EFFECTS OF EXTENDED WATER RESTRICTION ON PERFORMANCE, BEHAVIOR, HEALTH, AND WELFARE OF FEEDLOT STEERS

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Abstract: Climate change and a growing human population are expected to impact water availability globally, which could limit water resources for animal agriculture. As water resources become scarcer, methods to efficiently utilize water will be necessary for livestock production. The following experiments aimed to evaluate methods to identify animals that are more water efficient. These experiments restricted water intake to 50% of *ad libitum* water intake for 42 d using an Insentec Roughage Intake Control system. In the first experiment, cattle were assigned to a chute score (CS) and exit velocity (EV) rank, and performance was assessed during baseline intake and water restriction. During restriction, DMI, ADG, WI, and G:F were all decreased compared to baseline ($P \leq 0.05$). Low CS steers had greater ADG during baseline, but intermediate CS steers had greater ADG during restriction ($P \leq 0.05$). There were no EV by period interactions ($P \geq 0.13$). The next 2 experiments examined effects of water restriction between steers with different water efficiency utilization. Water efficiency (WE) was calculated as ADG (kg)/water intake as a percent of BW. High WE steers had greater DMI, ADG, and G:F during baseline, but measures were not different during restriction ($P \geq 0.22$). Red blood cell counts, hemoglobin, and hematocrit were lower in Medium WE steers, compared to Low and High ($P \leq 0.05$). There was no difference in morbidity between WE groups ($P \geq 0.14$). Low WE steers had higher rectal temperatures than Medium and High ($P \leq 0.01$). There was not a difference in agonistic behavior between WE groups ($P \geq 0.93$). During baseline, feeding behavior following a biphasic pattern, where feeding occurred during morning and evening hours, but during water restriction feeding and drinking behavior shifted to early morning hours. These experiments provide information about how beef cattle respond to water restriction, both behaviorally and physiologically. Results of these experiments indicate that there are differences in adaptability between cattle with differing CS and WE.

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ABBREVIATIONS

Abbreviation	Meaning
AII	Angiotensin II
Ach	Acetylcholine
ACTH	Adrenocorticotropic hormone
ADF	Acid detergent fiber
ADG	Average daily gain
ADH	Antidiuretic hormone
ADWI	Average daily water intake
ANS	Autonomic nervous system
BAS	Baseline water intake period
BASO	Basophil
BBM	Baseline behavioral measures
BCS	Body condition score
BGT	Black globe temperature
bpm	Breaths per minute
BW	Body weight
CCI	Cattle comfort index
Cl	Chloride
CS	Chute score
CSR	Chute score rank
CSR1	Chute score rank 1
CSR2	Chute score rank 2
CSR3	Chute score rank 3
CRH	Corticotropic releasing hormone
d	Day
DM	Dry matter
DMI	Dry matter intake
DWI	Daily water intake
EHL	Excessive heat load
EPA	Environmental protection agency
EO	Eosinophils
ET	Effective temperature
EV	Exit velocity
EVR	Exit velocity rank

EVR1	Exit velocity rank 1
EVR2	Exit velocity rank 2
EVR3	Exit velocity rank 3
FI%BW	DMI as a percent of body weight
G:F	Gain to feed efficiency
GR	Glucocorticoid receptor
GRE	Glucocorticoid response elements
h	Hour
HCT	Hematocrit
HGB	Hemoglobin
HPA	Hypothalamic-pituitary axis
HSD11β	11β hydroxysteroid dehydrogenase
HWE	High water efficiency
IPCC	Intergovernmental panel on climate change
K	Potassium
LTc	Lower critical temperature
LWE	Low water efficiency
LYMPH	Lymphocytes
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MONO	Monocytes
MWE	Medium water efficiency
MR	Mineralcorticoid receptor
Na	Sodium
NDF	Neutral detergent fiber
NEUT	Neutrophils
NEFA	Non-esterified fatty acids
N:L	Neutrophil to lymphocyte ratio
PER1	Period 1
PER2	Period 2
PER3	Period 3
PER4	Period 4
PER5	Period 5
PLT	Platelet
PVN	Paraventricular nucleus

RBC	Red blood cell
RBM1	Behavioral measures during early restriction
RBM2	Behavioral measures during late restriction
RET	Reticulocytes
RIC	Roughage intake control
RR	Respiration rate
RST	Restriction
RST1	First two weeks of restriction
RSTP1	First two weeks of restriction
RSTP2	Second two weeks of restriction
RSTP3	Last two weeks of restriction
RT	Rectal temperature
SMS	Sympathomedullary system
SNS	Sympathetic nervous system
SON	Supraoptic nuclei
TDN	Total digestible nutrients
THI	Temperature humidity index
TNZ	Thermoneutral zone
VP	Vasopressin
WA	Water allowance
WBC	White blood cell
WI	Water intake
WI%BW	Water intake as a percent od body weight
WIAL	Water intake during ad libitum period
WIEFF	Water intake efficiency
wk	Week

CHAPTER I

LITERATURE REVIEW

Introduction

Two conclusions of the Intergovernmental Panel on Climate Change (**IPCC**) Fourth Assessment Report were that the current warming of the climatic system is indisputable and that the increase in surface air temperature will range from 1.1 to 6.4°C in the 21st century, depending on the range of emissions assumptions (IPCC, 2007). The report also estimates that because of the increase in temperature there will be increases in heat waves, heavy rainfall, and incidences of drought. Drought has become more of a concern in the last decade. In 2012, 55% of the U.S. experienced moderate to great drought and 35% of the U.S. experienced severe drought (Folger, 2017). Although less severe in the extent of drought, this pattern has persisted over time, where the percentage of land in the U.S. affected by drought has ranged from 5 - 42% and 6 - 47% in 2017 and 2018, respectively (NOAA, 2019). An increase in future droughts can be detrimental for animal agriculture as decreased performance, increased morbidity, and decreased reproductive success of livestock occurs during drought conditions. This can be caused

by the animal's inability to mitigate heat stress without ample water availability or a result of general dehydration.

