THE EFFECTS OF EXTENDED WATER RESTRICTION ON THE HEMATOLOGICAL CELL DISTRIBUTION OF BEEF CATTLE IN CONFINEMENT

By

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Abstract: Climate change is likely to limit water availability and drought intensity in the future. The objective of this study was to assess the impacts of water restriction on the health of beef cattle. Four groups of cattle (n = 461) over the course of two years were water restricted with the use of the Insentec System. Baseline water intakes were calculated over a 70-day baseline phase, which was used to step animals down by 10% increments until animals were consuming 50% of their *ad libitum* intakes. Data collected included feed and water intake, blood samples, treatment records, respiration rates, and weather data. White blood cell (WBC) counts were higher during the restriction in the two winter groups in comparison to the summer groups ($P < 0.05$). The neutrophil: lymphocyte in the winter groups were also greater than the summer groups ($P < 0.05$). Hematocrit values were greater during the early restriction in all groups than during baseline ($P < 0.05$). Animals that had been treated at any point in the study had overall higher WBC than animals that were never treated ($P < 0.05$); however, there were no significant differences in hematocrit between the healthy and morbid animals. Animals that consumed higher amounts of water as percent of body weight had higher WBC than other intake categories while being stepped down ($P < 0.05$), but there was no difference during 50% restriction. While animals were able to handle the water restriction without increasing sickness, immunosuppression was evident at the 50% restriction and may leave animals more susceptible to increased illness.

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CHAPTER I

REVIEW OF LITERATURE

Climate Change

Global climate models have projected increases in atmospheric greenhouse gas (GHG) emissions over the next century (Rosenzweig et al., 2001). The GHG concentrations cause an overall rise in temperature and increasing variability and severity in weather patterns (Rosenzweig et al., 2001; Timmermann et al., 1999). Some of the changes predicted are the rise in global temperatures, an intensification of the hydrological cycle which increases the likelihood of floods and droughts, and an increase in winter rain rather than snow which decreases snow packs and intensifies spring and summer droughts (Rosenzweig et al., 2001). Small changes in mean temperatures can disproportionally increase the frequency of extreme weather events (Timmermann et al., 1999; Rosenzweig et al., 2001). Additionally, the fluctuation between the extremes can also negatively impact agriculture (Rosenzweig et al., 2001; Oleson and Bindi, 2002). For example, the constant back and forth between flooding and drought can cause erosion and lead to poorer soil quality (Rosenzweig et al., 2001). Many climate projection models have determined the type and extent of impacts these climate changes may have on crop production. A myriad of potential issues can arise such as increased pest populations due

to warm humid environments, and unsuitable conditions for crops in all phases of growth (Rosenzweig et al., 2001; Oleson and Bindi, 2002). While there are certain areas around the world that would not see the harmful effects of these climatic changes as severely, it is still unknown whether or not that scenario would only be short term (Rosenzweig et al., 2001). Schlenker and others (2005) determined that irrigated versus non-irrigated areas should not be treated as equivalent when assessing climatic impacts via simulation models, due to the differing climates for each technique. Land that is being irrigated is treated as such due to a lack of sufficient natural precipitation, whereas non-irrigated lands typically have adequate supplies of precipitation (Schlenker et al, 2005). This means that irrigated land could have substantially more costs associated with climate change than other models before have concluded from the additional inputs of irrigation water from groundwater or surface water (Schlenker et al., 2005).

In another facet of agriculture lies the livestock sector, and the impacts of climate change on this sector can also be detrimental. A simulation model by Baker et al. (1993) found that in the Southern Great Plains and California, the change in climate negatively impacted animal productivity. This decline in productivity was attributed to higher temperatures in those regions and decreases in forage quality (Baker et al., 1993). In another report, increased global temperatures led to impaired growth, meat and milk yield, and egg quantity and quality in livestock animals (Nardone et al., 2010). Additionally, hot environments negatively impacted reproductive performance and immune function (Nardone et al., 2010). More recently, Beach and others (2015) concluded that climate change is an overall negative stressor on U.S. agriculture. The authors predicted that while climate change will negatively impact livestock directly,

there will be an increase in forage availability on range and grasslands; however, that forage will likely have lower nutritional value for livestock (Baker et al., 1993; Beach et al., 2015). Additionally, as the climate changes the production of corn and other commodities used for grain fed operations may become limited and/or out of producers' price range (Beach et al., 2015). Therefore, if climate change continues at its current pace, producers may be forced to utilize extensive and forage based systems due to available commodities (Beach et al., 2015). Due to the modeled changes in climate, there may be an overall decrease in production efficiency and impacts on livestock well-being.

Cattle Health and Well-being

Animal Health and Climate Change

Climate change can potentially impact livestock production negatively in multiple ways; not only does it reduce the overall profitability of livestock production, but also the well-being of individual animals. While there is a deficiency in research explicitly looking at impacts of climate change on cattle health, many researchers believe there will be both direct and indirect effects (Nardone et al., 2010). Directly, extreme weather events typically increase morbidity and mortality (Belasco et al., 2015; Bishop-Williams et al., 2015; Maunsell 2015). Additionally, the direct effects of climate change include increased basal maintenance energy levels, decreased reproductive success, and decreased immune function. Indirectly, climate change can impact animals' ability to adapt to environments, influence microbial populations, and distribution of diseases and infections (Nardone et al., 2010).

Heat Stress

The increasing temperatures that are likely due to climate change can pose a significant threat to cattle health and well-being (Nardone et al., 2010). Heat stress in cattle occurs when environmental conditions add to the animal's overall heat load faster than the animal can dissipate it (Hahn and Becker., 1984; Bishop-Williams et al., 2015). A common method for assessing the thermal impact of weather conditions on cattle is the Temperature and Humidity Index (THI), which uses both ambient temperature and relative humidity to determine how hot it actually feels to the animal (Gaughan et al., 2008; Mader et al., 2006). However, it has recently been noted that there are many more factors besides temperature and humidity that affect the heat index, both climatically and when considering individual animal variation (Gaughan et al., 2008). Animal variations that allow for elevated heat tolerance include genetics and nutrition (Gaughan et al., 2008; Nardone et al., 2010). A newer index, Heat Load Index (HLI), was developed to include climatic conditions such as solar radiation, wind speed, black globe temperature, duration, and nighttime recovery (Gaughan et al., 2008). One of the more recent indexes is called the Comprehensive Climate Index (CCI), which includes the same factors from HLI but also includes cold weather conditions, such as temperature and wind speed to determine a wind chill factor, in the algorithms used to create the formula (Mader et al., 2014). This allows the CCI to be used in all climatic conditions, not just those of potential heat stress (Mader et al., 2014). Derived from these indexes are certain ranges in which cattle are considered to be experiencing varying degrees of heat stress, from "mild" to "severe" or "dangerous" (Gaughan et al., 2008; Mader et al., 2014; Mader and Davis, 2004). These ranges are meant to assist producers in monitoring their cattle for signs of heat stress, to aid in better management, and incorporate mitigation strategies.

Heat stress impacts cattle in many ways, including but not limited to decreased feed intake, growth and milk production, reproductive efficiency, and in severe cases, death (Hahn, 1999). Generally, cattle are able to cope with a wide range of environmental stressors, but it is when the climate exceeds those thresholds that impacts are seen in behavioral, physiological, and immunological functions (Hahn, 1999). There are several factors that can lower this tolerance level, including life stage, nutritional status, and body condition score (Hahn, 1999). An animal experiencing prolonged heat stress will have an increased core body temperature, increased respiration rates, and behavioral changes (Hahn, 1999). Many of these behavioral changes are attempts by the animal to minimize internal heat loads and include changes in eating behaviors, activity levels, and shade seeking behaviors (Nienabar et al., 1999; Mitloehner et al., 2002). The return to thermo-neutral conditions at night is critical for the ability of the animals to cope during heat events. When THI returns to a thermoneutral state, cattle are able to dissipate excess body heat that had built up during the day (Hahn, 1999). Brown-Brandl and others (2006) observed that dark hided cattle were 25% more heat stressed than light hided cattle, animals with a history of respiratory illness were 10% more heat stressed than healthy cattle, and by each increase in body condition score cattle became 10% more heat stressed. Having one or more of these characteristics can make it significantly more difficult for the animal to cope with temperatures over 25°C (Brown-Brandl et al., 2006).

There are multiple ways in which cattle can cope with the stresses of hotter climates. Firstly, an increase in respiration rate helps to dissipate some of the accumulated body heat via evaporation (Hahn, 1999). While certain factors, such as body condition and nighttime cooling, cause greater increases in respiration than others,

respiration rates increase as temperatures increase (Gaughan et al., 2000). Evaporative cooling is a significant means for cattle to dissipate excess heat and this occurs primarily through increased respiration rate, but also by sweating (Blackshaw and Blackshaw, 1994). The effectiveness of evaporative cooling is also dependent on several weather conditions, namely wind speed and humidity (Blackshaw and Blackshaw, 1994). Cattle will also seek shade and cooler places to rest, or spend more time standing in order to increase the surface area of their body exposed to wind (Mitloehner et al., 2002). Another mode of heat loss is through the skin, either by evaporation in sweating or conductivity of the skin (Finch, 1985). One study showed significant breed differences in skin heat loss between Brahman, Shorthorn, and Hereford-Shorthorn crosses (Finch, 1985). Brahman steers were able to release more heat via the skin than the other two breeds (Finch, 1985). It was also observed that skin conductivity accounted for approximately 55-65% of heat loss through the skin, while the rest is attributed to sweating (Finch, 1985). This varies considerably between breeds, and with different coat lengths and colors (Mader et al, 2006). Additionally, feed intakes will decrease during periods of heat stress, either overall or during the hottest parts of the day (Mader, 2003). Holter and colleagues (1996) showed that Jersey cows' dry matter intakes were significantly impacted by THI, with DMI decreased as THI increased due to the effects of heat stress. Animals that are higher producing, such as finishing beef cattle or lactating dairy cows, produce greater amounts of thermal energy and are more susceptible to the impacts of heat stress (Hahn, 1999). This stems from higher concentrate diets and overall DMI, in addition to higher percentages of insulating fat in finishing cattle, causing the peak of fermentation heat to be greater and likely peaking during the hottest part of the day (Mader, 2003). The rumen

produces significant amounts of heat during digestion, so eating during cooler parts of the day or not eating as much overall is a means for cattle to reduce their heat load (Mader, 2003). Lastly, cattle will increase their water intake; thus, the availability of waterer space is very important. According to Mader (2003), a 300% increase in water bunk space per animal from non-heat stressed conditions is necessary to allow all animals' equal access to water bunks. Temperature and DMI are the two most influential factors in water consumption, and as temperatures rise, water intake rises as well (Murphy et al., 1983).

Much research has been conducted on heat stress mitigation in order to provide producers with management strategies to minimize the impact of hot climates on cattle. Extensive research has been done on the impact of shade structures in confined cattle systems (Mitloehner et al., 2002, Brown-Brandl et al., 2005, Eigenberg et al., 2005). Many studies have seen a significant reduction in stress responses when cattle were given shade; however, others have seen no difference in heat stress symptoms between shaded and unshaded treatments (Mitloehner et al., 2002, Brown-Brandl et al., 2005). The speculated reason for these inconsistencies is the intensity of the heat event; thus when cattle are experiencing only moderate levels of heat stress, the shade structures are not utilized (Eigenberg et al., 2005). Eigenberg and colleagues (2005) saw no significant difference between the time *Bos taurus* feeder cattle spent in the shade versus in the sun when the THI was categorized as "normal" or "alert", however when weather reached "danger" and "emergency" the feeder cattle spent significantly more time in the shade. Additionally, at "alert" heat stress ranges body temperature and respiration rates of the feeder cattle were decreased by the addition of shade, while production measures such as

feed intake and feeding behavior were not impacted (Brown-Brandl et al., 2005). This indicates that body temperature and respiration rates may be more sensitive indicators of heat stress than feed intake and feeding behavior. In regions where the climate stays in the mild to moderate range, or below 69, shade was seen to have less of an impact compared to regions where the climate is frequently in the severe ranges (Eigenberg et al., 2005). Shade helps reduce the amount of solar radiation that an animal is exposed to, as well as provide the animal a cool place to lie down (Mitloehner et al., 2002). Mitloehner and others (2002) and Eigenberg and others (2005) both observed that providing shade decreased the respiration rates of cattle. Shade also decreased core body temperatures in cattle, indicating overall lower heat loads (Brown-Brandl et al., 2005; Kendall et al, 2007). However, measuring body temperatures on every animal is an unrealistic tool for management simply because it is much more time and labor intensive than other methods. Based on the data observed by Brown-Brandl and others (2005) when implementing shade, respiration rate would be the best early indicator of heat stress, as it was the most impacted at the lower critical temperatures.

The use of water to sprinkle or mist pens or soak cattle is another heat stress mitigation tool (Mader and Davis, 2004; Lin et al., 1998). Mader and Davis (2004) looked at the differences in heat stress management of sprinkled versus non-sprinkled cattle, as well as the time of day in which sprinklers were used. It was determined that sprinkling cattle has the benefit of evaporative cooling, assuming there is enough wind speed to promote evaporation (Mader and Davis, 2004). It was also observed that for sprinkling to be most effective, it must be sprayed on cattle before the peak temperature is reached and adequate wind or fans are present (Mader and Davis, 2004; Lin et al.,

1998). Additionally, ground that had been sprinkled with water was about 15°C cooler than ground that was dry, allowing for greater heat exchange between cattle and the ground (Mader and Davis, 2004).

Another recent potential method of heat stress management is the adjustment of feeding times (Mader and Davis, 2004). By limit feeding, or altering the time that feed is available, producers can ensure that the peak of fermentation will be less severe or will occur during a cooler part of the day (Mader and Davis, 2004). Mader and Davis (2004) observed that limit-fed cattle experience higher rates of compensatory gain as compared to control cattle, and indicated that limit feeding cattle can reduce the severity of heat stress while also minimizing the negative impacts heat stress can have on growth. An interesting note from the same study was that when bunk management was being used to either limit feed or alter feeding time, water intakes were significantly lower (Mader and Davis, 2004). Murphy and colleagues (1983) also saw decreased water intakes when feed intakes were limited. Additionally, it was found that as ambient temperature rises so does water intake per animal (Murphy et al, 1983). Conversely, as the temperature of the drinking water increases, drinking water intakes decrease (Murphy, 1992). Quality of water that cattle are offered also effects water intakes, shown by Willms and colleagues (2002) when cattle on pasture gained 9% better when given access to clean water rather than water of low quality. Several of the mitigation strategies both by the animal and by human intervention require the use of additional water, which can problematic when faced with more variable climates and increases in drought length and severity.

Cold Stress

While heat stress poses a significant threat to cattle health and well-being, cold stress also poses a threat. Despite both finishing cattle and lactating cows being very cold resistant and rarely enduring temperatures lower than their tolerance, instances can occur that may decrease productivity and overall efficiency (Young, 1981). Lactating dairy cows can endure temperatures of -10°C without much detrimental effects, and similar lower critical temperatures have been calculated among beef steers (Berman and Meltzer, 1973). While cattle are able to function at these temperatures, the efficiency of gain is compromised as are other productive functions (Berman and Meltzer, 1973). Cattle that are kept outside in colder climates experience increased feed intakes with reduced growth rates, as more feed energy is diverted for maintenance requirements rather than growth (Young, 1981). One study observed that calves born during lower temperatures and significant precipitation conditions had higher mortality rates than those born in warmer, dryer seasons (Azzam et al., 1993). These examples underscore the severity of cold stress on cattle and the necessity for producers to manage these animals accordingly through severe winter weather. The original indexes used to assess environmental stress (THI) mainly focused on heat stress and did not look as in depth at the impacts of factors like humidity and wind speed during colder climates (Gaughan et al., 2008). However, more recent indexes such as the HLI and CCI use many factors influencing climate to develop additional ranges for cold stress (Mader et al., 2004).

There are several management strategies to effectively manage cattle during cold weather, a common one being the addition of adequate shelter (Mader, 2003). Mader (2003) observed that physical structures and bedding provision were sufficient in helping cattle deal with cold stress. However, if the bedding is a fibrous feed source, animals may

eat the bedding material instead of their high energy diet. Nonetheless, the detriments of animals consuming bedding are often outweighed by the benefits of mitigating cold stress. The addition of bedding can increase gains by approximately 7% and feed efficiency by 6%, and increase profit by $11/animal compared to cattle not offered bedding in cold climates (Mader, 2003). The implementation of shelters or windbreaks helped cattle to outperform control cattle without wind breaks during severe winters (Mader, 2003). Yet, when the same structures were used in the summer months, cattle performance was hindered and offset the benefits gained during the winter (Mader, 2003). Cattle that were nearing slaughter weights, however, saw a significant enhancement of fat deposition when they were provided wind breaks, despite their decrease in performance (Mader, 2003).

Another management strategy that has been researched is a change in dietary energy concentrations (Mader et al, 2001). Maintenance energy requirements during winter months can increase between 30 to 70% from basal maintenance requirements (Young, 1981). Commonly, producers will switch cattle to a higher roughage diet during severe winter weather to increase the heat produced in the rumen (Birkelo et al, 1991). However, Mader et al (2001) observed that the opposite was true, and that a switch from a higher to a lower roughage diet was the most beneficial due to the increase in metabolizable energy (ME) available to the animal. During colder conditions, there is an increase in maintenance requirements of cattle and feeding a higher grain diet (higher in ME concentration) would help offset those differences (Mader et al., 2001).

