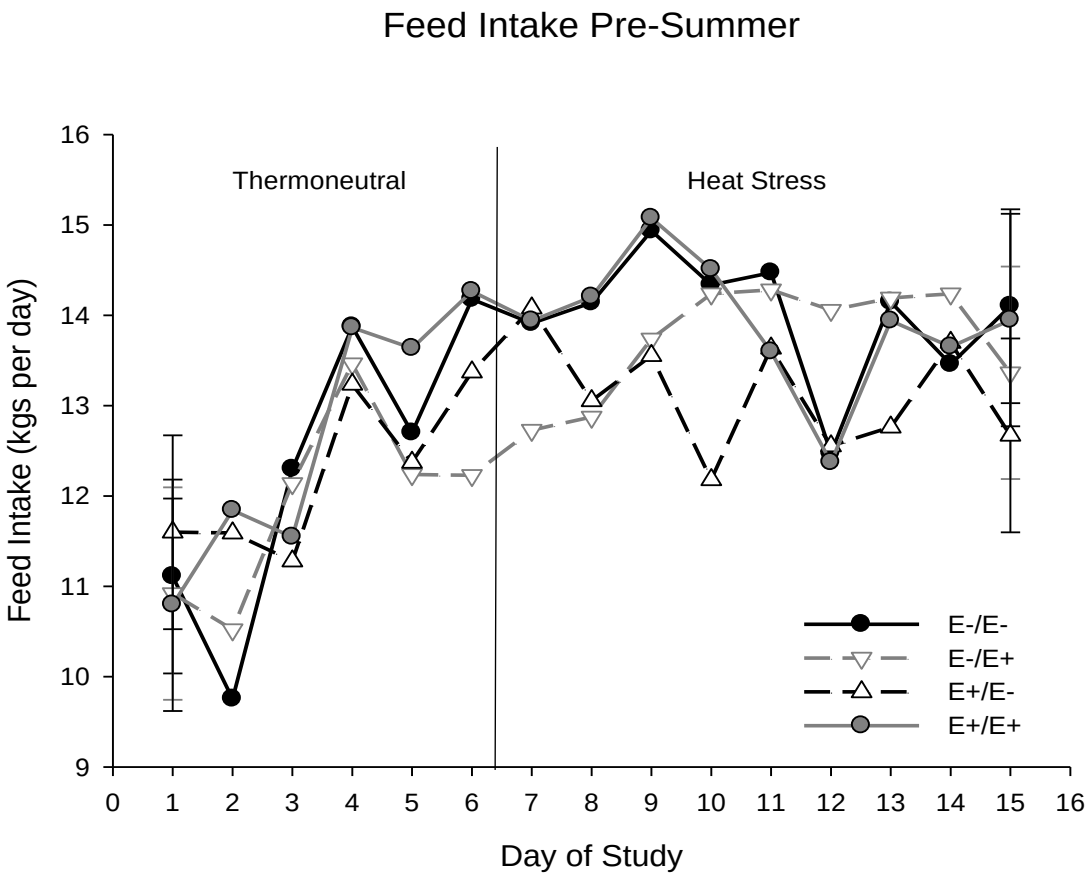

Figure 3.4 - Daily feed intake of steers shown as a function of time in days for the chamber exposure. Animals during the pre-summer exposures all received the same E- diet. However, treatments (E+/E+, E+/E-, E-/E+, and E-/E-) are shown. The solid vertical line separates thermoneutral and heat stress periods. The vertical line on top of each variable bar is +1 SEM.

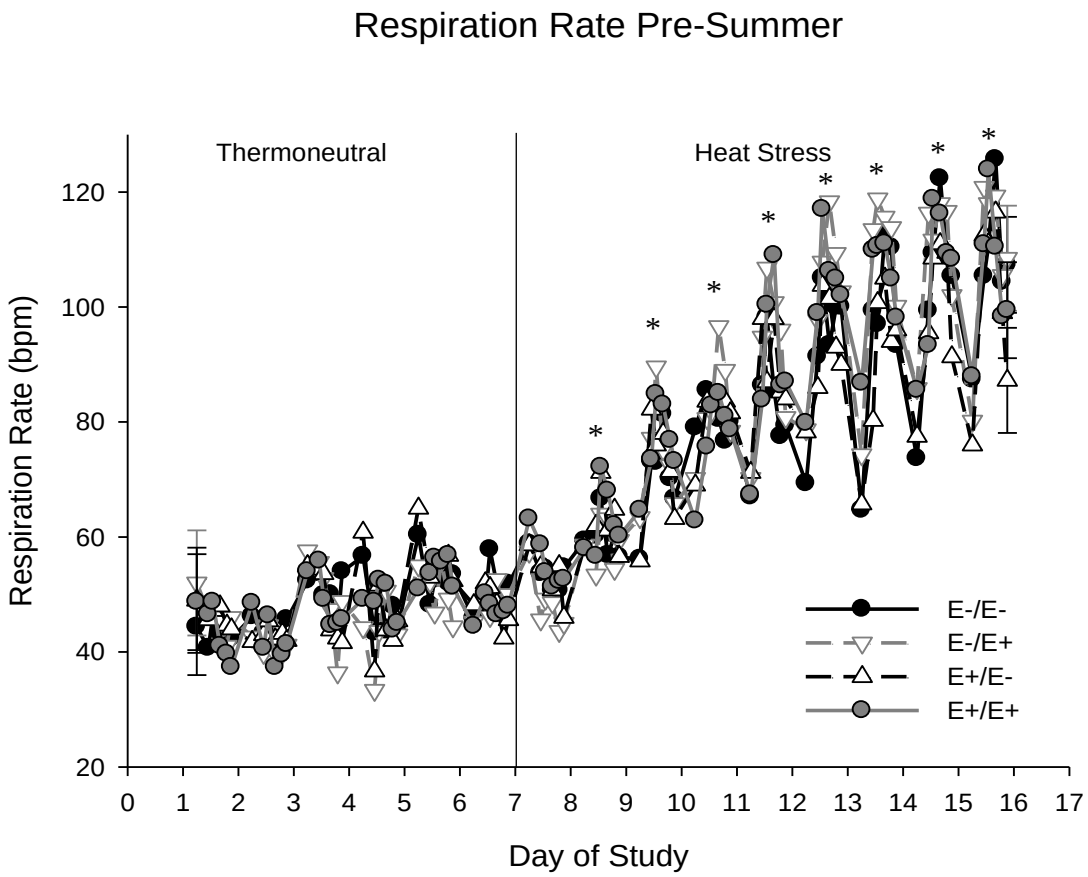

Figure 3.5 - Mean respiration rate (± 1 SEM) of steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. Animals during the pre-summer exposures all received the same E- diet. However, treatments (E+/E+, E+/E-, E-/E+, and E-/E-) are shown. An asterisk (*) signifies day is significantly different ($P < 0.05$) from thermoneutral.

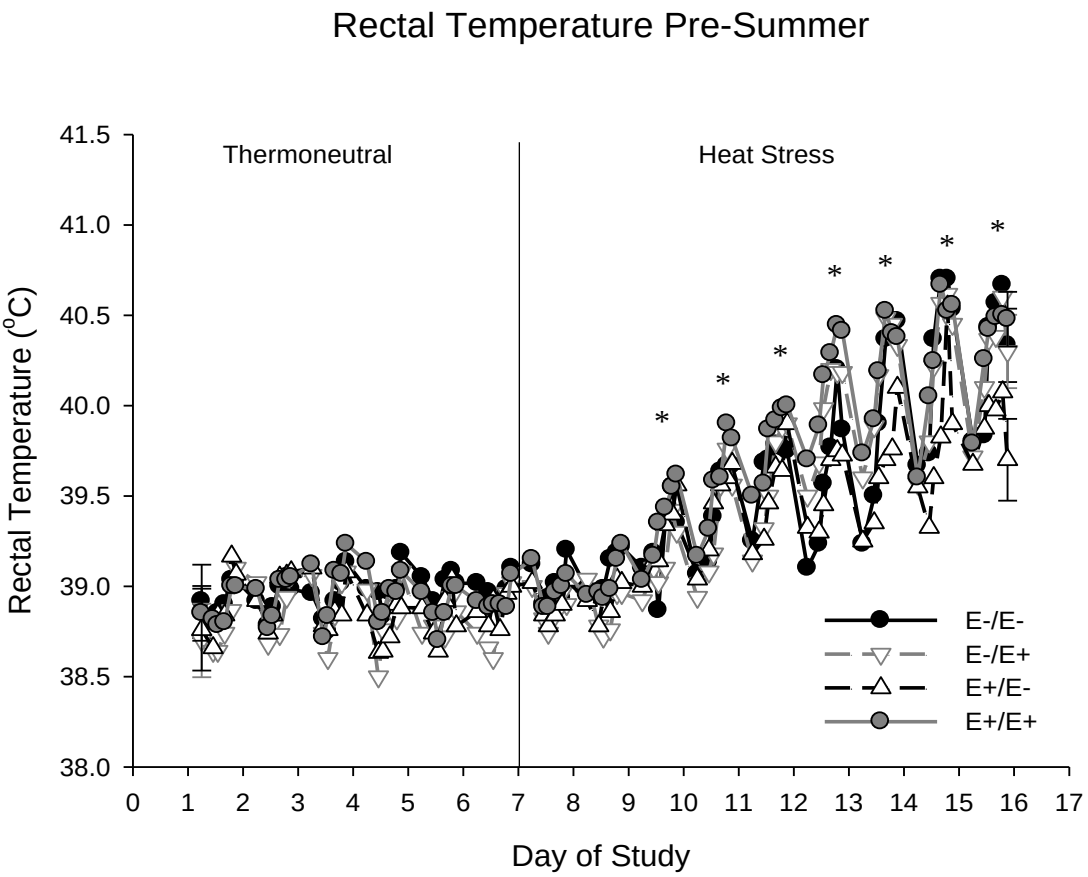

Figure 3.6 - Mean rectal temperature (± 1 SEM) of steers shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods. Animals during the pre-summer exposures all received the same E- diet. However, treatments (E+/E+, E+/E-, E-/E+, and E-/E-) are shown. An asterisk (*) signifies day is significantly different ($P < 0.05$) from thermoneutral.

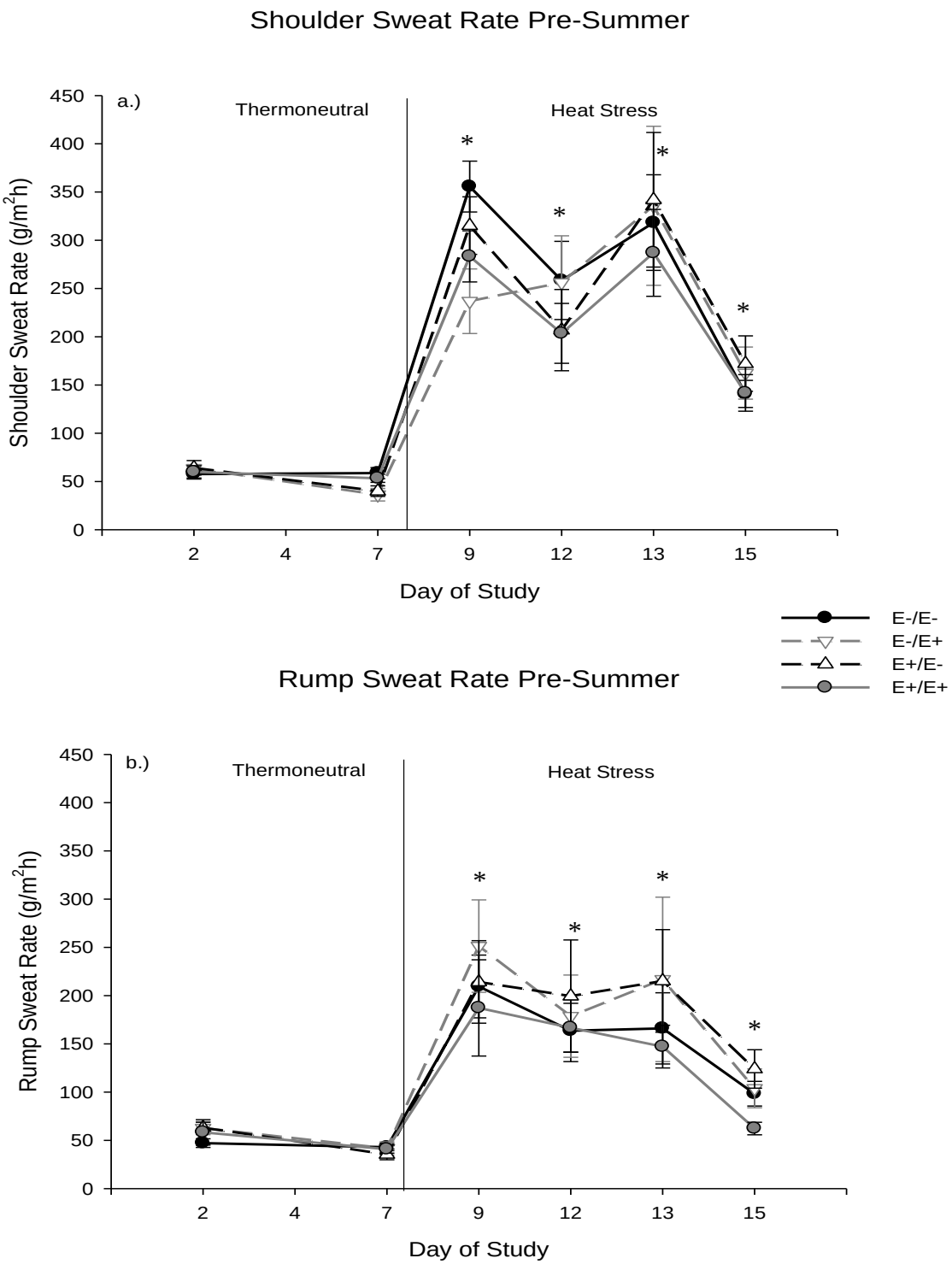

Figure 3.7 - Mean skin sweat rate (± 1 SEM) of shaved shoulder (a) and rump (b) skin sites of steers shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods. Animals during the pre-summer exposures all received the same E- diet. However, treatments (E+/E+, E+/E-, E-/E+, and E-/E-) are shown. An asterisk (*) signifies day is significantly different ($P < 0.05$) from thermoneutral.

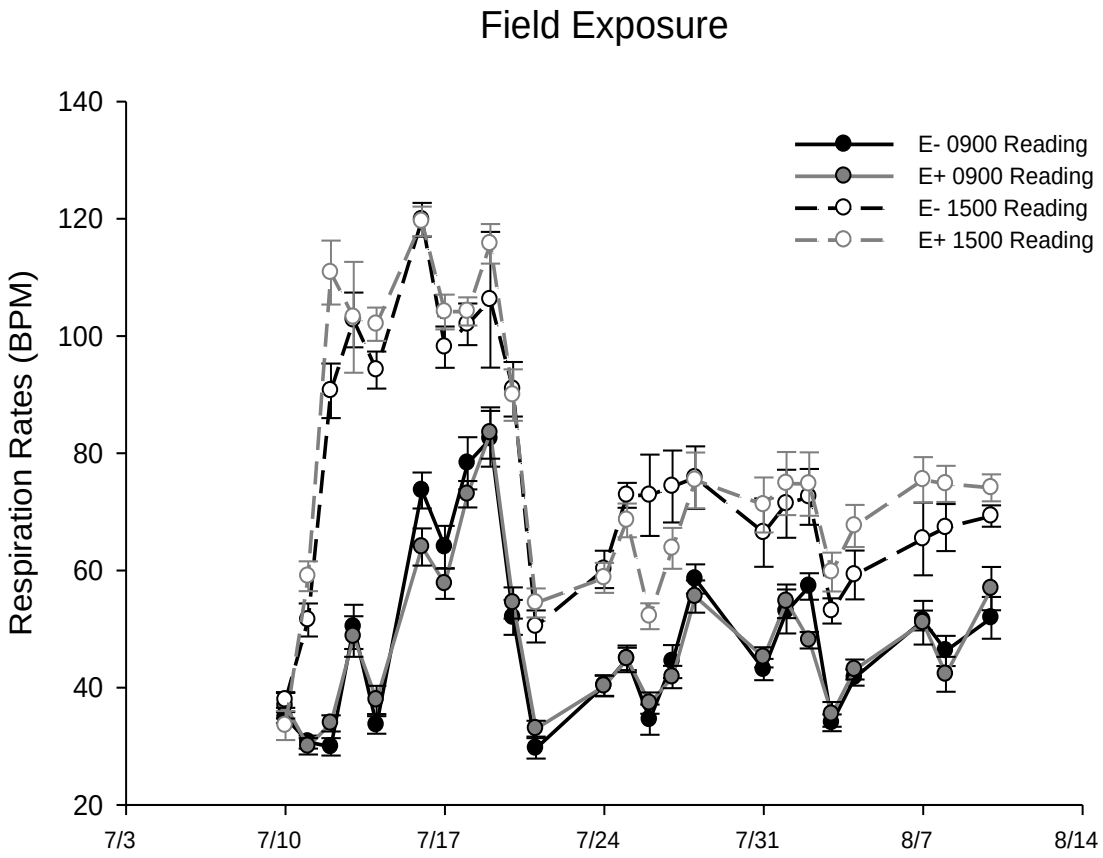

Figure 3.8 - Average values for respiration rate at 0800 and 1500 h are shown for field exposure. Solid lines represent 0900 reading and dashed lines represent 1500 readings. Standard error bars represent ±1SEM. 0900 readings significantly different from 1500 readings during all days ($P < 0.05$).

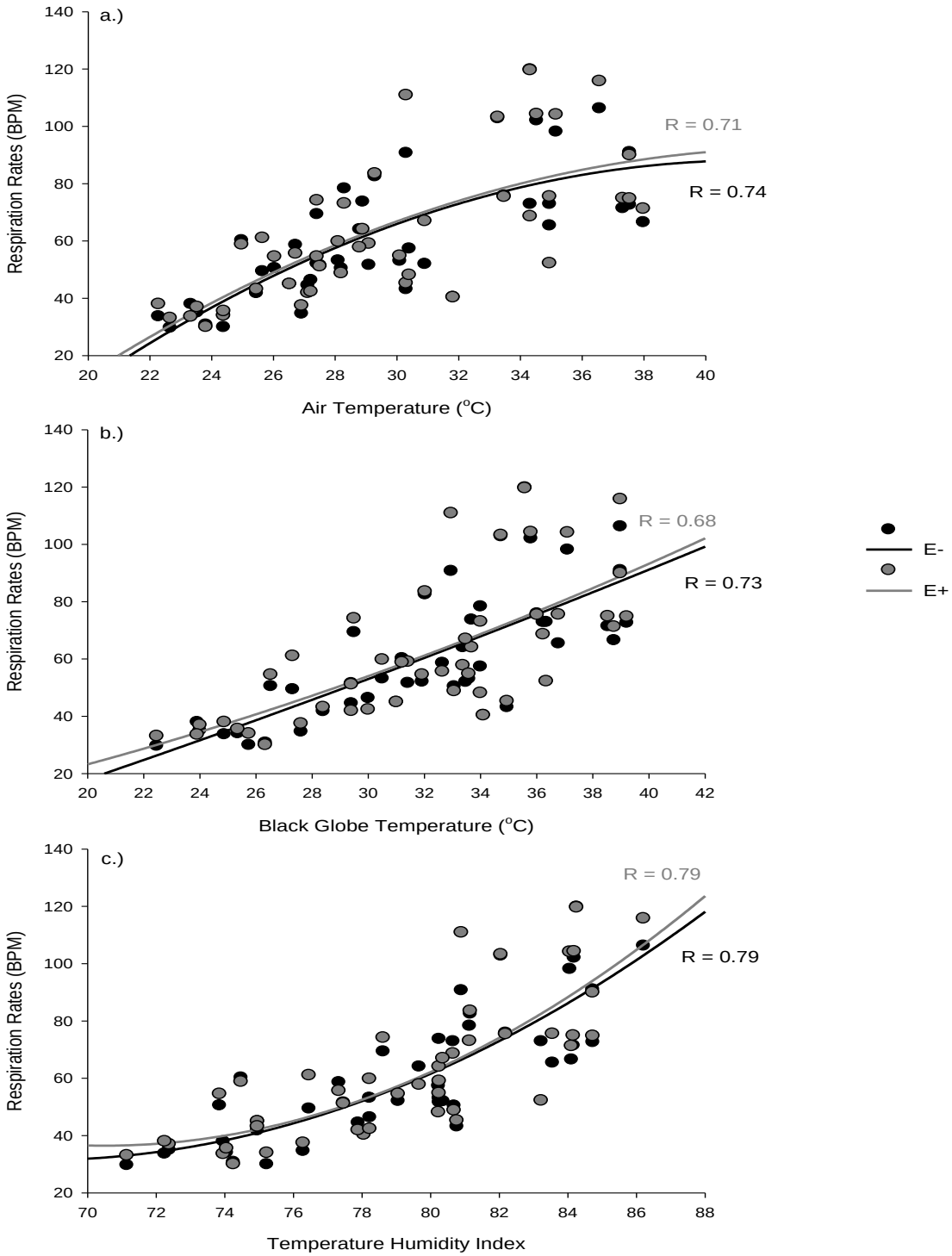

Figure 3.9 – Quadratic fit of respiration rate to (a) air temperature, (b) black globe temperature, and (c) temperature humidity index is shown for the field exposure. Correlation coefficient are given with in the graph.

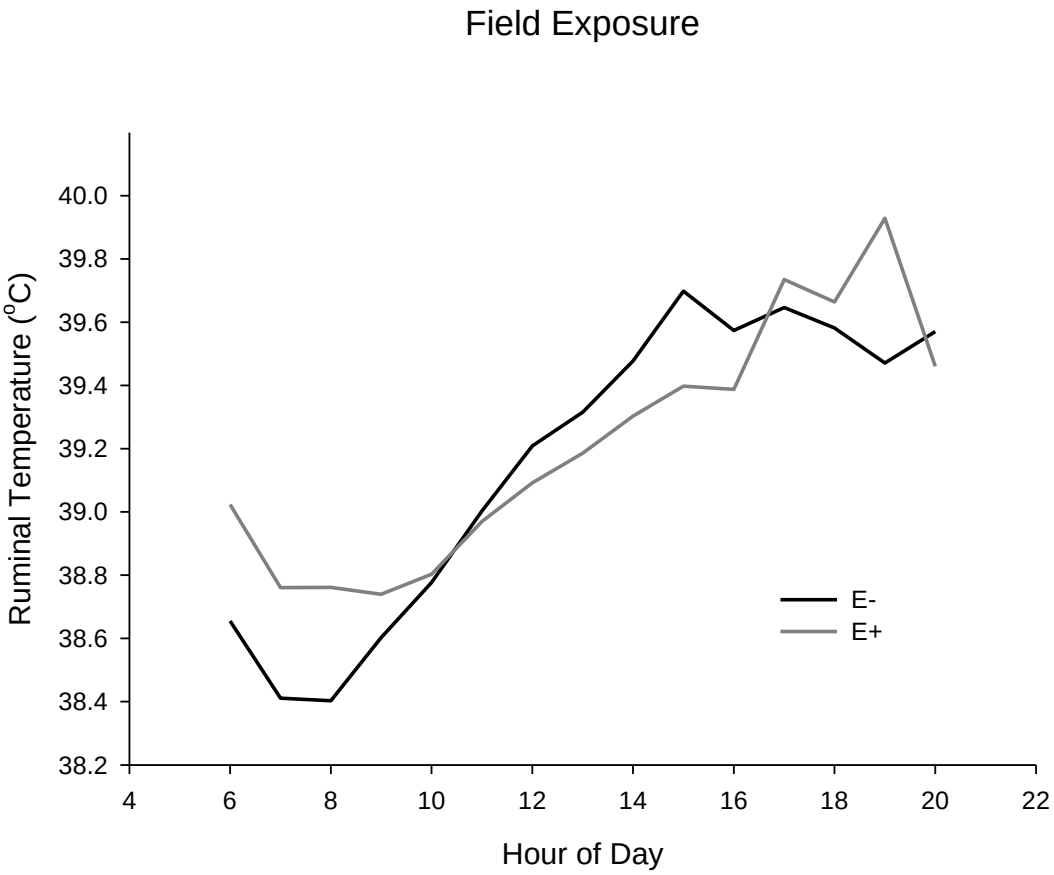

Figure 3.10 - Ruminal temperature by hour for E+ and E- animals during the field exposure. Only hours 060 through 2000 hours shown.

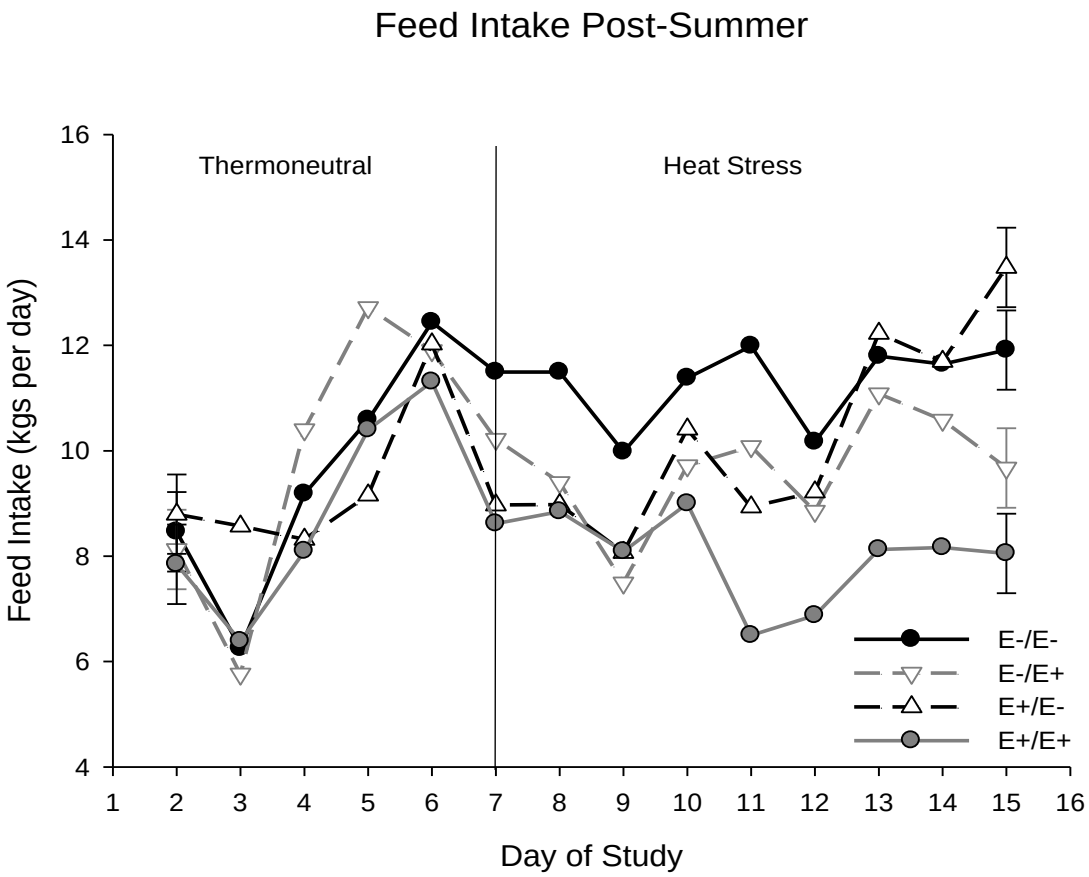

Figure 3.11 - Daily feed intake (+1 SEM) for E+/E+, E+/E-, E-/E+, and E-/E- steers shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods. Starting on Day 11 E+/E+ group is significantly different ($P < 0.05$) from E-/E- group. No other differences found.