Within the next few decades, global food and water demands are expected to increase in order to meet the requirements of a growing population. Depending on the model assumptions being made, global populations are expected to reach between 8.9-9.3 billion by 2050 (Cohen, 2001; Cohen, 2003). Global meat consumption is expected to double by that time, requiring increased food animal and crop production (Nardone et al., 2010). In order to meet such demands, the overall number of animals, individual animal output, or likely both will have to increase. Increasing production will most likely increase resource utilization, including land and water. As water becomes a more limited resource, efficient water utilization will be needed to continue animal production during water scarcity (Nardone et al., 2010).

Water is expected to be the common challenge across animal agriculture as climate change worsens. In addition to water scarcity, water quality is expected to be poor due to increased salination, chemical contaminants, heavy metal contamination, and biological contamination (Nardone et al., 2010). Drinking contaminated water can have biologically costly effects on the animal and hinder animal performance and production. Thus in addition to further understanding livestock water requirements, understanding alternative management techniques to efficiently use water will be imperative for animal agriculture.

Because beef cattle are an important aspect of the U.S.'s economy, trade, and livelihood, understanding how cattle are affected by water scarcity will be necessary for

the future of animal agriculture in the U.S. There is some previous research on the effects of water restriction on livestock, but there are limitations with the current water restriction literature. First, most of the published literature utilizes small ruminants (Jaber et al., 2004; Hamadeh et al., 2006; Alamer, 2005; Mengistu et al., 2016) as the experimental model and there is limited research using cattle, specifically beef cattle. This is not surprising since traditionally arid areas focus on small ruminant production, rather than cattle, due to the small ruminant's known adaptability potential. However, since areas that are projected to be affected by water scarcity are expanding to traditionally temperate areas, livestock that were not historically exposed to such environments may become vulnerable to the detrimental consequences of restricted water. Second, water restriction research commonly restricts water over short time intervals (8 – 14 d; Burgo et al., 2001; Parker et al., 2003; Alamer, 2005), which does not provide adaptation information for long-term water restriction. Lastly, the sample size for most previous research has been small (10 animals or less per treatment), but because water intake has considerable inter-animal variation, a larger sample size is necessary (Kaliber et al., 2016; Mengistu et al., 2016; Benatallah et al., 2019). Examining the effects of prolonged water restriction on a larger population of beef cattle will be helpful to understand the effects of water restriction.

Climate Change

Global climate models have projected that numerous greenhouse gas (GHG) emissions, which have been increasing dramatically for some time, will continue to increase. Increased GHG emissions can lead to overall rises in ambient air and water temperature with variable and severe weather patterns (Rosenzweig et al, 2001). A rise in

global temperature can result in an intensification of the hydrological cycle, which can increase the likelihood of floods, drought, and increases in winter rain rather than snow. This can decrease snow packs and possibly increase the likelihood for summer/spring droughts.

The direct effects of climate change on livestock include temperature related illness and death during extreme weather conditions (Nardone et al., 2010). Heat stress is the primary extreme weather condition that can negatively impact livestock. Further consequences of these impacts can include reductions in weight gain, milk production, and reproductive success (Beach et al., 2010). In addition to heat stress, an additional challenge is projected to be water availability. A decrease in available water could limit the animal's ability to respond to and overcome heat challenges (Myers et al., 2017). Indirect effects of heat stress and decreased water availability on the animal include distribution of vector-borne illnesses, host resistance to infectious agents, feed and water shortages, or food-borne illness and possible death (Nardone et al., 2010).

These effects could have far-reaching consequences as well, affecting overall feedstuff production, livestock productivity, and cause a greater need for adjusted management efforts so that livestock can better mitigate (Adams et al., 1998a). Baker et al. (1993) conducted a simulation model for the Southern Great Plains and California, and found that a changing climate could decrease animal productivity primarily because of increased temperatures and decreased forage quality. Adams et al. (1998b) reported that with a 5˚C increase in temperature, U.S. livestock yields were predicted to decrease 10% for cow/calf and dairy operations.

Thus, future climate change could be exceptionally stressful for agricultural animals due to the increase in heat, decrease in available water, and the associated stress on the animal. Information regarding the coping responses to water scarcity and heat stress will be imperative for best management practices and effective mitigation strategies.

Stress physiology

In 2003, Toscano et al. defined stress as "normal deviations from homeostasis, to which the body invokes the sympatho-adrenal (SA) and hypothalamic-pituitary-adrenal (HPA) systems to bring itself back to a steady state". Curtis (2009) defined stress as "resulting from an animal's failure to adapt to challenging environmental conditions – reducing an animal's fitness. Unless mitigated, stress inevitably leads to harm to and even the untimely death of the animal". Generally, stress can be defined as a state where the animal has deviated from homeostasis after encountering a stressor.

What's important to consider when thinking about stressors is that not all stressors cause physical harm or result in negative impacts and there is individual variation in how animals perceive stressors. A stressor can be any internal or external stimuli or threat that disrupts homeostasis (Burdick et al., 2011b). Many common management practices can act as stressors for cattle, such as thermal extremes, crowding, mixing unfamiliar animals, transportation, weaning, vaccination, and handling (Minton, 1994). Stressed cattle can be more economically costly to raise due to increased morbidity rates and decreased performance (Burdick et al., 2011b), and can increase risk of harm to humans and other animals during handling.

Stress can be broken into 3 categories: understress, eustress, and distress. Understress may occur when an animal is kept in a barren environment that is deficit in stimuli that are needed by that animal; this can result in psychological stress due to the deprivation of needed stimuli (Curtis, 2009). Eustress refers to "good stress" or stimuli that cause a positive biological response, such as exercise or sexual behavior (Curtis, 2009). Distress, or "bad stress", refers to a biological state where the stress response can have a deleterious effect on the animal's well-being, where the animal cannot adapt to the threat (Moberg and Mench, 2000).