Both heat and cold stress, and the variable climates that go with them cause stress on livestock and cause them to utilize more resources than they would normally use.

Resources that were once thought to be renewable may decrease in the near future, such as water availability and quality, and well as quality forage. This will put even more pressure on humans to help these animals cope with the changing climates.

Stress

The Stress Response

The concept of "stress" in livestock is an area of great interest to many researchers and producers alike. Hans Selye was the first person to actually define stress, which he defined as the bodies' physiologic response to a stimulus to maintain homeostasis (Carroll and Forsberg, 2007). Regardless if the stimuli, or stressor, is physical, emotional, or mental, the body reacts the same (Carroll and Forsberg, 2007). The stress response in cattle is largely mediated through two major systems: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) (Minton, 1994). Stressors that cattle experience can include management practices such as transportation, weaning, castration, dehorning, and mixing of new animals (Minton, 1994). Both the HPA axis and the SNS are precise and stimuli-specific in their reactions, allowing for an appropriate magnitude of response correlated to what is necessary to return to homeostasis (Carroll and Forsberg, 2007). The two types of stress, acute and chronic, both have the potential to cause significant biological distress (Mench and Moberg 2000b). In a study between acute and chronic stress in rats, there were significant differences between the hormone profiles, indicating a difference in responses by the animal depending on the type of stress (Chappell et al., 1986). Acute stressors, while shorter in duration, may hinder biological processes if the cost of responding to the stress is great enough (Mench and Moberg, 2000b). An acute stressor impacts the animals'

ability to function in one of two ways: either by disrupting necessary biological events or by using up biological resources that would normally be used elsewhere (Mench and Moberg, 2000b). An example of an animal's response to acute stress is the increase in respiration rates when an animal is under heat stress. The animal's increased respiration rate causes water loss via evaporation in an attempt to rid the body of excess heat (Blackshaw and Blackshaw, 1994). When acute stress disrupts a specific biological event, it has an impact on processes that require specific timing, such as ovulation, which is why animals' reproductive success can decline in the event of a heat wave, other climatic changes, or stressors (Mench and Moberg, 2000b). Acute stress can take away biological resources from other bodily functions, but the stressor either needs to be large in magnitude or compounded by other simultaneously occurring stressors (Mench and Moberg, 2000b; Rivier and Rivest, 1991). Another challenge for producers dealing with stressful situations is "subclinical stressors" (Mench and Moberg, 2000b). These are stressors that are mild enough to not elicit a visual stress response from the animal, leading observers to believe there is no perceived issue; however, it leaves the animal more susceptible to other stressors (Mench and Moberg, 2000b). An example of a subclinical stressor is confinement. While it may not explicitly cause signs of distress, it can limit normal behaviors and force greater human and animal interactions, and when combined with other stressors could disrupt normal function (Mench and Moberg, 2000b; Chirase et al., 2004).

Chronic stress occurs over extended periods of time, in excess of what time or resources would be needed to evade a stressor, and can have greater overall impacts on the animal (Mench and Moberg, 2000b). A study looking at the impacts of chronic stress

on dairy cow reproductive performance showed that cows exposed to chronic stress took longer to conceive, and had an overall longer calving-to-conception interval (Hernandez et al., 2005). Initially, the animal enters a pre-pathologic state, in which the chronic stressor suppresses the immune system and puts the animal at greater risk of contracting a disease or other illness (Mench and Moberg, 2000b). When the animal remains in a pre-pathologic state for extended periods of time, it eventually develops into a pathologic state and actually contracts the pathology. A common example of this concept would be cattle that have been recently transported and have greater occurrences of respiratory disease (Mench and Moberg, 2000b; Chirase et al., 2004). Other examples of responses to pathological states include stagnant growth rates, suppressed reproduction and deleterious behaviors such as tail biting in pigs (Mench and Moberg, 2000b).

The Endocrine System Response to Stress and its Interaction with the Immune System

The HPA axis and the sympathetic nervous system control many of the hormones released into the body, and have a large influence in energy regulation (Mormede et al., 2006). This control over allocation of energy is why the HPA axis is critical under conditions of stress. By shifting energy sources, the animal is able to adequately cope with the stressor (Mormede et al., 2006). The axis itself is a conglomeration of interactions between three endocrine glands, the hypothalamus, the pituitary gland, and the adrenal gland (Carroll and Forsberg, 2007).

Three critical hormones in the activation of the HPA axis are corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and vasopressin (VP) (Mormede

et al., 2006). Their response and interactions allow for the HPA axis to be highly sensitive to many stimuli (Mormede et al., 2006). Vasopressin and CRH are both produced in the hypothalamus, and VP is stored in the posterior pituitary (Carroll and Forsberg, 2007). Both CRH and VP act to stimulate the release of ACTH from the corticotrophic cells in the anterior pituitary (Carroll and Forsberg, 2007). Under normal, non-stressful conditions, CRH and VP are secreted in pulses throughout the day, leading to normal increases and decreases in concentrations (Charmandari et al., 2005). Outside the normal rhythm of hormone release, changes such as light, feeding schedules, and acute stress can stimulate ACTH and cortisol production in excess of normal concentrations (Charmandari et al., 2005). Cortisol is a glucocorticoid (GC) and its production in the adrenal cortex is stimulated by ACTH. ACTH will bind to its receptors on the adrenal gland to produce cortisol in the event of a stress response (Sapolsky et al., 2000). Thus, as plasma concentrations of ACTH rise, the release of cortisol from the adrenal cortex also increases (Carroll and Forsberg, 2007). Because of the positive relationship between the presence of a stressor and the activation of the HPA axis, cortisol is often used as an indicator of stress in cattle and many other species (Mormede et al., 2006).

In the 1930s, when Hans Selye was first investigating the stress response, it was thought that the activation of the HPA axis was very general, responding in the same way for any stressor (Carroll and Forsberg, 2007). However, the system is much more complex and sensitive than initially hypothesized (Carroll and Forsberg, 2007). For each hormone secreted, there are different types of cell receptors that will bind to it and enzymes that will interact with it, thus, allowing each stimuli to have varying biological

effects on the body (Carroll and Forsberg, 2007). An example of alterations in the stress response can be seen when compensatory increases in catecholamine enzymes such as tyrosine hydroxylase occur under prolonged stress. Under acute stress, tyrosine hydroxylase activity increases, without an increase in the numbers of enzyme molecules. However, during chronic stress, the actual number of enzyme molecules increase (Axelrod and Reisine, 1984). It has been shown that both CRH and VP can be down regulated or desensitized in the event of chronic stimulation (Carroll and Forsberg, 2007). This has the potential for severe impacts in the event of chronic stress situations, with desensitization causing a lack of appropriate response to future stressors. Minton (1994) observed that when sheep were exposed to repeated restraint stressor over the course of several days, initially both ACTH and cortisol increased, yet after several repetitions of the stressor ACTH continued to increase while cortisol concentrations did not increase. These findings are indicative of an acclimatization to the stressor. Similar results were observed in restrained pigs, where the plasma cortisol levels decreased after several days of restraint (Minton, 1994). However, in pigs that were subjected to the repeated use of a nose-snare, ACTH and cortisol levels were similar between day one of the study and day nine, indicating a difference in stress responses based on the stressor (Minton, 1994). In a previously mentioned study on chronic and acute stress in rats, it was observed that acutely stressed rats experienced elevated levels of both ACTH and corticosterone (the GC produced by the adrenal cortex in rodents), while chronically stressed rats only saw increases in corticosterone, not ACTH (Chappell et al., 1986). The chronically stressed rats were subjected to a constantly changing schedule of stressors, as to avoid acclimatization to the stress stimuli (Chappell et al., 1986). The authors hypothesized that

the ACTH levels decreased due to significant hypertrophy of the adrenal gland and concluded the chronically stressed rats were not able to acclimate to the stressor (Chappell et al, 1986).

Glucocorticoids are an integral part of the maintenance of homeostasis and overall survival, and cortisol is the most common GC in mammals (Carroll and Forsberg, 2007). Some of their functions include regulating carbohydrate and protein metabolism, control of growth rates and reproduction, and regulation of the stress response and immune function (Carroll and Forsberg, 2007). During a stressful scenario, certain GC are slower to take their effect than faster acting stress hormones such as catecholamines. The GC take a few minutes to be secreted and do not take effect on other bodily functions substantially until approximately an hour after the stressor, whereas catecholamines are secreted and act on target tissues within minutes (Sapolsky et al., 2000). Glucocorticoids have four broad actions: permissive, suppressive, stimulating, and preparative actions (Sapolsky et al., 2000). Permissive actions occur under normal, non-stress induced conditions and they prepare the immune system for any potential challenges (Sapolsky et al., 2000). The suppressive actions occur due to a stress induced rise in GC and are the actions responsible to limit the stress response and not overproduce immune cells (Sapolsky et al., 2000). Stimulating actions are very similar to permissive actions, except they occur after the onset of the stress response (Sapolsky et al., 2000). Finally, preparative actions do not impact the immediate stressor, but impact how the animal responds to future stressors, and these actions can be either permissive or suppressive (Sapolsky et al., 2000). Preparative actions are the actions that have the potential to suppress the immune system, as they are geared towards ensuring the animal survives in

the event the stressor continues (Sapolsky et al., 2000). It is speculated that the inverse relationship between the stress response and immune system functionality is an attempt to keep the immune system in check and limit the immune system from over producing cells and causing harm to the animal (Minton, 1994). Corticosteroids and catecholamines are mediators of the inflammatory response, and this response is an example of a function that can be harmful to the animal if left unchecked (Black, 2002). The suppression of the immune system via GC is mainly seen in chronic stress situations (Minton, 1994; Klemcke et al., 1990; Minton and Blecha, 1990). Conversely, for people with Addison's disease in which the body does not produce ACTH, there is chronic stimulation and excess white blood cell (WBC) concentrations (Sapolsky et al., 2000). Glucocorticoids also suppress inflammation and immune responses, which is necessary to avoid chronic stimulation (Carroll and Forsberg, 2007). Some of the other chronic problems caused by GC include excessive protein catabolism, immunosuppression, and depression, all of which lead to decreases in desirable production characteristics in livestock species (Carroll and Forsberg, 2007). One study showed that short-term supplementation of corticosterone beneficially enhanced rats' responses to a reaction called delayed-type hypersensitivity (DHT), a reaction that can either cause beneficial or detrimental immune function in the skin (Dhabhar and McEwen, 1999). However, when the corticosterone was supplemented over a longer period of time, the authors observed that the DHT reaction was immunosuppressive. In fact, GC were critical in the beneficial enhancement of the immune function of normal rats and adrenalectomized rats (Dhabhar and McEwen, 1999). Furthermore, increase in exogenous GC caused an increase in circulating WBC that acted as surveyors for any incoming threat (Dhabhar and McEwen, 1999).

Catecholamine production is controlled by the SNS, produced in the adrenal gland and has also been hypothesized to influence the HPA axis and ACTH production (Carroll and Forsberg, 2007; Plotsky et al., 1989). Catecholamines, most notably epinephrine and norepinephrine, are responsible for the biological processes involved in the fight or flight response. Some of these responses include dilation of pupils, increase in heart rate, vasodilation in leg muscles, vasoconstriction in the gut, and stimulation of the liver to produce glucose (Carroll and Forsberg, 2007). As the first defense to a stressor, catecholamines are released within minutes of initial stress exposure (Carroll and Forsberg. 2007). Mineralocorticoids, another type of adrenocortical steroid hormone, are responsible for maintaining the sodium balance in the body as well as overall extracellular fluid volume (Carroll and Forsberg, 2007). Aldosterone, the primary mineralocorticoid in mammals, signals the kidney to both conserve sodium and release potassium when animals are in a hot environment (Carroll and Forsberg, 2007). Mineralocorticoids are important mediators of water conservation in the body (Carroll and Forsberg, 2007). Beede and Collier (1986) reported that in instances of acute thermal stress, concentrations of anti-diuretic hormone increase and concentrations of aldosterone decrease. Anti-diuretic hormone aids in the conservation of water, which is why it increases during thermal stress (Beede and Collier, 1986).

In one of the earliest descriptions of the stress response, three phases were outlined: alarm, resistance, and exhaustion (Selye, 1956). The alarm phase has been studied extensively, and includes acute stress scenarios such as weaning, castration, parturition, transportation, and social mixing (Selye, 1956). Overall, these varying scenarios activate mostly similar responses that are considered to be non-specific in their

nature (Mormede et al., 2006). During the alarm phase, circulating concentrations of cortisol, epinephrine, and norepinephrine increase in order to meet the demands of the stressor (Mormede et al., 2006; Carroll and Forsberg, 2007). However, if the stressor is maintained for an extended period of time, the cortisol levels will return to baseline, even though other indicators point to the sustained activation of the HPA axis (Mormede et al., 2006). For example, in a study conducted in which pigs were randomly given electric shocks over the course of a month, their cortisol and ACTH levels were similar to the levels observed in the control animals by the end of the month. Based on that one would predict that the animals were not stressed; however, they still displayed the behavioral indicators of stress, such as attempting to flee and a visible pain response in the form of writhing (Jensen et al., 1996). Therefore, cortisol may not be an effective measure of chronic stress (Mormede et al., 2006). As the animal is continually exposed to stressors, the body mounts more sophisticated immune responses and diverts energy from nonessential functions like growth and reproduction towards essential functions for maintenance (Carroll and Forsberg, 2007). These events constitute the resistance phase. By the time the stress response and immune response reaches the exhaustion phase, certain reactions have become immunosuppressive and it becomes harder for the animal to cope with the energy requirements needed to defend against the stressor (Carroll and Forsberg, 2007). The constant presence of stressors can lead to the hyper activation of the stress response, which is only meant to be activated for short periods of time (Charmandari et al., 2005). When activated for short periods of time, the shutting down of unnecessary functions like growth and reproduction will not have lasting impacts, but when prolonged, these reallocations can have harmful effects (Charmandari et al., 2005).

It is also worth noting that there is a great deal of variability in stress responses between breeds, species, and individual animals (Mormede et al., 2006). Although there have been "normal" ranges reported for different stress indicators, an individual animal's "normal" indicator concentration might be outside of that range, skewing the outcome of a test (Mormede et al., 2006). Therefore, collecting baseline data for individual animals can be beneficial in remedying situations such as this (Mormede et al., 2006). Studies have demonstrated that there are many sources of variation in hormone levels, such as animal handling, prior genetic programming, and method of sample collection (Carroll and Forsberg, 2007; Liu et al., 1997).

Many animal caretakers in livestock production have made firsthand observations indicating stress's influence on immunity such as in situations of increased disease outbreaks in animals subjected to stressful environments (Mench and Blecha, 2000a). It is important to note that stress and its impact on an animal is also dependent on factors such as age, nutrition, genetics and previous experiences (Hahn and Becker, 1984). In older adult humans, Kiecolt–Glaser and colleagues (1996) showed that chronic stress negatively influenced the immune response to influenza vaccines, and indicated the potential vulnerability of older adults. Bretschneider (2005) reported that the younger bull calves were at the time of castration, the less they were impacted by the stress of the procedure. Hutchison and Cole (1986) found that feeder calves fed specifically formulated diets including 50% concentrates, and supplemented potassium and lactobacillus acidophilus, were better equipped to face the immune challenges of stressful events such as transportation. While these factors can all play a role, it is clear that the

management practices and environment cattle live in also have impacts on immunity and susceptibility to disease (Blecha, 2000; Mench and Blecha, 2000a).

A stressor that has long plagued the cattle industry is bovine respiratory disease (BRD; Mench and Blecha, 2000a). Not only does BRD impact the animals' health and well-being, but causes increasing costs to the industry economically (Mench and Blecha, 2000a). If the immune system is suppressed, say from excessive stress, the animal is going to be more susceptible to BRD (Mench and Blecha, 2000a). In cattle that were shipped for 10 hours, several changes in immune cells were seen, indicating the onset of stress and suppression of the immune system (Blecha et al., 1984). One of these cell changes reported was neutrophilia, an abnormally high number of neutrophils and GC induced observation in stressed animals that affected how neutrophils were moved and released from the bone marrow. Research has also demonstrated that a suppression of WBC called lymphocytes occurs in various breeds of cattle that have undergone transportation (Blecha et al., 1984; Webster-Marketon and Glaser, 2008). In another study, rats were supplemented with corticosterone at varying times to simulate acute and chronic stress before testing immune function, and it was observed that chronically stressed rats had more immunosuppressive activity (Dhabhar and McEwen, 1999).

While completely eliminating stress in an animals' life is impossible, there are management practices that can help prevent harmful levels of distress (Mench and Moberg, 2000b). According to Mench and Moberg (2000b), the best way to manage stress is to limit the biological cost of each stressor. This can be tackled in several ways, one of which being through behavior modification. If an animal can be desensitized into thinking a stressor is no longer a threat, they may not be affected by it (Mench and

Moberg, 2000b). An alternative approach may be to distract animals from a stressor. One study showed that if pigs are experiencing frustration waiting for food in between feeding times, producers can provide them with a toy to distract them, resulting in lowered cortisol concentrations (Dantzer and Mormede, 1981). Finally, stress can be reduced through breeding and genetics, to sort out animals that are less able to cope with stressful situations, or encourage traits that allow for greater adaptability to varying stressful situations (Mench and Moberg, 2000b). Hulbert and others (2011) observed that calm cattle were minimally affected by transportation stress and their immune systems were able to return to basal levels faster when compared to temperamental cattle.