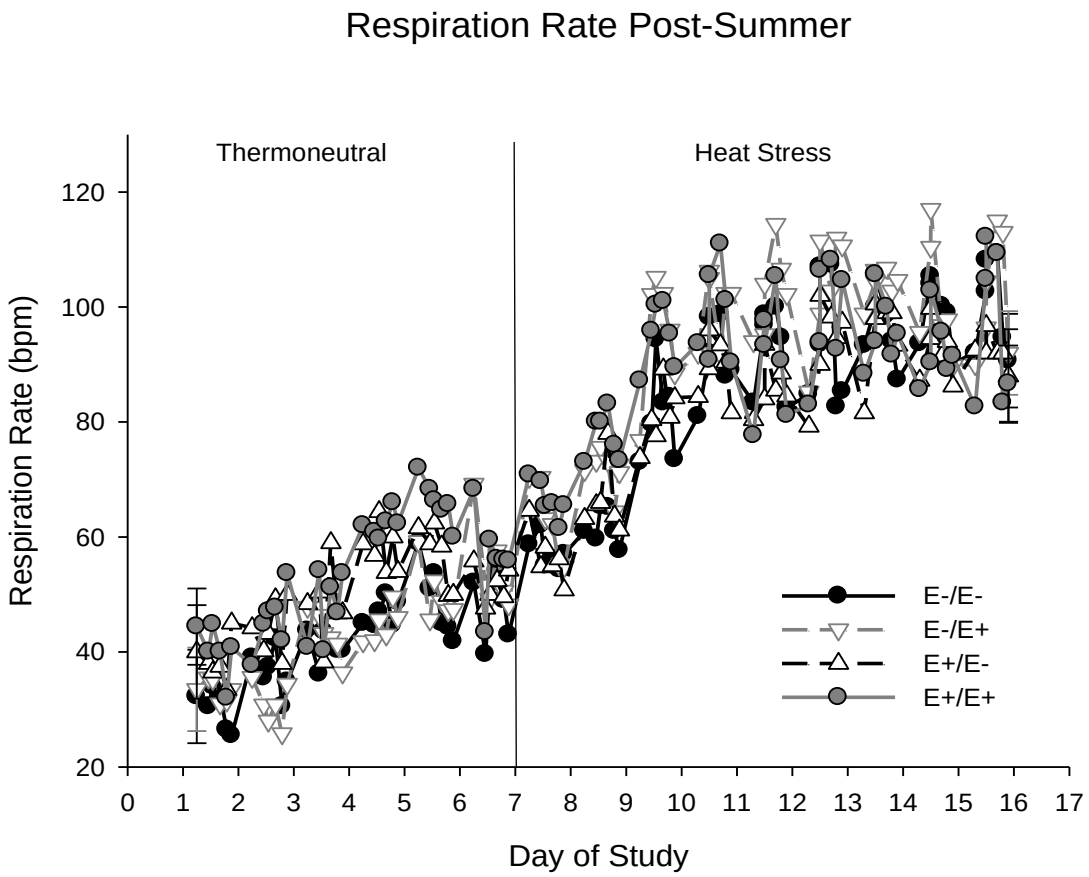

Figure 3.12 - Mean respiration rate (± 1 SEM) for E+/E+, E+/E-, E-/E+, and E-/E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. The solid vertical line separates thermoneutral and heat stress periods. Starting on Day 9 all groups were significantly different from thermoneutral. No treatment differences were found.

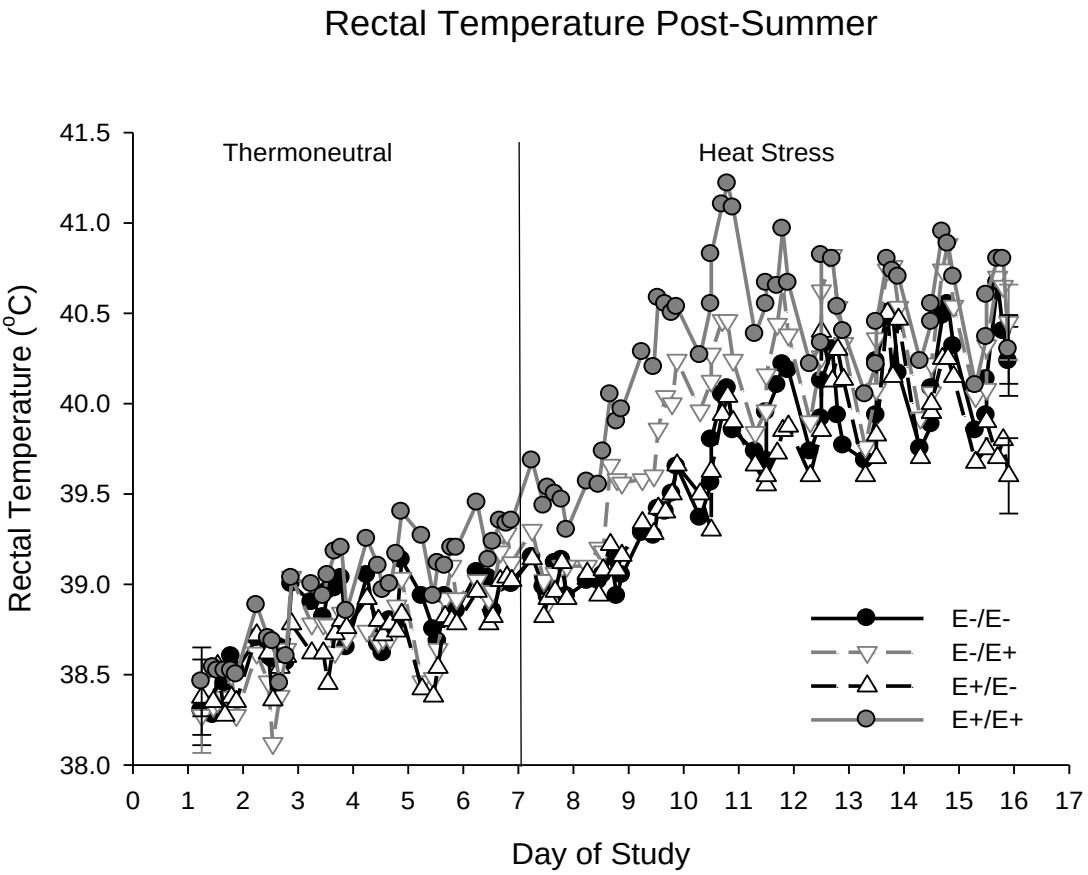

Figure 3.13 - Mean rectal temperature (± 1 SEM) for E+/E+, E+/E-, E-/E+, and E-/E- steers shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. The solid vertical line separates thermoneutral and heat stress periods. Starting on Day 9, all groups were significantly different from thermoneutral ($P < 0.05$). E+/E+ group was significantly different ($P < 0.05$) from E- groups on Days 9, 10, 11, and 12. E-/E+ group was significantly different ($P < 0.05$) from E- groups on Days 9 and 10. No other differences found.

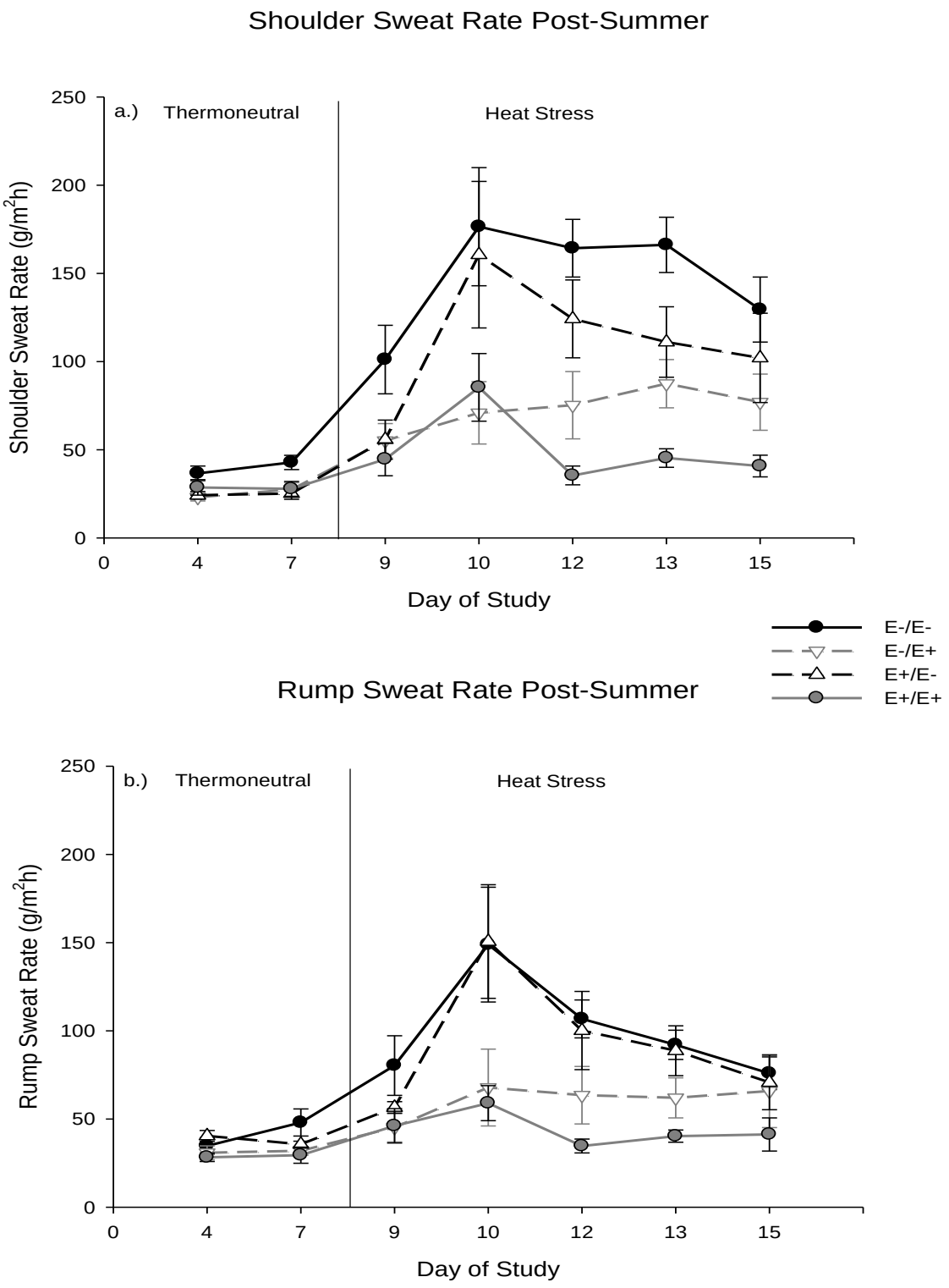

Figure 3.14 - Mean skin sweat rate (± 1 SEM) of shaved shoulder (a) and rump (b) skin sites of steers shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods.

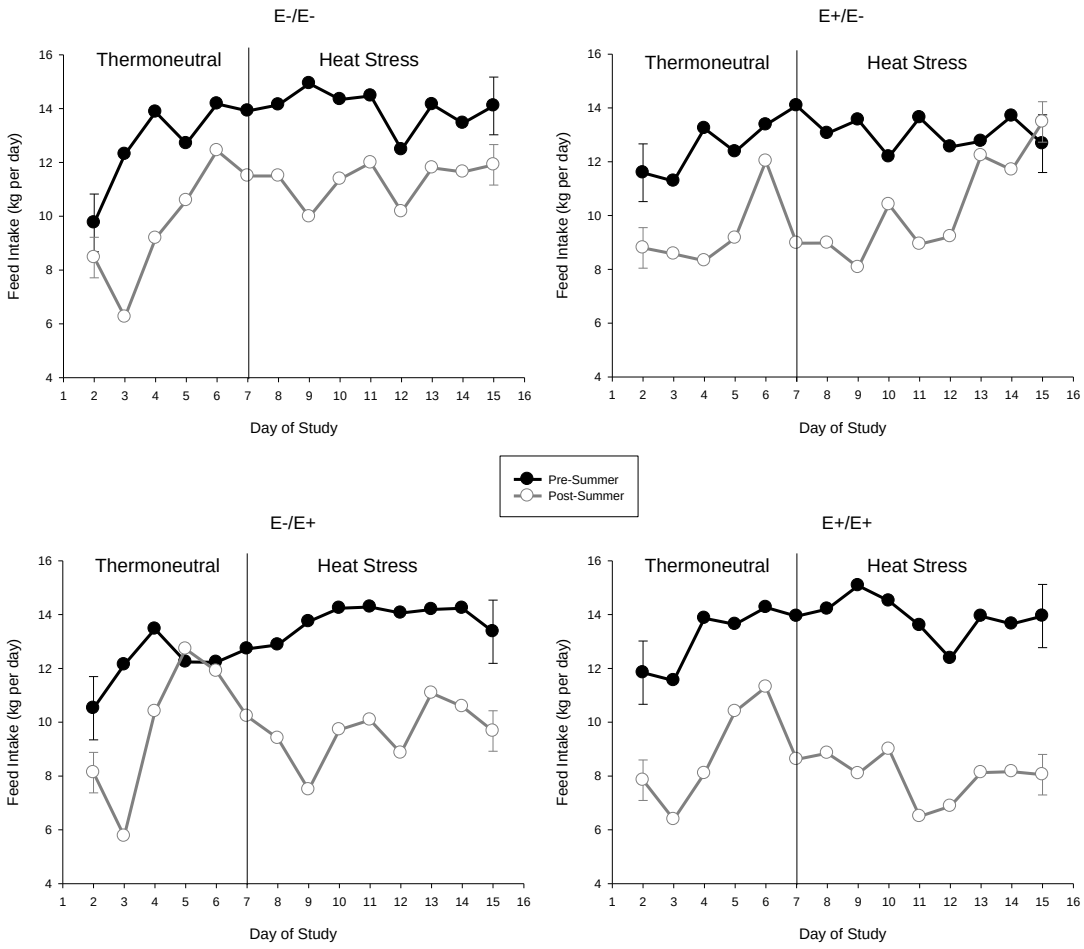

Figure 3.15 - Daily feed intake (+1 SEM) of steers shown as a function of time in days for the Pre-Summer and Post-Summer chamber exposures. Treatments E+/E+, E+/E-, E-/E+, and E-/E- are each shown on separate graphs. The solid vertical line separates thermoneutral and heat stress periods.

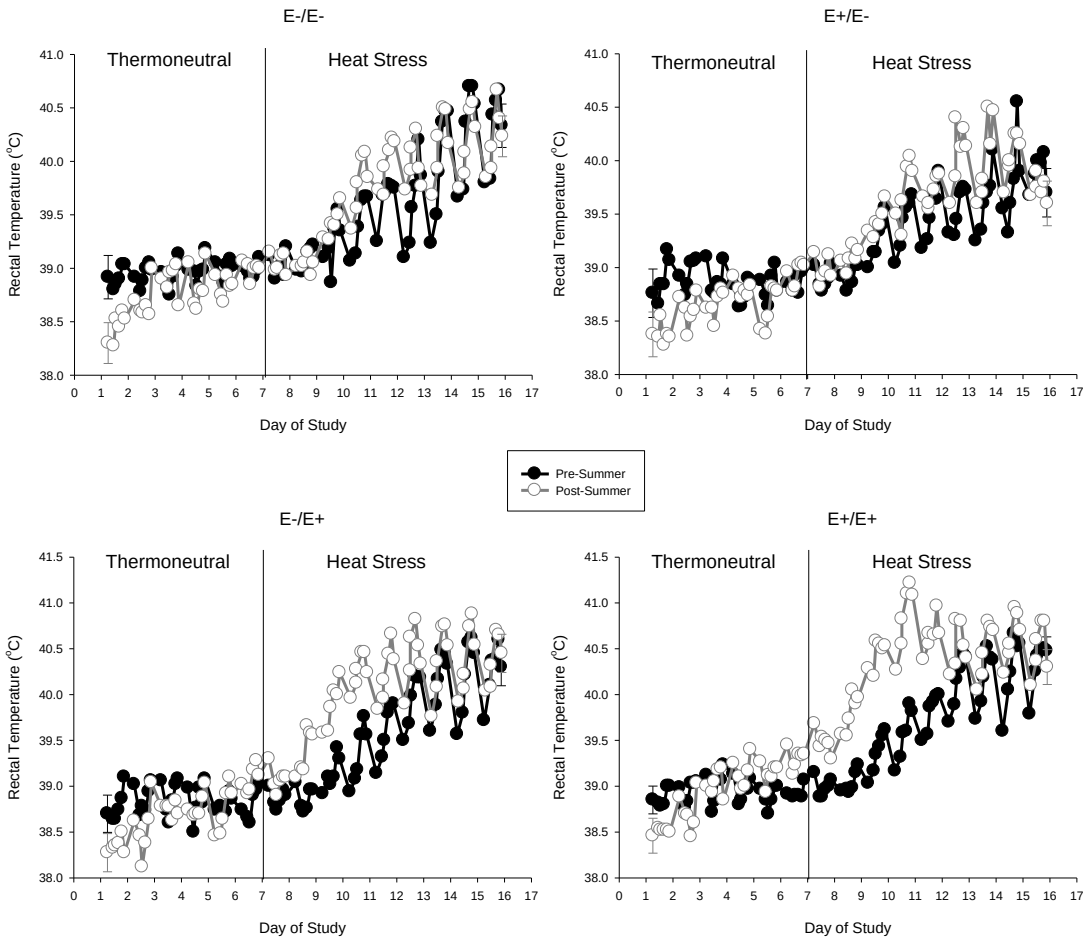

Figure 3.16 - Mean rectal temperature (+1 SEM) of steers shown as a function of time in days for the Pre-Summer and Post-Summer chamber exposures. Treatments E+/E+, E+/E-, E-/E+, and E-/E- are each shown on separate graphs. The solid vertical line separates thermoneutral and heat stress periods.

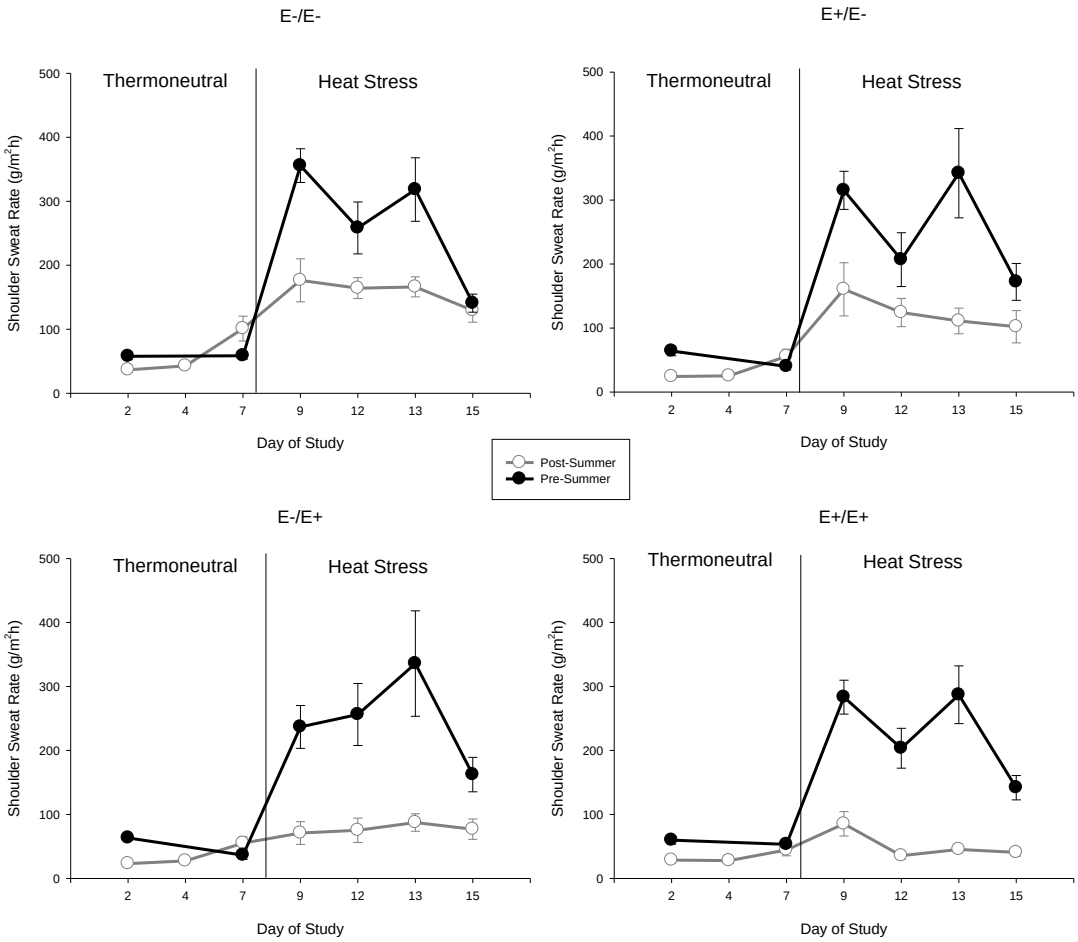

Figure 3.17 - Mean shoulder sweat rate (+1 SEM) of steers shown as a function of time in days for the Pre-Summer and Post-Summer chamber exposures. Treatments E+/E+, E+/E-, E-/E+, and E-/E- are each shown on separate graphs. The solid vertical line separates thermoneutral and heat stress periods.

Table 3.1 – Group mean serum biochemistry values for E-/E-, E+/E+, E-/E+, and E+/E- cattle under thermoneutral conditions and heat stress and heat stress. P-values given for Treatment (E+ vs. E-), Time (TN vs. HS), and Interaction (Treatment x Time). ALP – alkaline phosphatase

	Blood Parameters						
					P Values		
	Treatment	*TN*	*HS*	*± SE*	*Treatment*	*Time*	*Interaction*
Albumin g/dL	E-/E-	2.67	2.92	0.11	0.53	**0.001**	0.32
	E+/E+	2.78	3.18				
	E-/E+	2.80	3.06				
	E+/E-	2.78	2.94				
ALP U/L	E-/E-	111.50	74.50	13.54	0.09	**0.001**	0.12
	E+/E+	82.67	46.17				
	E-/E+	56.00	47.20				
	E+/E-	85.20	80.72				
Calcium mg/dL	E-/E-	9.07	9.23	0.23	0.77	0.12	0.9
	E+/E+	9.02	9.30				
	E-/E+	9.30	9.42				
	E+/E-	8.96	9.38				
Chloride mEq/L	E-/E-	98.50	104.50	1.75	0.91	**0.001**	0.32
	E+/E+	95.83	106.83				
	E-/E+	98.60	103.20				
	E+/E-	96.60	104.18				
Cholesterol mg/dL	E-/E-	37.33	51.17	8.55	0.38	0.53	**0.05**
	E+/E+	57.67	43.67				
	E-/E+	54.00	52.40				
	E+/E-	31.60	42.87				
Creatinine mg/dL	E-/E-	1.27	1.35	0.11	0.45	**0.05**	0.68
	E+/E+	1.37	1.53				
	E-/E+	1.18	1.32				
	E+/E-	1.24	1.31				
Globulin g/dL	E-/E-	3.18	3.53	0.18	0.22	**0.001**	0.29
	E+/E+	2.70	3.15				
	E-/E+	3.10	3.16				
	E+/E-	2.88	3.21				
Leptin ng/ml	E-/E-	5.30	6.92	0.85	**0.05**	0.62	0.08
	E+/E+	8.95	8.34				
	E-/E+	6.81	6.27				
	E+/E-	5.01	6.47				

Table 3.1 (Continued)

		Blood Parameters					
					P Values		
	Treatment	*TN*	*HS*	*± SE*	*Treatment*	*Time*	*Interaction*
Potassium mEq/L	E-/E-	3.82	4.23	0.11	0.27	**0.001**	0.07
	E+/E+	4.00	4.03				
	E-/E+	4.12	4.28				
	E+/E-	3.76	4.09				
Prolactin ng/ml	E-/E-	29.19	33.96	3.04	**0.001**	0.27	0.54
	E+/E+	12.45	12.90				
	E-/E+	13.88	14.58				
	E+/E-	30.39	30.50				
Sodium mg/dL	E-/E-	135.50	142.17	3.06	0.63	**0.01**	0.27
	E+/E+	130.67	142.00				
	E-/E+	138.80	139.00				
	E+/E-	132.60	141.88				
Total Protein g/dL	E-/E-	5.85	6.45	0.28	0.82	**0.001**	0.39
	E+/E+	5.48	6.33				
	E-/E+	5.90	6.22				
	E+/E-	5.66	6.16				
Triglyceride mg/dL	E-/E-	10.17	15.00	2.01	0.74	**0.01**	0.43
	E+/E+	13.33	14.33				
	E-/E+	10.60	12.80				
	E+/E-	10.00	15.00				
Urea N mg/dL	E-/E-	8.67	10.00	1.65	0.37	0.25	0.27
	E+/E+	8.83	11.17				
	E-/E+	5.80	9.00				
	E+/E-	10.20	7.88				

CHAPTER FOUR

PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS DURING THE PEAK OF HEAT

1. ABSTRACT

Heat stress studies are often conducted using controlled laboratory or field exposures. Each approach has limitations and provides a partial understanding of complex interactions between simultaneous environmental stressors. The question becomes, how similar are the responses under each situation in order to be able to transfer information from one source into another? We used numerous physiological measures of thermal status to compare responses of cattle to chamber "stress tests" and "naturally occurring" field conditions. Angus steers (N=23; 318±8 kg BW) were placed on either endophyte-infected (E+) or uninfected (E-) tall fescue pasture for the field exposure followed by a controlled heat challenge in the chambers. Consumption of E+ fescue is known to exacerbate the effects of heat stress resulting in a condition known as fescue toxicosis. During the controlled heat challenge, steers were assigned to diets of either 0 or 40µg ergovaline/kg/d to maintain the fescue toxicosis state. Respiration rate (RR) was measured via flank counting and telemetric temperature transmitters in the rumen of each animal transmitted core temperature ($T_{rum;}$ 20 minute interval). Linear regression fit models for RR, T_{rum}, and air temperature (T_a) was utilized to compare relationships between field and chamber exposure. Correlation coefficients for respiration rates were similar during both chamber (R =0.69) and field exposures (R = 0.72). Respiration rate showed greater responsiveness to change in T_a under field conditions having twice the slope in the chamber test (4.4 versus 1.75 bpm/°C) and a lower Y-intercept (-42.14

versus +30.97 °C) compared to the chamber run. Ruminal temperature was consistent between exposures showing a similar slope (0.04 °C versus 0.03°C T_{rum}/°C T_a) and Y-intercept (38.4 versus 39.3°C) for its relationship with air temperature. While respiration rate may be the more sensitive indicator of heat stress, ruminal temperature proved to be the more consentient variable between exposures.