HPA axis

When an organism encounters a stressor, the hypothalamic-pituitary-adrenal (**HPA**) axis is activated (Burdick et al., 2011b). Initially, the brain will activate neurons in the paraventricular nucleus (**PVN**) of the hypothalamus, which will stimulate the synthesis and secretion of corticotrophin releasing hormone (**CRH**) and vasopressin (**VP**) (Plotsky, 1991; Burdick et al., 2011b). Only a subset of PVN parvocellular neurons synthesize and secrete CRH and VP. During stress, this subset of neurons increases significantly (Charmandari, 2005). The primary hypothalamic regulator of the HPA axis is CRH, which stimulates the secretion of adrenocorticotropin hormone (**ACTH**) from the anterior pituitary (Charmandari, 2005). The anterior pituitary secretes ACTH into the blood circulation, where it travels to the adrenal cortex to stimulate production of glucocorticoids (Webster Marketon, 2008).

In normal biological rhythms, CRH and VP are secreted in the portal system in a circadian, pulsatile, and concordant fashion (Charmandari, 2005). They are released at

low frequencies (2-3 secretory episodes/hour), but the amplitude increases in early morning hours, resulting in increased ACTH and glucocorticoid secretions in the morning (Charmandari, 2005; Burdick et al., 2011b). Some management practices can also affect the release of CRH and VP, such as lighting activity and feeding schedules (Burdick et al., 2011b).

The main target of the circulating ACTH is the adrenal cortex, which regulates glucocorticoid and adrenal androgen synthesis and secretion in the zona fasiculata and reticularis. Additionally, other hormones, cytokines, and neuronal information from autonomic nerves of the adrenal cortex may contribute to the regulation of cortisol (glucocorticoid) secretion (Calogero et al., 1992).

The final step of the HPA axis is the secretion of glucocorticoids. Glucocorticoids apply their effects through their commonly distributed intracellular receptors (Charmandari, 2005). Glucocorticoids are carried through the circulatory system via carrier proteins that prevent degradation and allow increased bioavailability (Burdick et al., 2011b). The glucocorticoid receptors (**GR**) are located primarily in the cytoplasm of cells as a part of a larger multiprotein made up of the receptor polypeptide, two HSP90 molecules, and other proteins (Bamberger et al., 1996). After hormone binding, the receptor dissociates from the HSP90 molecules and other proteins and translocates to the nucleus where it binds as a homodimer to glucocorticoid-response elements (**GRE**) in the promoter region of target genes. This regulates the expression of glucocorticoid-responsive genes positively or negatively, depending on the GRE and promoter sequence (Bamberger et al., 1996). The effects of gene expression are tissue specific, but can

include effects on prostaglandins, pro and anti-inflammatory cytokines, and cell adhesion molecules (Burdick et al., 2011b).

The main glucocorticoid in cattle is cortisol (Burdick et al., 2011b). The primary carrier protein for cortisol is albumin, but cortisol can also be carried by binding to cortisol-binding globulin (transcortin) (Burdick et al., 2011b). Approximately 90% of all cortisol is transported by a carrier, while 1-10% travels as a free steroid; the free steroid is commonly converted to cortisone (Rhen, 2005). Cortisol levels in tissue are controlled by 11β hydroxysteroid dehydrogenase (**HSD11β**), which converts cortisone to cortisol and vice versa (Rhen, 2005). Cortisol can bind to GR as well as mineralocorticoid receptors (**MR**), which are located in some limbic brain areas (hippocampus), heart, kidney, and colon (Carrasco, 2003); whereas, the GR are more widely distributed throughout the body (Carrasco, 2003).

Glucocorticoids elicit a wide variety of biological effects, including lipid metabolism, regulation of the stress response, and overall immune function (Burdick Sanches et al., 2014; Carroll et al., 2007). Glucocorticoids can also increase synthesis and secretion of catecholamines, which will in turn affect heart rate, pupil dilation, skin and gut vasoconstriction, vasodilation in the leg muscles, and increased liver glucose production. There can also be secondary effects, such as the inhibition of immune function (Hulbert et al., 2011; Carroll et al., 2007). In cattle, increased concentrations of cortisol are associated with reduced reproductive success, poor growth, and decreased immune responsiveness (Carroll et al., 2007; Curley et al., 2006; Petherick et al., 2001).

Autonomic nervous system

Some effects of the autonomic nervous system (**ANS**) may be more useful for measuring stress because of the faster response time, such as epinephrine or heart rate. The ANS is made up of two branches: the parasympathetic and the sympathetic nervous system (**SNS**). During a relaxed state, parasympathetic pathways predominate and primarily function to restore energy reserves. When an animal is threatened, the sympathetic pathways predominate and function to stimulate catecholamines to mobilize energy stores to respond to a stressor (Carroll et al., 2007). This response will include: increased heart rate and blood pressure to pump more oxygen around the body rapidly, release of stored sugar from the liver and directed to the muscles, deepening respiration and dilation of bronchioles to increase oxygen intake, dilation of pupils, and increased lymphocytes to aid in possible tissue damage (Carroll et al., 2007). This response is rapid and can cause effects within seconds. Currently, research is lacking in measures of the SNS response system in livestock.

Prior to activation of the HPA axis, the sympathomedullary system (**SMS**) activates in response to a stressor. When stimulated, noradrenergic neurons in the brain and postganglionic sympathetic neurons in peripheral organs (heart, gut, kidneys, etc.) secrete norepinephrine into circulation, which in turn increases blood pressure, heart rate, and respiration rate (Burdick et al., 2011b). Higher cortical center nerve impulses within the brain send messages through the limbic system to release norepinephrine, serotonin, and acetylcholine (**ACh**). The release of ACh stimulates the PVN to activate the HPA axis (Black, 2002). Additionally, preganglionic sympathetic fibers innervating the adrenal medulla stimulate the synthesis and secretion of epinephrine and norepinephrine (Butcher

and Lord, 2004). In cattle, the primary catecholamine produced by the adrenal medulla is epinephrine (Tsigos and Chrousos, 2002).

The sympathetic nervous system regulates involuntary biological functions, such as cardiovascular, gastrointestinal, respiratory, and renal systems (Charmandari, 2005). When the SMS is activated, epinephrine concentrations begin to increase, sending an alert signal to the body, which can result in a decrease in eating and sleeping behavior and activation of the HPA axis. Additionally, increased concentrations of norepinephrine in the brain stimulate enhanced long-term memory and store adverse emotional reactions in the hippocampus (Tsigos and Chrousos, 2002).