The Immune System and its Cells

The immune system consists of two major branches: the innate and acquired immune systems (Carroll and Forsberg, 2007; Kindt et al., 2007). Innate immunity is the branch that each individual is born with, and has been developed evolutionarily (Carroll and Forsberg, 2007). It is the first line of defense in protecting against foreign objects. The cells that are part of the innate immune system are largely non-specific, but instead target certain cell structures (Carroll and Forsberg, 2007). This allows the innate system to cover a broad spectrum of potential invaders and keep infections and other stressors under control until the acquired immune system can fully activate (Carroll and Forsberg, 2007; Hulbert et al., 2011). Cattle have underdeveloped acquired immune systems from the time that they are born until approximately three weeks of age (Mallard et al., 1998). During this time, they can be protected by means of passive immunity, in the form of antibodies from the dam that are transferred via colostrum to the calf (Mallard et al., 1998). The acquiring of passive immunity through consumption of colostrum is only

effective if the calf nurses within the first 24 to 48 hours after birth (Brambell, 1970). Calves deprived of colostrum have higher mortality rates, increased incidence of scours, and poor weight gain when compared to calves that were offered colostrum (Nocek et al., 1984). Innate immunity is present when individuals are born, however, its strength and capabilities can be altered by factors such as nutrition, genetics, stress, and dehydration (Carroll and Forsberg, 2007). Within innate immunity, there are three types of cells: phagocytic cells, natural killer cells, and cells specialized in the initiation of the inflammatory response (Carroll and Forsberg, 2007; Kindt et al., 2007). Phagocytic cells target and destroy invading cells or particles before they are able to multiply and cause damage, whereas natural killer cells specialize in destroying abnormal or infected cells of the host (Carroll and Forsberg, 2007). Natural killer cells also aid in the release of inflammatory cytokines, which initiate the acute phase response (Carroll and Forsberg, 2007; Hulbert et al., 2011). This acute response phase usually involves a fever, systemic release of pro-inflammatory cytokines, an increase in circulating WBC, and other characteristic behaviors of a sick animal (Carroll and Forsberg, 2007; Hulbert et al., 2011). These behaviors can include lethargy, depression, a lack in appetite and water consumption, and decreases in social behaviors (Carroll and Forsberg, 2007). An invaluable benefit of the acute phase response is the impact a fever has on the body (Carroll and Forsberg, 2007). Higher body temperatures accelerate enzymatic processes that kill pathogens and most immune cells proliferate faster at these higher temperatures (Carroll and Forsberg, 2007). These cell types are known as neutrophils and in the event of an acute stressor, one study has shown a 13% increase in neutrophil levels (Dhabhar et al., 1995). Dhabhar and colleagues (1995) studied the difference between normal rats and

adrenalectomized rats undergoing acute stress and saw that baseline levels of neutrophils were higher in the adrenalectomized rats. The authors speculated that this was due to the lack of a negative feedback loop signaling for the body to stop releasing CRH and ACTH (Dhabhar et al., 1995). The authors concluded that adrenalectomized rats had abnormally high levels of hormones circulating in the blood without the corresponding stressor that typically initiates a stress response (Dhabhar et al., 1995).

Acquired immunity is developed throughout the lifespan of an individual, and the cells involved are highly specific (Carroll and Forsberg, 2007; Kindt et al., 2007). It is also known as the adaptive immune system as it is able to adapt to each new antigen it is exposed to (Carroll and Forsberg, 2007; Colditz, 2002). The cells of this system are able to recognize and remember pathogens, allowing the body to fight off the invading cells faster and with increasing intensity in subsequent exposures (Carroll and Forsberg, 2007). Within acquired immunity, there are two classifications: cell-mediated immunity and humoral immunity (Carroll and Forsberg, 2007; Colditz, 2002; Kindt et al., 2007). Cell-mediated immunity targets the body's own cells that have been infected by the pathogen, and humoral immunity targets the pathogenic cells themselves (Carroll and Forsberg, 2007). Lymphocytes are a specialized type of WBC and are an integral part of acquired and innate immunity (Carroll and Forsberg, 2007). T and B cells are lymphocytes that are part of the acquired immune system and Natural Killer (NK) cells are lymphocytes that are part of the innate immune system (Kindt et al., 2007). Lymphocytes of the acquired immune system ingest pathogens and are able to display the pathogen's specific antigen to other immune cells that are then able to find and target the corresponding pathogenic invaders (Carroll and Forsberg, 2007; Kindt et al., 2007). Dhabar and colleagues (1995)

reported a 54% decrease in lymphocyte counts in stressed rats within two hours of an acute stressor. When looking at the difference between normal and adrenalectomized rats undergoing acute stress, the normal rats had higher overall baseline levels of lymphocytes; after the two hours of restraint, while all treatments saw decreases in total lymphocyte counts, the normal treatment had significantly greater decreases. This was likely due to the corticosterone produced that redirected lymphocytes out of the blood stream and into tissues (Dhabhar et al., 1995).

It is accepted that acute stress can actually bolster the immune system, while chronic stress is immunosuppressive in most cases (Carroll and Forsberg, 2007). Davis (1998) hypothesized that cold stress can positively stimulate the immune system. It is known that cold temperatures increase thyroid activity, and the thyroid hormone is the only hormone that consistently stimulates both primary and secondary lymphoid tissue. Primary lymphoid tissue consists of the thymus and bone marrow, while secondary lymphoid tissue consists of circulating lymphocytes, the spleen, and lymph nodes (Davis, 1998). However, it has been reported that under chronic stressful conditions, the amount and activity level of natural killer cells decreases markedly, leading to a decreased response to infections (Webster-Marketon and Glaser, 2008). Lymphocytes increase overall cell numbers in the event of an infection; however, the presence of a stress response leads to a decrease in lymphocyte proliferation, leaving the individual more susceptible to infection (Webster-Marketon and Glaser, 2008). In ecological research, some researchers have been turning to leukocyte profiles in order to measure stress in animals; however, there had been some inconsistencies in the interpretation and utilization of the research until recently (Davis et al., 2008). Leukocyte profiles have been

increasing in popularity due to the difficulty in obtaining accurate baseline measurements for fast acting hormones like cortisol (Davis et al., 2008, Foote et al., 2016). When restraining and sticking animals with a needle to collect blood, the stress of processing can artificially raise the baseline concentrations of cortisol (Davis et al., 2008, Foote et al., 2016); whereas, analyzing WBC counts via blood smears can be more reliable due to the slightly longer response time for immune cells to stress (Davis et al., 2008; Dhabar et al., 1995). The benefit of using a leukocyte profile is its responsiveness to stress and direct relation to stress hormone levels (Davis et al, 2008). One of the most noteworthy changes to the profile when under stress is in the neutrophil and lymphocyte counts (Davis et al, 2008). Typically, an increase in neutrophils and a decrease in lymphocytes occurs in response to a stressor, and due to their opposite responses, the ratio of the two has become a composite measurement of stress (N:L; Davis et al, 2008). It has been shown that the change in ratio before and after a stressful event is positively correlated to both the magnitude of the stressor and the amount of circulating GC, making it a reliable measurement of the stress response (Davis et al, 2008). Another aspect that makes the N:L an adequate measurement of chronic stress is that the time it takes to see a response is several hours, therefore, making it much easier to get baseline values as well (Davis et al, 2008, Foote et al, 2016). Equine neutrophil and lymphocyte counts were compared in exercise versus non-exercise control groups, and the authors observed that neutrophil levels increased throughout the experiment in the exercise group (Cardinet et al, 1964). The lymphocyte counts for the exercise group remained lower than in control groups for the duration of the exercise, but had returned to normal levels after 14 hours (Cardinet et al., 1964).

There is also evidence of the neutrophil to lymphocyte ratio (N:L) being influenced by disease or infection in the animal; however, it is possible that this is from the stress hormones produced in response to the disease and infection (Davis et al., 2008). This uncertainty can be somewhat problematic when using leukocyte profiles as a predictive tool (Davis et al., 2008). It has been speculated that changes in an animal's leukocyte profile can be a predictor of stress or disease, but it would seem difficult to distinguish between the two (Davis et al., 2008). However, if the monocyte counts, a type of white blood cell involved in phagocytosis, increase along with the N:L ratio, then it would imply that the increase is caused by an infection (Davis et al., 2008).

A study looking at leukocyte levels in humans suffering from Cushing's disease (characterized by chronically high levels of cortisol) saw consistently high levels of neutrophils and low levels of lymphocytes in comparison to normal human levels (De la Balze et al., 1946). Based on that study and in other scenarios involving high levels of cortisol it was determined that these elevated stress hormone levels led to sustained increases in the neutrophil to lymphocyte ratio (Davis et al., 2008). The reason for the shifting ratio is redirection of cells, rather than destruction (Dhabhar, 2002). Circulating lymphocytes are directed into tissues such as lymph nodes or the spleen while neutrophils are directed into circulation from the bone marrow (Dhabhar, 2002). Interestingly, researchers have observed that newly hatched chicks with higher basal N:L ratios were more likely to have future susceptibility to disease and illness (Al-Murrani et al., 2006). This could potentially be due to an overactive immune system or insensitivity to certain stress hormones. Additionally, animals subjected to heat stress had altered concentrations

of circulating T-lymphocytes and an overall reduction of WBC, all which may indicate resistance to pathogens immediately following heat stress (Hahn, 1999).

Additional Blood Components and Measures

In addition to the WBC and other cells involved in the immune response, there is another essential group of cells and components in the blood, including but not limited to red blood cells (RBC; Kindt et al., 2007). Most of the blood cells in the body originate from the bone marrow, where they begin as hematopoietic stem cells (Kindt et al., 2007). Red blood cells are characterized by their donut-like shape, circular with a concaved center, and their ability to promote gaseous exchange in the body (Klinken, 2002). They are able to bring oxygen to cells throughout the body, while also removing any carbon dioxide waste generated (Kindt et al., 2007). These molecules are affixed to the cell by hemoglobin with assistance from iron molecules as well, which are present on every red blood cell (Kindt et al., 2007). In flounder, red blood cell numbers decreased during stressful events; however, the concentrations had returned to normal levels after twenty-four hours (Fletcher, 1974).

Hematocrit level, also known as mean cell volume, is the percentage of solids in the whole blood and the remainder is plasma (Sawka et al., 1984). When dehydrated, the total plasma volume decreases, causing an increase in the hematocrit percentage, making it an adequate indicator of hydration levels (Sawka et al., 1984), and a potential cause of immunosuppression (Carroll and Forsberg, 2007).

As with any cells, there can be pathologies that cause detrimental effects on the animal. When it comes to red blood cells, a common issue that can occur is the inability to adequately carry oxygen, also known as anemia (Klinken, 2002). This can occur in

three ways: low red blood cell counts, low hemoglobin levels, or poor gas exchange (Klinken, 2002). These issues have several different causes, many of them involving inadequate production or absorption of one element like hemoglobin or iron (Klinken, 2002). It has also been reported that infections can sometimes cause severe red blood cell destruction, and certain chronic diseases involving cytokines can suppress red blood cell production all together (Klinken, 2002).

The ability of the cells of the immune system and other blood cells to function properly is dependent on having adequate water reserves in the body. Without proper amounts of water, cells are unable to function from electrolyte imbalances (Murphy, 1990).

Water Metabolism

Water Metabolism and Restriction

Although it is often forgotten, water is an essential nutrient, and perhaps the most vital. For many years, people in the United States have thought of water as a renewable resource and not something that need be taken into account when preparing for adverse climates. But with the increase in extreme weather predicted in the future, drought and overall water unavailability may become a bigger issue than expected (Rosenzweig et al., 2001). Water is necessary for a vast majority of the biological processes occurring in animals on a daily basis (Murphy, 1990). On a molecular level, the properties of water make it extremely useful within the body. Its high heat of vaporization, heat capacity, ability to form hydrogen bonds, surface tension, and electrical conductivity are examples of the many beneficial properties of water. The high heat of vaporization allows animals to transfer heat to the environment, while the high heat capacity helps in keeping thermal

stability and the surface tension allows for easy uptake into capillaries. These properties among others make water the perfect liquid to make up a large portion of an animal's body composition, with dairy cattle specifically ranging anywhere from 56% to 81% water by body weight (Murphy, 1990).

A great deal of movement into and out of cells and interstitial space by water occurs every day. The body also contains "water pools" and the amount of water in these pools depends on environmental conditions and feeding practices (Murphy, 1990). Water metabolism overall can be looked at as a set of inputs and outputs, and the status of these pools dependent on each (Murphy, 1990). In a single day, cattle can turnover almost 34% of their total body water, whereas more heat and drought tolerant animals (such as camels) only turnover about 10% of their total body water, making them more efficient at using water (Siebert and McFarlane, 1968). Inputs can include the drinking of water or consumption of moisture in feed, or it can occur from the body's metabolic processes. Outputs can be losses from urine, feces, milk production, and evaporation (Murphy, 1990).

Metabolic water losses in cattle come from varying processes, a major one being milk production, especially in high producing dairy cows (Murphy, 1990). It is speculated that water intake and milk production are positively correlated; however, data to support this are variable (Murphy, 1990). Some researchers predicted that based on milk being composed of 87% water, it would require 0.87 kg of water to produce 1 kg of milk (Winchester and Morris, 1956). Yet when several studies were conducted, the results varied from study to study; some found correlations while others found no significant relationship (Murphy, 1990). It should also be noted that a strong correlation

was seen between milk yield and dry matter intakes, and between dry matter intakes and water consumption (Murphy, 1990). Water is also lost through the urine, although the amount of water lost varies based upon water consumption. In a study looking at urine volume under dehydration, it was observed that the baseline urine rate was 18 liters per day while after undergoing 54 hours of dehydration that rate had decreased to just 3.2 liters per day (Becker et al., 1985). Another form of water loss is through evaporation, whether it be via saliva, sweat, or nasal secretions (Murphy, 1990). Unfortunately, cattle are more likely to lose water through evaporation in the summer months when they may already be subjected to heat stress and water restrictive conditions (Murphy, 1990).

The main source of water to cattle is their drinking water. When deprived of water for three days, eight lactating Friesian cows lost 21% of their body weight (Little et al., 1984). This body weight loss percentage was more than double what steers lost in similar situations (Little et al., 1984). Dehydration of an animal can pose very serious health concerns and should be avoided at all costs (Little et al., 1984; Murphy, 1990). Under normal circumstances some factors that may impact water intakes include eating patterns, response to types of water receptacles, dominance hierarchies in the pens, and water temperature (Murphy, 1990).

Body fluids and their osmotic concentrations are largely controlled by the hormone vasopressin and its effects on the kidneys (Berl et al., 1976). Vasopressin works by altering the permeability of the kidney ducts in order to dilute or further concentrate the urine. Mammals are able to conserve water when necessary due to the hairpin loop between the proximal and distal tubules of the kidneys. This structure allows for the body to increase the concentration of the urine to be greater than that of the blood. When these

processes are interrupted there can be severe impacts on the body. A defect in the production or circulation of vasopressin can lead to suboptimal urinary concentration. When there is damage to the areas of the brain where vasopressin is produced, it can lead to a sudden flood in circulating vasopressin, which can lead to extreme water retention and cause water intoxication. An additional defect that can impact water metabolism is an imbalance of electrolytes, primarily potassium, which alter the osmolality in cells and potentially inhibit the cellular response to vasopressin. Electrolyte imbalance has been reported to cause humans to consume more than three liters of water a day, and it is theorized that electrolyte imbalance causes a thirst response (Berl et al., 1976). A correction of the electrolyte imbalances will restore the kidneys' concentrating ability in most individuals that experienced the imbalance (Berl et al., 1976; Beede and Collier, 1986). Another electrolyte that has an impact on water metabolism is sodium (Berl et al., 1976; Beede and Collier, 1986). When an individual is experiencing low total body levels of sodium, it is characterized by flat neck veins, hypotension, dry mucous membranes, and poor skin turgor (Berl et al., 1976). It is common to see an increase in hematocrit levels and serum protein concentrations simply because of hemoconcentration (Berl et al., 1976). In the event a human patient was to experience low sodium levels, an immediate action would be to treat with a dose of isotonic sodium chloride; however, it is necessary to address the original issue causing low sodium (Berl et al., 1976).

In a study done by Little and colleagues (1976), eight lactating cows were water restricted by either 100, 87, 73, or 60% for six days. Feed intake, milk yield and quality, water, serum and urine osmolalities, metabolic profiles, and sodium and potassium balances were recorded throughout the duration of the restriction and subsequent 8-day

recovery. Both milk yield and dry matter intakes decreased as restriction became more intense, yet there were no significant differences in the urine output (Little et al., 1976). Water restriction can also cause reductions in body weight, which were attributed to overall loss of body water in past studies (Siebert and McFarlane, 1975; Little et al., 1976). The more intense the restriction, the more concentrated the serum sodium and osmolality became (Little et al., 1976). In an additional study in which lactating Brown Swiss cows were restricted to either 25 or 50% of normal water intake, significant body weight loss was observed as well as reduced feed intake (Burgos et al., 2001). Interestingly, the depression in feed intake did not occur until day three of restriction, and the authors concluded it was most likely because the animals did not begin to feel the effects of the restriction until then. Also during the restriction phase, organic matter and crude fiber digestibility increased, yet nitrogen digestibility inexplicably decreased. Overall, the researchers concluded that lactating cows with higher energy requirements were able to adapt to water restriction (Burgos et al., 2001). However, this adaptation is not without costs that occur in the form of decreased feed intake, which if occurring over an extended period of time would limit growth of the animals (Berl et al., 1976). In the event of a period of water restriction, livestock are capable of slowing certain processes that can be detrimental (Robertshaw and Dmiel, 1982). For example, dehydrated Bedouin goats were observed to have reduced sweating, while panting rates still maintained. While both processes cause a loss of water, the amount of heat lost from panting is greater than heat loss from sweating, which is why panting still occurs while sweating does not (Robertshaw and Dmiel, 1982). Aside from the fact that dehydration has immunocompromising effects, the psychological stress or “frustration” caused by thirst

can also contribute to an immune response and potential activation of the stress response (Papini et al., 2015; Carbonaro et al., 1992).