2. INTRODUCTION

Cattle, as most adult mammals, rely on a delicate balance of heat production and heat loss to maintain their thermal status. A shift in this balance towards either increased heat production or decreased heat loss will result in heat strain as evidenced by hyperthermia, increased respiration rate, and reduced productivity (Dowling, 1956). Studies in environmentally-controlled chambers (Beatty et al., 2006; Gaughan et al., 2000; Wilson et al., 1998) have developed valuable models of response to heat stress. However, application of these models to field situations is often difficult due to the impermanent nature of critical environmental variables, such as ambient temperature and relative humidity. Traditionally studies in stress physiology have focused on only one of these two approaches. However, both approaches are necessary to develop a realistically useful model of the complex animal/ambient interactions as noted by Yousef (1989):

"The time is now right for field studies since many physiological responses can be measured simultaneously and continuously under field conditions using available microcomputer data loggers."

Now over 20 years later, still little is known about extrapolating laboratory data to naturally-occurring field conditions. Most notably a series of papers over a decade ago looked at selective brain cooling and found that thermoregulatory responses of animals in

their natural environment could not be predicted from measurements made on tame, captive, or restrained animals in the laboratory (Jessen et al. 1994; Mitchell et al. 1997; Fuller et al. 1999). Given the possibility of different responses by cattle to heat stress under field and laboratory conditions, a study was conducted utilizing telemetric temperature transmitters as a noninvasive way to measure core body temperature for comparison of both situations.

While the main focus of the experiment was to determine the thermoregulatory responses of cattle to heat stress; the effects of an additional environmental stressor that compromises thermoregulatory ability (i.e., fescue toxicosis) was also studied. Fescue toxicosis is common condition occurring during the summer months in southeast and mid-west regions of the United States due to consumption of endophyte-infected tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. –*Schedonorus arundinaceus* [Schreb.] Dumort). Within the United States, more than 8.5 million cattle graze on tall fescue, and more than half is infected by the endophyte *Neotyphodium coenophialum* (Hoveland, 1993). Consumption of this toxin exacerbates the heat strain on the animal, with notable symptoms including hyperthermia, decreased feed intake, and increased peripheral vasoconstriction (Strickland et al., 1993).

Few studies have looked at thermoregulatory responses of cattle to multiple stressors under both field and laboratory conditions (Legates et al., 1991). Therefore, the objective of this study was to determine how similar the thermoregulatory responses of cattle are under a field exposure followed directly by a controlled chamber run under similar conditions. A second objective was to determine the relationship between rectal temperature and ruminal temperature utilizing radio telemetric transmitters.

3. MATERIALS AND METHODS

3.1. Animals

Twenty-three Angus steers (318 ± 8 Kg BW) were obtained from the University of Missouri Beef Research Farm. These animals have been raised on tall fescue for many generations and represent animals that possibly that they have acquired some tolerance to the fescue endophyte indirectly through selection. Animals were randomly assigned to an endophyte-infected (E+; 344 ppb ergovaline; n=11) or endophyte-free (E-; n=12) tall fescue pasture. Ergovaline content in the pastures was measured by high-performance liquid chromatography (HPLC) (Hill et al., 1993). Pasture levels for E- was low ranging from 10 ppb to 55ppb while E+ pastures levels were 160 to 340 ppb in the grass with levels above 1100 ppb in the seed heads. Steers were placed on E+ and E- pastures for two months before being transported to the Brody Environmental Center during the peak of summer (July 25 – August 10th). The Brody Environmental Center consists of four 6.1 × 9.1 m chambers, two of which were used in the present study. For chamber exposure, steers remained on treatments receiving either E+ (40 µg ergovaline/Kg BW/day) or E- tall fescue seed top-dressed over a typical finishing diet at each feeding (0600 and 1600). Ergovaline is considered to be the primary ergopeptine alkaloid responsible for fescue toxicosis in an array of toxins found in E+ tall fescue. For ergovaline analysis, pasture samplings were collected by walk in a zigzag pattern through the 2 pastures. Approximately 30 samplings were collected for both E+ and E- pasture. Samplings included the entire plant above ground including some root material. Samples were freeze dried and ground for analysis. Ergovaline content was measured by HPLC (detection limit = 50 ppb and CV = 7%; Rottinghaus et al., 1993). Ergovaline concentrations prior

to the chamber exposure for the E+ pastures ranged from 280 ppb to 344 ppb. Ergovaline content for the E- pasture was almost negligible ranging from 10 to 30 ppb throughout the summer.

The two chambers were divided into six stanchions with each animal loosely restrained to the stanchion by a chain. Due to space requirements, the 23 animals were split into 2 groups (Group 1 = 12 animals; Group 2 = 11 animals) and run 2 weeks apart. Chamber temperatures used during this study represent were chosen to represent a typical day in late July in Missouri. Air movement in the chamber was held at 15 room changes per hour (4,462 cubic feet of volume). Light cycle during the chamber study was a 12-h light: dark (0600:2000 h) schedule. The experimental animal protocol and procedures were approved by the University of Missouri Animal Care and Use Committee.

3.2. General Procedure

Steers were housed in covered feedlots at the University of Missouri South Farm prior to being placed on either E+ or E- pastures. Calibrated data loggers (HOBO H8 Pro; Onset Computer, Bourne, MA; accuracy: ± 0.2°C and ± 3% RH) were used to record air temperature (T_a) and percent relative humidity (%RH), as well as black globe temperature (T_{bg}; hollow copper sphere; 15.24 cm diameter; flat black exterior, located between animal pastures; Bond and Kelly 1955;) for assessment of radiant heat load (Figure 4.1a). Determinations of respiration rate (RR) were made by counting flank movement over a 1-minute interval twice a day (i.e., 0800 and 1500 h). These points were selected as they represent both low and high points, respectively, of the daily core temperature cycle (Scharf et al., 2011).

A telemetric, temperature transmitting bolus (SmartStock LLC, Pawnee, OK) was placed into the rumen of each animal (oral administration using a standard bolus gun) prior to the study to record ruminal temperature (T_{rum}). Ruminal temperature measurements were transmitted, using a radio frequency of 900 MHz to an antenna placed approximately 10 m from the animals. The signal was then transmitted to the base receiver unit which was connected to the personal computer. The boluses were designed to transmit every 20 minutes. Along with the current reading, the bolus transmits the previous 11 readings to minimize the loss of data resulting from lost transmissions. Boluses were calibrated to a NIST (National Institute of Standards and Technology) thermometer prior to ingestion by the animal. The data was filtered for maximum hourly value, which was used for all analyses. This avoided the incorporation of thermal artifacts associated with water intake.

For chamber exposures, steers were housed for 7 d at a thermoneutral T_a (TN; approximately 20°C) prior to initiation of heat stress (HS). Heat stress consisted of daily cyclic air temperature (approximately 26°C night: 36°C day) for 7 d (Figure 4.1b). Chambers at TN had a controlled set-point of 20°C showing only slight fluctuations across days. During the HS cycle, chamber T_a increased daily as a step-up function with 3 set points throughout the rise phase, followed by a 4 hour stable period (36°C; 1200 to 1600 h). The decline phase consisted of 2 step-downs to reach the stable low T_a (26°C; 0000 to 0600 h). Relative humidity was maintained under 50% during the entire study (TN: 40 to 50%; HS: 35 to 45%) to minimize its influence on heat flow. Chamber environmental conditions were controlled using a Fisher-Porter Controller (698B179U01) and a Sensycon I/P Converter (Controller Type 27/06–65). This procedure has been used

in a number of previous studies (Spiers et al., 2001; Scharf et al., 2008). Environmental conditions were measured, as during field exposure, with calibrated HOBO H8 data loggers (Onset, Bourne, MA) to record T_a and %RH every 10 minutes.

Animal measurements, including respiration rate (RR), skin temperature (T_{skin}), and rectal temperature (T_{re}), were taken 6 times daily (0600, 1100, 1300, 1600, 1900, and 2100 h). Determination of RR was made as previously described. Skin temperatures, at 5 different shaved sites (ear, shoulder, rump, tail head, and lower tail), were measured using a calibrated infrared thermometer (Model RAYST80XB, Raytek Corporation, Santa Cruz, CA; Accuracy ± 1%). Readings were taken less than 35 cm away from each skin site with a thermometer target ratio of 50:1. Rectal temperature was measured using a calibrated thermistor thermometer (Model 8110–20, Cole-Parmer Instruments, Chicago, IL). This was accomplished by inserting a YSI probe (Model 400, YSI Inc., Yellow Springs, OH; Accuracy: 0.1°C) approximately 15 cm into the rectum for approximately 2 minutes. As during the field exposure, ruminal temperature was monitored every 20 minutes with the maximum value during the hour used in the analyses.

Blood (20 ml) was collected at 0900 on Days 6 (TN) and 14 (HS) via a jugular venous puncture. Samples were collected into a 15 ml tubes and allowed to clot prior to centrifugation. Serum was separated by centrifugation (2,300 x *g* for 25 minutes; 4°C) before being removed and stored at −20°C for later analysis. Serum analyses used standard procedures. Most serum measurements were components of a larger biochemical profile produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an auto-analyzer (Olympus AV400; Olympus America, Inc., Melville, NY). These include albumin, alkaline phosphatase, chloride,

cholesterol, creatinine phosphokinase, creatinine, globulin, potassium, sodium, total protein, triglyceride, and urea nitrogen. Serum concentrations of prolactin were determined by radioimmunoassay procedures previously validated at the University of Missouri (Lutz et al. 1991). Minimum detectable concentrations of prolactin in serum were 1.19 ng/tube.

3.3. Statistical Analysis

3.3.1. Field Exposure

The effects of period on ambient variables (T_a, T_{bg}, and Temperature Humidity Index (THI)) were modeled using the repeated measures ANOVA procedures of JMP® (SAS Institute; Cary, NC) with the ambient variable modeled as the dependent variable, with period, time of day, and period by time of day interaction as independent variables that were modeled as fixed effects. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD (Steele and Torrie, 1980) adjustment procedures for multiple mean comparisons.

A repeated measure ANOVA (Steele and Torrie, 1980), constructed using JMP, was also used to test the effects of period on T_{rum}. The model included T_{rum} as the dependent variable, with the independent variables of treatment, hour (0700 to 1800 h), and period fit as fixed effects, and animal within period, treatment by period, treatment by hour, and period by hour interaction as random effects.

Respiration rate, T_{rum}, and T_a observations were averaged for each of the two days within period observed at 0800 and 1500 h. These means were tested by way of ANOVA, using JMP to determine if any statistical differences existed.

Simple linear regression procedures of JMP were utilized to establish the linear relationships between T_a, RR, and T_{rum} during each period. Regression coefficients for slope and model correlation coefficients (R) are reported, as well as P values for the hypothesis test that the time regression coefficients are significantly different from zero. Regression models were constructed using JMP with a time delay of 0, 1, 2, and 3 hours for response variable, T_{rum}, to explore the relationships with ambient conditions. Model R values are reported and were used in the determination of model sufficiency.

3.3.2. Chamber Exposure

All evaluations at TN were performed using the last 6 d (i.e., Days 2 through 6) before the increase in T_a. The analysis included RR, skin temperatures, T_{rum}, or T_{re} as the dependent variables. Skin temperatures were included as an average of the shoulder and rump sites (T_{trunk}), or an average of ear, tail head and lower tail ($T_{appendage}$). Treatment, time, and treatment by time effects were set as fixed, with animal nested within breed as a random effect. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD adjustment procedures for multiple mean comparisons.

Simple linear regression procedures of JMP were utilized to establish relationships between animal variables (RR, T_{trunk}, $T_{appendage}$, T_{re}, and T_{rum}) and T_a. Regression coefficients for slope and model R, as well as P-values for the hypothesis test that the regression coefficients were significantly different from zero, are reported. Similarly, simple linear regressions were constructed to determine the relationship for early heat (Days 8 to 10) and late heat exposure periods (Days 12 to 14) using the regression procedures of JMP. Blood analyses were performed using the repeated measures ANOVA procedure in JMP® with fixed and random effects as described above.

4. RESULTS

4.1. Field Exposure

Average daily T_a for entire field exposure period was 24.6°C (Min: 13.9°C; Max: 34.3°C). Six periods were selected where the complete data set was analyzed. Average daily T_a was relatively consistent across periods. Overall, minimum daily T_a for the six periods was similar to TN conditions during chamber exposure (20°C; Figure 4.1a). Maximum daily T_a was lower across all periods compared to chamber temperature (36°C; Figure 4.1a). Daily minimum values occurred at approximately 0600 h for T_a and T_{bg}. Solar heating resulted in a greater increase in T_{bg} after 0700 h, which was more rapid than the increase for T_a alone in the chambers. Daily peak values in T_a and T_{bg} were at 1500 h, with rapid reductions after this time. Average daily THI during the periods was 78, with a maximum of 82 that occurred during Periods 3 and 6.

4.2. Animal Values

Average respiration rate was not different between E+/E- treatment groups over the six periods ($P = 0.88$; Figure 4.2a) in the field. All treatment periods showed similar time of day differences having a lower RR at 0800 versus 1500 h ($P < 0.01$; 70 versus 91 bpm; Figure 4.2b). Respiration rates were very similar across all periods, with only Period 2 showing differences ($P < 0.05$). Animals during Period 2 showed a lower RR at both 0800 (61 versus 72 bpm) and 1500 h (86 versus 92 bpm) compared to the other 5 Periods ($P < 0.01$). Linear relationships between RR and T_a, T_{rum}, T_{bg}, and THI were evaluated to determine which variable was the more reliable predictor for RR in the field. Air temperature showed the greatest correlation with RR ($R = 0.72$; Figure 4.2a), followed by T_{bg} ($R = 0.72$) and THI ($R = 0.64$). Using T_{rum} matched with the 2 points a day where RR was taken (i.e., 0800 and 1500 h), showed that T_{rum} was poorly correlated

with RR (R = 0.24). Ruminal temperature correlations with T_a, T_{bg}, and THI showed similarly low correlations for all variables (R = 0.15 – 0.22). To determine if a delay would improve the linear relationship with RR, a delay of 1, 2, and 3 hours was evaluated. However, correlations showed no benefit of adding a delay (R = 0.69 – 0.72).

Use of only 2 time points during the day had limitations for T_{rum}. Therefore, hourly values from 0700 to 1800 h were used to incorporate more sample times into the analysis. Hourly T_{rum} was not different between E+/E- treatment groups over the six periods (P = 0.52; Figure 4.3a). Minimum T_{rum} across all groups occurred at 0900 h (2 hours after T_a) and reached a maximum at approximately 1800 h (3 hours after T_a). No differences in T_{rum} were found between Periods 3 and 5 or Periods 2 and 4 (39.6°C and 39.8°C, respectively; P=0.12). Period 6 showed a lower T_{rum} than all other periods (39.5°C; P < 0.05) and Period 1 had the highest T_{rum} (39.9°C; P < 0.05). Interestingly, E- animals tended to have a higher T_{rum} than E+ animals during the peak hours of the day (1500 to 1700 h; P < 0.10). Linear relationships between T_{rum} and T_a, T_{bg}, and THI showed low correlations combining all periods (R = 0.30 or lower). Running the correlation by period increased the linear fit for all variables (Figure 4.3a; R = 0.30 – 0.43). Across all periods, T_{bg} showed the greatest correlation with T_{rum} (i.e., R = 0.43) followed closely by T_a (R = 0.38) and THI (R = 0.36). Unlike RR, adding a 3 hour lag to the hourly T_{rum} showed an improvement in correlations with T_{bg} (R = 0.47) and T_a (R = 0.48; Figure 4.3b). A lag of 1, 2, 3, 4, and 5 hours were analyzed with a 3 hour lag showing the greatest improvement in correlation coefficient.

4.3. Chamber Exposure

4.3.1. Thermoneutrality

After field exposure, animals were moved to the chambers where they remained on their respective treatments. Feed intake was significantly lower for E+ animals (5.1 versus 8.1 kgs/d) during TN ($P < 0.001$; Figure 4.4). It started to decline on Day 2, being significantly different from E- treatment from Day 3 until the end of the TN period ($P < 0.001$; Figure 4.4). Respiration rate at TN was not different between E+/E- treatment groups ($P = 0.45$; 41 versus 43 ± 2.61 bpm; Figure 4.5a). Both groups showed a small increase in RR at TN, being lowest on Day 2 (39 bpm) and stabilizing by Day 4 (45 bpm; Fig 5a). Both T_{trunk} (Figure 4.6) and $T_{appendage}$ (Figure 4.7) showed no treatment differences at TN ($P = 0.49$). While both treatment groups showed variation in skin temperatures during the first 2 days of chamber exposure, the E- animals showed largest changes in skin temperature sites during Days 2 and 3 of chamber exposure ($P < 0.05$; Figures 4.6 and 4.7). This variation decreased beginning of Day 4 and then was not different between treatments ($P = 0.15$; Figures 4.6 and 4.7). Skin temperature regions were approximately 1.1°C different, with T_{trunk} having the higher value. Rectal temperatures were also not different between E+/E- treatment groups ($P = 0.11$; 38.7 versus 38.5 ± 0.06°C; Figure 4.5b). Similarly to RR, T_{re} was lowest on Day 2 (38.5°C) before stabilizing after Day 3 (38.7°C; Figure 4.5b). Ruminal temperature, like T_{re}, was not different between treatment groups ($P = 0.41$; 39.6 versus 39.5 ± 0.13°C) and was stabile by Day 4 (Figure 4.8a). The T_{rum} - T_{re} difference was also not significantly different across treatments ($P = 0.19$), with T_{rum} maintained approximately 1°C higher than T_{re} (Figure 4.8a).

4.3.2. Heat Stress

Feed intake for E- animals did not differ between TN (8.2 ± 0.38 kgs) and HS (8.6 ± 0.29 kgs) periods ($P = 0.21$). In contrast, E+ animals showed a further reduction in feed intake to below TN values ($P < 0.05$) ending the chamber exposure period at half that of E- animals (Figure 4.4). The transition from TN to HS environments produced a rapid increase in RR ($P < 0.01$), with E+ animals being approximately 43 bpm and E- approximately 28 bpm higher than the last day at TN (Figure 4.5a). Animals in the E+ group maintained a higher RR than E- animals for the first 3 days of HS ($P < 0.05$; 90 versus 72 bpm). However, RR for E+ and E- animals came together as HS continued, with no significant difference starting after Day 11 ($P = 0.12$; Figure 4.5a).

Transition to HS resulted in a large increase in skin temperature, as expected, which was strongly associated with T_a ($R = 0.89$; Figures 4.6 and 4.7). Skin temperatures at the two different regions showed no mean E+ versus E- treatment differences during HS (T_{trunk} 38.2 versus 38.1°C; $T_{appendage}$ 37.5 versus 37.3°C, respectively). However E+ animals displayed a significantly lower skin temperature for both regions at 0600 and 2100 h ($P < 0.01$; Figures 4.6 and 4.7). This decrease reduces the temperature gradient for heat loss and may be responsible for some of the shown treatment responses. While no differences were found in mean skin temperatures, E+ animals showed the greater variation throughout HS, with approximately a 2°C daily increase between 0600 and 1600 h ($P < 0.05$; approximately 1.5°C for E- animals; Figures 4.6 and 4.7).

There was also a sharp increase in T_{re} during the transition period for both treatment groups, with E+ animals showing the greatest increase ($P < 0.001$; 1.1°C E+ animals versus 0.5°C E- animals from TN; Figure 4.5b). After the large increase, E+ animals maintained the higher T_{re} throughout HS ($P = 0.12$). In contrast, E- animals

gradually increased T_{re} during HS ($P < 0.05$). Like RR, both E- animals increased to the same level as E+ animals not being significantly different from each other ($P = 0.28$), however not until Day 13 (2 days after RR; Figure 4.5b).

Ruminal temperature displayed a smaller increase during transition to HS compared to T_{re} ($P < 0.01$; approximately 0.3°C less), and was not different between treatments ($P = 0.51$; Figure 4.8a). Ruminal temperature quickly stabilized with no treatment or day of study differences after the first day of HS ($P = 0.27$; Figure 4.8a). The T_{rum} - T_{re} difference decreased from TN to HS (1.1 versus 0.6 ± 0.1°C), with a large treatment effect. A small T_{rum} - T_{re} difference (0.11 ± 0.1°C) was seen in E+ animals, due almost entirely to the large increase in T_{re} with only a minimal change in T_{rum} during HS. In contrast, E- animals showed a significantly higher T_{rum} - T_{re} value (0.67 ± 0.1°C) than for E+ animals ($P<0.05$) due to a smaller increase in T_{re} and a similar increase in T_{rum} (Figure 4.8a). However, due to the increase in T_{re} for E- animals throughout HS, the T_{rum} - T_{re} rose to a value that was similar to the E+ animals by Day 13. Ruminal temperature showed a strong correlation with T_{re} for both treatment groups (E- R = 0.86, E+ R = 0.79; Figure 4.8b). While E- animals showed the greater correlation, E+ animals had the greater slope for T_{re} versus T_a (1.768 °C versus 1.008 °C T_{re}/°C T_{rum}), probably due to the large increase in T_{re} during transition to HS (Figure 4.8b).

4.3.3. Temperature Relationships

Temperature relationships during the shift from TN to HS (days 4 to 10) were analyzed in greater detail to determine the early changes between E+/E- treatment groups. Correlations for E+ animals were higher than E- animals (E+ R = 0.68 vs. E- R = 0.55) for all variables with exception of T_{rum} (E+ R = 0.14 vs. E- R = 0.37). Respiration

rate was highly correlated with T_a (R = 0.92), T_{re} (R = 0.92), T_{trunk} (R = 0.84), and $T_{appendage}$ (R = 0.84) regardless of treatment. Ruminal temperature showed poorer correlations with T_{re} for E+ animals (R = 0.53) compared to E- animals (R = 0.84). This low correlation only occurred during the transition period, with high T_{rum} correlations for E+ animals throughout the rest of the chamber exposure (R = 0.80). On the other hand, T_{rum} for E- animals showed a good correlation with T_{re} during all chamber exposure periods (R = 0.84).

As during the field exposure, a lag in T_a was analyzed to determine if it would improve the linear fit. Respiration rate showed a small improvement for both treatment groups by adding a 1 hour lag in T_a (R = 0.90 versus 0.93). Rectal temperature, however, showed the greatest improved when adding a 3 hour lag effect with T_a (E- R = 0.78 versus 0.89; E+ R = 0.85 versus 0.94). Ruminal temperature, like during the field exposure, showed the best improvement when a 3 hour lag effect was added to the analysis for both treatment groups (E- R = 0.70 versus 0.85; E+ R = 0.50 versus 0.73).

Differences in thermoregulatory ability between the beginning (Days 8 to 10; early heat) and end of HS (Days 12 to 14; late heat) were compared to assess short-term acclimation (Figs 7a and 7b). Linear regression was used to determine the change in response of RR, skin temperature, T_{re}, and T_{rum} to T_a between early and late heat periods. Respiration rate during both periods paralleled each other showing similar responses to T_a, with E+ animals having the higher rates (Figure 4.9a). Late heat showed an upward shift in the y-intercept ($P < 0.05$) for E- animals, moving toward E+ animals (Figure 4.9a). Rectal temperature was poorly correlated with T_a (R = 0.12). Like for RR, E- animals showed a shift upward in y-intercept toward the E+ animals ($P < 0.05$) during

late heat (Figure 4.9b). Skin temperatures were not different between regions or between early and late heat periods (P = 0.42; not shown). However, there was a difference in slopes between treatment groups with E+ having the greater slope (0.23 versus 0.13°C T_{skin}/°C T_a). Ruminal temperatures relationship with T_a showed no treatment differences or shifts between early heat and late heat periods as found by RR or T_{re} (not shown).

4.3.4. Chamber vs. Field Comparison

Late heat values were used for comparison with field data since it was the more stable period compared to the first 4 days of HS in the chambers. Only T_a, and not T_{bg}, was used for comparison with T_{rum} and RR since T_{bg} in the chambers would be equal to T_a. Linear regression for RR and T_a under field conditions had an R of 0.72, with a slope of 4.4 bpm/°C and an y-intercept of -42.14°C. Under chamber conditions, the line fit correlation was similar (R = 0.69), however, the slope was less than half that of the field exposure (slope = 1.75 bpm/°C), with a y-intercept of +30.97°C. A similar linear fit was performed to evaluate similarities for T_{rum} versus T_a. Ruminal temperature showed similar correlation coefficients with T_a during the 2 exposures (Field R = 0.43; Chamber R = 0.44). However, unlike RR which showed very different responses in the field environment, T_{rum} showed a similar slope (Field 0.04°C T_{rum}/°C T_a; Chamber 0.03 °C T_{rum}/°C T_a) and y-intercept (Field 38.4°C; Chamber 39.3°C) for both exposures.

4.3.5. Blood parameters

Several blood analyses, including albumin, calcium, total protein, and urea nitrogen showed no treatment or thermal effects ($P \geq 0.14$). Other blood parameters including alkaline phosphatase, cholesterol, globulin, and potassium revealed no

treatment differences, but exhibited HS-induced decreases ($P \leq 0.05$). Prolactin showed large treatment differences with E+ animals being significantly lower than E- animals ($P < 0.0001$; 10.7 vs. 36.1 ± 3.1). Prolactin also showed a trend to increase with HS ($P < 0.10$; TN: 19.3 ± 3.2 vs. HS: 27.5 ± 3.2). Creatinine (E+ 1.44 vs. E- 1.29), chloride(E+ 95.0 vs. E- 97.6), and triglyceride (E+ 19.69 vs. E- 15.8) also showed treatment differences with E+ animals have the higher values ($P < 0.05$). Creatinine also showed a HS-induced increase ($P < 0.05$; 1.28 vs. 1.44 ± 0.04).