Stress assessment

Assessment of stress in animals has historically used a combination of physiological (endocrine, immune, etc.) and behavioral responses (intake, activity, etc.). One drawback to hormonal and catecholamine measures is that they can be short lived and do not indicate a severity of stress or pain. Rather, these measures indicate that a response is present or not. Behavioral responses have the advantage that they can occur immediately, be long-lasting, be measured non-invasively, and indicate intensity of stress (Mellor et al., 2000).

During an acute stressor, an animal may first show behavioral signs of adaptation, typically displayed as orientation reactions (stomping, kicking, tail swishing, change in orientation during temperature stress, etc.). Orientation changes may not be indicative of a stressor by themselves, but can be followed by a startle response (escape or fighting behavior) indicative of a fight or flight reaction. Other behavioral indicators of stress are

reduced feed or water intake and reduced activity levels. These behaviors are fairly obvious and a good indicator of how an animal is coping with a stressor. However, behavioral indicators can be scrutinized for the subjectivity in their assessment and may vary depending on the stressor and animal. Previous work has reported that the animal's behavioral response to a stressor may vary greatly among different temperaments (Bruno, 2015).

Physiological measures may also be helpful in assessing animal well-being during a stressor. There are many measures that can indicate a short-term or acute stress response. These measures include changes in heart rate, respiration rate, adrenal activity, and brain chemistry (cortisol, epinephrine) (Fraser and Broom, 1997). Body temperature may also be an indicator that the animal is coping with a stressor, as body temperature increases or certain areas experience differences in blood flow (specifically to the head and vital organs). Measures of immune function may also be useful in assessing a stress response. An acute stressor can cause increases in neutrophil counts and a decrease in lymphocyte counts, resulting in an altered neutrophil to lymphocyte ratio. This measure may be important because it is a simple test that can be performed cheaply and quickly. However, this test is not as sensitive as other measures.

A chronic stress response may appear differently. This occurs when an animal is continuously exposed to a stressor and cannot return to a homeostatic state. Thus, the biological stress response persists over long periods of time. One way to measure a chronic stress response would be with growth rate, as reduced growth rate may be indicative of chronic stress (Carroll et al., 2007). Some researchers have speculated that

differences in growth between cattle with different temperaments is caused by underlying differences in stress levels or stress reactivity (Bruno et al., 2018; Petherick et al., 2002)

Temperament

In cattle, temperament is defined as the animals' response to humans and novel objects or environments and is commonly measured in a handling setting (Burrow, 1997a; Grandin, 1997; Burdick et al., 2011a). Behavioral responses can range from "wild" or escape behavior to docile or non-responsive behavior. Poor temperament, or "wild" temperament, can have costly effects on the beef industry through decreased production of the animal (Burrow, 1997a).

The two most common restraint measurement methods are chute score and exit velocity; however, there are many temperament tests, both in a restrained and unrestrained setting. Burrow (1997a) reviewed different temperament measures as well as the benefits and limitations of each. Chute score is commonly measured on a 1 - 5 or a 1 – 4 point scale that assesses animal movement while the animal is restrained in a squeeze chute. Chute score is a subjective measure that can account for many behavioral indicators of stress (stepping, tail swishing, movement, vocalizations, etc.), but can differ between observers. Exit velocity (also known as flight speed) is the time taken to travel a specified distance upon exiting a squeeze chute and is expressed as m/s. Flight speed is an objective measure of a specific behavior that excludes observer bias. These measures tend to be repeatable over time in individuals, but show some acclimation to human handling (Vetters et al., 2013; Bates et al., 2014).

Temperament can be affected by many factors in cattle, such as age, breed, sex, and past experience (Burrow, 1997). Some studies have found that heifers are more temperamental than both bulls and steers (Voisinet et al., 1997; Café et al., 2011); whereas steers have been reported to be more temperamental than bulls (Vanderwert et al., 1985). *Bos indicus* cattle tend to have longer flight distances, faster flight speeds, and overall "poorer" temperament than *Bos taurus* cattle (Burrow, 1997; Café et al., 2011). Past experience can also affect temperament measures. Cooke et al. (2009) stated that acclimated heifers had a reduced chute score compared to heifers that were not acclimated. Curley et al. (2006) reported that cattle acclimated to handling over time, showed a more favorable temperament response to handling with repeated exposure.

Temperament is related to performance in cattle, where less temperamental (calm) cattle typically have a more favorable performance compared to more temperamental (wild) cattle. Relationships are commonly seen between temperament and average daily gain (**ADG**), health, and feed conversion efficiency. Cattle with a calm temperament have increased ADG compared to more temperamental cattle (Voisinet et al., 1997). Petherick et al. (2002) reported that cattle with poor temperament had decreased ADG, feed conversion, body conditions, and dressing percentage compared to calmer cattle. Differences in performance may be due to higher cortisol levels, because of a more active stress response (Bates et al., 2014). However, some research has not reported differences between temperament groups. Graham et al. (2001) stated that there was no difference seen between temperament measures (chute score and flight speed) in live weight or growth rate in Angus cattle. The authors attributed the lack of differences to cattle being generally docile and Angus.

Temperamental cattle tend to have differences in metabolism and physiological response as well. Burdick Sanchez et al. (2013) reported that between calm, intermediate, and temperamental bulls, the intermediate bulls had the highest feed intake and that non-esterified fatty acids (**NEFA**), cortisol, and epinephrine were highest in the temperamental bulls following a stress challenge. These results support the theory that cattle with high temperament scores have a more active stress response and utilize fat stores to meet energy needs. Petherick et al. (2009) stated that hemoglobin and NEFA were increased in cattle that were minimally handled compared to cattle that had "good" and "poor" handling experiences following a handling event. The authors also stated that "good handled" cattle had lower cortisol measures and that there was no difference in ADG between treatments. The authors speculated that infrequent handling can increase stress because the stimulus continues to be novel, leading to a more pronounced stress response. As such, cattle that were minimally handled habituated slower and displayed a higher flight speed over a longer period of time compared to other cattle handled more frequently.