It is undoubtable that at some point in the future there may be periods of drought or limited water availability. With the many stressors that livestock are subjected to daily, lack of water availability is likely going to add to those stressors, putting increasing pressure on the immune system and overall health of the animal. It will be beneficial to have a knowledge base in how water unavailability will impact the overall health of cattle as we head into the future.

CHAPTER II

THE EFFECTS OF EXTENDED WATER RESTRICTION ON THE HEMATOLOGICAL CELL DISTRIBUTION OF BEEF CATTLE IN CONFINEMENT

Introduction

Climate change, its subsequent variability, and increasing intensity are well-researched and proven (Rosenzweig et al., 2001). With the increasing intensity of climate events, come increasing incidence of drought and water insecurity which are lasting longer than previous records indicate (Timmermann et al., 1999). Water insecurity has tremendous negative implications for the livestock and cattle industries, both from an economic and welfare perspective. With the potential scenario of water scarcity, it is important to understand the implications of water availability on cattle health. Overall, there is a lack of water restriction research in cattle, with much of the research that has been done either on a pen basis or a non-group housed individual animal basis (e.g., cattle housed in stalls or crates). The group housed research poses potential downfalls due to the variability in individual animal water consumption and competition around the water bunk, while individually housed research trials can significantly impact the animals' normal behaviors. This underscores the need for research in water restriction that maintains normal pen dynamics, while also obtaining individual animal data. The

Insentec Bunk Management System is an excellent tool for measuring and controlling individual animal feed and water intakes, while allowing the animals to be housed in a traditional setting.

The purpose of this study was to simulate conditions in which cattle would not have access to adequate water for extended periods of time. The objectives included evaluating animal performance during water restriction to assess adaptability to drought via genetic markers, to analyze behavior during drought, and to study the impacts of drought on health and immunity. The objective of this thesis is to assess the impacts of extended water restriction and chronic stress on the overall health and the immunological blood parameters in crossbred steers.

Materials and Methods

Housing and Management

This study was in accordance with Animal Care and Use Protocol #AG-13-18, which was approved by the Institutional Animal Care and Use Committee at Oklahoma State University.

The study was conducted at the Willard Sparks Beef Research Center of Oklahoma State University, in Stillwater, Oklahoma from May of 2014 through March of 2016. In total, 541 crossbred steers were enrolled in the study, with 461 steers exposed to water restriction and 80 steers serving as controls. The study consisted of 4 groups total: groups 1 and 3 during the summer months (May-September) and groups 2 and 4 during the winter months (November- March). Group 1 consisted of 117 animals on trial and 20 control animals, group 2 consisted of 115 animals and 20 control animals, group 3 consisted of 117 animals and 20 control animals, and group 4 consisted of 112 animals

and 20 control animals. The cattle were housed in groups in a 12.2 m by 30.5 m semi-open barn with approximately 6.2 m^2 per head of shade, and were housed in groups of 25-31 steers. The barn had four separate dirt pens, each with shaded and unshaded areas. A concrete slab surrounded the bunks in each pen that was approximately 12 m by 6 m. There were automated curtains on the north side of the barn; and on the east and west sides of the barn were rolling doors. Both of these structures could be opened in the summer for ventilation or closed in the winter for added shelter. All animals were fed a total mixed ration (TMR) *ad libitum* and the ingredient details of that diet can be seen in Tables 1, and 2. Cattle were fed between two and three times daily, depending on the amount of feed being delivered. If there were two feedings, they were at approximately 0730 and 1400. If there were three feedings, they were at approximately 0730, 1130, and 1400. Cattle were visually inspected for any sick animals during bunk calling by feedlot management.

Insentec Bunk Management System

The animals were provided feed and water via the Insentec Bunk Management System (Hokofarm Group, Netherlands). Each pen had access to six Insentec feed bunks and one Insentec water bunk, for a total of 24 feed bunks and four water bunks. The Insentec Bunk Management System works through the use of individualized electronic identification tags (EIDs), sensors in the bunks, and a receiving computer. There is one programmable system unit for every two bunks and each bunk has a gate that is raised and lowered via an air compressor. Every bunk sits on its own scale that reads out the weight on the system monitor.

All cattle had a unique EID placed in their left ear. When they entered the bunk, a sensor overhead read their EID number. Once the EID was read, the head gate on the bunk lowered and allowed them access to either feed or water. The system recorded the starting weight of feed and water in the bunk, the ending weight, the starting time and the ending time of the feed or drinking bout and would then transmit the data to the receiving computer. From that information, the total amount ate or drank and the total time spent in the bunk were recorded. Water restriction is accomplished by programming a daily water weight allotment in the receiving computer. Therefore, once the animal reached its daily water allotment, the gate would no longer be lowered for the animal by the system until the following 24 hr period.

Study Timeline

Each group consisted of 5 phases: a 21 day acclimation phase, 70 day baseline phase, 35 day step down phase, 35 day extended restriction phase, and a 6 day step up phase (Figure 1).

The acclimation phase began the first full day that the cattle entered into the Insentec facility and is denoted as study day (SD) -21. The purpose of this phase was to allow the cattle to learn how to use the Insentec System bunks and adapt to the pen environments and penmates.

The baseline phase commenced the day immediately following the acclimation phase and lasted for 70 days (SD 0 through SD 70). The purpose of this phase was to collect feed and water intake data for each animal under *ad libitum* conditions. According to Beef Improvement Federation (BIF) guidelines, the minimum days on feed required to determine an accurate baseline for daily feed intake is 35 days (Hohenboken, 2010). The

baseline phase was chosen to be the length of 70 days due to the unavailability of individualized daily water intake guidelines in the literature. Upon the conclusion of the baseline phase, a "baseline intake" for water was calculated for each animal. This was done by excluding any days in which there were possibilities for error, and averaging the remaining days. Possible reasons for exclusion included mechanical breakdowns, excessive water puddles in the pens, loss of an EID for the majority of a day, or any other reason that may have limited or otherwise altered the animals' natural intake. The baseline water intake for each animal was used during the step down and restriction phases.

The step down phase includes five weeks (SD 71 to SD 105) in which the cattle were transitioned from *ad libitum* to 50% of their calculated baseline intake. The water intake of each animal was reduced by 10% each week until each animal was limited to 50% of their baseline daily water intake. They were acclimated to this water restriction level for one week before the restriction phase began. An effort was made to keep all animals within ± 10% of the current restriction level.

After cattle were acclimated to the 50% restriction level, the restriction phase began (SD 106 to SD 140). This phase lasted for 35 days and cattle were processed biweekly for a total of three processing dates. Cattle were closely monitored for any signs of significant and serious deterioration of health, and respiration rates were taken daily to monitor cattle health and well-being.

Upon completion of the restriction phase, the step up phase began and lasted six days (SD 141 to SD 146). This phase was similar to the step down phase, but in truncated form. Briefly, on the first day after the restriction phase, the animals' allotted water

intakes were increased to 60%. On day two of the step up phase animals' intakes were increased to 70%. On the third day the intakes were increased to 85% and then maintained for two days. On the sixth day, all animals were allowed *ad libitum* access to water. After completion of the sixth day, cattle were processed for final weights and removed from the Insentec facility.

Behavior, Blood, and Environmental Data Collection

Cattle health, behavior, and environmental data were collected either during the processing days, or on a daily or weekly basis in the Insentec facility. In a typical processing day, the cattle would be moved to the processing barn starting at approximately 0500, and restrained using a hydraulic chute. Once a weight was obtained and the animal's head had been caught, a blood sample was drawn via jugular venipuncture using 18 gauge multi-use needles and 4 mL vacutainers containing ethylenediamine tetraacetic acid (EDTA). Blood samples were immediately stored on ice until analysis was completed. On SD 56 and 140, two additional samples were collected on each animal: a rumen fluid sample and a fecal sample. These samples were taken two weeks before the start of the step down phase and on the last day of the extended restriction phase. The additional sampling on those two days required the cattle to be restrained approximately 4 minutes longer than normal processing days, with a normal processing day taking approximately 2 minutes in the chute per animal and the processing days with additional samples requiring animals be in the chute for 6 minutes.

Several other data measures were collected on differing intervals within the Insentec facility. Ambient temperature and relative humidity were recorded every 15 minutes using HOBOware data loggers (Onset, Bourne, MA). There were four loggers

placed in varying locations: two were under the shade in the Insentec barn (one on the east side and one on the west side), and the remaining two loggers were placed outside of the Insentec barn (the east or west side). The temperature and humidity data collected was used to calculate a daily Temperature Humidity Index (THI). The formula used to calculate THI is shown below as described by Mader et al (2006):

THI= 0.8 × ambient temperature + [(% relative humidity ÷ 100) × (ambient temperature – 14.4) + 46.4

Morning and evening respiration rates were recorded for each animal during each group, and either taken twice per week or daily depending on the phase of the study. During the baseline phase, respiration rates were recorded twice per week; during the restriction phase, respiration rates were recorded daily. The morning data collection was recorded between the hours of 0700 and 0900 and the afternoon data collections were recorded between 1200 and 1400. When respiration rate data were collected twice weekly during the baseline phase, the two days selected were the hottest projected days based on weekly weather forecasts. Any respiration rates are reported as amount of breaths taken in one minute. One breath was described as an inhale and exhale of air. It was also recorded whether or not the individual animal was under the shade. There were between 4 and 5 observers per day collecting respiration rates, however, it was not the same observers throughout all 4 groups.

In the event that any animal was suspected with a health issue, the animal was brought up to a chute to collect rectal temperature. Animals were treated accordingly based on rectal temperature, the advice of the attending veterinarian, and all approved treatment protocols. Records of all treatments were filed and included body weight, rectal

temperature, type of clinical symptom, medication used, medication dose, and any other action taken.

Blood Analysis

Blood samples were handled and analyzed via two separate techniques and sets of equipment. Samples were analyzed using either the Idexx Procyte Hematology Analyzer (IDEXX Laboratories, Westbrook, ME) to assess complete blood cell (CBC) counts or using a microcentrifuge to manually obtain hematocrit (HCT) values.

A total of 6 blood samples were processed through the Procyte analyzer, starting on SD 70, continuing biweekly until SD 140, and lastly at the conclusion of the restriction phase. Purple top vacutainers containing EDTA were used to collect individual blood samples. Samples were stored on ice until analyzed by the Procyte machine, which occurred no longer than six hours after sample collection. The Procyte hematology analyzer uses laser flow cytometry and optical fluorescence to obtain blood parameters. The data collected from the Procyte analyzer included white and red blood cell counts, white blood cell differentials, hematocrit percentages, and a neutrophil: lymphocyte ratio. Prior to analyzing blood samples, a quality control sample was used each sampling day to ensure that the analyzer was properly calibrated and as accurate as possible. Each blood sample took approximately two minutes to process before results were loaded into the IDEXX VetLab Station, which is the accompanying computer program. Data were exported directly onto an Excel spreadsheet for summary and analysis.

Manual hematocrit analysis was performed once during the baseline phase (SD 56) and during the step down phase on the weeks when but the Procyte analyzer was not being used for sample analysis. In order to run manual hematocrit, a microcentrifuge,

capillary tubes, and critoseal were needed. The tops were removed from the vacutainers and two capillary tubes were placed in each vacutainer. The blood was allowed several minutes to travel up the tube. Once the tube was at least halfway full, the tube was quickly removed and critoseal was pressed into one end to form a seal. Once 24 tubes had been filled and sealed, they were spun in the microcentrifuge for five minutes at approximately 2200 RPM. A spiral hematocrit reader was used to obtain the values for each blood sample. Blood samples were spun and measured in duplicate to check for consistency.

Statistical Analysis

All data were recorded in Excel (Microsoft, Redmond, WA) and analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC). For the respiration rate and environmental data, the CORR procedure of SAS 9.4 was used as well as PROC GLM. For all blood data, the GLIMMIX procedure in SAS was used to analyze the data with animal as the experimental unit. The model included restriction level (baseline, 80, 60, or 50%) and group (1-4) as fixed effects, and their interactions as well as the random intercept of animal identification within group. Interactions were significant and thus were kept in the model. Any P value < 0.05 was considered statistically significant.

For analysis based on individual water intake, animals were categorized into either high, medium, or low drinking water intakes. This was based on each animals calculated average daily water intake divided by their metabolic mid weight. Each group was divided based on standard deviations from the mean; with the low group containing animals with intakes as a percent of body weight 2 standard deviations below the mean, the medium group being 1 standard deviation above or below the mean, and the high

group being 2 standard deviations above the mean. The GLIMMIX procedure of SAS was used and animal was the experimental unit. Group, restriction level, and intake category (high, medium, or low) were included in the model as well as interactions and the random intercept of animal identification within group. The three-way interaction between group, restriction level, and intake category was found to be insignificant and was removed from the model. All two way interactions were kept in the model. Any *P* value < 0.05 was considered statistically significant.

For analysis of morbidity, animals were categorized as either "healthy" or "morbid" based on their treatment records. Table 19 summarizes the percentage of animals treated for different health issues. If an animal was treated at any point during the study, they were considered morbid. The GLIMMIX procedure of SAS was used and animal was the experimental unit. The model included group, restriction level, and health status (healthy or morbid), as well as all interactions and the random intercept of animal identification within group. The three-way interaction of group, restriction level, and health status was insignificant and was removed from the model. All two way interactions were kept in the model. The P Diff option with a Tukey adjustment in SAS was used to separate least-squares means. Any *P* value < 0.05 was considered statistically significant.

Results

Animal Performance

All feed and drinking water intake data were calculated per group and by restriction level, and are reported in Tables 3 and 4, respectively. Figure 2 illustrates the

intake trends throughout the study, but for significance of interactions, refer to tables 3 and 4. While only water was artificially restricted, overall feed intakes followed the same decreasing pattern as the water intakes were restricted (Figure 2). As expected based on the imposed restriction of water, groups 2 and 3 saw significant decreases in water intake as restriction increased ($P < 0.05$). However, group 1 saw less consistent decreases in feed and water intakes, with water intakes from 90% and 70% being statistically similar ($P < 0.05$) and both greater than baseline ($P < 0.05$). At restriction levels 90, 80, and 50 %, group 3 had the lowest feed intakes ($P < 0.05$); as well as numerically lower at restriction levels 70 and 60 % restriction ($P > 0.05$). The ADGs were calculated for each group and by phase of the study (baseline, stepdown, and restriction; Table 5). For Groups 2 and 3, ADG decreased throughout the study as water intake was restricted ($P < 0.05$). A decrease in ADG was observed during the step down phase for group 1 as compared to baseline ($P < 0.05$); however, ADG was higher during the restriction phase than in the step down phase ($P < 0.05$).

Environmental Data

An illustration of the average daily weather parameters for each group is summarized in Figure 3 (A-D). The average Temperature Humidity Index (THI) and standard deviation (SD) for each restriction level and group is presented in Table 6. The Pearson Correlation Coefficients (PCC) between THI and respiration rate was 0.665. Table 7 summarizes the means of respiration rates through the different restriction levels. The highest respiration rates were during the step down phase, which is also when the hottest THI values were reported for the two summer groups (Table 6). Table 8 shows the R^2 values for the four blood parameters and their relationship to THI, by group. The

relationship between N:L and THI was insignificant in all groups ($P > 0.05$) except for group 3. Group 3 had significant relationships for all blood parameters and THI ($P < 0.05$). Table 9 shows the PCC values for the blood parameters and their relationship to THI and group. While none of the PCCs are very strong, group 3 blood parameters all had significant relationships to THI. For both RBC and HCT, there was a negative relationship to THI, so as THI increased, RBC and HCT decreased ($P < 0.05$). For the groups in which WBC and N:L had significant relationships, they were positively related to THI ($P < 0.05$).

Blood Parameter Data

The Least squares means of all blood parameters by restriction level are summarized in Table 16. Overall, from baseline to restriction, HCT increased ($P < 0.05$), RBC and WBC were not significantly different, N:L and neutrophil counts decreased ($P < 0.05$), and an increase in lymphocyte counts was observed ($P < 0.05$).