5. DISCUSSION

Previous research comparing field and chamber exposures is very limited. Both field and chamber approaches to study large animals have separate limitations, with neither offering the perfect scenario for assessment of long-term response to environmental stressors. However, it is imperative that there be some understanding of the similarities and differences between the two scenarios if one is to derive some understanding of the thermal stress response. The present work served to create a hybrid approach by combining both field and chamber environments at different times throughout the study. This allows for an assessment first under "natural" conditions, followed by a shorter period of testing in a controlled laboratory environment. Two studies have tested ruminants under both field and chamber conditions. Borut et al. (1979) found that under similar ambient conditions, resting metabolism of goats was the same under both conditions. Legates et al. (1991) looking at the heat tolerance of dairy cattle showed that chamber values were higher for respiration rate and rectal temperature than under field conditions. The same study also found significant breed differences under field conditions, but no differences during the chamber study. In the present study,

one primary objective was to determine if the thermoregulatory responses of beef cattle to heat stress and fescue toxicosis would be similar in field and chamber environments.

In order to make this comparison, it was important to understand the relationships between environment and thermal status of the animal. Currently, thermal status is assessed using determinations of core body temperature at rectal, vaginal, tympanic, or ruminal sites. The most widely used method of body temperature measurement is a rectal thermometer (Brown-Brandl et al. 2003). Recently vaginal, tympanic, and rectal data loggers have become popular, however these can only be used for a short periods before it becomes uncomfortable for the animal or leads to an infection (Berman and Morag, 1970; Hillman et al., 2005; Reuter et al., 2010). Newly developed radio-telemetric transmitters are now available which can be either surgically implanted into the abdominal cavity or ingested by the animal (resting in the rumen) allowing for undisturbed measurements in a variety of environments without the previously mentioned obstacles. This study used both a rectal thermometer during the chamber exposure and a telemetric transmitter placed in the rumen of the animals for field and chamber exposures.

A major goal of the present study was to determine the relationship between ruminal and ambient temperatures in the field environment. Many researchers have shown that core temperature may lag behind T_a due to the large body size of most cattle (Gaalaas, 1945; Scott et al., 1983; Hahn, 1999). However, researchers have reported lags of 1–5 hours behind T_a that is highly dependent on ambient conditions. In the present study, a 3 hour lag during both the field and chamber exposure yielded the greatest correlation with T_a. Black globe temperature, which rises rapidly to a higher level

compared to T_a, also yielded a greater correlation with T_{rum} when adding a lag effect. In contrast, there was lack of a delayed effect for RR during the field exposure. However, there were only 2 sample times per day, making it difficult to assess whether a lag would help correlations without more data. In a study by Brown-Brandl et al. (2005), researchers found that respiration rate lagged behind ambient conditions by slightly under 1 hour. This is consistent with the chamber exposure regressions in the current study which measured RR six times daily and found only slight improvement in adding a 1 hour delay. Since RR is an effector response to T_a that ultimately contributes to the core temperature response, one would expect less of a delay and possibly no lag effect.

Respiration rate for E+ and E- cattle showed no differences under field conditions. Only Period 2 showed any RR differences (lower than all other periods regardless of treatment). This was the only period which showed a decrease in Ta from the previous day, and this may have been responsible for the reduced RR. It is not surprising that RR differences may be difficult to detect as it is the first outward sign of HS, with sharp increases as T_a rises above 21°C (Gaalaas, 1945; Hahn et al., 1997). In the present study, all mean daily T_a values were above this value. In contrast, core body temperature rises as T_a increases above 25°C, or nearly 4°C higher than RR (Hahn et al., 1992; Lefcourt and Adams, 1996). The question then is how useful is RR in assessment of the thermal status of cattle. Respiration rate is extremely easy to measure and is a sensitive indicator of HS; however, it may be too sensitive. In a study by Scharf et al. (2011), minimum core temperature was found to be the most important variable for assessing maximum RR or maximum core temperature during the day. Finally, RR was measured in the current study by flank movement. It may be that other RR measurement

techniques, such as panting scores (Gaughan et al., 2008), which is a visual assessment of flank movements and respiratory pattern, would have a better comparison between chamber and field exposures. More research is needed to determine how reliable RR is for determining heat strain.

Another objective of the current study was to look at how these relationships would change when you add an additional stressor such as fescue toxicosis. No treatment differences in T_{rum} occurred during the field exposure, despite the added stressor of fescue toxicosis. In fact, E- animals tended to have higher T_{rum} than E+ animals during the peak hours of the day during the field exposure. This may be attributed to the fact that cattle grazing E+ pastures tend to shift their grazing time to cooler periods of the day or at night (Bond et al., 1984; Stuedemann et al., 1985), whereas E- animals graze throughout the day. Also, E+ animals spend more time idling and standing under shade (Howard et al., 1992). Even with the difference during the peak hours, the relationship between T_{rum} and T_a or T_{bg} was the same for both treatments. Ruminal temperature was highest during Periods 1, 3, and 5 when mean daily T_a was above 26°C and maximum daily T_a surpassed 31°C. Periods 2 and 4 with lower mean and maximum daily T_a resulted in little increase in T_{rum}. However, Period 6 ambient conditions were similar to Periods 1, 3, and 5, yet showed the lowest T_{rum} values. This may be attributed to the to the previous days exposures which were high causing the animals to reduce metabolism and remain under shade rather than graze.

Although measurements during the field exposure showed none of the typical signs of fescue toxicosis (i.e., increased RR and hyperthermia; Strickland et al., 1993), it is possible that such treatments under controlled conditions would identify differences. In

addition, animals were given a larger dosage of the toxin (i.e., ergovaline) to potentially increase symptoms of fescue toxicosis even if there was adaptation to this condition in the field environment. The chamber study would also allow for measurement of other notable symptoms of fescue toxicosis, which include decreased feed intake and increased peripheral vasoconstriction (Strickland et al., 1993; Paterson et al., 1995). Paterson et al. (1995) reviewed 11 trials and found that steer gains were 30 to 100% lower when steers consumed E+ rather than E- tall fescue, due to decreased feed intake, as shown in the present study, with E+ consuming half as much as E- animals.

During the chamber exposure, RR doubled and skin temperatures rose 3°C with an increase in T_a from 20 to 36°C, allowing the animals to increase heat flow from the body. Respiration rate only showed treatment effects during the first 3 days of HS, with E+ animals having the greater rate. This is a different result from the field exposure. However, it was short lived with E- animals rising to the same level by the end of the chamber exposure. This is not surprising as many variables, including T_a, relative humidity, radiation heat load, and air velocity, impact RR (Eigenberg et al., 2000) and are not easily controlled during chamber exposures. The transient nature of the RR treatment effect is one reason why it may not have been possible to detect an RR difference in the field studies. It is also possible that the RR taken in the field did not represent the significant changes that occurred during transition from TN to HS in the chambers. Perhaps if measurements were taken during a day when a large temperature swing had taken place, the values would be more consistent between the chamber and field measurements.

It is known that ingestion of ergot alkaloids can cause peripheral vasoconstriction, producing reduced heat loss and increased heat retention (Rhodes et al., 1991). This might also result in a faster response for E+ animals that would result in an increase in RR ahead of E- animals. Rhodes et al. (1991) hypothesized that reduced blood flow to the periphery would reduce the ability to move heat from core tissue to the surface. Vasoconstriction was not found during periods when T_a was increasing or already high in the present study. However, during the evening hours of the chamber study, E+ animals showed a reduced skin temperature suggesting a lower gradient for heat loss. This could result in some of the notable changes found in T_{re}.

Overall rectal temperature in the current study, during TN or late HS, was not different across treatments. During the early HS period, however, E+ and E- animals were very different. The E+ animals showed a rapid increase in T_{re}, but surprisingly only a minimal increase in T_{rum}. Control E- animals displayed a steady increase in T_{re} throughout HS, and the same minimal increase in T_{rum}. This suggests, first, that T_{rum} is a poorer indicator of fescue toxicosis compared to T_{re}, which showed a much more dynamic response. In addition, the relationship between T_{re} and T_{rum} is not well known. Only two studies have documented the correlation between the two variables with very different results. Prendiville et al. (2002) found an R of 0.34, whereas Dye et al. (2007) found a much higher R of 0.89. In the current study, there was a different R for each treatment group (E- R = 0.86 and E+ R = 0.79), but both were very similar to the study conducted by Dye et al. (2007). It is clear, that although T_{re} and T_{rum} can be highly correlated T_{re} is a much more dynamic responder to HS than T_{rum} and this might lead to many differences in correlation.

While T_{rum} shows promise as a site to easily measure core temperature without disturbing the natural behavior of the animal, little is known about temperature change within the rumen. Currently, the effect heat of fermentation on T_{rum} is not well understood (Beatty et al., 2008). Rumen heat production is known to account for as much as 8% of total heat production (Czerkawski, 1980). Dale et al. (1954) showed that there is a 2°C difference between T_{rum} and T_{re} under normal conditions, but this difference is reduced to 0.7°C during fasting. Beatty et al. (2008) found that the difference between T_{rum} and T_{ip} did not change despite a 50% reduction in feed intake. In the current study, a similar result was found at TN, with E+ animals having a lower feed intake yet showing no $T_{rum} - T_{re}$ difference (approximately 1°C) from E- animals. Beatty et al. (2008) found that T_{rum} also exceeded intraperitoneal temperature by 1°C with a range from 1.6 to 0.8°C throughout the day. This is consistent with the present study under TN conditions, but not under HS conditions.

Under HS conditions in the present study, feed intake continued to decrease in E+ animals, and they showed a much smaller $T_{rum} - T_{re}$ difference (0.6°C) due mainly to the rise in T_{re}. Beatty et al. (2008) stated that the temperature difference between the rumen and the body core depends on the rate of heat production and rate of loss to the body. Drinking water acts as an additional element of difficulty, as it is cooler than T_{rum} and acts as a heat sink for the rumen. This heat exchange between the rumen and the body surface occurs via conduction and convection (or blood flow). Ingestion of E+ fescue causes vasoconstriction, and as a result of the buildup of internal body heat, there is a more homogenous internal thermal environment. However, the relationship between the rumen and core body is clearly complex and warrants further study.

Decreased Serum prolactin levels have been reported in nearly all animals consuming E+ fescue (Waller et al., 2009). This has led researchers to suggest that a change in prolactin level is the most sensitive indicator of fescue toxicosis. In the present study prolactin was decreased during both the TN and HS period which is consistent with previous research. Aldrich et al. (1993) showed a reduction in prolactin levels in cattle consuming an E+ diet during exposure to heat (32ºC) and thermoneutral conditions (22ºC). Serum prolactin is known to increase in response to an increase in air temperature (Schams, 1972; Head et al., 1976; Johnson, 1985; Wetteman and Tucker, 1974). In the present study prolactin only showed a trend to increase with HS. This could be due to the fact that animals were already heat adapted.

Other signs of fescue toxicosis include a reduction in serum alkaline phosphatase (Schultze et al., 1999), triglyceride (Oliver et al., 2000), and cholesterol (Thompson et al., 2001). In the present study alkaline phosphatase was not different between treatments however; E+ animals were numerically lower then E- animals (77 vs. 68). Triglyceride on the other hand has been reported to be lowered in animals consuming E+ fescue was increased above E- animals in the present study. It is known that HS reduces triglycerides (Abeni et al. 2007). It is possible that this difference is due to the animals being heat adapted prior to the blood draws. Serum cholesterol has also been shown to be reduced by feeding an E+ diet to cattle (Stuedemann et al., 1985). Similarly to the triglycerides, in the present study there was no reduction in cholesterol. Stuedemann et al. (1985) reported an association between high-nitrogen fertilization of tall fescue and reduced serum cholesterol which may account for the lack of decrease found in the current study. Serum

creatinine is also used as an indicator of fescue toxicosis. In the present study creatinine increased which is consistent previous research (Schultze et al., 1999; Oliver et al., 2000).

The current study measured RR between 2 and 6 times a day with measurement of T_{rum} to determine the relationship with ambient conditions under both field and chamber conditions. Respiration rate, measured 2 times a day, showed little change over the 6 Periods and no treatment differences during the field exposure. However, RR during the chamber exposure showed a rapid rise for E+ animals and a gradual increase for E- animals. Even with the difference in response during the early HS period, it appears that chamber studies underestimate RR during HS with field exposures showing having higher RR at the same T_a. Because body temperature arises from the balance heat loss and heat production it seems logical that T_{rum} would be consistent between chamber and field exposures. While RR differs between environments, T_{rum} is an integrator of all the stressors and as long as the strain is not severe the end-point is the same.

Measurement of ruminal temperature with SmartStock boluses allowed for minimally invasive real-time data collection for cattle maintained in a natural environment. How T_{rum} responds during conditions of elevated body temperature and reduced feed intake is unknown and needs further study. Additional studies are also needed to evaluate the relationship between ruminal temperature and rectal temperature, and the impact of an additional environmental stressor such as fescue toxicosis.

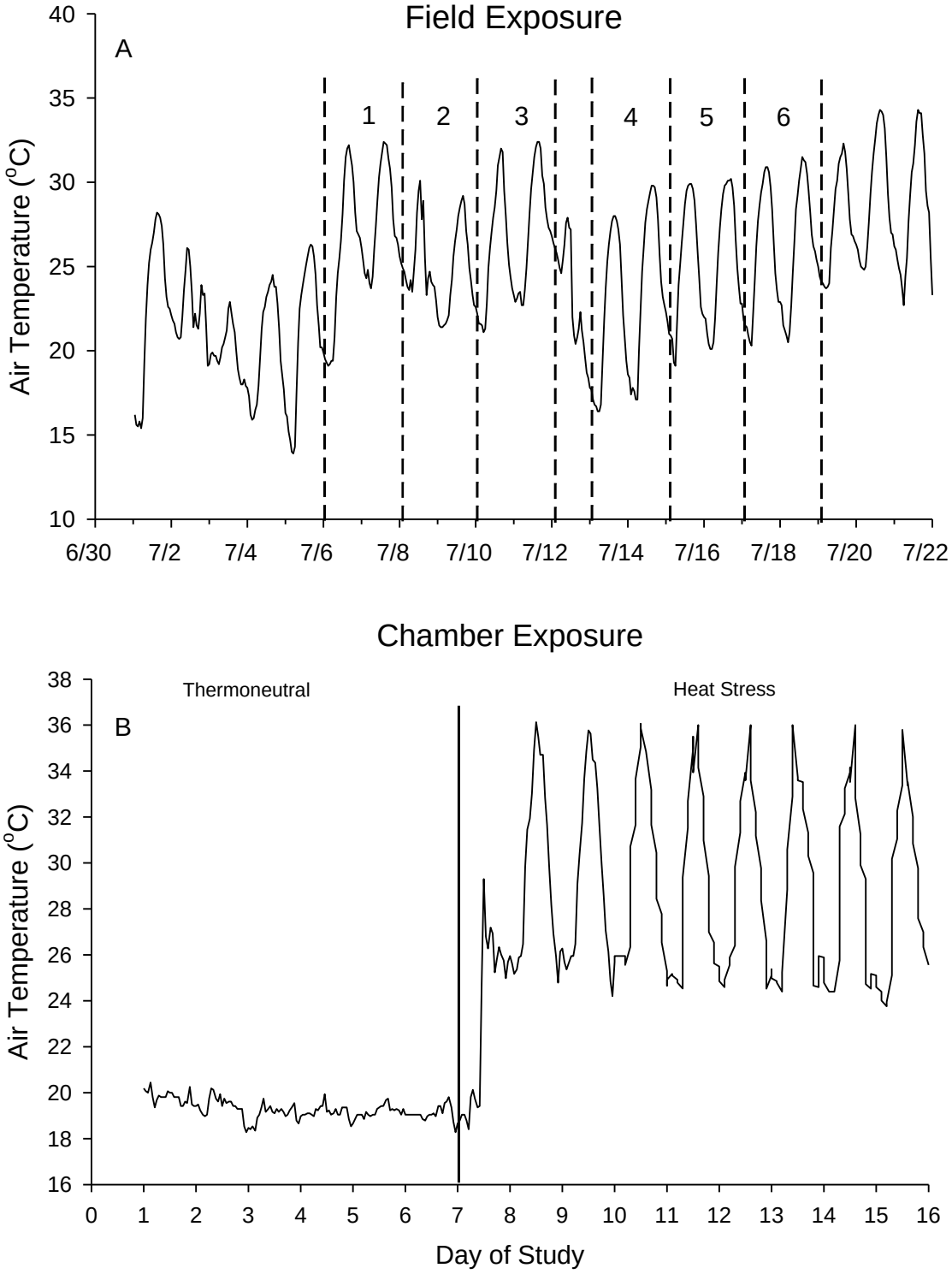

Figure 4.1 - (a) Hourly air temperature (T_a) of the field environment during the experiment. Dashed, vertical lines designate the 6 periods used for analysis. Numbers designate the 2 days within each. (b) Mean room air temperature for the chamber study beginning on Day 1 at thermoneutrality (19-21°C) and continuing through Day 14 which was the last day of heat stress (Night: 26°C; Day: 36°C). Values were collected hourly for each day.

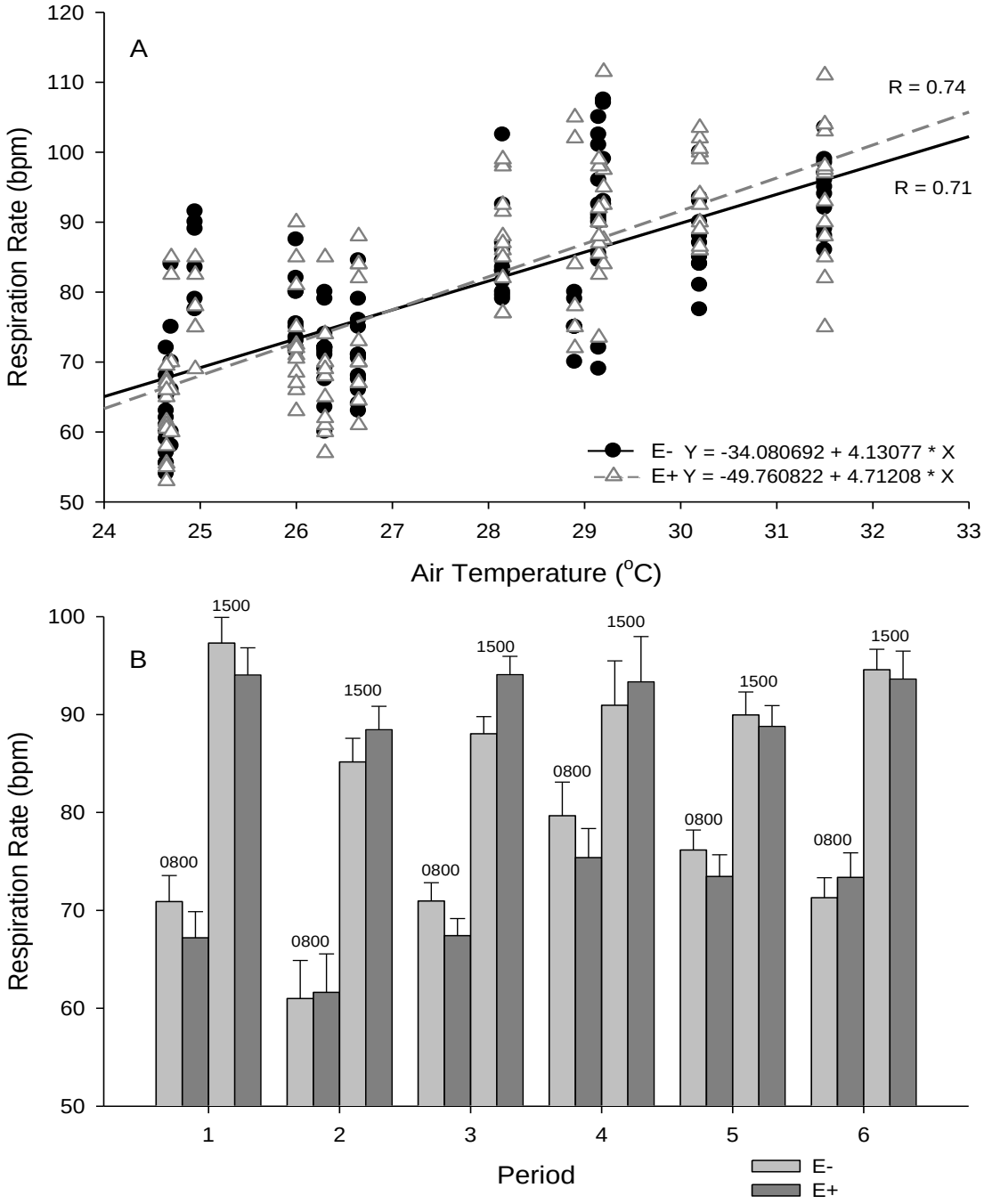

Figure 4.2 - (a) Linear relationships of respiration rate to air temperature using the six periods. Values used in the calculations were hourly averages for each animal across the 2 days in each period. Correlation of determination values and linear regression formulas are given with the graph with Y-variable equaling respiration rate and X-variable equaling air temperature. (b) Average values for respiration rate at 0800 and 1500 h are shown for each period. Dependent variables with the same letter over the bar are not significantly different within the variable. The vertical line on top of each variable bar is +1SEM.

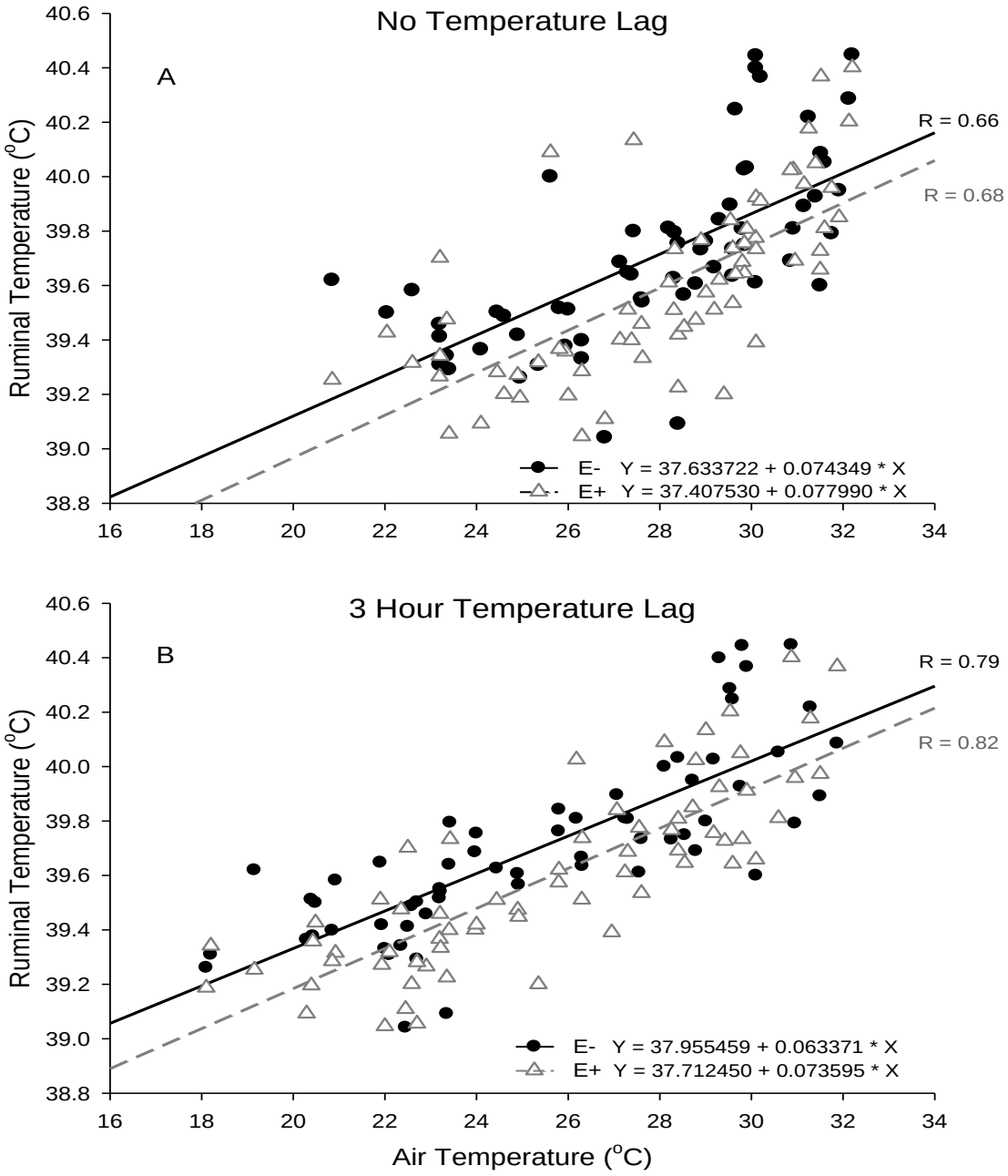

Figure 4.3 - Linear fit of ruminal to air temperatures both without (a) and with (b) a 3 hour lag is shown using the 6 periods during the field exposure. Values used in the calculations were hourly averages for each animal across the 2 days in each period. Correlation of determination values and linear regression formulas are given with the graph with Y-variable equaling ruminal temperature and X-variable equaling air temperature.