Temperament may also alter the response to an immune challenge and sickness behavior in cattle. Burdick et al. (2011a) reported that temperamental cattle may display less sickness behavior than calm or intermediate cattle. Thus, it may be more difficult to identify and treat more temperamental animals when they are sick. Temperamental cattle may not respond to vaccines as well, as temperamental cattle have been reported to have decreased vaccine titers over time compared to calmer cattle (Oliphint, 2006; Bruno et al., 2017). Burdick et al. (2011a) also reported that temperamental cattle have altered neutrophil action following an immune challenge. Hematocrit and WBC counts did not

differ among calm and temperamental bulls following transportation stress, while cortisol was lower in calm bulls (Hulbert et al., 2011).

Agonistic Behavior

Dominance is an important aspect of social behavior in cattle. Dominance is typically defined as an attribute of a relationship between two or more individuals whenever an asymmetry in the outcome of agonistic interactions is measured (Drews, 1993). Previous interactions between individuals may affect future interactions of the same individuals and individual recognition is required to form a stable hierarchy (Drews, 1993). As a hierarchy stabilizes, agonistic interactions tend to decrease or shift from physical to non-physical threats (Kondo, 1990).

Dominance hierarchies develop as animals learn which animal is dominant over which others. Hierarchies can be simple (linear) or complex (triangular linear). Small groups (6 - 10 cattle) tend to have linear relationships, whereas larger groups tend to have more complicated hierarchies (Craig, 1986; Price, 2008). The time requirement to form a stable hierarchy is not well defined, but Bruno et al. (2018) found that pens of 4 steers take approximately 7 days to form a stable hierarchy. In larger groups, there may be increased frequency of role reversals, where an individual's role in the hierarchy changes, causing the group's hierarchy to lose stability. Barroso et al. (2000) reported 8% and 5.5% of interactions were role reversals in groups of domestic goats and American bison, respectively. Reversals may occur when certain resources are restricted and biological motivation for food or water supersede the hierarchy.

Dominant animals typically have priority access to resources, whereas lower ranking animals will most likely become excluded (Craig, 1986), but this may also depend on the resource and the environment. These results have not been reported in a pasture setting where the limited resource was mineral supplement (Wagnon, 1965). Intermediate animals typically do not lose access to resources when restricted (Craig, 1986; Bruno et al., 2018), but generally, agonistic interactions increase, as resources are restricted. Val-Laillet et al. (2008) reported that more dominant dairy cows spent more time at the feeder and had a higher milk yield than low-ranking cows. Coimbra et al. (2012) reported that dominance did not play a role in access to a water bunk on pasture, but in a smaller setting, more dominant cows had greater access to water over subordinate cows. Thus, the overall environment that animals are in can determine the response to a limited resource.

Previous research has also found that feed barrier type can influence frequency of agonistic interactions. Bouissou (1970) reported that partitions that protect the head in a side-by-side feed bunk can increase the feeding time for subordinate dairy cattle. Similarly, Holmes et al. (1987) reported that a solid or wired partition at the feed bunk can increase feeding time for subordinate mares. However, Huzzey et al. (2006) reported that these differences may rely on stocking density, as feeding time decreased with increasing stocking density of dairy cows even with feed bunk partitions. Herlin and Frank (2007) also investigated protective feeding barriers and found that adding barriers decreased aggressive interactions up to 65%. Since limiting resources and bunk design can both greatly affect social interactions in groups of cattle, it is worth exploring how

social interactions change when water is restricted by an Insentec system, which has bunk partitioning.

Thermal Stress

Thermoregulation

The thermoneutral zone (**TNZ**) is defined as the range of ambient temperature within which metabolic rate is at a minimum and temperature regulation is achieved by non-evaporative physical processes alone (Bligh and Johnson, 1973). The lower critical temperature and the upper critical temperature define the temperature range for each species. The width of the TNZ depends on many factors, such as age, breed, sex, species, level of nutrition, previous temperatures or temperature acclimation, housing conditions, insulation, behavior, etc. Heat gain and loss affect the animal as it approaches the upper and lower critical temperatures. Heat can be transferred through conduction, convection, or evaporation. Conduction refers to the process by which heat or electricity is transmitted through a substance when there is a difference in temperature between adjoining regions, without movement of the material. Convection refers to movement of a hotter and less dense fluid to rise while colder and denser materials sinks, resulting in transfer of heat. Evaporation refers to vaporization of a liquid as it changes from a liquid to a gaseous state.

Heat gain

Conduction of heat transfer results from physical contact of the animal with a surface, whether that surface is gaseous or liquid. The rate of heat flow is dependent on the area of contact, the conductivity of said material, the distance of heat flow, and the

temperature gradient (Sparke et al., 2001). For cattle to gain heat by conduction, their skin or mucosal linings must be in contact with a hotter material. The main source of conduction for cattle that are standing occurs with the air around them when the air is cooler. Since air has modest thermal conductivity, this form of conduction will play a small role in heat transfer for cattle within their environment. But, if the animal is lying on the ground, which has a greater thermal temperature than the skin of the animal, this can cause heat conduction and add to the metabolic heat load (Robertshaw, 1985).

Although all solid objects emit some level of electromagnetic radiation in the infrared range, warmer objects emit shorter wavelengths as well as more emissions per unit time than do cooler objects. When these emissions come in contact with another object (like an animal), some of those wavelengths are absorbed and transferred as heat (Cunningham, 2002). If the temperature of the environment or surrounding objects is greater than the temperature of the animal, a greater quantity of heat can be radiated to the body than from the body (Guyton and Hall, 1996).

Heat loss

Evaporation is the primary route of heat loss in cattle. For every gram of water evaporated, 2.43 joules of energy is lost (Guyton and Hall, 1996). Evaporative heat loss can occur through diffusion of water through the skin and by loss of water vapor from the respiratory tract. Mammals lose heat as a result of evaporation from respiration passages, commonly referred to as respiratory cooling, because inspired air is cooler than expired air; thus, heat is released from the lung surface (Feldhamer, 2007). The rate of heat loss can greatly depend on air temperature, humidity, and movement of air (Sparke et al.,

2001). Evaporative cooling is the only form of heat loss available once the ambient temperature exceeds body temperature (Cunningham, 2002). The effectiveness of evaporative cooling becomes reduced as humidity increases, in which the air becomes more saturated with water vapor. If the inhaled air is already near core temperature and saturation, the scope for respiratory cooling is limited (Sparke et al., 2001).