Hematocrit: All data regarding the blood parameters are reported in three ways: overall by group, categorically based on baseline water intake, and categorically based on health. The HCT values collected are illustrated in Table 10 as Least squares means. The HCT percentages across all groups were not significantly different during the baseline phase ($P > 0.05$). There were differences between groups seen at restriction levels 50%, 60%, and 80% ($P < 0.05$). At 80% restriction, group 2 HCT levels were significantly higher than all other groups ($P < 0.05$). At the same restriction level, group 3 was significantly lower than all the other groups ($P < 0.05$). At 60% restriction, group 2 had significantly higher HCT values than all other groups ($P < 0.05$), while group 1 had

significantly lower HCT values than all other groups ($P < 0.05$). Lastly, at 50% restriction group 2 and 3 saw the highest HCTs ($P < 0.05$) and group 4 had the lowest HCT values ($P < 0.05$). There were no differences between the summer groups (1 and 3) and the winter groups (2 and 4) during the baseline period ($P = 0.8913$), yet at 50% restriction HCT values were higher in the summer groups as compared to the winter groups ($P = 0.0002$). The winter groups saw the highest HCT percentages during the 60% restriction ($P < 0.001$), while the summer groups saw the highest HCT during the 50% restriction ($P < 0.05$). Overall, there was a significant increase in HCT when the cattle were water restricted to at least 60% of their baseline amounts in all groups ($P < 0.05$). There was no significant difference between baseline and 80% in groups 1 and 4 ($P = 0.108$, $P = 0.249$, respectively), indicating a restriction of 60% or greater was likely to cause changes in the hydration status of the experimental steers in those groups. However, in groups 2 and 3, baseline and 80% restriction were significantly different ($P < 0.05$) indicating a restriction level of 80% elicited signs of altered hydration in the experimental steers of those groups.

Red blood cells: The RBC Least squares means are shown in Table 11. During the baseline phase, RBC values were significantly different across groups, with group 4 having the numerically highest counts, but not significantly higher than groups 1 and 2. Group 3 had the lowest RBC counts and was significantly lower than both of the winter groups ($P < 0.05$). There was also a significant difference between seasons at the baseline level, with observed RBC counts higher in the summer months as compared to the winter months ($P < 0.05$). During the 50% restriction phase, seasonal differences were also seen, however it was the winter versus the summer groups that had higher RBC counts ($P <$

0.05). Throughout the duration of the study, the winter groups saw higher RBC counts during the 50% restriction compared to the baseline; and the summer groups saw lower RBC counts during the 50% restriction compared to the baseline ($P < 0.05$). However, the decrease in cell counts throughout the restriction during the winter can be attributed to a larger drop in cell counts from baseline to restriction during group 4. During group 4, the 50% restriction was significantly lower than all other restriction levels ($P < 0.05$).

White blood cells: White blood cell (WBC) Least squares means are reported in Table 12. During the baseline phase, there were no significant differences between groups ($P > 0.05$). Throughout all restrictive phases of the study, group 4 had significantly higher WBC counts than groups 1 and 2 ($P < 0.05$). Group 3 and 4 had no significant differences at all restriction levels except for 50% restriction, in which group 4 was significantly higher than group 3 ($P <0.05$). There were no significant differences in WBC counts between the summer and winter groups during baseline ($P > 0.05$), however, at 50% restriction the winter groups had higher counts of WBC as compared to the summer groups ($P < 0.001$). During 50% restriction, group 2 was not significantly different than the summer groups ($P > 0.05$), however group 4 had significantly higher WBC counts than all three other groups ($P < 0.05$), likely causing the overall differences between seasonal groups. Group 2 saw numerical increases in WBC counts as restriction level increased to 60%, at which point the 50% restriction level was significantly lower than all other levels ($P < 0.05$). Group 3 saw similar numerical increases, however 60% restriction had significantly higher WBC counts ($P < 0.05$), before the 50% restriction level WBC returned back to levels statistically similar to baseline. Group 4 saw an increase in WBC counts from baseline to 60% restriction ($P < 0.05$), after which 50%

restriction WBCs remained at the same levels as 60% restriction. Group 1 had higher WBC counts during baseline in comparison to 60% restriction ($P < 0.001$), yet there was no difference between the 60% and 50%. While groups 2 and 3 had increasing trends from baseline to 60% restriction, and group 1 had decreasing changes in WBC at the same levels, they were all statistically similar by 50% restriction.

Neutrophils: Table 13 shows the Least squares means of neutrophil counts by group and restriction level. Groups 2 and 3 followed similar trends, with 60% and 80% restriction having higher counts than 50% restriction ($P < 0.05$). Group 4 saw significantly higher values at both 60% and 50% restriction in comparison to all other groups ($P < 0.05$). Group 1 was the only group to see the highest neutrophil counts during the baseline, which decreased at each restriction level until the 50% restriction was significantly lower than the baseline ($P < 0.05$).

Lymphocytes: The Least squares means of lymphocyte cell counts by group and restriction can be found in Table 14. In groups 1 and 2, there were no differences between restriction levels ($P > 0.05$). Both groups 3 and 4 saw higher cell counts during 50% restriction compared to baseline ($P < 0.05$). Group 1 saw lower lymphocyte values in comparison to all other groups at both 50% and 60% restriction ($P < 0.05$).

Neutrophil-to-lymphocyte ratio: Table 15 shows the Least squares means of Neutrophil: Lymphocyte (N:L) calculations by group and restriction level. During the baseline phase, group 1 had a significantly higher N:L than the other three groups ($P < 0.05$). However, by 80% restriction, all groups were statistically similar to each other ($P > 0.05$). Values remained unchanged up until 60% restriction but by the 50% restriction,

groups 2 and 4 were significantly higher than groups 1 and 3 ($P < 0.05$). Furthermore, group 3 was significantly lower than all other groups ($P < 0.05$) at 50% restriction. There was no significant difference between baseline and 50% restriction in the winter groups ($P = 0.994$). However the summer groups saw lower N:L values at 50% restriction in comparison to baseline ($P < 0.05$).

Blood parameters by baseline water intake category: Intake categories were calculated based on individual animal water intakes as a percent of metabolic body weight. All animals within a group were then placed into one of three categories; high, medium, or low. Analysis using intake categories did not include group 4 due to *ad libitum* feed not being available at all times. When intake categories were included in the model assessing WBC, there was no significant interaction between group and intake category, however the interaction between restriction level and intake category was significant ($P < 0.05$). Table 17 shows the Least squares means of WBC by intake category. There was no significant difference between the baseline and the 50% restriction for any of the intake categories ($P > 0.05$). The high intake category had significantly higher WBC counts at 60% restriction than at 50% restriction or during baseline ($P < 0.05$). Including the three intake categories in the model did not significantly impact the analysis of N:L of the cattle as intake category was not significant ($P = 0.819$) nor were its interactions with group ($P = 0.141$) and restriction level ($P = 0.908$). In the RBC analysis, both the interaction between intake category and restriction level, and the interaction between intake category and group, were insignificant ($P = 0.287$ and $P = 0.913$, respectively). When including the intake category in the model analysis of HCT levels, the interaction between group and intake category

was not significant ($P = 0.859$). When HCT percentages were analyzed by intake categories, all categories saw higher HCT values at 50% restriction level in comparison to baseline ($P < 0.05$; Table 18).

Blood parameters by animal health category: Morbidity rates and percentages of health issues within morbid animals by group can be found in Table 19. When health categories were included in the HCT model analysis, the interactions between health and restriction ($P = 0.094$) and health and group ($P = 0.831$) were both insignificant. All interactions involving group, restriction, and health status were significant in the analysis of WBC. Table 20 shows the Least squares means of WBC counts between restriction level and health status, and Figure 4 illustrates the same interaction graphed by group. The "morbid" animals had significantly higher WBC counts for all restriction levels except for 60% ($P < 0.05$; Figure 5). There were no significant differences between restriction levels for the morbid animals ($P > 0.05$). However, group 2 had the most notable differences between health statuses among all restriction levels, with morbid animals having higher WBC at all restrictions ($P < 0.05$). When focusing on morbid animals across groups and restriction levels, the animals in group 2 had higher WBC counts at baseline, 80%, and 60% restriction than groups 1 and 3 ($P < 0.05$; Table 21). At 50% restriction there were no significant differences in WBC between any of the groups ($P > 0.05$), however when compared by season the winter groups had higher cell counts than the summer groups ($P = 0.004$). In the N:L model, the interaction between restriction level and health was not significant ($P = 0.113$). Figure 6 shows the Least squares means for the N:L by group and health status. Healthy animals were not significantly different from healthy animals in other groups ($P > 0.05$), however morbid

animals in group 2 had the lowest N:L compared to the other groups ($P < 0.05$). There were also differences between healthy and morbid animals within group 2, with morbid animals having lower N:L values ($P < 0.05$). Table 22 compares a standard reference range of hematological values for beef cattle compared to the values found for each blood parameter and group at baseline and 50% restriction.

Discussion

Animal Performance

As water was restricted during the step down and restriction phases, feed intakes decreased as well. This is in agreement with previous literature (Silanikove, 1985; Balch et al., 1953; Koknaroglu et al., 2008). Silanikove (1985) found that longer periods of water deprivation would result in greater decreases in feed intake in goats. Balch and others (1953) reported that reduced water intakes inhibited the rate of passage of digesta out of the rumen of dairy cattle. A study in which Comisana sheep were water restricted found that feed intakes did not decrease with water restriction (Casamassima et al., 2008). However, those animals were only restricted to either 80% or 60%, and over a period of 7 days. The increased restriction level and duration of restriction in this study likely contributed to the decrease in feed intakes observed. Similarly, Abdelatif and Ahmed observed decreased feed intakes in water restricted sheep when the interval between drinking was increased to every 48 hours and 72 hours (Abdelatif and Ahmed, 1994).

During groups 2 and 3, ADG decreased during the stepdown and restriction phase which agrees with decreased feed intakes during the same phases. Similarly to feed intakes, previous studies have observed that feed efficiency and ADG decrease under

adverse conditions such as water restriction or heat stress (Blackshaw and Blackshaw, 1994; Burgos et al., 2001; Mader and Davis, 2004). However, during group 1 the ADG remained at the same level, if not slightly higher. In group 1, this may be due to greater variation in the actual restriction levels of the animals as well as a heavy rain event that occurred the week before the first 50% restriction blood sample was taken. There were puddles of standing water and it was observed that some animals drank from those puddles. The water consumed from that source was not able to be quantified but may have contributed to increased feed intake and efficiency. In several water restriction studies, researchers have observed increased feed intakes soon after rehydration (Abdelatif and Ahmed, 1994; Burgos et al., 2001; Robertshaw and Dmi'el, 1983). The increase in SEM for the ADG during stepdown and restriction could be explained by differences in animal performance and adaptability. It is already well researched that differing breeds and species of livestock are better able to adapt to water restriction and hotter climates via genetic variations (Silanikove, 1985; Seibert and McFarlane, 1968; Finch, 1985). This would indicate the possibility that certain animals within the study were better able to adapt to drought-like conditions in terms of feed efficiency.

Environmental Data

The average temperature and humidity levels reported did not indicate any abnormally severe or mild conditions in comparison to other recent years' averages (OCS, 2016). Any THI values over 69 are considered to cause heat stress in cattle, and both of the summer groups experienced hot enough average climates to induce heat stress during all restriction levels (Dikmen et al., 2012). Based on the average THI for each restriction level, both summer groups were above the heat stress inducing threshold at all

restriction levels. The hottest months of the summer occurred while the studies were in the step down phase, potentially causing increased incidences of heat stress than during the 50% restriction phase. At no point during group 4 did THI rise enough to induce heat stress, and group 2 only experienced two days in which the THI reached that threshold. It is well documented in the literature that as THI increases, respiration rates also increase (Hahn, 1999; Mader et al., 2014; Mitloehner et al., 2002). This knowledge was confirmed by the present study in the positive PCC seen between respiration rate and THI as well as the increase in respiration rate means during the step down periods. The stepdown periods had higher overall THI in groups 1, 2, and 3. The negative relationship between THI and HCT can be explained by the increase in respiration rates as a coping mechanism for heat stress. Heat stress typically causes an increase in respiration, which adds to total water loss in the animal (Robertshaw and Dmi'el, 1983). A study of Bedouin black goats under dehydration saw decreased sweating as a physiological effort for the goats to retain water, while the rate of panting was not altered (Robertshaw and Dmi'el, 1983). This indicates that the evaporative cooling benefits of panting outweigh the detriments of any water loss in terms of maintaining internal body temperature. However, this increased water loss during the summer months could be a contributor to the differences in coping capabilities of animals between seasonal groups and their overall health status. While none of the PCC values between the blood parameters and respiration rates were very strong, the negative and positive relationships seen are interesting. As THI increased, WBC increased, which would indicate the water deprivation was strong enough of an environmental stressor to elicit an immune response. Additionally, the relationship between WBC and THI was only significant in groups 1

and 3, in which the animals were under heat stress conditions. The WBC counts for groups 1 and 3 were slightly lower in comparison to group 2, which is potentially due to the immunosuppressive nature of heat stress. Mashaly and colleagues (2003) found that laying hens that were heat stressed had significant WBC suppression. However, none of the WBC levels ever approached the outer limits of the healthy range that is considered "normal" for beef cattle.

Blood Data

Many of the overall changes seen in the collected blood parameters can be indicators of the immune system mounting a response or the body reacting to the lack of water; however, the findings may not have enough of a difference to consider an animal to have abnormal cell counts. As dehydration persisted, HCT percentages increased. This is commonly seen and well documented due to an over concentration of the blood due to use of the water pools within the body for other necessary functions (Berl et al., 1976; Jaber et al., 2004). It is noteworthy to mention that while there were statistical differences between groups and restriction levels, the Least squares means never exceeded outside ranges considered normal for beef cattle. While the water restriction was severe enough to cause a marked difference between certain groups' restriction levels, it may not have been severe enough to severely alter the animals' physiological state. Both summer groups saw an increase in HCT levels throughout the restriction, however the winter groups HCT percentages did not have as clear cut of a response. In the winter groups, the 60% restriction saw the highest HCT levels, with 50% following secondly to 60% levels. This could suggest that the designated 50% restriction level is not adequate in achieving the "drought-like" conditions that were intended, specifically in the winter groups; this

may also be true for the summer groups due to their HCT levels remaining within normal ranges for beef cattle. Also, because the cattle were not under heat stress conditions during the winter groups, they were not losing any body water to panting or sweating, thus preserving more water throughout the restriction. Another potential cause to the higher HCT levels during the 60% restriction could be an acclimation to the restriction and the cattle becoming more water efficient. It is already known that the body undergoes physiological changes in order to conserve water, including reduced sweating and more concentrated urine (Robertshaw and Dmi'el, 1983; Schmidt-Nielson et al., 1983). Thus as the restriction level was being decreased by 10% each week, the cattle were physiologically more impacted by the water restriction, but once the restriction level reached 50% and remained at 50% for 5 weeks, cattle may have adapted to that water allotment. Kaliber and colleagues (2016) water restricted goats in heat stress conditions for approximately two months and found that the goats were able to adapt in those conditions. The animals did not perform at optimal levels, but they were able to maintain themselves and stay relatively healthy (Kaliber et al., 2016). Additionally, the summer groups may have seen a sharper increase in HCT due to the increased occurrence of heat stress and respiration rates, which likely led to increased panting and overall water loss.

Some research has shown a decrease in overall RBC counts in the presence of chronic disease, infections or other pathologies (Klinken, 2002; Fletcher, 1974). With that in mind, it would appear that even without the presence of drought conditions, the increased THI may negatively impact RBCs. A study involving human patients assessed the correlation between RBC distributions and inflammatory biomarkers and found that there is a strong correlation between the two (Lippi et al., 2009). This phenomenon could

explain the significant increase in RBCs of group 1 and 3 between baseline and 50% restriction. While there were statistically significant differences between groups, the RBC counts did not fall outside of normal ranges at any point in the studies in any groups.

Group 4 experienced higher percentages of morbid animals in comparison to the other groups, and higher incidences of respiratory related morbidity, all of which can contribute to the higher WBCs (Blecha et al., 1984; Carroll and Forsberg, 2007; Maunsell, 2015). Additionally, this could explain the overall occurrences of higher WBC counts seen in group 4 throughout all restriction levels, especially at 50% restriction. Group 1 had an uncharacteristic drop in WBCs at 60% restriction that differs than the pattern followed by the other groups. This could be caused by the heavy rainfall previously mentioned in which cattle were able to drink from puddles, and potentially supplying their immune system with extra water resources. In groups 2 and 3 there is a peak increase of WBC at 60% restriction before they experience a sharp decrease in WBC counts. It has been observed that chronic stress is detrimental to the immune system and can cause an overall decrease in WBC counts if the stress is not mitigated (Mench and Moberg, 2000b). This may explain the significant decrease seen in WBC counts at 50% restriction, as chronic stress situations may lead to an initial increase in WBC counts before hormones (such as glucocorticoids) elicit their immunosuppressive capabilities (Minton, 1994; Sapolsky et al., 2007).

Groups 2, 3, and 4 saw increases in neutrophil levels from baseline to 60% restriction, which is in agreement with the literature that reports that as an animal is exposed to a stressor, neutrophils counts increase (Earley et al., 2006). However, by 50% restriction the neutrophil levels had decreased to levels similar to or less than baseline in

groups 2 and 3, indicating potential immunosuppression. The normal range of cattle neutrophil levels is between 0.6 and 4.0 ($x10^3/\mu L$), and the animals in this study had values near the top end of that range or slightly over at all restriction levels and groups. This may suggest that there might be perceived stressors in addition to the water restriction.

Lymphocyte counts did not act in accordance with results found in previous research that reported significant decreases in lymphocyte counts (Earley et al., 2006). In this study, while there were some statistically significant differences, the values remained within normal ranges. This would indicate that the change in N:L is likely due in most part to changes in neutrophil levels rather than lymphocyte levels.