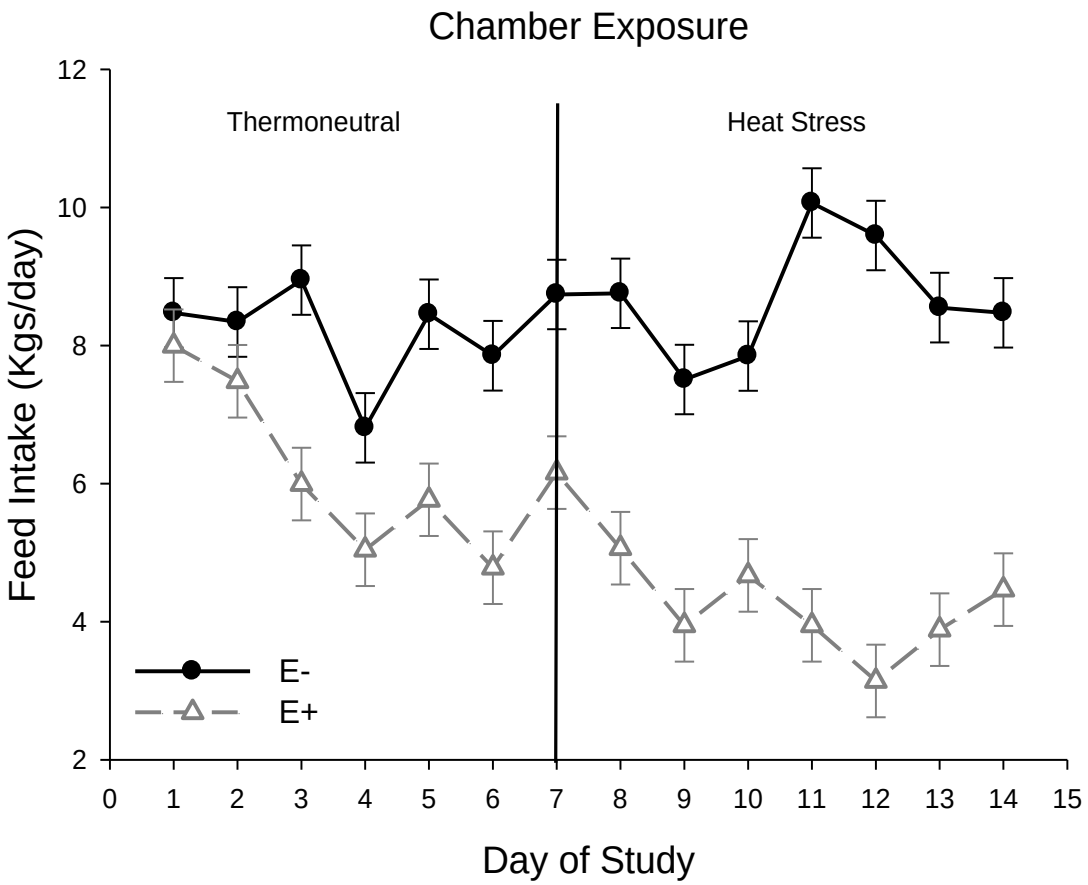

Figure 4.4 – Daily feed intake of E+ and E- steers is shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods. The vertical line on top of each variable bar is +1 SEM.

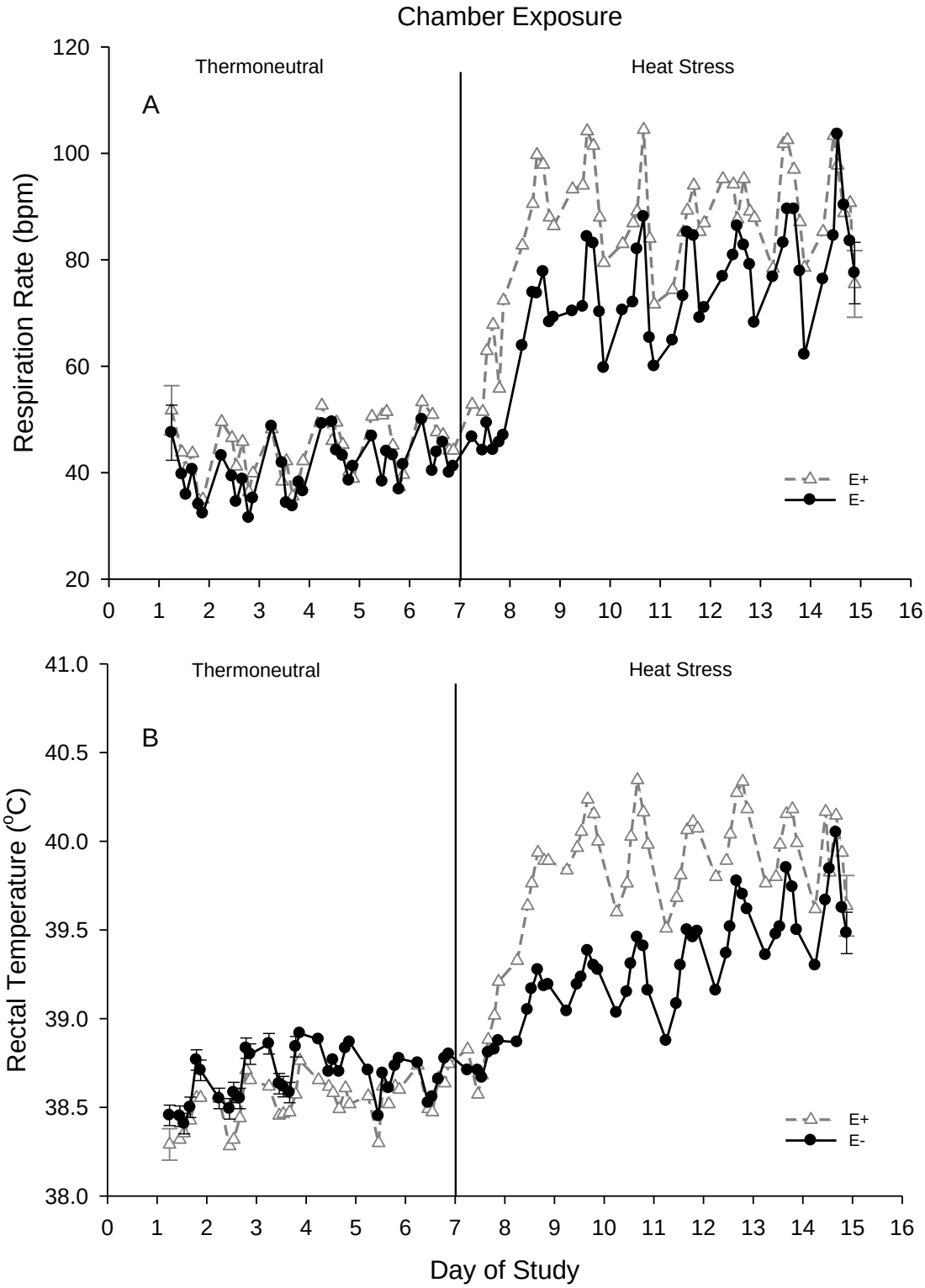

Figure 4.5 – (a) Mean respiration rate (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. (b) Mean rectal temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods.

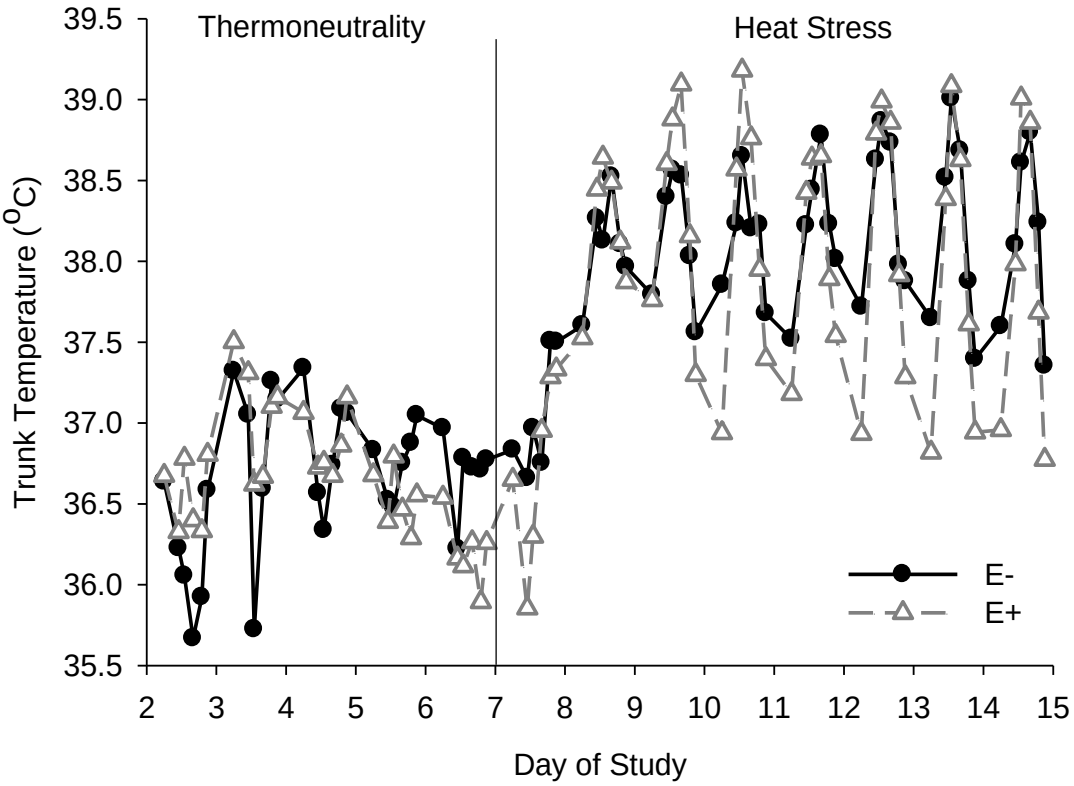

Figure 4.6 - Mean trunk skin temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. Trunk temperature represents a combination of shoulder and rump temperatures.

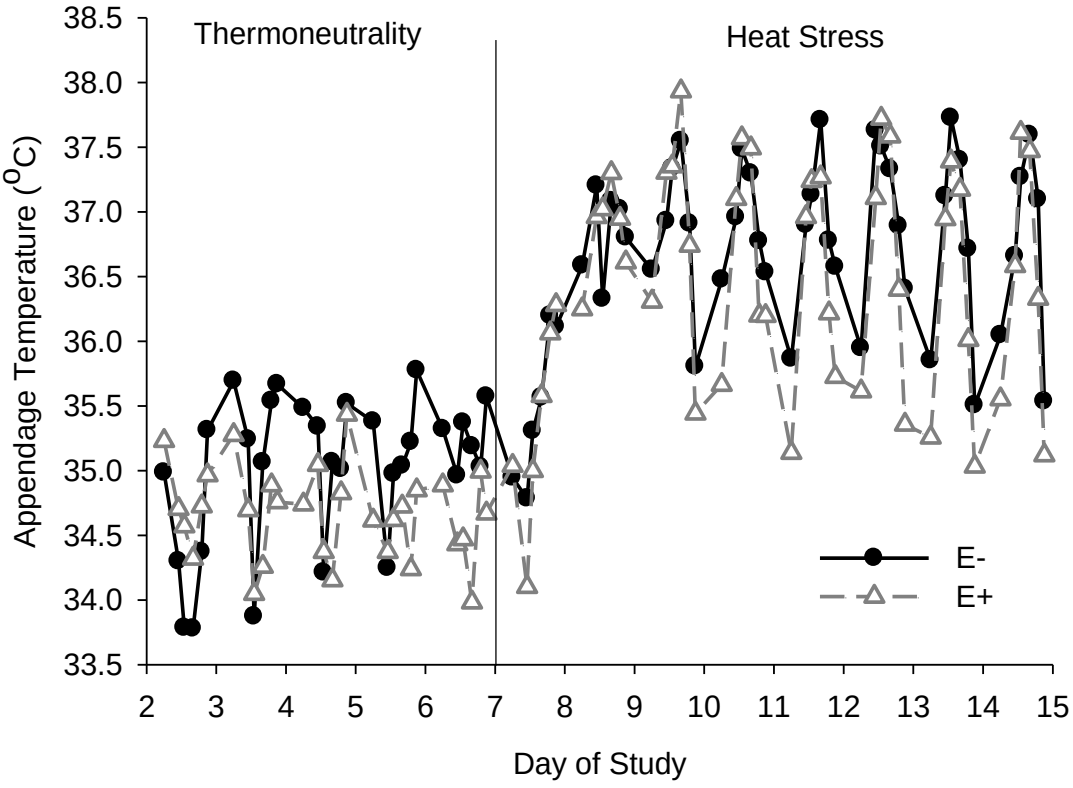

Figure 4.7 - Mean appendage skin temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. Appendage temperature represents a combination of ear, upper tail, and lower tail temperatures.

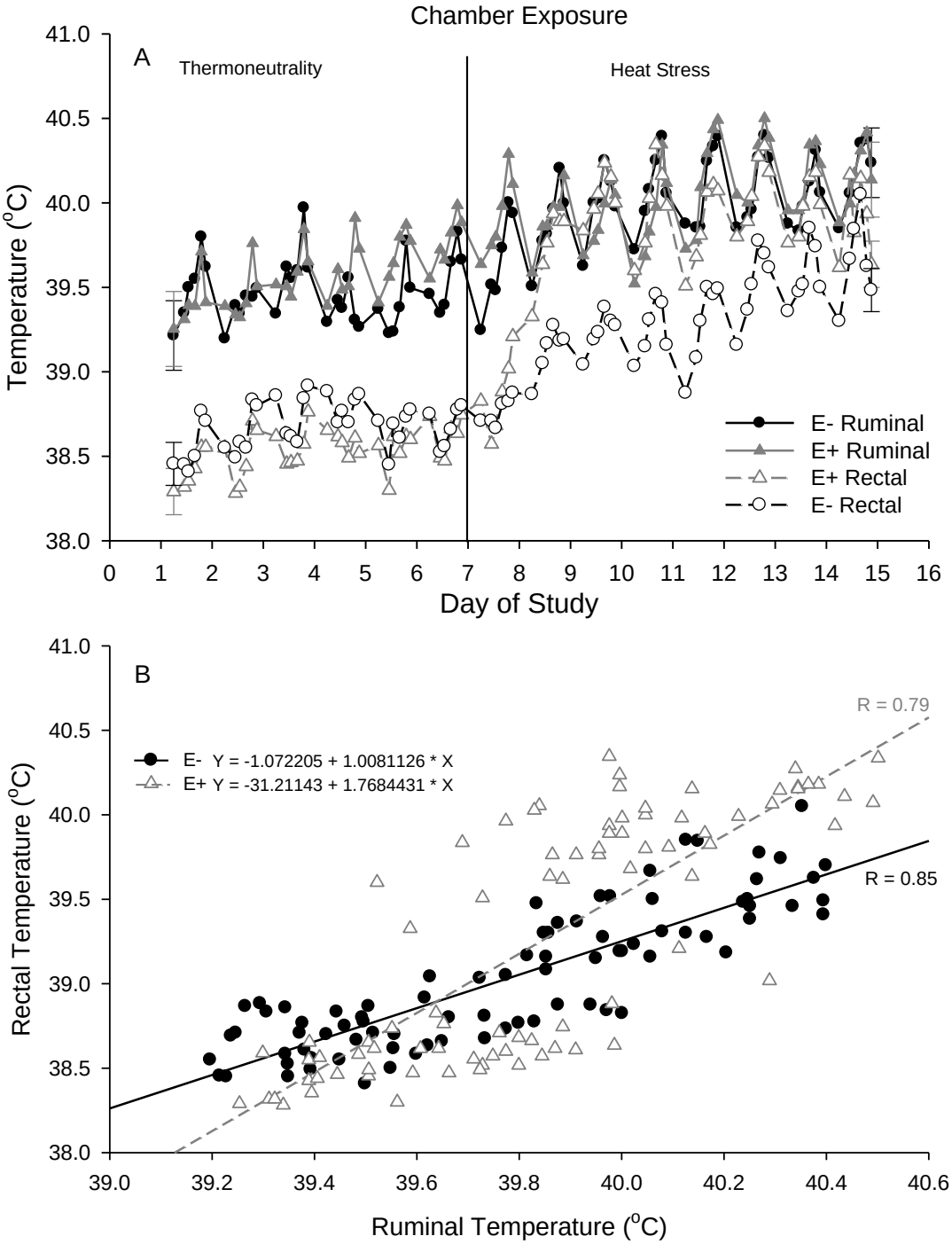

Figure 4.8 - (a) Mean ruminal temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. The solid vertical line separates thermoneutral and heat stress periods. (b) Linear relationship of rectal temperature to ruminal temperature is shown using all data during the chamber exposure. Values used in the calculations are averages of the 6 time measurements for each animal over the 14 day exposure. Correlation of determination values and linear regression formulas are given with the graph with Y-variable equaling rectal temperature and X-variable equaling air temperature.

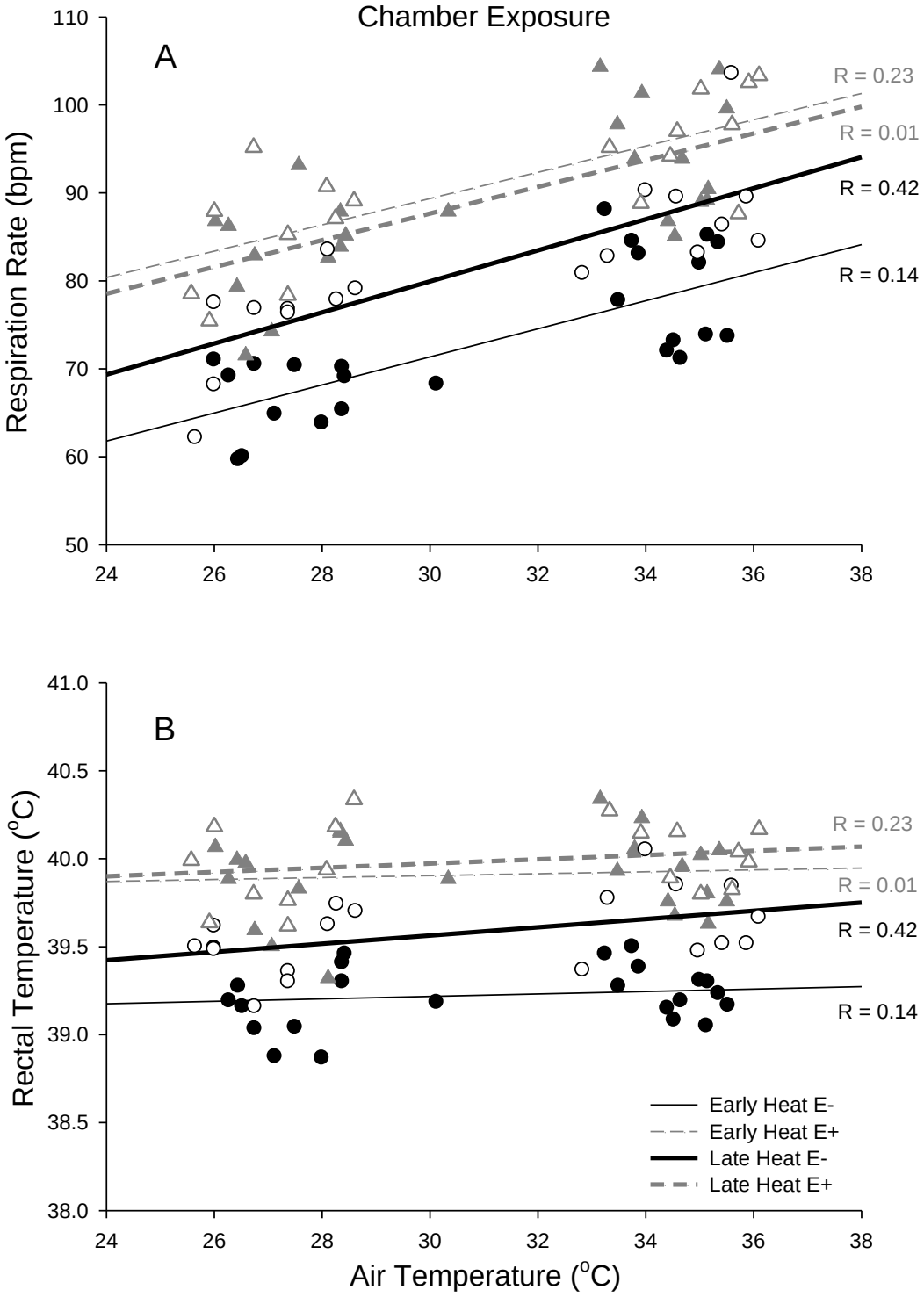

Figure 4.9 - Heat stress-induced changes in respiration rate (a) and rectal temperature (b) are shown for E+ and E- steers from early (i.e., Days 8 - 10) to late heat exposures (i.e., Days 12 -14). A linear fit is shown through points for each variable, treatment, and set of days (T_a: 26-36°C).

Table 4.1 – Group mean serum biochemistry values for cattle under thermoneutral and heat stress conditions. P-values given for Treatment (E+ vs. E-), Time (TN vs. HS), and Interaction (Treatment x Time).

Blood Parameters							
					P Values		
	Treatment	*TN*	*HS*	*± SE*	*Treatment*	*Time*	*Interaction*
Albumin	E-	3.47	3.31	0.07	0.23	0.27	0.13
g/dL	E+	3.28	3.31				
ALP	E-	82.41	72.42	6.32	0.21	**0.001**	0.16
U/L	E+	82.18	54.81				
Calcium	E-	9.53	9.27	0.19	0.19	0.25	0.96
mg/dL	E+	9.30	9.01				
Chloride	E-	95.75	94.41	1.17	**0.05**	0.59	0.11
mEq/L	E+	96.27	98.91				
Cholesterol	E-	64.67	49.33	4.63	0.66	**0.001**	0.18
mg/dL	E+	66.09	42.18				
CPK	E-	147.41	107.73	19.70	0.64	**0.05**	0.98
U/L	E+	157.45	118.27				
Creatinine	E-	1.25	1.33	0.06	**0.05**	**0.001**	**0.05**
mg/dL	E+	1.32	1.56				
Globulin	E-	3.47	3.05	0.13	0.56	**0.001**	0.77
g/dL	E+	3.35	3.00				
Potassium	E-	4.39	4.01	0.11	**0.05**	**0.001**	0.39
mEq/L	E+	4.54	4.32				
Prolactin	E-	36.39	38.03	5.11	**0.001**	0.1	0.55
ng/ml	E+	16.93	6.47				
Sodium	E-	140.00	137.00	5.73	0.29	0.51	0.25
mg/dL	E+	127.34	137.81				
Total Protein	E-	6.94	6.35	0.16	0.29	**0.01**	0.38
g/dL	E+	6.63	6.31				
Triglyceride	E-	18.92	13.41	1.5	0.07	**0.001**	0.24
mg/dL	E+	20.45	18.18				
Urea N	E-	8.41	8.41	0.81	0.49	0.2	0.2
mg/dL	E+	8.00	10.00				

CHAPTER FIVE

PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS DURING THE END OF SUMMER

1. ABSTRACT

Heat stress and fescue toxicosis, resulting from intake of endophyte-infected fescue, have significant impacts on physiological processes with multiplicative interaction. To study this impact, we used numerous physiological measures of thermal status to compare responses of cattle to chamber "stress tests" and "naturally occurring" field conditions. A two month summer field exposure during which Angus steers (N=23; 318±8 kg BW) consumed either endophyte-infected or uninfected fescue pasture was conducted followed by a controlled heat challenge. During this heat challenge, steers were assigned to diets of either 0 or 40µg ergovaline/kg/d to maintain the fescue toxicosis state. During the chamber challenge, feed intake quickly decreased more than 50% in the E+ animals ($P < 0.05$), while E- animals FI did not change during the challenge. HS resulted in a further decrease in FI for E+ animals ($P < 0.05$). Respiration rate was significantly higher for E+ animals under TN conditions ($P < 0.05$). During HS, E+ animals continued to have the higher rate, however it was short-lived, with E- animals rising to the same level by the end of the chamber exposure. Rectal temperature was also higher for E+ animals at TN ($P < 0.01$). HS resulted in an increase in rectal temperature for both groups with E+ animals showing the greatest increase ($P<0.01$), but like respiration rate both groups came together and were not different by the end of the trial. Surprisingly, T_{rum} showed no differences between groups during TN

or HS. This is likely due to a decline in feed intake and heat production associated with consumption of endophyte infected seed.

2. INTRODUCTION

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. –*Schedonorus arundinaceus* [Schreb.] Dumort) is an important cool-season perennial grass grown in the United States that occupies over 35 million acres nationwide. More than 8.5 million cattle graze these acres, and more than half is infected by the endophyte *Neotyphodium coenophialum* (Jones et al., 2004). Intake of fescue infected with the fungus can result in a series of symptoms known as fescue toxicosis. Studies have shown that animals with fescue toxicosis have increased respiration rate, rough hair coat, and increased core temperature during heat stress, along with decreased feed intake (Bond et al., 1984, Hemken et al, 1981; Rhodes et al., 1991; Osborn et al. 1992; Strickland et al., 1993). Economic losses associated with fescue toxicosis exceed $600 million annually (Hoveland, 1993), with the majority of losses stemming from decreases in milk production and a reduction in average daily gain that is often attributed to hyperthermia and reduced feed intake.

Many different symptoms associated with fescue toxicosis and heat stress have been identified in both laboratory and field studies. However, there have been few attempts to identify the differences between the 2 environments, and there is little understanding of their interactions especially over long periods of time (Spiers et al., 2012). Furthermore, there is large variation in the response of cattle during periods of rapidly changing ambient temperature. Many studies have recorded core body temperature to relate it to overall performance; however, core body temperatures in these

studies were taken at infrequent times during the day (Mendal et al., 1971; Thompson, 1973), or under feedlot conditions (Lefcourt and Adams, 1996; Scharf et al., 2011). No studies have continuously measured core body temperature over a significant portion of the summer on pasture to look at adaptation to both heat and fescue toxicosis. By simultaneously collecting core body temperature and respiration rate under both field and chamber conditions, it allows for the opportunity to identify individual responses and differences between the 2 environments. Therefore, the objective of this study was to determine how similar the thermoregulatory responses of cattle are under a field exposure followed directly by a controlled chamber run under similar conditions. A second objective was to determine the relationship between rectal temperature and ruminal temperature utilizing radio telemetric transmitters.

3. MATERIALS AND METHODS

3.1. Animals

Twenty-three Angus steers (345 ± 10 Kg BW) were obtained from the University of Missouri Beef Research Farm. These animals have been raised on tall fescue for many generations and represent animals that possibly that they have acquired some tolerance to the fescue endophyte indirectly through selection. Animals were randomly assigned to an endophyte-infected (E+; 344 ppb ergovaline; n = 11) or endophyte-free (E-; n = 12) tall fescue pasture. Steers were placed on E+ and E- pastures for approximately two month (August 7th through October 3rd) before being transported to the Brody Environmental Center at the end of the summer. The Brody Environmental Center consists of four 6.1 × 9.1 m chambers, two of which were used in the present study. For chamber exposure, steers remained on treatments receiving either E+ (40 µg ergovaline/Kg BW/day) or E-

tall fescue seed top-dressed over a typical finishing diet (39% each of corn and soybean hulls, 20% dried corn distillers grains, and 2% mineral supplement). Ergovaline is considered to be the primary ergopeptine alkaloid responsible for fescue toxicosis in an array of toxins found in endophyte-infected tall fescue. For ergovaline analysis, pasture samplings were collected by walk in a zigzag pattern through the 2 pastures. Approximately 30 samplings were collected for both E+ and E- pasture. Samplings included the entire plant above ground including some root material. Samples were freeze dried and ground for analysis. Ergovaline content was measured by HPLC (detection limit = 50 ppb and CV = 7%; Rottinghaus et al., 1993). Ergovaline concentrations prior to the chamber exposure for the E+ pastures ranged from 305 ppb to 340 ppb. Ergovaline content for the E- pasture was almost negligible ranging from 15 to 80 ppb throughout the summer.