Heat loss due to convection can occur when air or water is warmed by the body (Cunningham, 2002). For heat loss from convection to occur, the heat must be conducted to the air from the skin, then carried away by convection currents. Free-convection is when heat rises from an animal as the temperature and density decreases (Robertshaw, 1985). Forced-convection involves a cool fluid moving over the skin surface of the animal. Forced-convection can be more effective than free-convection because the thermal gradient will be maintained by the cooler liquid as it covers the surface of the skin (Cunningham, 2002).

For heat loss to occur through conduction, the animal's skin or mucosal membranes must come in contact with a colder surface. This surface could be the air or the ground, in which case the animal could be laying or standing. If the animal is lying on the ground and this surface is cooler than the skin of the animal, the animal will lose heat to the cooler surface (Cunningham, 2002). This method of heat loss is important for cattle during heat stress and can be achieved when temperatures drop overnight and in shaded housing systems. Decreased night temperatures allow the animal to dissipate some heat.

Heat Stress

Heat stress refers to an external stressor stemming from the thermal environment where heat production causes a strain on the animal, causing displacement of various internal parameters from their resting or basal state (Beatty, 2005). Excessive heat load (**EHL**) has also been used to describe stress in cattle (Sparke et al., 2001; Young, 1993). Excessive heat load occurs when there is a combination of environmental conditions and individual animal characteristics that exceed an animal's ability to adequately release its heat load, which leads to an increase in body temperature beyond the animal's normal physiological range (Sparke et al., 2001).

Typically, the normal body temperature for mature cattle living in temperate climates ranges between 36.7 and 39.1°C (Cunningham, 2002). This is the temperature when the animal's biological and cellular activities operate most effectively. If the body is above its neutral temperature, metabolism will speed up; as the metabolic rate increases, there is greater metabolic heat production, causing body tissues to continually increase in temperature. The consequence of this is called uncontrolled metabolism and can lead to a situation called "run-away hyperthermia", which can result in death (Young and Hall, 1993). However, animals may attempt to adapt to heat conditions, which may not always be possible. Additionally, when body temperatures rise too high, there is risk for the denaturing of proteins, disruption of cell membrane integrity, and possible permanent tissue damage resulting in long-term morbidity and poor performance (Guyton and Hall, 1996).

Heat stress measures

Numerous environmental measures can be used to determine heat load, such as ambient temperature, relative humidity, wind speed, and direct or indirect solar radiation. These are the typical factors of thermal stress that can impose strain on cattle (Finch, 1984). Measures of heat stress can also range from individual ambient temperature measures to complex indices that attempt to provide a weighted estimation of all environmental factors. These indices will be discussed throughout this section. Nevertheless, because we know that many factors are involved in thermal stress, it seems improper to solely use ambient temperature as the measure of heat load.

The temperature humidity index (**THI**) has been widely used for heat stress research as an index in the beef and dairy industries (Mader et al., 2002). The THI takes ambient temperature and relative humidity into account and is expressed as:

$$\mathbf{THI = 0.8T_a + RH \times [(T_a - 14.3) + 46.3]}$$

where T_a refers to ambient temperature (Celsius) and RH refers to relative humidity expressed in decimal form (Thom, 1959). Temperature humidity index values of 70 or less are considered comfortable, 75 - 78 are considered stressful, and values greater than 78 can cause extreme distress, where those animals may not be able to maintain thermoregulatory mechanisms (Silanikove, 2000). However, THI does not take into account wind speed and solar radiation, limiting this approach to measure heat load for cattle.

A similar heat load index has been developed in dairy heifers (Yamamoto et al., 1994) utilizing effective temperature (**ET**; calculated from ambient temperature) and radiation (black globe temperature, **BGT**) as described in the equation:

$$\mathbf{ET = 0.24\ T_a + 0.76\ BGT}$$

where T_a refers to ambient temperature (Celsius) and BGT stands for black globe temperature. The black globe temperature assimilates the influence of air temperature, radiation, and air movement. The equation assumes that solar radiation will contribute more to the heat load on cattle than the ambient temperature. Of course, this approach's weakness is measuring heat load when cattle have strong shade options.

Mader et al. (2010) also developed a comprehensive climate index (**CCI**), which can be used for both hot and cold climates. This method makes adjustments for RH, wind speed, and radiation, which may give a better measure of a "feels like" temperature index. The authors also developed threshold categories to assist producers in knowing when to intervene.

Physiological response to heat stress

Body temperature

Because a majority of heat transfer occurs at skin level, cattle have adapted to regulate body temperature using a variety of temperature sensors located within the body. Specifically, cattle have temperature-sensitive neuronal structures located in the skin and mucosal surfaces, in regions of the spinal cord, and in the hypothalamus of the midbrain (Bligh, 1985). These relay temperature information to the preoptic area of the hypothalamus, which is believed to be the main center of temperature regulation. After the message is sent to the preoptic area, this area will initiate secondary mechanisms that will either increase or decrease the animal's heat loss or production (Cunningham, 2002).

These responses occur when the body temperature rises or falls above average normal temperature for that animal.

Core body temperature has been frequently used as an indicator of heat stress and welfare for cattle (Finch et al., 1982; Mader et al., 2002). Tympanic temperature probes (Mader et al., 2002), rectal temperature probes (Gaughan et al., 1999), and carotid artery thermocouples (McLean et al., 1982) can allow for continuous or individual measurements of temperature in cattle. Additionally, surgically implanted temperature data loggers have been placed in the abdomen of impalas, which facilitates less handling stress and time, while still collecting accurate readings (Kamerman et al., 2001). Newer technologies in animal science now continuously measure temperature in the rumen or ear with an automated logger. Dye et al. (2007) reported that rumen temperature measured using rumen boluses was accurate and matched changes in core body temperature, following an immune challenge.