The N: L is an effective indicator of chronic stress, as it takes several hours to see the effects of stressors, versus the mere minutes in which many stress hormones take effect (Davis et al., 2008). It is also thought to be modulated by stress hormones, with the ratio positively correlated to GC concentrations and its levels decreasing as GC concentrations return to normal levels (Davis et al., 2008). Davis and colleagues (2008) saw that the relationship between the N:L and GC levels was strong enough to use the N:L as a proxy for GC measurement. Based on those conclusions, there was a stress response occurring in groups 2, 3, and 4. However it appears that by the time 50% restriction is reached, the animals have adapted to the stressor and it the N: L began to decrease again. Group 1 saw an uncharacteristically high baseline value for N: L. There is no clear explanation that may have caused that very high baseline value, except that group 1 had nearly the lowest morbidity rates of all the groups. It is possible that there were animals in that group coping with sub-acute illnesses that went undetected. It has

also been reported that continued exposure to stressors can cause lymphocyte desensitization to GC stimulation, leading to an inability to mount an immune response in the future and compromising cell mediated immunity (Bauer et al., 2000; Dhabar, 2000).

Blood parameters by baseline water intake category: Water intake category seemed to have a minimal impact on how the cattle coped with the drought-like conditions indicating that water intake does not benefit or harm cattle's ability to cope with the lack of water. The RBC and HCT values follow the characteristic trends of increasing values during a water restriction event but there were minimal differences between intake categories and in terms of biologically relevant ranges (Robertshaw and Dmi'el, 1983; Schmidt-Nielson et al., 1983).

Blood parameters by animal health category: Across all groups, the occurrences of morbidity were inconsistent. The likely explanation for this is the variation in farm of origin and background, as each group was ascertained from a different farm but were exposed to the same protocol upon arrival. Group 2 saw the largest differences in WBC counts between health categories with the other three groups seeing insignificant differences between restriction level and health status. And while WBC were higher in the morbid animals of group 2, the N:L ratio of group 2 morbid animals was lower than both the healthy animals and the animals from all other groups. This is likely attributed to a shifting in the WBC distribution, with neutrophils being released into the peripheral blood while lymphocytes are directed into tissues (Al-Murrani et al., 2006; Carroll and Forsberg, 2007; Davis et al., 2008).

Conclusion

The restriction of water intake by beef cattle was significant and elicited the indicators of stress and dehydration in the blood parameters measured. However, the overall changes in blood parameters at the 50% restriction level are indicative of immunosuppressive qualities and the detrimental impacts of chronic stress. Based on the initial increase in HCT levels, before they decreased back to baseline levels, it would appear that cattle require a period of adaptation for water metabolism to adjust to the decrease in available water. The animals coped with the extended water restriction by altering normal activities such as decreased feed intakes, however it is suspected the animals would poorly withstand immune challenges and additional stressors in their restricted state due to overall decreases in WBC counts at the 50% restriction. The cattle were able to cope with limited water intake, however animal performance was impaired due to increased WBC and N:L requiring the diversion of energy from growth to the immune system and decreases in feed intakes as a coping mechanism. While there were indications of a stress response due to the characteristic changes in cell counts, the large majority of measurements did not exceed normal ranges for cattle. This underscores the resiliency of cattle in inclement conditions. It would be of interest to assess individual differences within a population to see if certain animals exceeded the normal ranges. While the overall cell counts were altered throughout the study, the additional collection of indicators of cell activity would be ideal in parsing out the functionality of the immune response and the animals overall immune function throughout the extended period of time. Based on the changes in cell counts throughout restriction, it would be interesting to expose animals to an immune challenge during the 50% restriction to assess the functionality of the animals' immune systems during that chronic stress. Further analysis

would be needed to compare individual animals within the entire group to determine if certain animals are better able to adapt to the restriction of water. While this study demonstrated the hardiness of beef cattle, it would also be of interest to select for those animals that were best able to adapt to the reduction in available water.

Table 1. Ingredients included in the total mixed ration fed during the experiment.

Diet Composition (Dry Matter)	
Item	Amount (%)
Cracked Corn	17
Sweet Bran[a]	45
B-273[b]	6
Prairie Hay	32

[a]Cargill, Inc., Minneapolis, MN.

[b] Formulated to contain: 27.86% limestone, 0.95% MgO, 0.353% salt, 6.445% urea 41.03% corn grain, 21.68% wheat midds, 0.11% copper sulfide, 0.05% selenium premix, 0.57% zinc sulfate, 0.286% vitamin A, 0.078% vitamin E. 0.29% Rumensin-90, 0.178% Tylan-40

Table 2. Dietary nutrient composition fed throughout the duration of the experiment.

Item	Value[1]
DM, %	73.3
$NE_{m,}$ Mcal/45 kg.	73.98
$NE_{g,}$ Mcal/45 kg.	46.5
TDN %	74.8
Fat	4.16
Crude Fiber	10.17
ADF	20.26
NDF	39.02
eNDF	32.39
Crude Protein %	16.05
Potassium %	1.19
Calcium %	0.94
Phosphorus %	0.76
Magnesium %	0.34
Sulfur %	0.28
Cobalt ppm	0.1
Copper ppm	21.9
Iron ppm	127.4
Manganese ppm	86.8
Selenium ppm	0.05
Zinc ppm	165.9

[1]All values are % of diet on dry matter basis unless specified.

Table 3. The Least squares means of dry matter feed intakes (kg) per group by restriction level.

	Group 1		Group 2		Group 3	
Water restriction level[1]	LS mean	SEM	LS mean	SEM	LS mean	SEM
Baseline	$10.6^{b,x}$	0.124	10.0^{ab}	0.118	10.1^{ab}	0.117
10	$10.9^{a,w}$	0.135	10.8^{a}	0.132	9.66^{b}	0.129
20	$10.5^{a,x}$	0.138	9.98^{a}	0.135	8.88^{b}	0.130
30	$9.13^{a,y}$	0.139	8.91^{b}	0.129	8.22^{a}	0.130
40	$8.99^{a,y}$	0.140	$7.84^{b,x}$	0.130	7.73^{b}	0.132
Restriction	$8.38^{b,z}$	0.126	$8.22^{a,x}$	0.122	5.96^{c}	0.121

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).
^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).
[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

Table 4. The Least squares means of the daily drinking water intakes (kg) per group by restriction level.

Water restriction level[1]	Group 1		Group 2		Group 3	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	$40.5^{a,x}$	0.401	23.5^{c}	0.416	$36.2^{b,v}$	0.402
10	$43.4^{a,w}$	0.633	36.3^{b}	0.641	$35.2^{b,v}$	0.634
20	$35.5^{a,y}$	0.480	25.0^{c}	0.486	$28.7^{b,w}$	0.481
30	$43.4^{a,w}$	0.630	19.6^{c}	0.638	$24.3^{b,x}$	0.633
40	$41.1^{a,wx}$	0.737	16.7^{c}	0.466	$22.1^{b,y}$	0.463
Restriction	$20.9^{a,z}$	0.386	12.4^{c}	0.391	$16.2^{b,z}$	0.386

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).
^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).
[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

Table 5. The Least squares means of ADG reported by phase of study.

Water restriction level[1]	Group 1		Group 2		Group 3	
	LS Means[2]	SEM	LS Means	SEM	LS Means	SEM
Baseline	$1.428^{b,x}$	0.028	$1.733^{a,x}$	0.030	$1.459^{b,x}$	0.030
Stepdown	0.912^{y}	0.048	0.702^{y}	0.052	0.295^{y}	0.051
Restriction	1.517^{x}	0.051	0.653^{y}	0.055	0.294^{y}	0.055

[1] Baseline being at restriction level of 100%, stepdown being all days between 90% and 60%, and restriction being all days at 50%.
[2] Means are reported in kilograms of gain per day.
[a,b,c] LS means within a row with differing superscripts are different ($P < 0.05$).
[w,x,y,z] LS means with different superscripts within a group are different ($P < 0.05$).

Table 6. Mean daily Temperature Humidity Index (THI)[1] by restriction level

Water restriction level[2]	Group 1		Group 2		Group 3		Group 4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	73.8	4.10	42.6	8.44	71.6	6.68	45.3	9.21
10	75.2	2.88	46.1	4.61	79.3	1.93	38.2	4.51
20	74.7	2.91	49.4	5.96	80.3	0.68	38.9	6.11
30	78.1	1.09	40.7	7.93	77.2	1.35	32.9	2.41
40	78.5	1.15	47.0	5.56	80.0	2.14	44.8	5.93
Restriction	70.3	6.43	45.5	11.63	73.4	4.03	51.6	6.44

[1] THI equation: THI=(0.8*Ambient Temp) + ((Relative Humidity/100) * (Ambient Temp-14.4)) + 46.4 (Mader, 2006)

[2] Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

Table 7. The Least squares means of respiration rate by restriction level.

Water restriction level	Baseline	10	20	30	40	Restriction
Mean	46.9[b]	38.2[c]	52.2[b]	50.7[b]	50.4[b]	39.7[c]
Standard Deviation	19.8	14.7	24.8	27.5	23.9	16.9

[a, b, c] ls means with different superscripts within a restriction level are significantly different ($P < 0.05$).

Table 8. The R^2 values for the relationship between each blood parameter and THI presented by group.

	Group 1	Group 2	Group 3	Group 4
Blood parameters[1]	R^2	R^2	R^2	R^2
HCT	0.026*	0.001*	0.030*	0.099*
WBC	0.005*	0.001	0.047*	0.001
RBC	0.013*	0.004	0.032*	0.141*
N:L	0.001	0.0002	0.062*	0.003

1 HCT = hematocrit, WBC = white blood cells, RBC = red blood cells, N:L = neutrophil to lymphocyte ratio.

* Indicates an R^2 that is significantly different than zero ($P < 0.05$).

Table 9. The Pearson Correlation Coefficients (PCC) between the blood parameters and THI by group.

	Group 1	Group 2	Group 3	Group 4
Blood parameters[1]	PCC	PCC	PCC	PCC
HCT	-0.166*	0.017	-0.177*	-0.316*
WBC	0.083*	0.017	0.220*	-0.011
RBC	-0.120*	-0.073	-0.183*	-0.378*
N:L	0.046	-0.041	0.252*	-0.065

[1] HCT = hematocrit, WBC = white blood cells, RBC = red blood cells, N:L = neutrophil to lymphocyte ratio.

* Indicates PCC that is significantly different than zero ($P < 0.05$).

Table 10. The Least squares means of hematocrit percentages by group and by water intake restriction level.

Water restriction level[1]	Group 1		Group 2		Group 3		Group 4	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	38.4^{y}	0.353	38.3^{y}	0.358	38.2^{z}	0.350	38.3^{y}	0.360
20	$38.1^{ab,y}$	0.336	$38.9^{a,y}$	0.339	$37.6^{b,z}$	0.334	$38.1^{ab,y}$	0.344
40	$38.1^{c,y}$	0.369	$41.2^{a,x}$	0.375	$39.5^{b,y}$	0.366	$39.9^{b,x}$	0.376
Restriction	$39.9^{b,x}$	0.357	$40.6^{ab,x}$	0.361	$41.3^{a,x}$	0.355	$37.9^{c,y}$	0.365

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).

^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).

Table 11. The Least squares means of red blood cells by group and by restriction level.

Water restriction level[1]	Group 1		Group 2		Group 3		Group 4	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	8.75^{a}	0.068	8.98^{a}	0.071	$8.61^{b,y}$	0.067	$9.08^{a,x}$	0.070
20	8.66^{ab}	0.065	8.98^{a}	0.068	$8.57^{b,y}$	0.065	$8.84^{ab,x}$	0.067
40	8.75^{b}	0.068	9.24^{a}	0.073	$8.90^{ab,x}$	0.069	$9.06^{ab,x}$	0.070
Restriction	8.85^{b}	0.067	9.03^{ab}	0.070	$9.20^{a,x}$	0.068	$8.51^{c,y}$	0.069

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).

^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).

Table 12. The Least squares means of white blood cell counts by group and water intake restriction level

Water restriction level[1]	Group 1		Group 2		Group 3		Group 4	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	11.3	0.230	11.2^{x}	0.233	11.1^{y}	0.227	11.9^{z}	0.234
20	11.2^{b}	0.223	$11.4^{b,x}$	0.223	$11.8^{ab,y}$	0.220	$12.6^{a,yz}$	0.228
40	10.4^{c}	0.225	$11.9^{b,x}$	0.230	$12.7^{ab,x}$	0.224	$13.3^{a,x}$	0.230
Restriction	10.5^{b}	0.203	$10.6^{b,y}$	0.205	$11.4^{b,y}$	0.201	$13.1^{a,xy}$	0.207

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

[a,b,c] LS means within a row with differing superscripts are different ($P < 0.05$).

[w,x,y,z] LS means with different superscripts within a group are different ($P < 0.05$).

Table 13. The Least squares means of neutrophils by group and by water intake restriction level.

	Group 1		Group 2		Group 3		Group 4	
Water restriction level[1]	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	$4.02^{a,x}$	0.113	$3.27^{b,xy}$	0.115	$3.00^{b,yz}$	0.112	$3.47^{ab,y}$	0.115
20	3.64^{xy}	0.111	3.45^{x}	0.111	3.46^{xy}	0.110	3.96^{xy}	0.114
40	$3.30^{b,yz}$	0.105	$3.66^{b,x}$	0.108	$3.63^{b,x}$	0.104	$4.20^{a,x}$	0.107
Restriction	$2.95^{b,z}$	0.086	$3.05^{b,y}$	0.087	$2.86^{b,z}$	0.085	$4.07^{a,x}$	0.087

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

[a,b,c] LS means within a row with differing superscripts are different ($P < 0.05$).

[w,x,y,z] LS means with different superscripts within a group are different ($P < 0.05$).

Table 14. The Least squares means of lymphocytes by group and restriction level.

Water restriction level[1]	Group 1		Group 2		Group 3		Group 4	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	5.93^{b}	0.150	6.32^{a}	0.152	$6.21^{ab,y}$	0.148	$6.79^{a,y}$	0.153
20	6.04^{b}	0.141	6.66^{a}	0.142	$6.64^{ab,xy}$	0.140	$6.98^{a,xy}$	0.144
40	5.88^{b}	0.145	6.97^{a}	0.148	$7.25^{a,x}$	0.144	$7.32^{a,xy}$	0.148
Restriction	6.25^{b}	0.136	6.38^{b}	0.137	$7.05^{a,x}$	0.135	$7.38^{a,x}$	0.139

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).

^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).

Table 15. The Least squares means of neutrophil:lymphocyte by group and by water intake restriction level.

	Group 1		Group 2		Group 3		Group 4	
Water restriction level[1]	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	$0.712^{a,x}$	0.021	0.541^{b}	0.021	$0.506^{b,x}$	0.021	0.526^{b}	0.021
20	0.614^{y}	0.019	0.537	0.019	0.545^{x}	0.019	0.581	0.019
40	0.586^{y}	0.018	0.545	0.019	0.525^{x}	0.018	0.608	0.019
Restriction	$0.493^{ab,z}$	0.015	0.503^{a}	0.015	$0.430^{b,y}$	0.015	0.564^{a}	0.015

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

^{a,b,c} LS means within a row with differing superscripts are different ($P < 0.05$).

^{w,x,y,z} LS means with different superscripts within a group are different ($P < 0.05$).

Table 16. The Least squares means of all blood parameters by water intake restriction level.

Water restriction level[1]	Baseline		20%		40%		Restriction	
Item[2]	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
HCT	38.3[b]	0.178	38.2[b]	0.169	39.7[a]	0.186	39.9[a]	0.180
RBC	8.85[b]	0.035	8.76[c]	0.033	8.99[a]	0.035	8.90[b]	0.034
WBC	11.4[c]	0.116	11.7[b]	0.112	12.1[a]	0.114	11.4[c]	0.102
N:L	0.571[a]	0.010	0.569[a]	0.009	0.566[a]	0.009	0.497[b]	0.008
NEUT	3.44[b]	0.059	3.65[a]	0.058	3.73[a]	0.058	3.25[c]	0.046
LYMPH	6.31[c]	0.075	6.58[b]	0.071	6.86[a]	0.073	6.76[a]	0.068

[1]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

[2] HCT = hematocrit, WBC = white blood cells, RBC = red blood cells, N:L = neutrophil to lymphocyte ratio, NEUT = neutrophils, LYMPH= lymphocytes.

[a,b,c] LS means within a row with differing superscripts are different ($P < 0.05$).

Table 17. The Least squares means for white blood cells by restriction level and intake category.

Water restriction level[2]	Intake Category[1] Low		Medium		High	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	11.3	0.416	11.3	0.179	10.9^{yz}	0.376
20	11.2	0.376	11.5	0.164	11.8^{xy}	0.344
40	11.0	0.359	11.7	0.155	11.9^{x}	0.325
Restriction	10.8	0.325	10.8	0.140	10.9^{z}	0.293

[1] Intake category based upon the animal's water intake as a percentage of their metabolic body weight. Each group was divided into three categories; high (1 standard deviation greater than the mean or greater), medium (within 1 standard deviation of the mean), and low (1 standard deviation lower than the mean or greater).

[w,x,y,z] LS means with different superscripts within an intake category are different ($P < 0.05$).

[2]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

Table 18. Least squares means of hematocrit by intake category.