Feed intake (FI; kg/steer/day) was manually recorded daily at 0600 h by subtracting any remaining feed from the amount given on the previous day. Steers were fed daily at 0600 and 1600 h. In order to ensure consumption of ergovaline the finishing diet was placed in individual feed bunks and then top-dressed with fescue seed. Water was available for *ad libitum* intake from individual water bowls. The two chambers were divided into 6 stanchions with each animal loosely restrained to the stanchion by a chain. Due to space requirements, the 23 animals were split into 2 groups (Group 1 = 12 animals; Group 2 = 11 animals) and run 2 weeks apart. Air movement in the chamber was held at 15 room changes per hour (127 cubic meters of volume). Light cycle during the chamber study was a 12-h light: dark (0600:2000 h) schedule. The experimental

animal protocol and procedures were approved by the University of Missouri Animal Care and Use Committee.

3.2. General Procedure

Steers were housed in covered feedlots at the University of Missouri South Farm prior to being placed on either E+ or E- pastures. Calibrated data loggers (Hobo H8 Pro; Onset Computer, Bourne, MA; accuracy: ± 0.2°C and ± 3% RH) were used to record air temperature (Ta) and percent relative humidity (RH), as well as black globe temperature (BG; hollow copper sphere; 15.24 cm diameter; flat black exterior, located between animal pastures; Bond and Kelly 1955;) for assessment of radiant heat load (Figure 5.1a). Temperature humidity index (THI) was calculated using recorded ambient values (Thom, 1959; THI= ($t_{dry\ bulb}$ x 0.81) + RH x ($t_{dry\ bulb}$ – 14.4) + 46.4). Determinations of respiration rate (RR) were made by counting flank movement over a 1-minute interval twice a day (i.e., 0800 and 1500 h). These points were selected as they represent both low and high points of the daily core temperature cycle (Scharf et al., 2011).

A calibrated, telemetric, temperature transmitting bolus (SmartStock LLC, Pawnee, OK) was placed into the rumen of each animal (oral administration using a bolus gun) prior to the study to record ruminal temperature (T_{rum}). Ruminal temperature measurements were transmitted, using a radio frequency of 900 MHz to an antenna placed approximately 10 m from the animals. The signal was then transmitted to the base receiver unit which was connected to the personal computer. The boluses were designed to transmit every 20 minutes. Along with the current reading, the bolus transmits the previous 11 readings to minimize the loss of data resulting from lost transmissions. Boluses were calibrated to a NIST (National Institute of Standards and Technology)

thermometer prior to ingestion by the animal. The data was filtered for maximum hourly value which was used for all analyses. This avoided the incorporation of thermal artifacts associated with water intake.

For chamber exposures, steers were housed for 7 d at a thermoneutral T_a (TN; 20^{o}C) prior to initiation of heat stress (HS). Heat stress consisted of daily cyclic air temperature (26°C night: 36°C day) for 7 d (Figure 5.1b). Chambers at TN had a controlled set-point of 20°C showing only slight fluctuations across days. During the HS cycle, chamber T_a increased daily as a step-up function with three set points throughout the rise phase, followed by a 4 hour stable period (36°C; 1200 to 1600 h). The decline phase consisted of two step-downs to reach the stable low T_a (26°C; 0000 to 0600 h). Relative humidity was maintained under 50% during the entire study (TN: 40 to 50%; HS: 35 to 45%) to minimize its influence on heat flow. Chamber environmental conditions were controlled using a Fisher-Porter Controller (698B179U01) and a Sensycon I/P Converter (Controller Type 27/06–65). This procedure has been used in a number of previous studies (Spiers et al., 2001; Scharf et al., 2008) Environmental conditions were measured as during field exposure with calibrated Hobo H8 data loggers (Onset, Bourne, MA) to record T_a and %RH every 10 minutes.

Animal measurements, including respiration rate (RR), skin temperature (T_{skin}), and rectal temperature (T_{re}), were taken 6 times daily (0600, 1100, 1300, 1600, 1900, and 2100 h). Determination of RR was made as previously described. Skin temperatures, at 5 different shaved sites (ear, shoulder, rump, tail head, and lower tail; 8 x 8 cm area), were measured using a calibrated infrared thermometer (Model RAYST80XB, Raytek Corporation, Santa Cruz, CA; Accuracy ± 1%). Readings were taken less than 35 cm

away from each skin site with a thermometer target ratio of 50:1. Rectal temperature was measured using a calibrated thermistor thermometer (Model 8110–20, Cole-Parmer Instruments, Chicago, IL). This was accomplished by inserting a YSI probe (Model 400, YSI Inc., Yellow Springs, OH; Accuracy: 0.1°C) approximately 15 cm into the rectum for approximately 2 minutes. As during the field exposure, ruminal temperature was monitored every 20 minutes with the maximum value during the hour used in the analyses.

Blood (20 ml) was collected at 0900 on Days 6 (TN) and 14 (HS) via a jugular venous puncture. Samples were collected into a 15 ml tubes and allowed to clot prior to centrifugation. Serum was separated by centrifugation (2,300 x *g* for 25 minutes; 4°C) before being removed and stored at −20°C for later analysis. Serum analyses used standard procedures. Most serum measurements were components of a larger biochemical profile produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an auto-analyzer (Olympus AV400; Olympus America, Inc., Melville, NY). These include albumin, alkaline phosphatase, chloride, cholesterol, creatinine phosphokinase, creatinine, globulin, potassium, sodium, total protein, triglyceride, and urea nitrogen. Serum concentrations of prolactin were determined by radioimmunoassay procedures previously validated at the University of Missouri (Lutz et al. 1991). Minimum detectable concentrations of prolactin in serum were 1.19 ng/tube.

3.3. Statistical Analysis

3.3.1. Field Exposure

For field exposure analyses, animal and temperature data was reduced to hourly averages for each day. Six periods within the data was selected where complete RR and T_{rum} data was collected. Each period of 2 consecutive days that was similar in ambient conditions (Figure 5.1a). This process was used to minimize potential variance homogenizing days within a period, individual animal responses, and limiting transmitter artifacts associated with particular animals. The effects of period on ambient variables (T_a, T_{bg}, and THI) were modeled using the repeated measures ANOVA procedures of JMP® (SAS Institute; Cary, NC) with the ambient variable modeled as the dependent variable, with period, time of day, and period by time of day interaction as independent variables that were modeled as fixed effects. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD adjustment procedures for multiple mean comparisons.

A repeated measures ANOVA, constructed using JMP, was also used to test the effects of period on T_{rum}. The model included T_{rum} as the dependent variable with the independent variables of treatment, hour (0700 to 1800 h), and period fit as fixed effects, and animal within period, treatment by period, treatment by hour, and period by hour interaction as random effects.

Respiration rate, T_{rum}, and T_a observations were averaged for each of the two days within period observed at 0800 and 1500 h. These means were tested by way of ANOVA using JMP to determine if any statistical differences existed.

Simple linear regression procedures of JMP were utilized to establish the linear relationships between T_a, RR, and T_{rum} during each period. Regression coefficients for slope and model correlation coefficients (R) are reported, as well as P values for the

hypothesis test that the time regression coefficients are significantly different from zero. Regression models were constructed using JMP with a time delay of 0, 1, 2, and 3 hours for response variable, T_{rum}, to explore the relationships with ambient conditions. Model R values are reported and were used in the determination of model sufficiency.

3.3.2. Chamber Exposure

All evaluations at TN were performed using the last 6 d (i.e., Days 2 through 6) before the increase in T_a. The analysis included RR, skin temperatures, T_{rum}, or T_{re} as the dependent variables. Skin temperatures were included as an average of the shoulder and rump sites (T_{trunk}), or an average of ear, tail head and lower tail ($T_{appendage}$). Treatment, time, and treatment by time effects were set as fixed, with animal nested within breed as a random effect. Experiment-wise Type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer HSD adjustment procedures for multiple mean comparisons.

Simple linear regression procedures of JMP were utilized to establish relationships between animal variables (RR, T_{trunk}, $T_{appendage}$, T_{re}, and T_{rum}) and T_a. Regression coefficients for slope and model R, as well as P-values for the hypothesis test that the regression coefficients were significantly different from zero, are reported. Similarly, simple linear regressions were constructed to determine the relationship for early heat (Days 8 to 10) and late heat exposure periods (Days 12 to 14) using the regression procedures of JMP.

4. RESULTS

4.1. Field Exposure

Average daily T_a for entire field exposure period was relatively low at 18.9°C (Min: 10.5°C; Max: 27.4°C) with only a couple of days HS. The 4 periods selected for

analysis are shown in Figure 5.1a. Average daily T_a ranged from 15.3°C during Period 1 (Min: 12.5, Max: 16.5) increasing during each period to 24.0°C during Period 4 (Min: 21.7, Max: 27.4). Overall, minimum daily T_a for the 4 periods was lower (~4 to 9°C lower) than TN conditions during chamber exposure (20°C; Figure 5.1). Maximum daily T_a across the periods was also much lower (~8 - 17°C lower) compared to chamber temperature during HS (36°C; Figure 5.1b). Daily minimum values occurred at approximately 0700 h for T_a and T_{bg}. Black globe temperature increased at a faster rate than Ta being higher by 0800. Daily peak values in T_a and T_{bg} were at 1600 h, with rapid reductions after this time. Average daily THI during the periods was 64.8, with a maximum of 75.2 that occurred during Period 4. THI values never achieved danger levels only reaching alert levels (i.e. THI above 74) during Periods 3 and 4.

4.1.1. Animal Values

Respiration rate showed no treatment (E+/E-), treatment by time (0900 or 1500) or treatment by period differences during this field exposure ($P \geq 0.65$; Figure 5.2). All treatment periods showed similar time of day differences having a lower RR at 0800 versus 1500 h ($P < 0.01$; Figure 5.2). Respiration rate was lowest during Period 1, being significantly different from all other periods ($P < 0.05$). Periods 2 and 3 showed similar rates (59 versus 64 bpm), while Period 4 showed the highest rate being significantly different from all other periods ($P < 0.05$). Animals during Period 4 showed a highest RR at both 0800 (57 bpm) and 1500 h (98 bpm) compared to the other 4 Periods ($P < 0.05$). Period 1 had the lowest RR at both 0800 and 1500 h ($P< 0.05$). A period by time effect was found with the 0900 readings during Periods 1 and 2 being significantly lower than

Periods 3 and 4 ($P < 0.05$). Readings at 1500 h were all significantly different from each other with Period 4 having the highest rate ($P < 0.05$)

Linear relationships (using all data points) between RR and T_a, T_{rum}, T_{bg}, and THI were evaluated to determine which variable was the more reliable predictor for RR. Black globe temperature showed the greatest correlation with RR ($R = 0.75$) followed by T_{bg} ($R = 0.71$) and THI ($R = 0.69$). Ruminal temperature taken twice daily (0900 and 1500 h) to match RR measurements, showed a poor correlation with RR ($R = 0.31$). Ruminal temperature correlations with T_a, T_{bg}, and THI also showed low correlations for all variables ($R = 0.42 – 0.52$). A lag or delay (1, 2, and 3 hours) in ambient temperature was evaluated to see if it would improve the linear relationship between RR and T_a. A 1 hour lag only showed a minimal increase ($R = 0.75$ vs. $R = 0.78$), while a 2 and 3 hour lag decreased the correlation coefficient ($R = 0.71$ vs. $R= 0.75$).

Because using only the 0900 and 1500 readings gave a poor correlation, hourly values from 0700 to 1800 h were used to incorporate more sample times into the analysis. Similarly to RR, hourly T_{rum} showed no treatment (E+/E-), treatment by time, or treatment by period effects were found ($P \geq 0.45$; Figure 5.3). Minimum T_{rum} occurred at 0900 h (2 hours after Min T_a) and reached a maximum at approximately 1800 h (3 hours after max T_a). Periods 1 and 3 were not different from each other, but were significantly different from Periods 2 and 4 ($P < 0.05$; 39.3 vs. 39.6°C). Likewise Periods 2 and 4 were not significantly different from each other ($P = 0.72$; 39.6 vs. 39.7°C). Even though there were no treatment differences across periods, E+ animals tended to have a lower T_{rum} during the morning hours and a higher T_{rum} than E- animals during the peak hours of the day (1500 to 1700 h; $P < 0.10$). Linear relationships between T_{rum} and T_a, T_{bg}, and THI

showed marginal correlations combining all periods (R = 0.45 or lower). Running the correlation by period showed little improvements for all variables (Figure 5.3a; R = 0.40 – 0.51). Across all periods, T_{bg} showed the greatest correlation with T_{rum}, (R = 0.51) followed closely by T_a (R = 0.40) and THI (R = 0.36). Unlike RR, adding a 3 hour lag to the hourly T_{rum} showed an improvement in correlations with T_{bg} (R = 0.67) and T_a (R = 0.66). A lag of 1, 2, 3, 4, and 5 hours were analyzed with a 3 hour lag showing the greatest improvement in correlation coefficient.

4.2. Chamber Exposure

4.2.1. Thermoneutrality

Feed intake was significantly lower for E+ animals (6.1 versus 8.1 kgs/d) during TN ($P < 0.05$; Figure 5.3). It started to decline on Day 1, being significantly different from E- treatment from Day 2 until the end of the TN period ($P < 0.001$; Figure 5.3). The E- animals also displayed a decline in feed intake after Day 2 stabilizing at 7.5 kgs/d by the end of the TN period ($P < 0.05$; Figure 5.3). Respiration rate was also different at TN, with the E+ animals having a higher rate than E- animals ($P < 0.001$; 48.3 vs. 65.8 ± 2.4 bpm; Figure 5.4a). Both groups showed a small increase in RR ($P < 0.05$) at TN, however, E+ animals maintained the higher rate ($P < 0.05$). A circadian rhythm was present during TN for RR with 1300 h being the highest rate and 1900 h the lowest rate. Both T_{trunk} (Figure 5.5) and $T_{appendage}$ (Figure 5.6) showed no treatment differences at TN ($P = 0.32$). During the first 2 days of chamber exposure skin temperature was quite variable (Figures 5.5 and 5.6). This variation decreased after Day 4. Skin temperature regions were approximately 1°C different, with T_{trunk} having the higher value. Similarly to RR, T_{re} was different between E+/E- treatment groups with E+ animals having the

greater temperature ($P < 0.05$; 39.3 versus 38.5 ± 0.09°C; Figure 5.4b). Rectal temperature, like T_{skin} and RR, was variable during the first 3 days of the study, but stabilized by the end of the TN period. The E+ animals maintained the higher temperature throughout TN, with exception of Days 4 and 5 which were not significantly different ($P = 0.15$; Figure 5.4b). Rectal temperature also displayed a circadian rhythm with hours 1600, 1900, and 2100 showing the higher temperature (E-: 38.8°C, E+: 39.3) and hours 1100 and 1300 showing the lowest ($P < 0.05$; E-: 38.6°C, E+: 39.1°C). Ruminal temperature, unlike T_{re}, was not different between treatment groups ($P = 0.75$; 39.7 versus 39.7 ± 0.12°C). Similarly to all other variables, T_{rum} showed variation on the first 2 days of the study, but was stabilized by Day 3 ($P < 0.05$; Figure 5.7a). There was no circadian rhythm present for T_{rum}, unlike T_{re}. The T_{rum} - T_{re} difference showed significant differences across treatments ($P < 0.01$), with E+ animals having the lower temperature gradient (0.53 vs. 0.94 ± 0.08°C) These treatment differences were maintained throughout the TN period, with a trend for the T_{rum} - T_{re} difference to increase from Days 1 to 6 ($P < 0.10$). While a circadian rhythm was present for T_{re}, no rhythm was found for T_{rum} and taking the difference between the 2 measurements resulted in the absence of an identifiable rhythm.

4.2.2. Heat Stress

Feed intake for E- animals was not different between TN (8.1 ± 0.38 kgs/day) and HS (8.3 ± 0.29 kgs/day) periods ($P = 0.21$). In contrast, E+ animals showed a further reduction in feed intake to below TN values ($P < 0.05$; TN: 6.1 kgs/day vs. HS: 4.1 kgs/day) ending the chamber exposure period at half that of E- animals (Figure 5.4; 8.3 vs. 4.1 kgs/day). The transition from TN to HS environments produced a rapid increase in

RR ($P < 0.01$), with E+ animals being approximately 25 bpm and E- approximately 36 bpm higher than the last day at TN (Figure 5.4a). Animals in the E+ group maintained a higher RR than E- animals for the first 2 days of HS ($P < 0.05$; 89 versus 82 bpm). However, RR for E+ and E- animals came together as HS continued, with no significant difference after Day 9 ($P = 0.12$; Figure 5.4a). While E+ animals had the higher rate during early HS, it was interesting to note that E- animals showed the greater increase from TN to HS. As during the TN period, a circadian rhythm was present, but followed the T_a cycle, being lowest from 0600 to 2100 h and highest from 1300 to 1600 h, and suggesting that T_a may be the principle determinate for RR rhythms during HS. No treatment differences were found for circadian rhythm ($P = 0.34$).

Transition to HS resulted in a large increase in skin temperature, as expected, which was strongly associated with T_a ($R = 0.78$; Figures 5.5 and 5.6). Skin temperatures at the two different regions (trunk and appendage) showed no mean E+/E- treatment or regional differences during HS (T_{trunk} 37.9 versus 37.6°C; $T_{appendage}$ 37.1 versus 37.0°C; Figures 5.5 and 5.6). However, E+ animals displayed a significantly lower skin temperature (~0.3°C) for both regions at 0600 and 2100 h ($P < 0.05$; Figures 5.5 and 5.6). This decrease reduces the temperature gradient between the skin and air resulting in a decreased ability to dissipate heat. During the peak heat of the day, this vasomotor response is masked resulting in no mean skin temperatures differences, however E+ animals showed the greater change throughout the day, with approximately a 2.3°C daily increase between 0600 and 1600 h ($P < 0.05$; approximately 1.3°C for E- animals; Figures 5.5 and 5.6).

Similarly to RR and T_{skin}, there was an increase in T_{re} during the transition period for both treatment groups, with E+ animals showing the greatest increase ($P < 0.001$; 1.2°C E+ animals versus 0.7°C E- animals from TN; Figure 5.4a). The E+ animals maintained the higher T_{re} throughout the first 5 days of HS ($P < 0.01$). In contrast to E+ animals which displayed a large increase on the first day of HS, E- animals showed a gradual increase in T_{re} throughout HS ($P < 0.05$). Like RR, both treatment groups increased to the same level of heat strain (in terms of T_{re}) during HS, however not until Day 13 (3 days after RR; Figure 5.4a). As during TN, a visible circadian rhythm was present with temperature being the lowest from 0600 to 1100 h and highest from 1600 to 1900 h. Despite the T_{re} differences between treatments, the circadian rhythm was similar suggesting T_a was the major determinate of the rhythm (Figure 5.4b).

Ruminal temperature displayed a smaller increase during transition to HS compared to T_{re} ($P < 0.01$; approximately 0.4°C less), and was not different between treatments ($P = 0.56$; Figure 5.7a). Ruminal temperature showed an increase throughout HS ($P < 0.05$), with no treatment differences (Figure 5.7a). Similarly to results found at TN, no visible circadian rhythm was found for T_{rum}. The T_{rum} - T_{re} difference decreased for both groups from TN to HS (0.75 versus 0.4 ± 0.1°C), with a large treatment effect. A small T_{rum} - T_{re} difference (0.26 ± 0.1°C) was seen in E+ animals, due almost entirely to the large increase in T_{re} with only a minimal change in T_{rum} during HS. In contrast, E- animals showed a significantly higher T_{rum} - T_{re} value (0.68 ± 0.1°C) than for E+ animals ($P<0.05$) due to a smaller increase in T_{re} and a similar increase in T_{rum} (Figure 5.7a). However, due to the increase in T_{re} for E- animals throughout HS, the T_{rum} - T_{re} difference decreased to as low as 0.45 ± 0.1°C as HS continued. Ruminal temperature

showed a strong correlation with T_{re} for both treatment groups (E- R = 0.90, E+ R = 0.86; Figure 5.7b). While E- animals showed the greater correlation, both treatment groups showed a similar slope for T_{re} versus Ta (1.13 °C versus 1.12 °C T_{re}/°C T_{rum}), suggesting the relationship is the same under these conditions (Figure 5.7b).

4.2.3. Temperature Relationships

Temperature relationships during the shift from TN to HS was analyzed in greater detail to study the early changes between E+/E- treatment groups. Correlation coefficients for E+ animals were higher for all variables with exception of RR. Respiration rate was highly correlated with T_a (R = 0.88), T_{re} (R = 0.82), T_{trunk} (R = 0.88), and $T_{appendage}$ (R = 0.86), regardless of treatment. Ruminal temperature showed the poorest correlations with all other variables regardless of treatment. This low correlation only occurred during the transition period, with high T_{rum} correlations for E+ animals throughout the rest of the chamber exposure (R = 0.86). On the other hand, T_{rum} for E- animals showed a good correlation with T_{re} during all chamber exposure periods (R = 0.82).

As during the field exposure, a lag in T_a was analyzed to determine if it would improve the linear fit. Respiration rate showed a no improvement regardless of treatment when adding a lag in T_a (R = 0.88 versus 0.89). Rectal temperature, however, showed the greatest improvement when adding a 3 hour lag effect with T_a (E- R = 0.76 versus 0.82; E+ R = 0.83 versus 0.87). Ruminal temperature showed the greatest improvement when a 3 hour lag effect was added to the analysis for both treatment groups (E- R = 0.57 versus 0.69; E+ R = 0.75 versus 0.83). A lag of 1, 2, 3, 4, and 5 hours were analyzed with a 3 hour lag showing the greatest improvement in correlation coefficient.

Thermoregulatory responses between the beginning (Days 8 to 10; early heat) and end of HS (Days 12 to 14; late heat) were compared to assess short-term acclimation (Figs 6a and 6b). Linear regression was used to determine the change in response of RR, skin temperature, T_{re}, and T_{rum} to T_a between Early and Late Heat periods. Respiration rate during the Early Heat period showed similar treatment slopes (E-: 1.38, E+: 1.35), with E+ animals maintaining the higher rate (Figure 5.8). During the Late Heat period, E+ showed a downward shift in the Y-intercept; however, the slope was similar to the Early Heat period suggesting the response to temperature change is the same. The E- animals showed a shift in both the slope and Y-intercept for RR during the Late Heat period. This response suggests that the E- animals have a greater response to a change in T_a than during the Early Heat period (Figure 5.8). Rectal temperature was poorly correlated with T_a. Similarly to RR, E- animals showed a shift upward in y-intercept and slope during Late Heat for T_{re} (Figure 5.8). The E- and E+ animals showed a large difference in Y-intercepts during the Early Heat period, but displayed a shift during the Late Heat period. The E+ animals showed a downward shift and E- animals exhibited an upward shift. Surprisingly, both treatment groups showed an increase in the responsiveness to change in T_a during the Late Heat period (Figure 5.8). There was no difference for skin temperature of both trunk and appendage regions or between Early (Days 8 to 10) and Late Heat (Days 12 to 14) periods (not shown). Ruminal temperature showed similar treatment differences and shifts between Early and Late Heat periods as found for T_{re} (not shown).

4.2.4. Chamber vs. Field Comparison

Late Heat values were used for comparison with field data, since it was the more stable period compared to the first 4 days of HS in the chambers. Only T_a, and not T_{bg}, was used for comparison with T_{rum} and RR, since T_{bg} in the chambers would be equal to T_a. Linear regression for RR and T_a under field conditions had an R of 0.76, with a slope of 3.4 bpm/°C and a y-intercept of -14.97°C. Under chamber conditions, the line fit was similar (R = 0.75), however, the slope was less than half that of the field exposure (slope = 1.74 bpm/°C), with a y-intercept of +32.65°C. A similar linear fit was performed to evaluate similarities for T_{rum} versus T_a. Ruminal temperature showed similar correlation coefficients with T_a during the 2 exposures (Field R = 0.31; Chamber R = 0.34). However, unlike RR, which showed very different responses in the field environment, T_{rum} displayed a similar slope (Field 0.03 °C T_{rum}/°C T_a; Chamber 0.02 °C T_{rum}/°C T_a) and y-intercept (Field 38.7°C; Chamber 39.6°C) for both exposures.

4.2.5. Blood parameters

Several blood analyses, including albumin, globulin, potassium total protein, and triglyceride showed no treatment or thermal effects ($P \geq 0.10$). Two blood parameters (urea nitrogen and chloride) exhibited HS-induced increases with no treatment differences ($P < 0.01$). Alkaline phosphatase, cholesterol, creatinine, and sodium showed both treatment and HS effects. The E+ animals showed a lower alkaline phosphatase than E- animals ($P < 0.01$; 53.4 vs. 79.5 ± 6.4 U/L). In addition, alkaline phosphatase decreased from TN to HS periods ($P < 0.001$; 78.1 vs. 54.8 ± 5.3 U/L). Cholesterol showed a trend for E+ animals having lower values than E- animals ($P < 0.10$; 42.2 vs. 49.1 ± 2.6 mEq/L) and being significantly lower during HS ($P < 0.01$; 49.1 vs. 42.1 ± 2.1 mEq/L). Like cholesterol, E+ animals tended to have the higher creatinine levels (P <

0.10; 1.58 vs. 1.40 ± 0.07 mg/dL), however, HS induced an increase in levels unlike the previous two variables ($P < 0.05$; 1.42 vs. 1.57 ± 0.06 mg/dL). Unlike other blood parameters, E- animals showed the higher sodium levels ($P < 0.05$; 140.6 vs. 138.8 ± 0.45 mEq/L). Serum sodium levels decreased during HS ($P < 0.05$; 140.5 vs. 138.9 ± 0.43 mEq/L). Both CPK and calcium showed treatment differences with E+ animals have lower values ($P < 0.05$), but no HS differences ($P \geq 0.21$). Prolactin showed a significant treatment difference, with E+ animals being lower than E- animals ($P < 0.05$; 16.0 vs. 25.1 ± 3.1 ng/ml). Surprisingly, prolactin showed no HS effects ($P = 0.33$; TN: 18.0 vs. HS: 22.4 ± 3.3 ng/ml).