Vasodilation

The initial physiological reaction of cattle to hot temperatures is vasodilation, which increases skin and limb blood flow, consequently increasing skin and core temperatures. Thus, the temperature gradient between skin and the external environment will be greater, leading to more heat loss through radiation and convection (Cunningham, 2002). If the skin temperature matches core temperature, resistance of heat removal must decrease or the heat will accumulate causing the body temperature to increase (Finch, 1986). An increase of only 0.5°C in skin temperature can cause a sevenfold increase in skin blood flow (Cunningham, 2002).

Skin temperature and sweating

Skin temperature can be a good indicator of heat stress, although skin color must also be considered as skin color can greatly affect skin temperature, where skin temperature can be higher and weigh gain can be lower in darker hided cattle (Finch et al., 1984). In 1961, Allen showed that *Bos taurus* and *Bos indicus* cattle displayed a similar response in skin temperature to increased ambient temperature, resulting in increased sweating. However, when skin temperature was correlated to sweating rate, *B. taurus* showed increased sweating when skin temperature increased to between 32 to 38°C, but *B. indicus* did not increase until a temperature of 35°C. These results are not surprising as *B. indicus* cattle are typically more heat resistant.

If heat load cannot be mediated through vasodilation, evaporative cooling will be increased by sweating, panting, or a combination. Evaporative cooling is the primary mechanism of heat dissipation at high temperatures and when ambient temperatures exceed the skin temperature (Cunningham, 2002). Studies evaluating sweating to maintain the TNZ have found mixed results due to a variety of factors, such as site of measurement (Dowling, 1955), method of measures (Johnson, 1970), breed (Finch et al., 1982), shape of sweat gland (Carvalho et al., 1995), nutrition (Dowling, 1955), climate conditions (Johnson, 1970), whether cattle are inside or outside (Gaughan et al., 1999), closeness to other cattle, and availability of drinking water (Sparke et al., 2001).

Sweating occurs from apocrine glands located in the dermis (Cunningham, 2002). Each hair follicle is accompanied by an apocrine gland that has a duct opening onto the skin surface at the mouth of the follicle. There is some anatomical association between

capillary beds and apocrine glands such that the amount of blood directed to the capillary beds will affect the rate of sweat production (Schleger and Bean, 1971; Finch, 1986.).

Dowling (1955) reported that *B. indicus* cattle tend to have a greater capacity to sweat because they have greater densities of apocrine glands. However, there is some variation in which breeds have more or less sweat gland densities. Allen (1961) reported that sweating rate showed some differences among breeds, with correlations to skin temperature. *B. taurus* cattle tended to have an increased sweating rate at lower temperature (from 18°C and 32°C), whereas *B. indicus* cattle showed a significant change in sweating rate at higher temperatures (at least 29°C), comparatively. Johnson (1970) found that sweating rates were greatest on the shoulder and the lowest part of the lumbar region on both *B. indicus* and *B. taurus;* the authors did not see a species difference until temperatures of 40-45 °C were reached, where *B. indicus* sweating rates were greater at all body locations. Gaughan (1999) reported little difference in sweating rate between Brahman and Hereford steers (171 and 175 $g \cdot m^{-2} \cdot h^{-1}$), although sweating rate by Brahman x Hereford cross steers were significantly increased (221 $g \cdot m^{-2} \cdot h^{-1}$).

Respiration

Respiration is the other form of evaporative heat loss. A common indicator used to measure heat load for cattle during hot weather is respiration rate, which will increase as animals try to maintain homeothermy by dissipating excess heat (Hahn, 1999). Respiration rate (**RR**) is primarily influenced by ambient temperature, solar radiation, relative humidity, and wind speed (Sparke et al., 2001). The normal RR of cattle under thermoneutral conditions is typically 20 - 60 breaths per minute (**bpm**; Smith, 2014). A

RR of 80-120 bpm usually indicates moderate thermal stress and cattle with a rate of over 120 bpm are considered to be under EHL (Gaughan et al., 1999). Cattle with a RR over 140 bpm are considered to be under considerable strain and additional cooling is required. In shaded cattle that are acclimated to heat, RR is poorly correlated with ambient temperatures less than 21°C. Above 21°C, RR is strongly associated with air temperatures and increases about 4.3 bpm per degree Celsius above baseline and has a lag of roughly 2 hours behind air temperature (Hahn et al., 1997).

An increased respiration rate, also known as panting, is a process by which the aqueous mucous secretions of the buccal area are assisted with evaporation by the constant movement of inhaled and exhaled air over the buccal surfaces (Robertshaw, 1985). Panting in cattle is characterized by vascular engorgement of the respiratory and oral mucosa as well as increased salivation. The goal of panting is increasing heat loss through evaporation. During rapid and shallow panting (under mild heat stress), the dead space ventilation will increase more than the alveolar ventilation in order to avoid hyperventilation and respiratory alkalosis (Cunningham, 2002). When the animal is under more severe heat stress and its body temperature approaches 41°C, panting changes to a lower respiratory frequency and an increase in tidal volume. This panting increases alveolar ventilation up to five-fold and leads to increased loss of carbon dioxide and respiratory alkalosis (Mount, 1979).

To maximize evaporative heat loss in the respiratory tract, air should be inspired through the nose and expired through the mouth, bypassing any countercurrent heat exchange (Taylor, 1977). Respiratory rates in hydrated and healthy animals typically increase with increasing ambient temperature, resulting in increased respiratory

evaporation. Comparatively, dehydrated animals tend to have lower respiration rates and initiate panting at higher temperatures than hydrated animals (Cain et al., 2006). Animals might breathe slowly and deeply during the evening or early morning hours to extract more oxygen per breath, reducing total air movement and respiratory water loss (Taylor, 1969).

Panting and sweating each have advantages and disadvantages. Animals that pant can have a lower thermal gradient by maintaining higher skin temperatures and limiting the amount of inward flow of heat from the environment (Cain et al., 2006). Panting also allows for airflow over evaporative surfaces, while sweating animals must rely on wind activity. Panting animals also lose significantly less electrolytes than sweating animals (Taylor, 1977). However, panting animals have an increased energy expenditure associated with metabolic heat production, which could increase overall heat load of the animal.