Water restriction level[2]	Intake Categories[1]					
	Low		Medium		High	
	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM
Baseline	39.3^{y}	0.559	38.2^{y}	0.244	38.0^{z}	0.514
20	39.4^{y}	0.532	38.2^{y}	0.235	37.0^{y}	0.494
40	40.4^{xy}	0.586	39.7^{x}	0.255	38.9^{xy}	0.539
Restriction	41.8^{x}	0.552	40.6^{x}	0.241	39.6^{x}	0.506

[1] Intake category based upon the animal's water intake as a percentage of their metabolic body weight. Each group was divided into three categories; high (1 standard deviation greater than the mean or greater), medium (within 1 standard deviation of the mean), and low (1 standard deviation lower than the mean or greater).

[2]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

[a,b] LS means with different superscripts between restriction levels are different ($P < 0.05$)

[x, y, z] LS means with different superscripts within an intake category are different ($P < 0.05$).

Table 19. The morbidity rates, expressed as a percentage of total animals, and percentages of health issues for each group.[2]

	Group 1	Group 2	Group 3	Group 4
Morbidity[1]	12.8	31.3	29.9	37.5
Respiratory Illness	13.3	25.0	22.8	95.2
Pink Eye	20.0	16.6	54.3	0.0
Hoof Rot	33.3	25.0	17.1	2.4
Other	33.3	33.3	5.8	2.4

[1] morbidity is presented as a percentage of total animals on the study within a group
[2] Respiratory illness, pink eye, hoof rot, and "other" are the percentages of morbid animals within a group that were treated for that respective health issue and are expressed as percentages.

Table 20. The Least squares means of white blood cells by restriction level and health status.

Water restriction level[2]	Health Status[1]			
	Healthy		Morbid	
	LS Mean	SEM	LS Mean	SEM
Baseline	11.3[b,y]	0.121	13.0[a]	0.429
20	11.6[b,x]	0.117	13.6[a]	0.430
40	12.0[x]	0.119	13.1	0.462
Restriction	11.3[b,y]	0.107	12.8[a]	0.382

[1] Health status is defined as whether the animal was treated at any point during the study, categorized as either "healthy" or "morbid".

[2]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted

[a, b, c] LS means with different superscripts within a restriction level are different ($P < 0.05$).

[x, y, z] LS means with different superscripts within a health category are different ($P < 0.05$).

Table 21. The Least squares means for white blood cells of morbid animals by restriction and group[1].

Water restriction level[2]	Group 1		Group 2		Group 3		Group 4	
	LS Means	SEM	LS Means	SEM	LS Means	SEM	LS Means	SEM
Baseline	12.3[b]	0.922	16.2[a]	1.22	11.6[b]	0.520	12.0[b]	0.575
20	12.3[b]	0.894	16.0[a]	1.26	12.5[b]	0.504	13.5[ab]	0.571
40	10.1[b]	0.906	15.1[a]	1.42	13.3[ab]	0.511	13.9[ab]	0.572
Restriction	11.8	0.819	13.5	1.09	11.8	0.462	14.05	0.511

[1] Health status is defined as whether the animal was treated at any point during the study, categorized as either "healthy" or "morbid".
[2]Values expressed as a percentage. Indicates how much of the animal's baseline was restricted
[a,b] ls means with different superscripts within a restriction level are different ($P < 0.05$)

Table 22. A comparison of Least squares means of the blood parameters during baseline and 50% restriction to normal reference ranges[1].

	Reference Range[2]	Group 1		Group 2		Group 3		Group 4	
		100%	50%	100%	50%	100%	50%	100%	50%
HCT (%)	24–46	38.4	39.9	38.3	40.6	38.2	41.3	38.3	37.9
RBC ($x10^6/\mu L$)	5.0–10.0	8.75	8.85	8.98	9.03	8.61	9.2	9.08	8.51
WBC ($x10^3/\mu L$)	4.0–12.0	11.3	10.5	11.2	10.6	11.1	11.4	11.9	13.1
Neutrophil ($x10^3/\mu L$)	0.6-4.0	4.02	2.95	3.27	3.05	3	2.86	3.47	4.07
Lymphocyte ($x10^3/\mu L$)	2.5-7.5	5.93	6.25	6.32	6.38	6.21	7.05	6.79	7.38
N:L	0.2-0.5	0.712	0.493	0.541	0.503	0.506	0.43	0.526	0.564

[1] HCT = hematocrit, WBC = white blood cells, RBC = red blood cells, N:L = neutrophil to lymphocyte ratio.

[2] Reference ranges courtesy of Merck Animal Health with data compiled from Latimer KS. *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology*, 5th ed., Wiley-Blackwell, 2011; and Weiss DJ, Wardrop KJ, *Schalm's Veterinary Hematology*, 6th Ed., Wiley-Blackwell, 2010. Reference ranges vary between laboratories. Values provided by the reference laboratory should be used.

Figure 1. Illustration of the study timeline and blood collection schedule[1]. [a] Length of 21 days. [b] Length of 70 days to collect *ad libitum* intakes. [c] Length of 35 days in which water level is restricted by 10% each week.[d] Length of 35 days in which water level is maintained at 50%[e] Length of 6 days in which animals are acclimated to *ad libitum* access.[1] The red arrows indicate days on which blood samples were collected for Procyte analysis and gray arrows indicate days in which blood was collected for manual hematocrit.

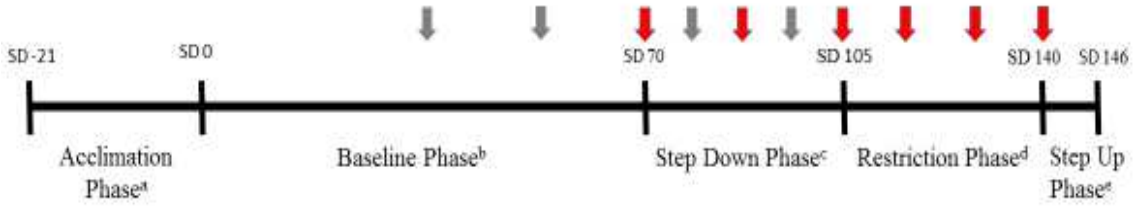


Figure 2. The Least squares means of feed and drinking water intake across all restriction levels and groups. Error bars represent the standard error of the mean.

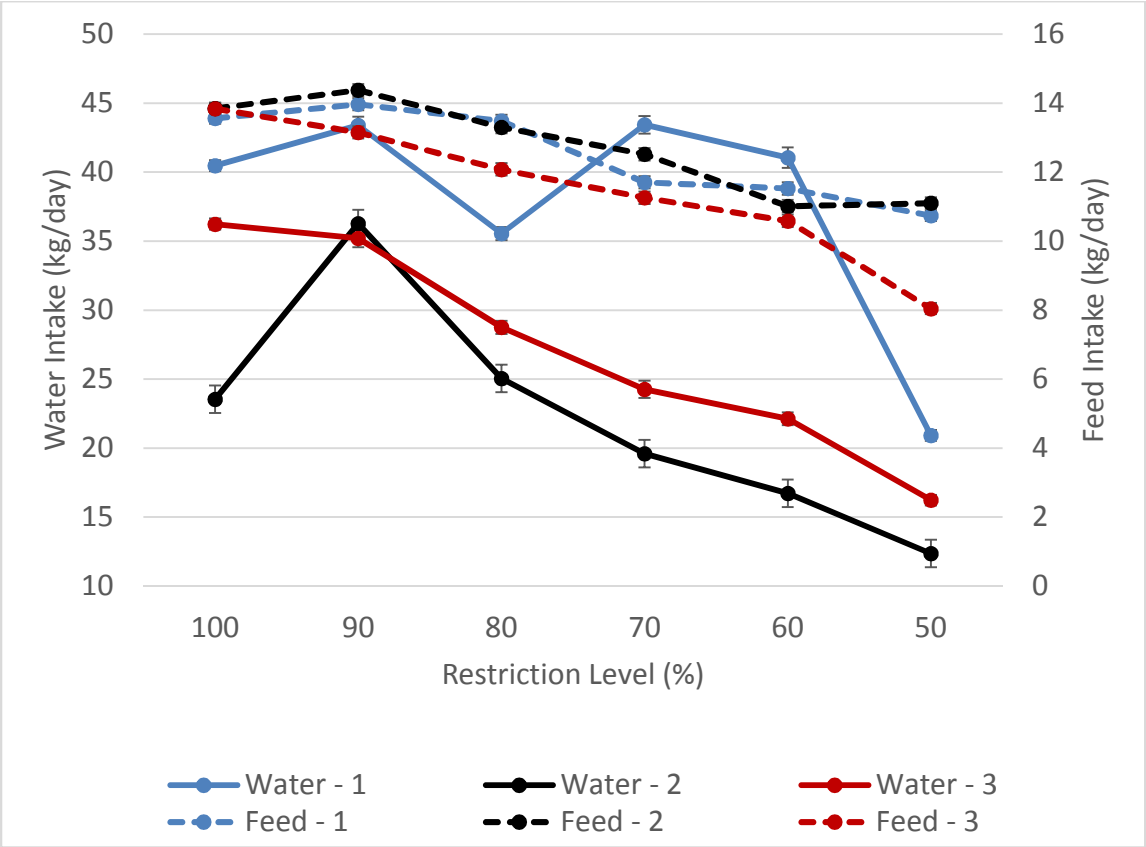
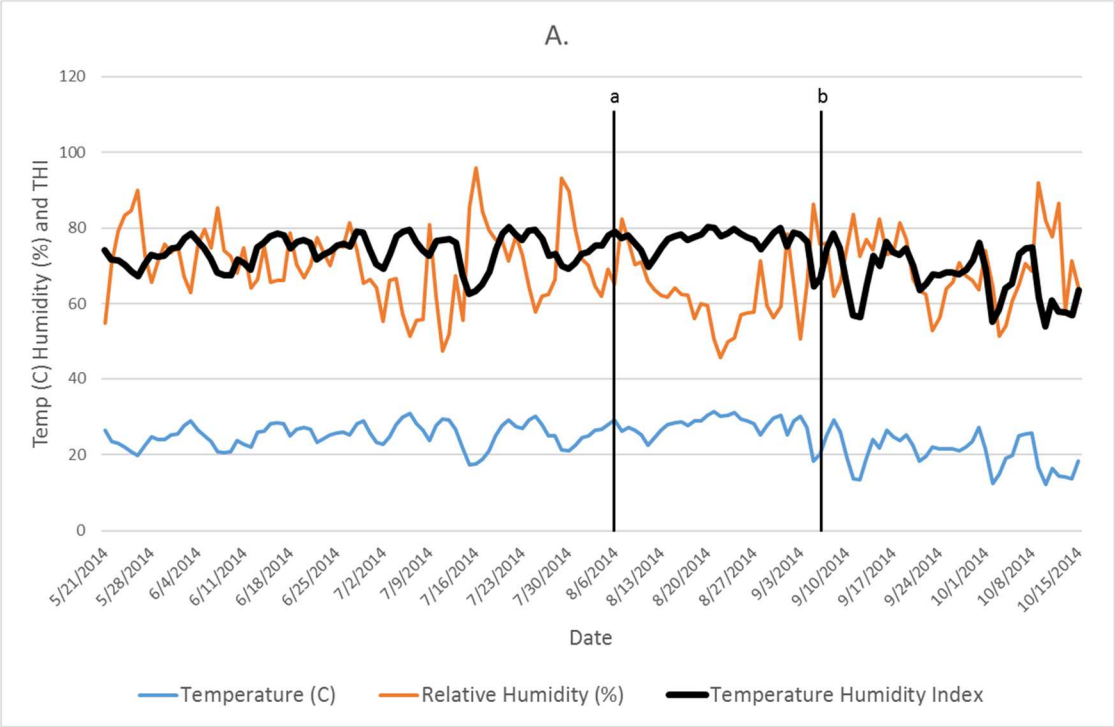
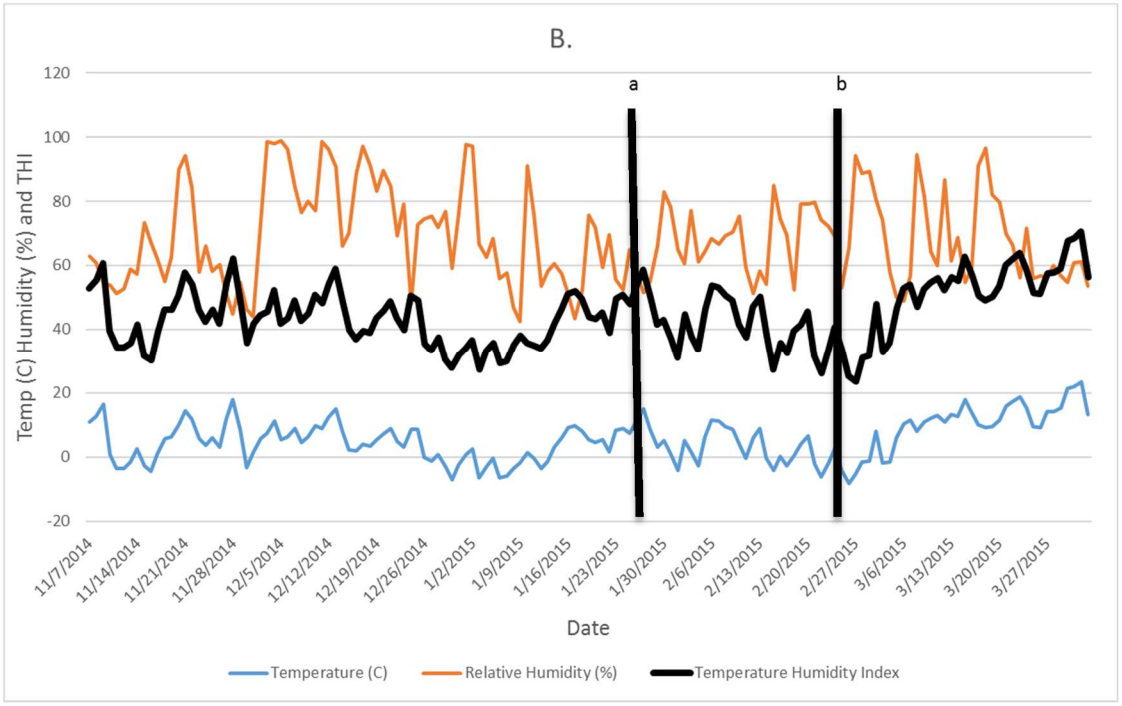


Figure 3. Weather parameters collected and calculated by group. A) Group 1 B) Group 2 C) Group 3 D) Group 4. [a] Denotes the end of the baseline phase and the beginning of the step down phase. [b] Denotes the end of the step down phase and the beginning of the restriction phase.

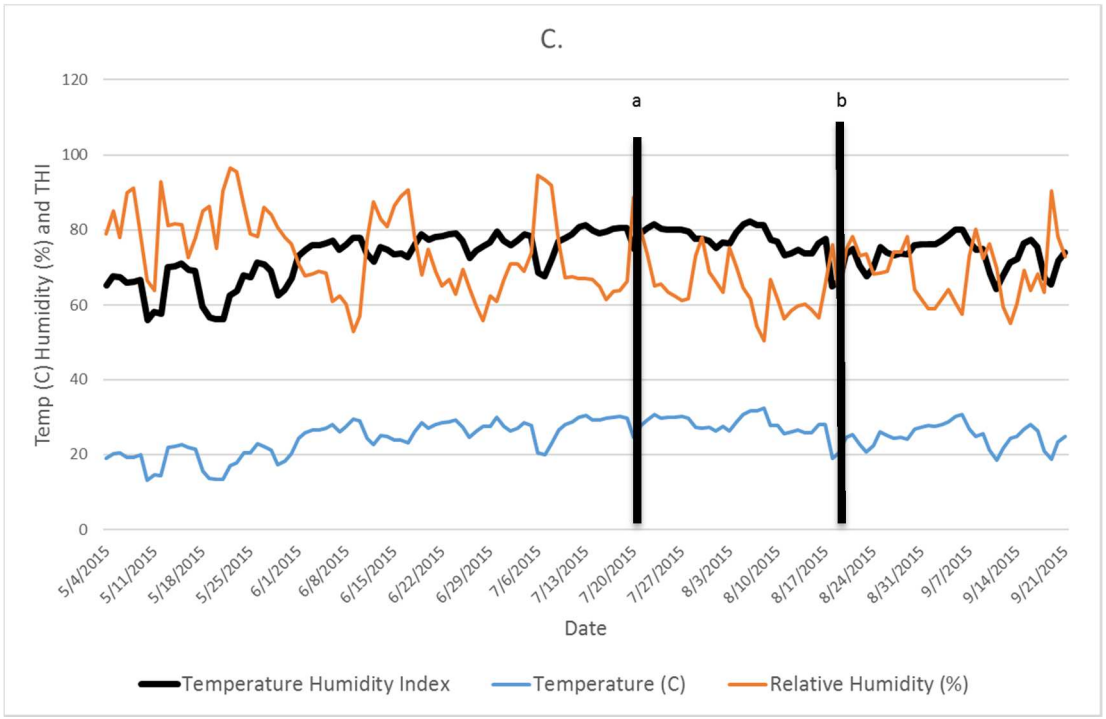
A.



B.