5. DISCUSSION

The thermal environment (i.e. a combination of air temperature, radiation, wind, precipitation, and relative humidity) is widely considered the most important factor affecting animal production. With so many combinations of these variables, it is difficult to predict how cattle will respond to stressful conditions. When confronted with wide differences in ambient variables, cattle must compensate by altering behavior, heat gain or loss, and feed intake. Seasonal changes also have an influence on production in cattle. Dry matter intake can be more than 20% greater in winter than summer in some cases (Kreikemeier and Mader, 2002). As the temperature cools off at the end of the summer, heat acclimation gained throughout the summer begins declines. Very few studies have followed animals under pasture conditions as the summer temperatures drop. The large swings in temperature at the end of the summer can be stressful for animals that are gaining hair coats and preparing for cooler temperatures. The same responses are found with regard to fescue toxicosis

As found in the previous chapter (Chapter 4), respiration rate under field conditions was not different between the E+ and E- treatments. This is not surprising as ambient temperatures were well within the thermoneutral range for most of the periods. Period 4 showed the highest respiration rate as well as the highest THI; however the stress level only reached alert levels. It is likely that the ambient temperatures to which the cattle were exposed were not stressful enough to produce the increase in RR normally found with fescue toxicosis. Respiration rate is extremely easy to measure and is a sensitive indicator of HS. It also exhibits a reliable relationship with air temperature and core body temperature (Scharf et al., 2010). In the current study, RR correlations core temperature ranged from R= 0.52 to 0.75, suggesting it is a sensitive indicator of heat stress consistent with previous research (Hahn, 1999; Eigenberg et al., 2000). If RR is effective enough to dissipate all the heat gained, then core body temperature will not increase. Since RR was never increased above 100 bpm or alert level (Mader, 2003) and there were no differences between treatments; it would suggest that core body temperature would not be different either. As expected ruminal temperatures were not different between E+/E treatment groups. Using multiple data sets (only 0800 and 1500 h, 0700 to 1800 h, and all 24 hours), showed a tendency for E+ animals to have a lower T_{rum} during the morning hours and a higher T_{rum} than E- animals during the peak hours of the day. This larger swing in core body temperature is possibly due to an inability to control heat dissipation due to increased peripheral blood flow (Strickland et al., 2009).

Because ambient temperature was not at a high level of stress, it is not surprising that the traditional signs of fescue toxicosis were not found in this study. However, there is no way to tell, under pasture conditions, if the animals have adapted to the endophytic

toxins or if the ambient temperature was masking the effects. Knowing that heat acclimation can alleviate some of the adverse effects found after consumption of E+ (Strickland et al., 2009), it is possible that heat acclimation was lost due to the cool temperatures, they would become more sensitive to the toxin. Therefore a controlled heat challenge was given to see if the animals had adapted to the endophyte or if the ambient conditions were the reason for the responses found under field conditions. In addition, during the heat challenge, we provided a larger dosage of the toxin (i.e., ergovaline) to potentially increase symptoms of fescue toxicosis and induce evidence of change. The chamber study also allowed for measurement of other notable symptoms of fescue toxicosis that include decreased feed intake and increased peripheral vasoconstriction with a change in skin temperature (Strickland et al., 1993; Paterson et al., 1995).

During the chamber challenge, feed intake quickly decreased more than 50% in the E+ animals even under thermoneutral conditions. Reduced feed intake has been reported in a number of animals and studies (Hannah et al., 1990; Fiorito et al., 1991; Redmond et al., 1991; McCann et al., 1992). Feed intake reductions are found in the literature, ranging from 22% to more than 60% depending on the thermal conditions (Aldrich et al., 1993). Unlike results found during the field exposure, RR was significantly higher for E+ animals even under thermoneutral conditions. During HS, E+ animals continued to have the higher rate, however it was short lived with E- animals rising to the same level by the end of the chamber exposure. As stated above, RR is a sensitive indictor of HS, however the transient response found in this study may suggest it is not a good indicator of fescue toxicosis and may be is one reason why it may not have been possible to detect an RR difference in the field studies.

Skin temperatures showed no differences under TN condition or during the peak hours of the day during HS. However, during the evening hours when ambient temperatures were cooler, signs of fescue toxicosis were evident with E+ animals have a lower skin temperature. Non-evaporative heat loss such as radiant heat from the skin increases at night, which normally is the coolest time of the daily cycle (Watts 1977). This allows animals to dissipate more body heat resulting in a reduction in internal body temperature (Finch, 1986). Because non-evaporative heat loss depends on a temperature gradient, a higher skin temperature at night is an advantage to dissipate heat. It is well known that consumption of endophyte infected fescue results in peripheral vasoconstriction and that this leads to an increase in heat retention (Rhodes et al., 1991; Oliver, 2005). This heat retention could account for the increase in RR and T_{re} found during the study under heat stress condition. However, differences were also found at TN for RR and T_{re} despite a drop in feed intake and no differences in skin temperature. It has been suggested that vasoconstriction happens throughout the body suggesting that it may not only be heat loss away from the body that is the problem, but also moving heat throughout the body (Klotz et al., 2006; Klotz et al., 2007; Klotz et al., 2008). This could result in some of the notable changes found in T_{re}. However, more research is necessary to determine this.

Similarly to RR, rectal temperature in the current study was greater for E+ animals than E- animals. Typically no difference is found under thermoneutral conditions between treatments (Strickland et al., 2009). However, studies have reported increased rectal temperatures in steers grazing endophyte infected tall fescue, as well as infected tall fescue hay (Schmidt et al., 1982). It is possible that this increased core body

temperature is due to the large dosage of ergovaline provided in our diets. However, it is the same dosage used in previous experiments (Scharf et al., 2012; Spiers et al., 2012) where no differences were found. It is also possible that the animals perceived thermal comfort had changed to a lower temperature than 20°C. During transition to HS, E+ and E- animals remained different. The E+ animals showed a rapid increase in T_{re}, but surprisingly only a minimal increase in T_{rum}. Control E- animals displayed a steady increase in T_{re} throughout HS, and the same minimal increase in T_{rum}. The relationship between T_{re} and T_{rum} is not well known. However, results from this study suggest that T_{rum} may be a poor indicator of fescue toxicosis due to the lack of responses found during both the chamber and field studies. Only a few studies have determined the relationship between rectal and ruminal temperature, and no research has looked at the impact of fescue toxicosis (Prendiville et al., 2002; Dye et al., 2007). It is clear, that although T_{re} and T_{rum} are correlated, T_{re} is a much more dynamic responder to HS than T_{rum}. Differences were still detected between TN and HS exposures, making T_{rum} a prime indicator of a sensitive core temperature measurement the does not disturb the animal's natural behavior. Rumen temperature is believed to be controlled partially by heat of fermentation. However, the effect heat of fermentation on T_{rum} is not well understood (Beatty et al., 2008). It is known that rumen heat production accounts for as much as 8% of total heat production in the body (Czerkawski, 1980). Heat produced in the rumen creates a load that must be dissipated to the rest of the body, or else it contributes to an increase in T_{rum}. Because conductive heat loss remains relatively constant within the body, the major changes in heat removal from the rumen must be due to changes in blood perfusion (Beatty et al., 2008). This is believed to be the reason why different sites within

the core yield different body temperatures (Maloney et al., 2001). Since fescue toxicosis is known to accept vasoconstriction and blood flow, the differences between the rumen and T_{re} are similar compared to that of the E- animals. The E+ animals maintain this higher core body temperature despite the fact they have 50% of the feed intake of the E- animals. This may suggest that fescue toxicosis affects blood flow relative to heat production as well. This is a topic that warrants further study.

Serum prolactin is a very sensitive indicator of fescue toxicosis, with animals showing a rapid decrease in serum levels (Waller et al., 2009). In the present study, prolactin was decreased in E+ animals during both the TN and HS period which is consistent with previous research.

Serum prolactin is known to increase in response to an increase in air temperature (Schams, 1972), however in the present study prolactin showed little change between TN and HS. It is unclear why serum prolactin did not change with HS; however, serum prolactin level is known to be reduced as ambient temperature drops and the days become shorter (Dahl et al., 2000). Other signs of fescue toxicosis include a reduction in serum alkaline phosphatase (Schultze et al., 1999), triglyceride (Oliver et al., 2000), and cholesterol (Thompson et al., 2001). In the present study, alkaline phosphatase was reduced in E+ animals, as well as being reduced during transition from TN to HS, which is consistent with the literature (Schultze et al., 1999; El-Nouty et al., 1990). Triglyceride, on the other hand, has been shown to exhibit no treatment or heat stress differences. Serum cholesterol showed a trend for E+ animals to have reduced serum levels which is consistent with previous reports (Stuedemann et al., 1985). Cholesterol also showed HS differences, with HS having lower levels than TN conditions. Though not extensively

studied in cattle, it has been reported that cholesterol increases during heat stress (Brody, 1956). It is unclear why the present results differ from previous research. Serum creatinine is also used as an indicator of fescue toxicosis. In the present study, creatinine showed a trend to for E+ animals to exhibit an increase, which is consistent with previous research (Schultze et al., 1999; Oliver et al., 2000).

The present study measured two parameters (RR and T_{rum}) during both chamber and field environments to determine the difference in the responses. Respiration rate was measured between 2 and 6 times a day, while T_{rum} was measured 24 hours a day. A linear regression between RR and air temperature under field conditions yielded a slope of 3.4 bpm/°C and a Y-intercept of +14.97 °C. Chamber exposure yielded a very different response with a slope of 1.74 bpm/°C and a Y-intercept of +32.65 °C. These results suggest the chamber exposures underestimate the RR response to a change in air temperature. This cannot be definitively stated, as RR was measured only twice during the field exposure compared to 6 times under chamber conditions. However, it is not surprising that chamber measurements would differ, as RR is a very sensitive indicator of heat strain increasing as air temperature increases above 21°C (Gaalaas, 1945; Hahn et al., 1997). Since RR has a very high correlation with air temperature, as well as solar radiation which cannot be provided in the chambers, one would predict that RR would have a greater response under field conditions. It is interesting to point out that treatment differences were only found under chamber conditions, showing that RR responses to air temperature are not the only differences between the 2 exposures. Because core body temperature arises from the balance between heat loss and heat production, it seems logical that T_{rum} would be more consistent than RR between exposures. Ruminal

temperature was measured the same amount of times throughout both exposures. As expected, both field and chamber slopes were similar (0.03 °C T_{rum}/°C T_a versus 0.02 °C T_{rum}/°C T_a) and Y-intercepts (38.7°C versus 39.6°C). As long as the strain is not severe, T_{rum} which is an integrator of all the stressors should not change between environments. This is consistent with the data in the present study.

Most studies of the effect of fescue toxicosis on thermoregulation have been only a few hours or days, with few controlled long-term studies that would represent a more realistic scenario. The current study looked at both field and chamber exposures to develop a long-term study using both scenarios. This is a difficult task with long-term studies being more complex than short-term ones because they must consider the time element and the possibility of adaptations to heat and/or the toxins (Spiers et al., 2012). Additional studies are also needed to evaluate the relationship between ruminal temperature and rectal temperature, and the impact of an additional environmental stressor such as fescue toxicosis.

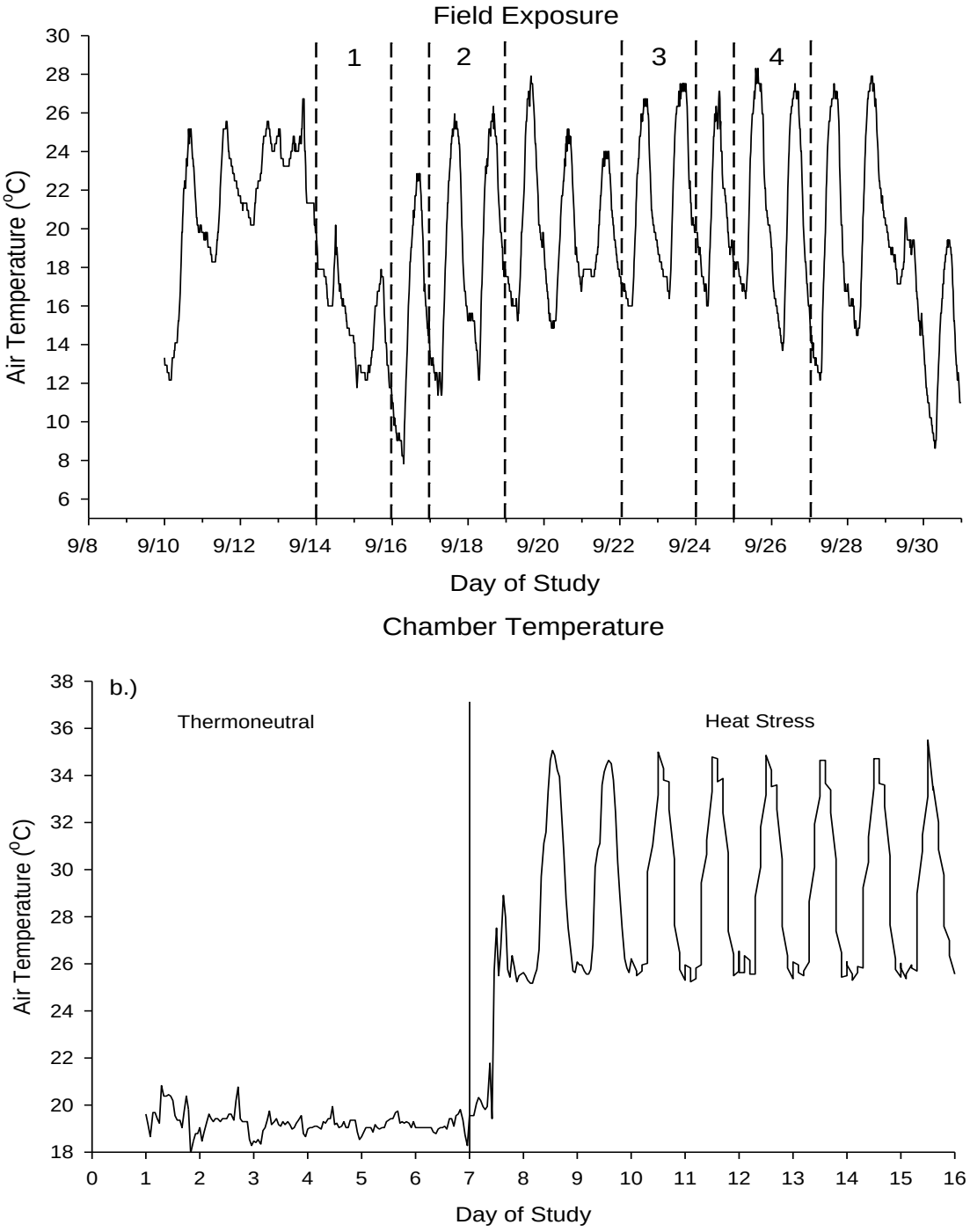

Figure 5.1 – (a) Hourly air temperature (T_a) of the field environment during the experiment. Dashed, vertical lines designate the 6 periods used for analysis. Numbers designate the 2 days within each. (b) Mean room air temperature beginning on Day 1 at thermoneutrality (19-21°C) and continuing through Day 14 which was the last day of heat stress (Night: 26°C; Day: 36°C). Values were collected hourly for each day.

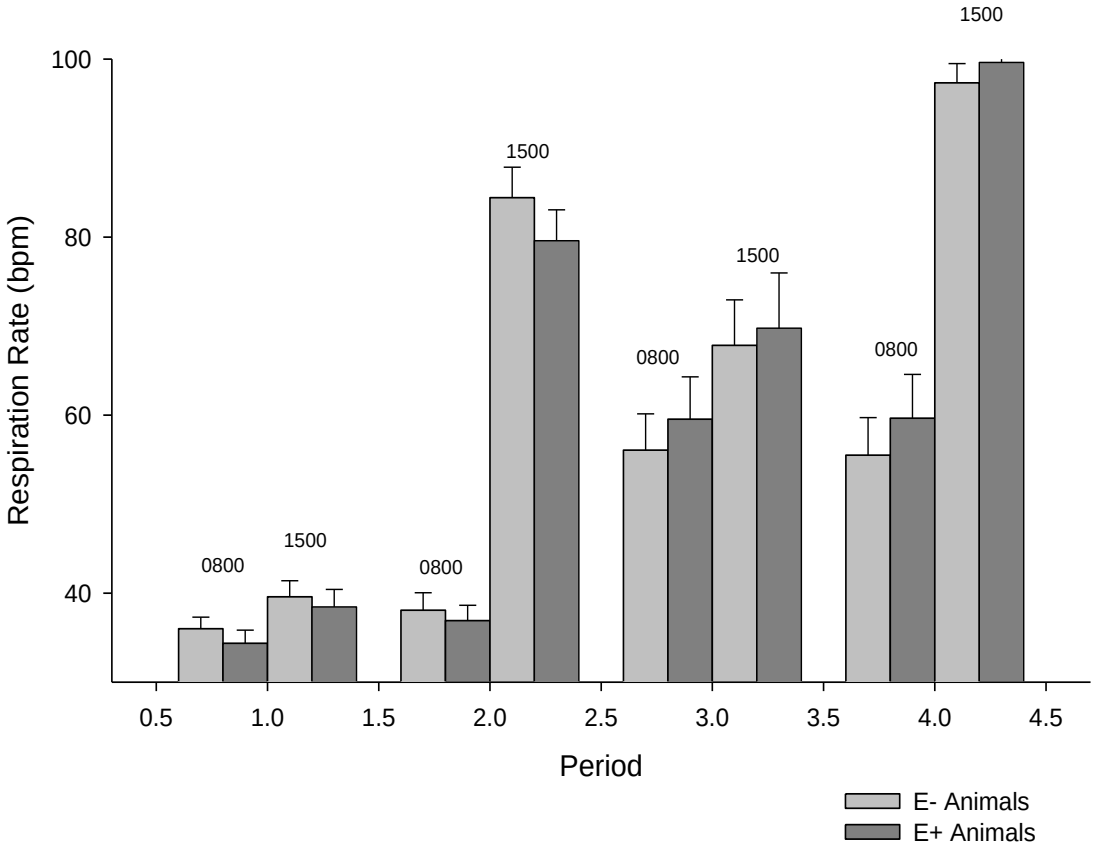

Figure 5.2 - Average values for respiration rate at 0800 and 1500 h are shown for each period. Dependent variables with the same letter over the bar are not significantly different within the variable. The vertical line on top of each variable bar is +1SEM.

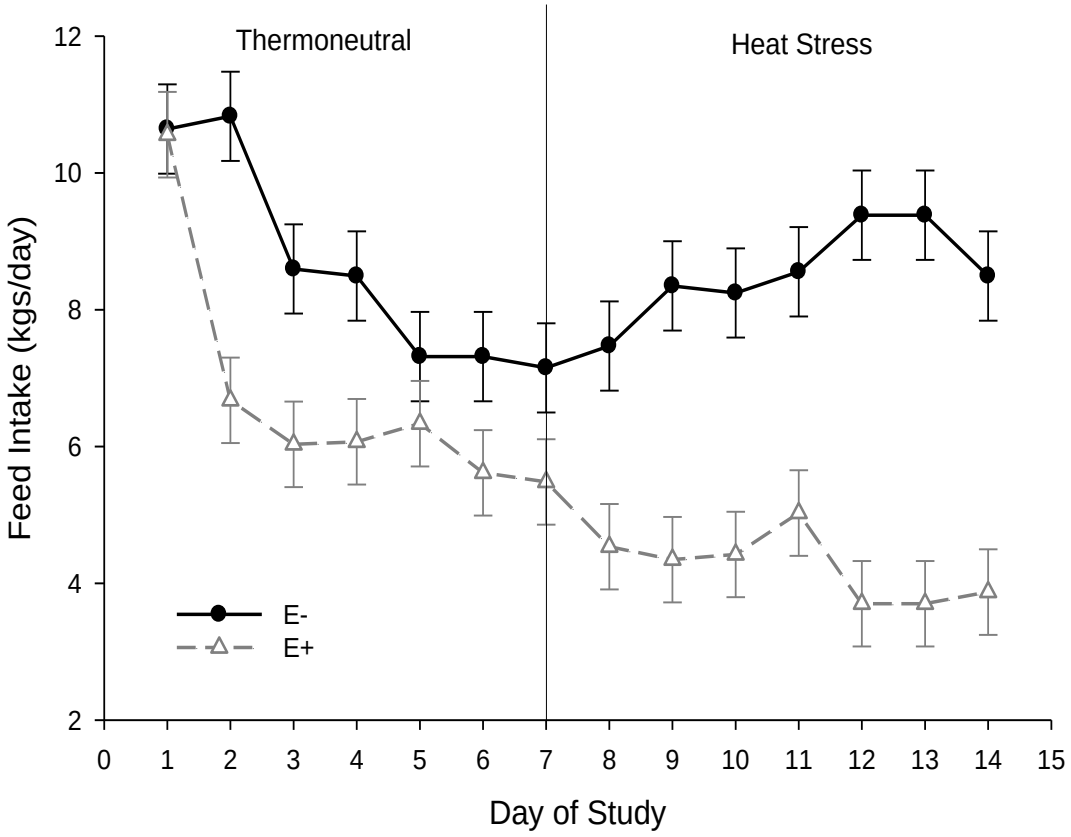

Figure 5.3 – Daily feed intake of E+ and E- steers is shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods. The vertical line on top of each variable bar is +1 SEM.

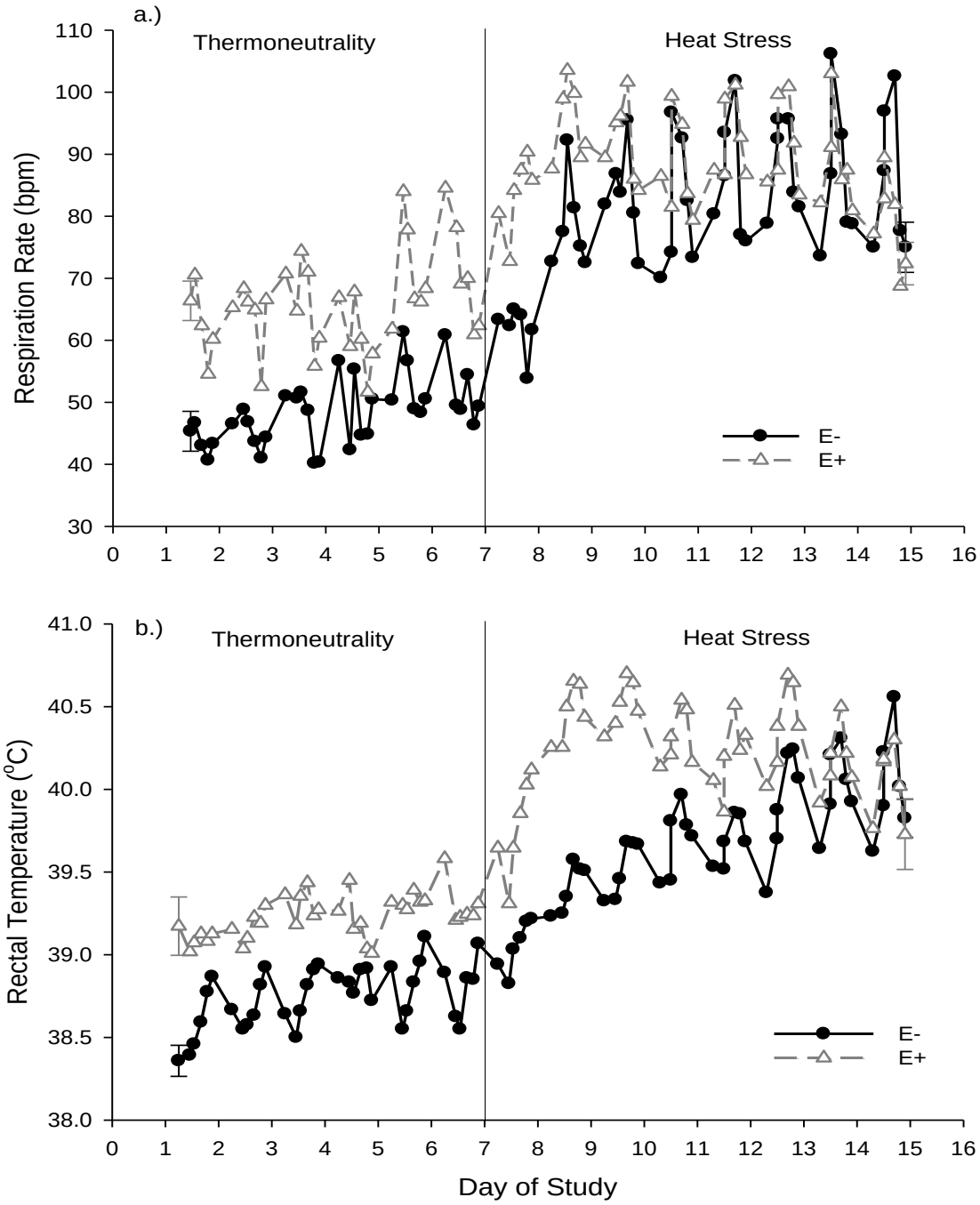

Figure 5.4 – (a) Mean respiration rate (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. (b) Mean rectal temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. The solid vertical line separates thermoneutral and heat stress periods.

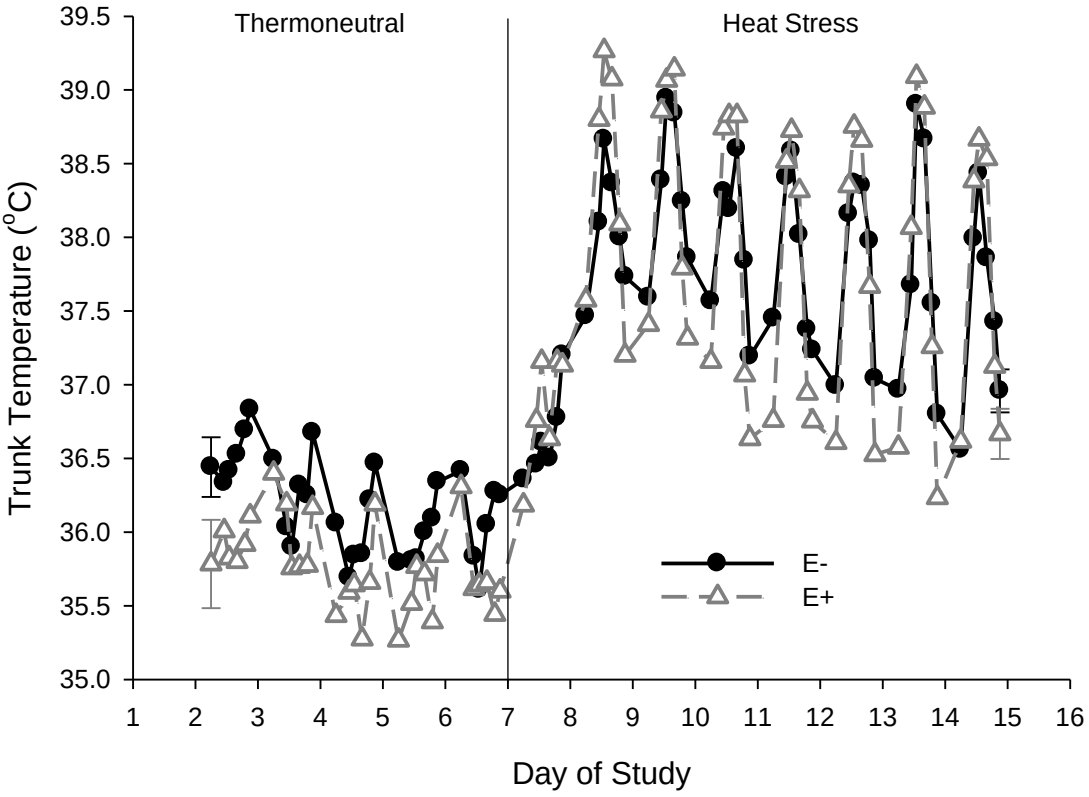

Figure 5.5 - Mean trunk skin temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. Trunk temperature represents a combination of shoulder and rump temperatures.