B.
a
b
120
100
80
60
40
20
0
-20
Temp (C) Humidity (%) and THI
11/7/2014
11/14/2014
11/21/2014
11/28/2014
12/5/2014
12/12/2014
12/19/2014
12/26/2014
1/2/2015
1/9/2015
1/16/2015
1/23/2015
1/30/2015
2/6/2015
2/13/2015
2/20/2015
2/27/2015
3/6/2015
3/13/2015
3/20/2015
3/27/2015
Date
Temperature (C)
Relative Humidity (%)
Temperature Humidity Index

C


C.
a
b
120
100
80
60
40
20
0
Temp (C) Humidity (%) and THI
5/4/2015
5/11/2015
5/18/2015
5/25/2015
6/1/2015
6/8/2015
6/15/2015
6/22/2015
6/29/2015
7/6/2015
7/13/2015
7/20/2015
7/27/2015
8/3/2015
8/10/2015
8/17/2015
8/24/2015
8/31/2015
9/7/2015
9/14/2015
9/21/2015
Date
Temperature Humidity Index
Temperature (C)
Relative Humidity (%)

D

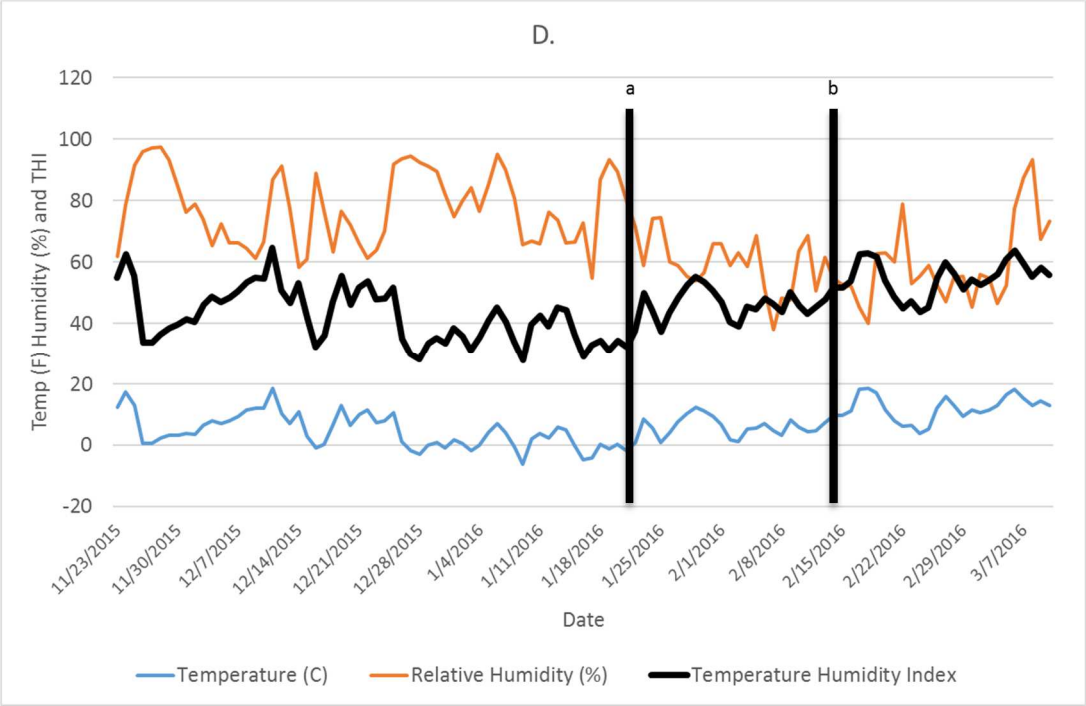
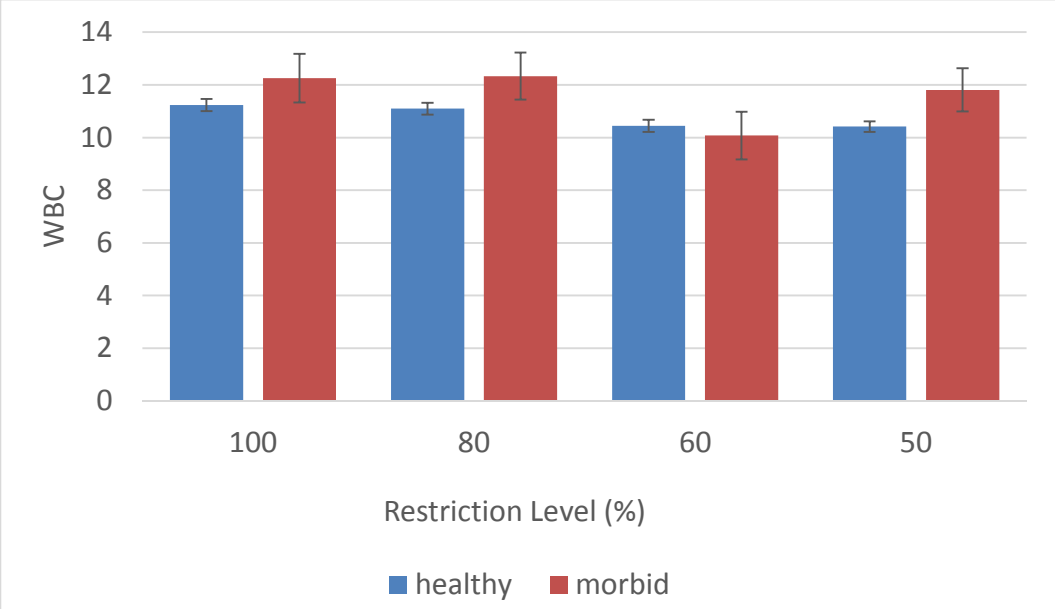
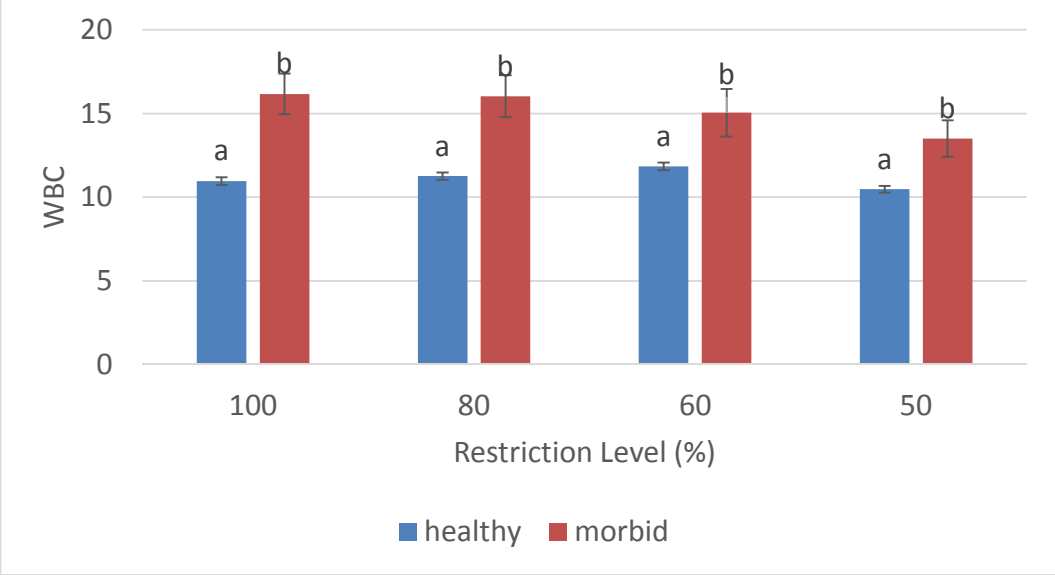

D.
120
100
80
60
40
20
0
-20
Temp (F) Humidity (%) and THI
a
b
11/23/2015
11/30/2015
12/7/2015
12/14/2015
12/21/2015
12/28/2015
1/4/2016
1/11/2016
1/18/2016
1/25/2016
2/1/2016
2/8/2016
2/15/2016
2/22/2016
2/29/2016
3/7/2016
Date
Temperature (C)
Relative Humidity (%)
Temperature Humidity Index

Figure 4. The Least squares means of white blood cells between restriction level and health status. A) Group 1 B) Group 2 C) Group 3 D) Group 4. Error bars represent the standard error of the mean. [a,b] differing superscripts within a restriction level indicate significant difference of $P < 0.05$

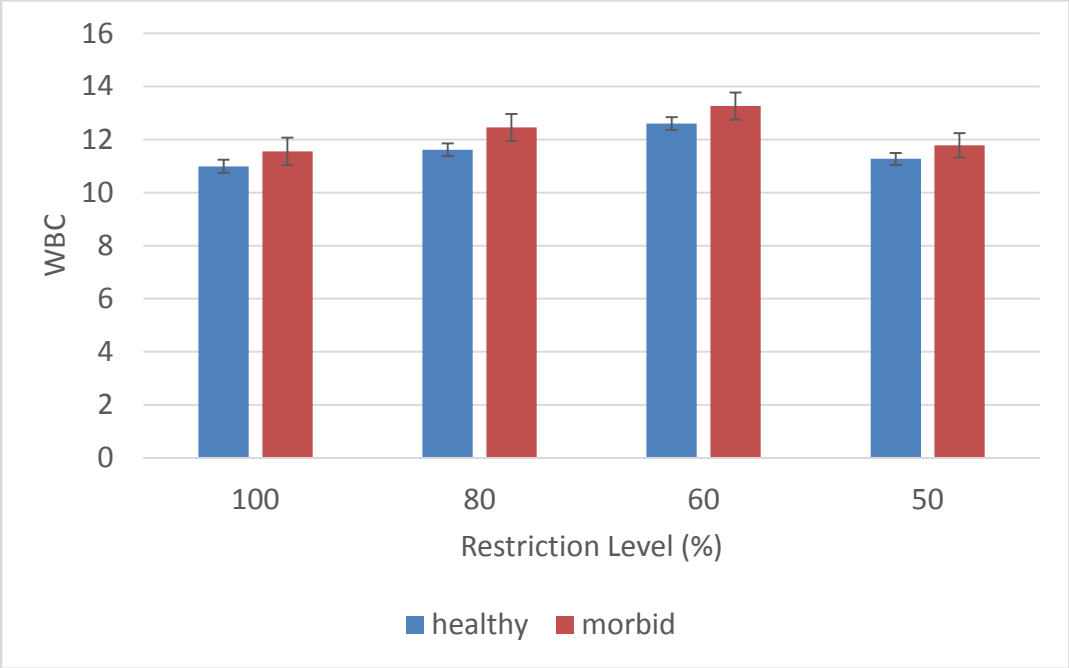
A.



B.



C.


16
14
12
10
8
6
4
2
0
WBC
100
80
60
50
Restriction Level (%)
healthy
morbid

D.

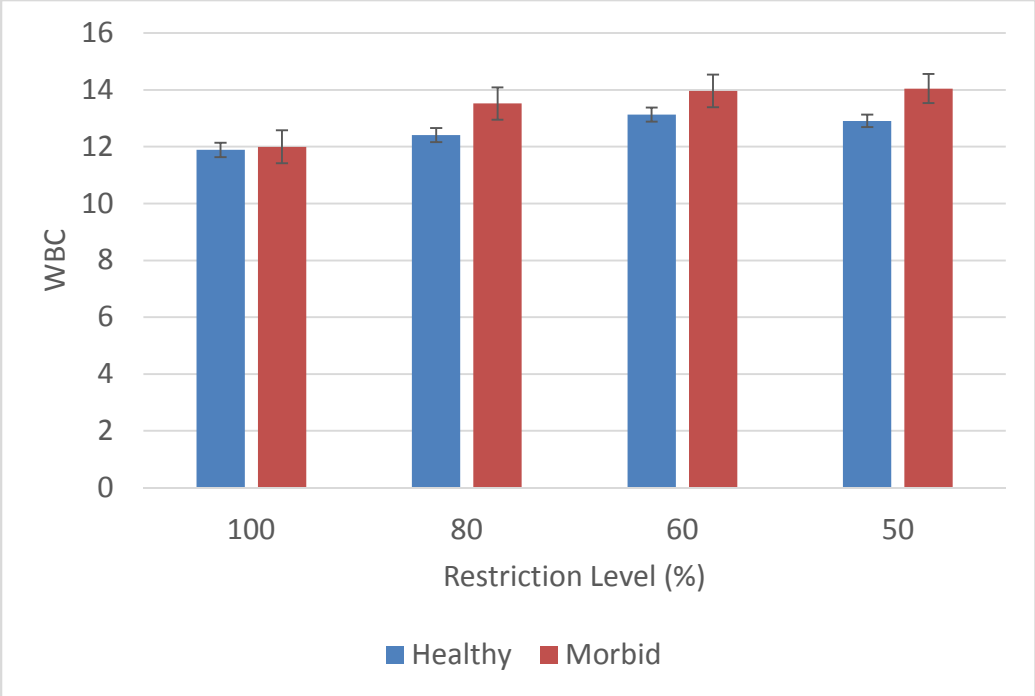

16
14
12
10
8
6
4
2
0
WBC
100
80
60
50
Restriction Level (%)
Healthy
Morbid

Figure 5. Least squares means of white blood cells by restriction level and health status. Health status is defined as whether the animal was treated at any point during the study, categorized as either "healthy" or "morbid". Standard error of the means denoted by error bars.* denotes differences between health categories within a restriction level ($P < 0.05$).

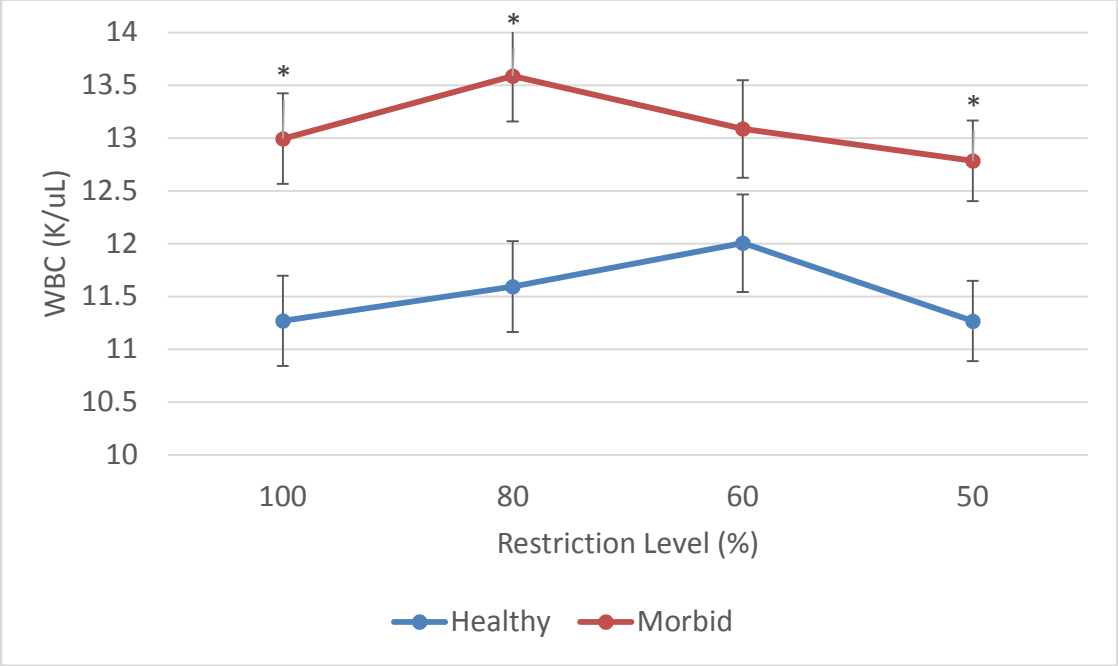
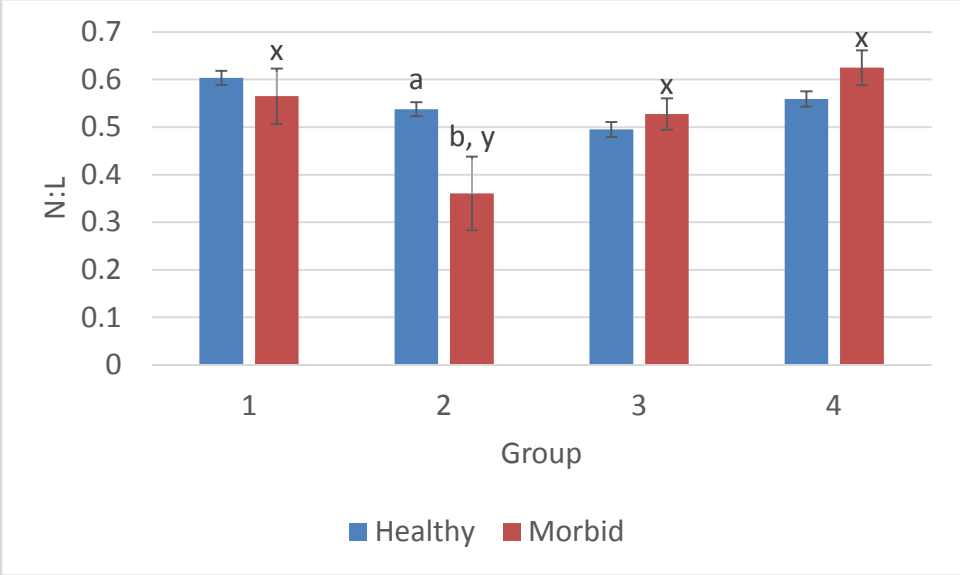


Figure 6. Least square means of neutrophil: lymphocyte by health status. Health status is defined as whether the animal was treated at any point during the study, categorized as either "healthy" or "morbid". Standard error of the means denoted by error bars. [a,b] LS means with different superscripts within a group are different ($P < 0.05$). [x, y] LS means with different superscripts across all groups are different ($P < 0.05$).



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