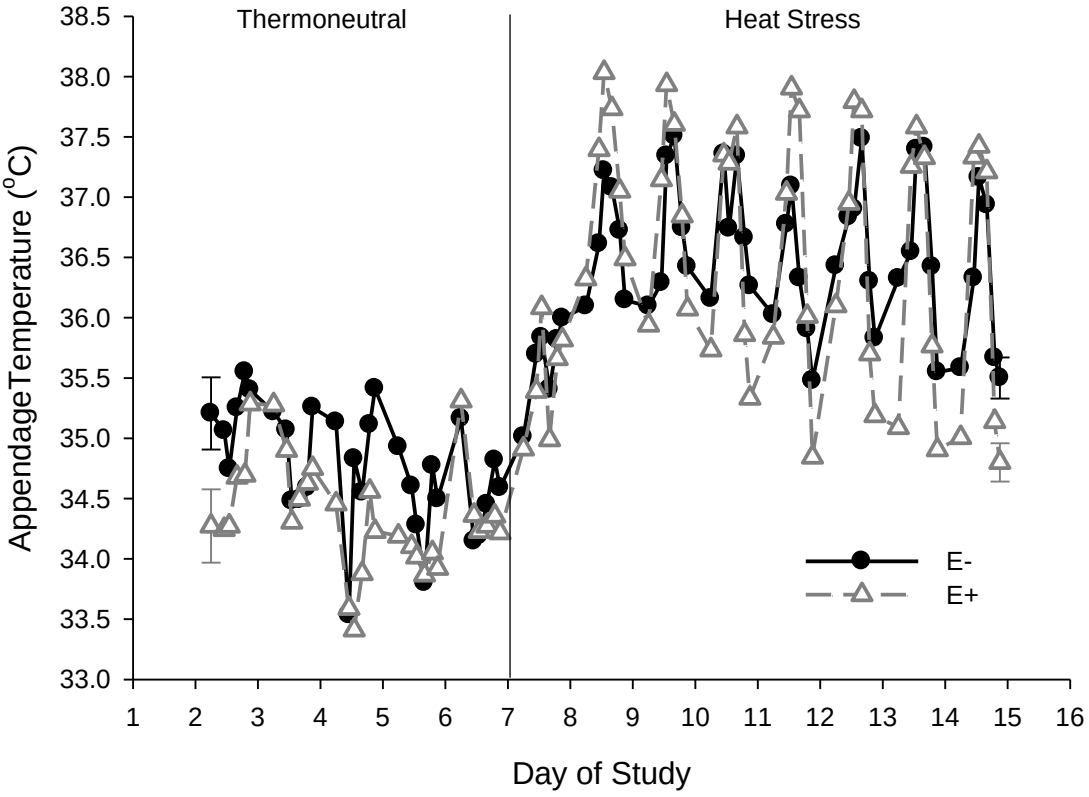

Figure 5.6 - Mean appendage skin temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. Appendage temperature represents a combination of ear, upper tail, and lower tail temperatures.

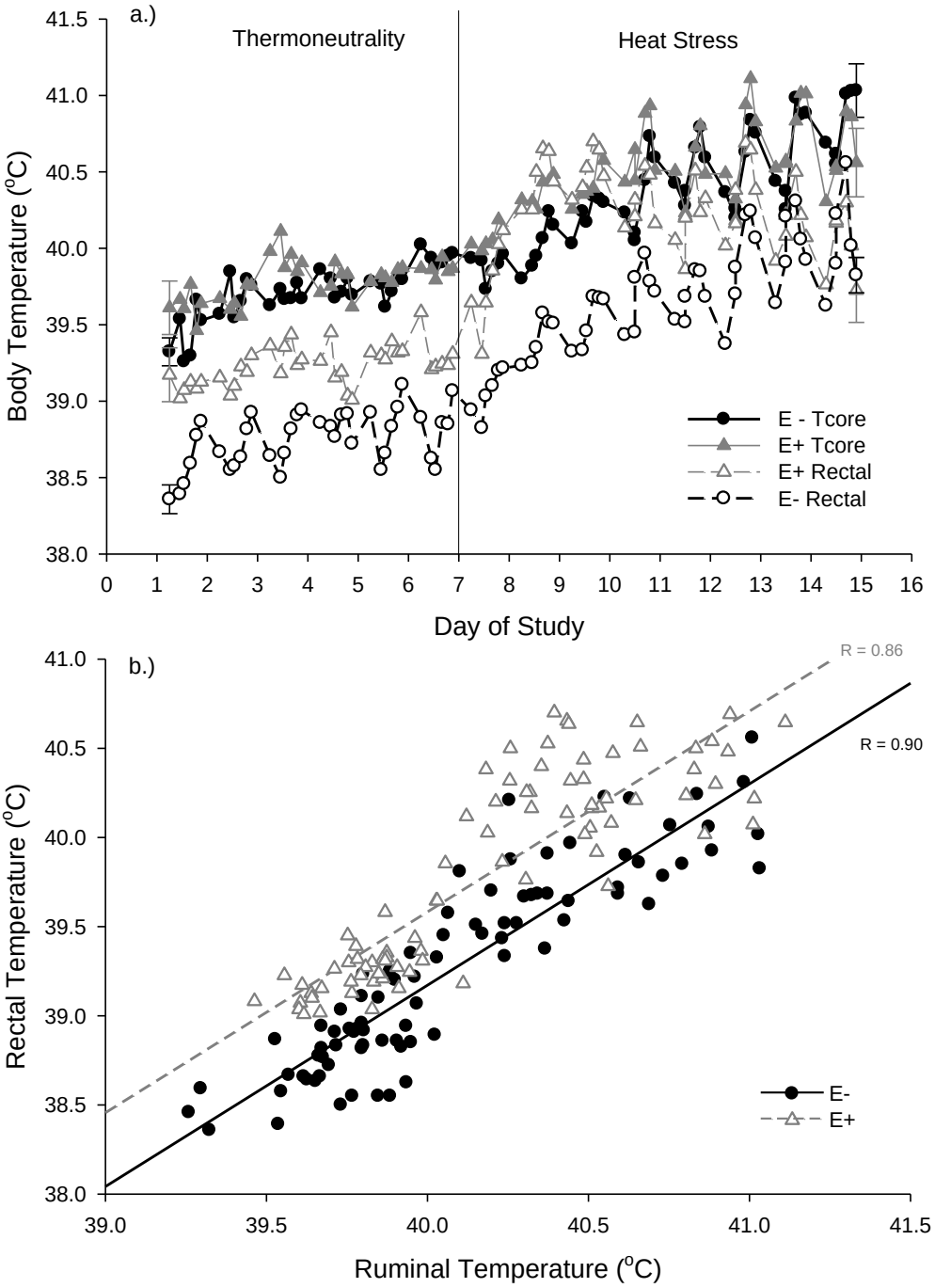

Figure 5.7 - (a) Mean ruminal temperature (± 1 SEM) of E+ and E- steers is shown as a function of time in days for the chamber exposure. All six sample times during a day are shown. The solid vertical line separates thermoneutral and heat stress periods. (b) Linear relationship of rectal temperature to ruminal temperature is shown using all data during the chamber exposure. Values used in the calculations are averages of the 6 time measurements for each animal over the 14 day exposure. Correlation of determination values are given with in the.

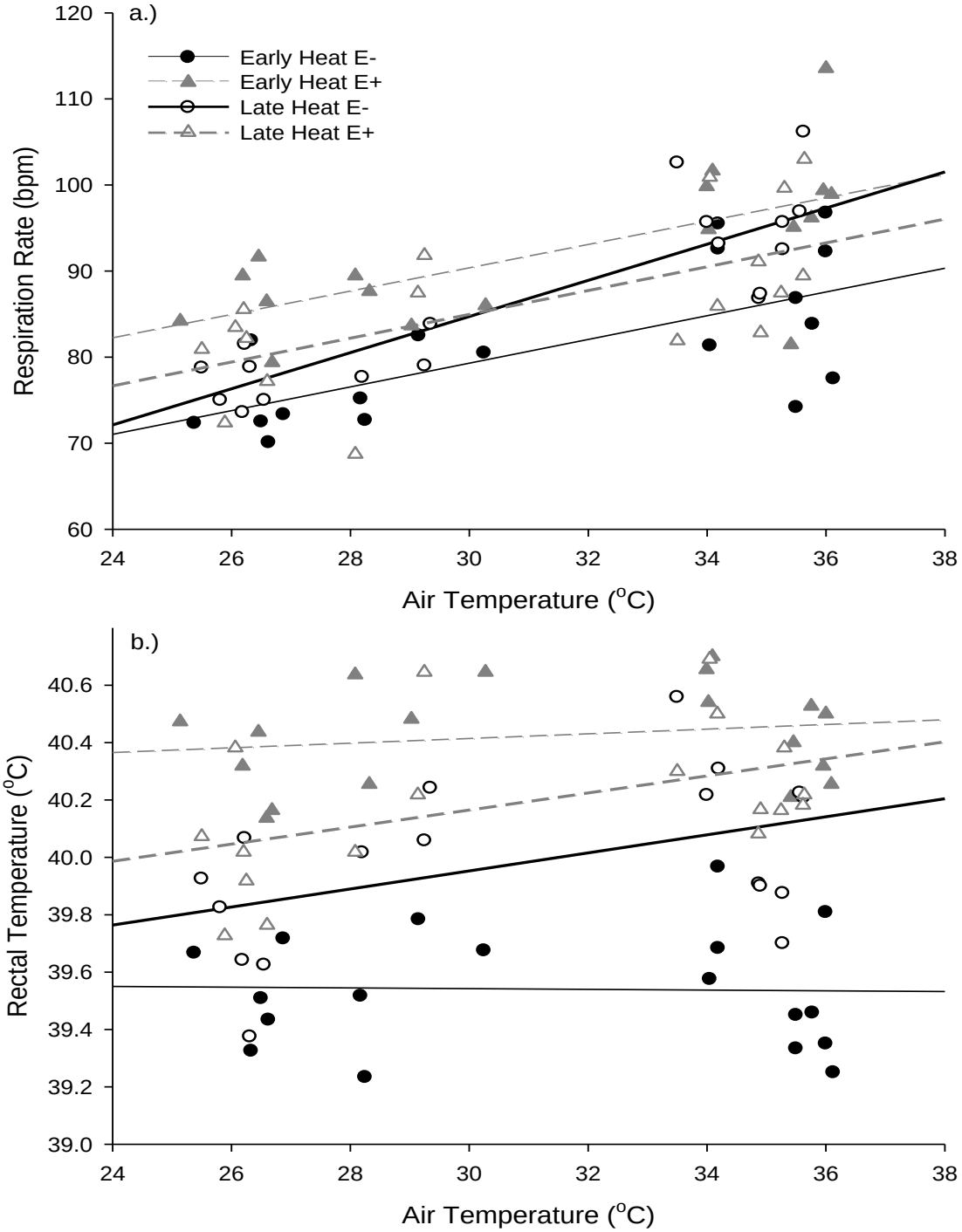

Figure 5.8 - Heat stress-induced changes in respiration rate (a) and rectal temperature (b) are shown for E+ and E- steers from early (i.e., Days 8 - 10) to late heat exposures (i.e., Days 12 -14). A linear fit is shown through points for each variable, treatment, and set of days (T_a: 26-36°C).

Table 5.1 – Group mean serum biochemistry values for cattle under thermoneutral and heat stress conditions. P-values given for Treatment (E+ vs. E-), Time (TN vs. HS), and Interaction (Treatment x Time)

Blood Parameters

					P Values		
	Treatment	*TN*	*HS*	*± SE*	*Treatment*	*Time*	*Interaction*
Albumin	E-	3.25	3.31	0.59	0.43	0.11	0.76
g/dL	E+	3.19	3.26				
ALP	E-	96.13	63	7.41	**0.001**	**0.001**	0.09
U/L	E+	60.11	46.72				
Calcium	E-	9.65	9.48	0.17	**0.05**	0.12	0.58
mg/dL	E+	9.37	9.02				
Chloride	E-	96.58	99.08	0.86	0.52	**0.001**	0.54
mEq/L	E+	96.84	100.09				
Cholesterol	E-	51.08	47.00	3.22	0.09	**0.01**	0.17
mg/dL	E+	47.14	37.27				
CPK	E-	107.50	87.41	9.62	**0.05**	0.11	0.52
U/L	E+	81.25	72.54				
Creatinine	E-	1.37	1.44	0.09	0.13	**0.05**	0.30
mg/dL	E+	11.48	1.69				
Globulin	E-	3.31	3.34	0.12	0.47	0.33	0.22
g/dL	E+	3.53	3.31				
Potassium	E-	3.97	4.09	0.12	0.09	0.08	0.38
mEq/L	E+	4.03	4.38				
Prolactin	E-	25.31	26.58	4.53	**0.05**	0.29	0.56
ng/ml	E+	12.22	16.49				
Sodium	E-	140.92	140.25	0.66	**0.05**	**0.05**	0.15
mg/dL	E+	140.08	137.63				
Total Protein	E-	6.57	6.63	0.12	0.69	0.58	0.3
g/dL	E+	6.73	6.57				
Triglyceride	E-	15.16	15.5	1.49	0.14	0.45	0.31
mg/dL	E+	18.79	16.45				
Urea N	E-	8.25	8.75	1.12	0.87	**0.01**	**0.05**
mg/dL	E+	5.69	11.63				

CHAPTER SIX

COMPARISON OF FIELD EXPOSURES AND CHAMBER STUDIES TO DETERMINE ADAPTIVE CHANGES IN ANGUS CATTLE

1. INTRODUCTION

The objective of the present studies was to determine if exposure to the summer environment would result in adaptation of cattle to heat stress and/or fescue toxicosis (e.g., lower core temperature, respiration rate, and sweat rate). It is well-known that some cattle respond very differently during exposure to environmental variables, such as ambient temperature and relative humidity. Part of this variation may be attributed to differences in their adaptation to heat. Homeotherms acclimate to heat by altering physiological or behavioral mechanisms which reduce the strain. It is not known how cattle adapt to heat stress, with the addition of a second stressor such as water restriction or fescue toxicosis. It has been suggested that acclimation to heat stress will result in a cross-adaption that will improve the performance of cattle on fescue toxicosis. It has also been suggested that long-term exposure to ergot alkaloids will result in an animal becoming resistant to the toxins.

The collections of studies reported in this dissertation were designed to see if heat acclimation and repeated exposure to the endophytic toxins would improve the performance of cattle on tall fescue. To study this, experiments using both environmental chambers and field exposures were created. Controlled chamber runs are ideal for repeatedly measuring many variables under the same conditions to look at acute adaptation. Unfortunately, these studies do not recreate the "real-world." Therefore, field

exposure periods were placed in-between chamber exposures in several of the experiments described in this dissertation. While field exposures contain less frequent measurements and inconsistent environmental conditions, they offer the ability to look at chronic long-term exposure in the animal's natural environment. The goal of any chamber or field experiment is to be able to transfer information from exposure to another condition; however, how similar the responses are under each situation still need to be studied. Therefore, a second goal of these studies was to determine how similar the thermoregulatory responses of cattle are under a field exposure, followed directly by a controlled chamber run under similar conditions.

2. CHAPTER THREE: ACCLIMATION OF ANGUS STEERS TO LONG-TERM HEAT STRESS IN THE FIELD USING CONTROLLED HEAT CHALLENGE - SUMMARY

The purpose of this experiment is to study the long term-effects of both heat stress and the grazing of endophyte infected pasture during the summer months. Four treatment groups were used to determine if: 1) there would be improved overall performance to heat at study end, 2) placement on E+ pasture would improve responses to E+ under controlled conditions, 3) placement of E+ pasture would alter the animals response to heat stress alone, and 4) summer heat acclimation would alter the animal's response to an E+ challenge.

A reduction in feed intake proved to be a reliable indicator of fescue toxicosis with both the E+/E+ and E-/E+ groups, having a lower intake than E- groups during heat stress. However, only the E+/E+ group was significantly different from the E-/E- group. The E-/E+ group was only numerically lower suggesting that summer heat stress may have aided them in their response to the E+ seed during POST. All groups increased

respiration rate during transition to heat stress with no treatment differences. No differences were found during the field exposure, suggesting respiration rate may not be a reliable indicator of fescue toxicosis. Rectal temperature showed no treatment differences under thermoneutral conditions in the second chamber trial, however, transition to heat stress resulted in both E+ groups (E+/E+ and E-/E+) having higher rectal temperature than the E- groups (E-/E- and E+/E-). Interestingly, by the end of the trial (~day12), all treatments came together resulting in no differences in rectal temperature. Of the parameters measured, sweat rate at both the shoulder and rump regions showed the greatest treatment response. Similarly to rectal temperature, there were no treatment differences during thermoneutral conditions. Transition to heat stress resulted in a large increase for both shoulder and rump regions only for the E-/E- and E+/E- groups. The groups receiving E+ seed (E+/E+ and E-/E+) did not increase above thermoneutral levels which could result in the rectal temperatures responses shown in the present study.

Results from this experiment showed only a few signs of adaptation. With exception of feed intake, animals in the two groups that switched (E-/E+ and E+/E-) treatments responded to the current diet rather than previous exposure suggesting no adaptation to the toxin. Feed intake was lower for all treatments during the final chamber run which could signify acclimation to heat stress. Sweat rate showed the greatest change between chamber tests, as well as within chamber runs with a reduction after several days in the heat. This reduction occurred even though rectal temperature and respiration rate were still elevated, suggesting that reduction of sweat rate, and possibly water loss, is more important than reduction of body temperature during heat stress. Further research is

necessary to study the short-term heat response and adaptation of sweat rate overtime in regard to heat stress and fescue toxicosis.

3. CHAPTER FOUR: PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS DURING THE PEAK OF SUMMER – SUMMARY

This chapter represented a similar type of experiment utilizing both the field and chamber to look at acclimation to heat. However animals were placed on pasture prior to a chamber run and moved to the controlled environment during the peak of summer. Both endophyte-infected and endophyte-free pastures and seed were used however; no groups switched treatments as in Chapter 3. While the study was conducted to continue to look at heat acclimation and the responses to the endophytic toxins; the study also focused on determining the similarities and differences in responses between the two environments with the goal to be able to transfer information from exposure to another.

Results from the field exposure showed no symptoms of animals having fescue toxicosis in terms of increased respiration rate or ruminal temperature over E- animals. In fact, E- animals tended to have higher ruminal temperature than E+ animals during the peak hours of the day during the field exposure. During the chamber exposure, a reduction in feed intake was found with E+ animals showing a decrease feed intake starting on the second day of the chamber exposure which was further reduced during heat stress equaling about half as much as E- animals. Respiration rate only showed treatment effects during the first 3 days of heat stress, with E+ animals having the greater rate. However, it was short lived with E- animals rising to the same level by the end of the chamber exposure. This result possibly explains the lack of a difference found during the field exposure. Daily skin temperature showed no treatment differences throughout

the study. However during the evening hours when ambient temperature was low (~26°C), E+ animals showed a significantly lower skin temperature, possibly due to vasoconstriction. This reduction in skin temperature would result in a lower gradient between the skin and air temperature suggesting a reduced ability to dissipate heat. Overall rectal temperature in the current study, at thermoneutrality or during late heat stress, was not different across treatments. During the early heat stress period, however, E+ animals showed a rapid increase in rectal temperature, but surprisingly only a minimal increase in ruminal temperature. Control animals (E-) displayed a steady increase in rectal temperature throughout heat stress, and the same minimal increase in ruminal temperature. This suggests, first, that ruminal temperature is a poorer indicator of fescue toxicosis, and second that rectal temperature is a much greater responder to heat stress than ruminal temperature.

Relationships between respiration rate, ruminal temperature, and air temperature were used to compare between field and chamber exposures. Correlation coefficients for respiration rates were similar during both chamber (R =0.69) and field exposures (R = 0.72). Respiration rate showed greater responsiveness to change in T_a under field conditions having twice the slope in the chamber test (4.4 versus 1.75 bpm/°C) and a lower Y-intercept (-42.14 versus +30.97 °C) compared to the chamber run. Ruminal temperature was consistent between exposures showing a similar slope (0.04 °C versus 0.03°C T_{rum}/°C T_a) and Y-intercept (38.4 versus 39.3°C) for its relationship with air temperature. These results suggest that chamber studies underestimate respiration rate during heat stress with field exposures showing a higher respiration rate at the same air

temperature. Whereas, ruminal temperature (i.e., an integrator of heat gain and heat loss) proved to be more consentient between exposures.

4. CHAPTER FIVE: PHYSIOLOGICAL RESPONSES OF CATTLE TO MULTIPLE ENVIRONMENTAL STRESSORS DURING THE END OF SUMMER – SUMMARY

The experimental design for this study was similar to that in Chapter 4. The difference being that this experiment was conducted at the end of the summer when animals have lost some of the heat acclimation gained throughout the summer. It has been suggested that cattle are more sensitive to the effects of fescue toxicosis at the end of the summer when temperatures are cooling off. While cattle are known to demonstrate the ability to adapt to increasing heat during the middle of summer, how much of the acclimation carries-over to a different thermal environment is still unknown.

As found in Chapter 4, respiration rate under field conditions was not different between the E+ and E- treatments. Similarly, ruminal temperature also showed no treatment differences throughout the field exposure. Using multiple data sets (0800 and 1500 h, 0700 to 1800 h, and all 24 hours), there a tendency for E+ animals to have a lower ruminal temperature during the morning hours and a higher ruminal temperature than E- animals during the peak hours of the day. This is possibly due to behavioral changes such as increased time under shade or decrease foraging or could be due to an alteration of the circadian rhythm of E+ animals. Because ambient temperature was not at a high level of stress, it is not surprising that the traditional signs of fescue toxicosis were not found in this study. Knowing that heat acclimation can alleviate some of the adverse effects found after consumption of E+ (Strickland et al., 2009), it is possible that as

animals lose their heat acclimation due to the cooler temperatures, they would become more sensitive to the toxin.

During the chamber challenge, feed intake quickly decreased more than 50% in the E+ animals even under thermoneutral conditions. Unlike Chapters 3 and 4, respiration rate was significantly higher for E+ animals under thermoneutral conditions. During heat stress, E+ animals continued to have the higher rate, however it was short lived with E- animals rising to the same level by the end of the chamber exposure. Skin temperatures showed no differences under thermoneutral condition or during the peak hours of the day during heat stress. However, similar to results found in Chapter 4, during the evening hours when ambient temperatures were cooler, signs of fescue toxicosis were evident with E+ animals have a lower skin temperature. Rectal temperature, like respiration rate was greater for E+ animals than E- animals under thermoneutral conditions. During transition to heat stress, both E+ and E- animals showed an increased rectal temperature but remained different. Interestingly, while the respiration rate and rectal temperature showed significantly different response from what was reported during Chapter 4, the response to heat stress was very similar between experiments. Animals increased approximately the same amount during initiation of heat stress; the difference was the starting and ending points. This could suggest that animals are regulating there rectal temperature at a different set-point than they were during Chamber 4. It is also possible that the increased toxin level present to the animals versus the field exposure overloaded the response. Similarly to Chapter 4, ruminal temperature showed no treatment differences and very little response to heat stress again suggesting that it is a poor indicator of fescue toxicosis.

5. CONCLUSION

Finding animals that are fescue tolerant has been the focus of research for a number of years. It is possible that there are animals within the population that are less responsive to the toxic effects of fescue, however identifying those animals is difficult since there is no diagnostic test is available. Due to this, our focus was to see if heat acclimation and repeated exposure to the endophytic toxins would improve the performance of cattle on tall fescue. Results throughout this dissertation however, showed little evidence that repeated exposure to the endophytic toxins gives animals a tolerance to the endophytic toxins. Feed intake, rectal temperature, sweat rate, and skin temperature responded similarly for E+ animals suggesting a lack of adaptation. However, heat acclimation did show some differences. When E+ animals were brought in during the peak of summer and tested, the endophyte responses were reduced compared to a similar challenge at the end of the summer. A similar response was found to a lesser degree in the E- animals. Sweat rate in particular showed a decreased response in acclimated animals as well as a decrease after several days in the heat. Sweat rate in the E+ animals was completely stopped from increasing. While, loss of heat acclimation is not well studied, results from the above experiments suggest that sweat rate may play a key role in determining an animal's ability to adapt to the changing environment.

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VITA

Bradley A. Scharf was born in Indianapolis, Indiana on March 15, 1983. At the age of 5, his family moved to Millstadt, Illinois, a small farming town just across the river from St. Louis, Missouri. He attended high school at Belleville West Township High school where he became interested in biology and animal sciences. After high school, Brad decided to attend the University of Missouri where he could pursue a degree in animal sciences and possibly go on to veterinary school. While working toward his bachelor's degree he worked during several summers in a PCR laboratory which began his interest in research. Brad completed his bachelor's degree at the University of Missouri in May 2005. While completing his final semester at the university, he was given the opportunity to be a teaching assistant for a class on the physiology of domestic animals. During this class, Brad met Dr. Don Spiers who gave him the opportunity to work in his laboratory for a Master's degree. He received his Master's degree in December 2008. Brad decided to continue on at the University of Missouri for his PhD in Dr. Spiers laboratory to research the effects of long-term exposure of cattle to heat stress and fescue toxicosis. He actively participated in numerous scientific meetings and is author of several scientific abstracts and peer-reviewed publications. Brad received the Doctor of Philosophy degree from the University of Missouri in July 2012. In December of 2011, Brad wrote a grant to obtain a post-doctoral fellowship from the USDA to continue his research. He was awarded the USDA post-doctoral fellowship in September 2012 where he will continue to work the area of stress physiology in the development of a smartphone application to identify, monitor, and ameliorate heat stress in beef and dairy cattle.