Behavioral response to heat stress

Increased water intake

Cattle typically acquire water from free water, water in feed, and metabolic water. Water intake from feed and consumed as free water is approximately equivalent to the water requirements for cattle (NASEM, 2016). Water requirements for cattle can be influenced by many factors, such as ambient temperature, stage of production, type of diet, and feed intake. However, Arias and Mader (2011) reported that ambient temperature, THI, and average daily minimum temperature were the principal drivers of water intake, more so than feed intake or solar radiation. Water loss in cattle is primarily

due to urine, feces, and evaporation from respiration and skin. Because evaporative cooling is the primary method for cooling in cattle during heat stress, the replenishment of lost water supplies is imperative for health. Water requirements can rise 1-2-fold during heat stress, compared to requirements during times of thermoneutrality (Beede and Collier, 1986). The organ responsible for increased water intake seems to be the hypothalamus, as warming the pre-optic area has been reported to increase water intake (Bianca, 1965), but the kidney, pituitary, and adrenal glands will also be important for regulation.

Shade seeking

An important way to alleviate heat stress in cattle during hot times of year is to provide shade that will protect the cattle from radiant heat and improve heat loss from the body to the environment. Providing cattle with shade reduces the radiation load by up to 30% and can also be a cheap and easy method to implement for cattle producers (Aggarwal, 2013). Blackshaw and Blackshaw (1994) reported that during hot weather, cattle would use shade if it were available, although *B. taurus* will typically use shade more than *B. indicus* cattle. A study by Gaughan et al. (1998) reported that cattle would not seek shade below 30°C and that when given the choice between 4 types of shade, galvanized iron roofing was preferred to vines on a trellis, 70% shade cloth, or natural tree shade. Roman-Ponce et al. (1977) reported cows that were kept in a shaded environment had lower rectal temperatures, decreased respiration rate, and a 10% higher milk yield than cows kept in an unshaded environment.

Decreased feed intake

Because feed intake relates directly to metabolism and heat production (Finch, 1986), feed intake will change during heat stress. Metabolism accounts for roughly one third of the heat produced by an animal (Finch, 1976). Because higher feed intakes will increase metabolic rate and thus exacerbate the effects of heat stress, a reduction in feed intake is the immediate behavioral response to heat stress (NASEM, 2016). The decrease in feed intake will result in a decreased metabolic rate, which will help to balance the amount of heat produced with heat lost. Bianca (1965) reviewed the physiological mechanisms underlying the behavioral response to adaptation and proposed that the hypothalamus acts to regulate feed intake and other energy balance functions. Anderson and Larsson (1961) also reported that heating the pre-optic area of the brain in hungry goats immediately stopped feeding activity.

Other behavioral changes

One behavioral adaptation of animals during heat stress is to change duration or timing of daily activities. Desert ungulates may shift to crepuscular behavioral patterns and perform most of their foraging and activity in the cooler early morning or late evenings during dry and hot periods (Cain et al., 2006). Some animals also increase their feed and water intake at night, rather than feeding during the day. In areas where humidity stays high but ambient temperature decreases and dew forms on plants, animals may intake up to 30% more water content by feeding on forages. Additionally, feeding on plant materials can decrease the overall need for free water intake and increase water gained from feeding behavior (Cain et al., 2006), depending on the water content of the available forage.

Animals that do not have access to shade may adapt to heat conditions by changes in body orientation to reduce total amount of solar radiation absorbed or adjust to sun and wind direction. Animals may stand or lay with the long axis of their body parallel to the sun, rump to the sun, and/or head down to shade the body (Cain et al., 2006). Animals may lay in small groups as well, utilizing the body shade that other animals offer and reducing surface area exposed to solar radiation (Sowls, 1997).

Cold Stress

Although heat stress is the more commonly discussed thermal extreme, cold stress may be common in northern areas where cattle are managed. Within the range of the thermoneutral zone, there is the lower critical temperature (**LTc**), which is the temperature below which an animal must increase its rate of metabolic heat production to maintain homeothermy (Young, 1983). Below the LTc, metabolic heat production becomes more associated to ambient temperature (Young, 1983). At extreme low temperatures, maximum heat production is reached and even lower temperatures can result in hypothermia. At these extremes, the animal will not be able to produce enough metabolic heat to survive. Each animal has a different LTc, depending on the amount of thermal insulation, hair coat, and rate of heat production. Some animals will be more vulnerable to cold temperatures, such as animals with low thermal insulation, newborns, or animals with restricted feed (Blaxter, 1977). In contrast, cold-adapted ruminants with adequate thermal insulation and full access to feed generally have low LTc's and can survive dry, cold conditions well (Webster, 1974; Young and Christopherson, 1974).

Physiological response to cold stress

The physiological strategies to withstand cold stress have been well documented in small mammals (Jansky, 1971) and the same types of changes have been observed in ruminants (Webster et al., 1970; Young, 1975). Adaptation to cold by ruminants is characterized by increases in thermal insulation, appetite, and basal metabolic intensity (Young, 1980). Additionally, during periods of cold stress ruminants have increased passage rate, rumination activity, and reticulorumen motility, further driving increased feed intake and utilization (Young, 1983). These actions limit the negative effects that both acute and chronic cold stress can cause.

Thermal insulation can be in the form of increased hair coat or tissue insulation. Animals that are adapted to cold environments increase metabolic intensity to create more heat, as opposed to the acute changes observed in animals that are not well adapted (Smith et al., 1972). Thus, animals that are adapted to cold stress have a better chance at survival and suffer less than those not adapted to cold conditions (Smith et al., 1972). One indication of an animal that is adapted to cold is decreased shivering activity throughout cold conditions or extended winters (Young, 1983). One study from Young and Degen (1981) concluded that for every 1° C decrease in ambient temperature, resting metabolic rates of cattle increased by approximately 0.69 kcal/kg$^{0.75}$. Additionally, the change in metabolic intensity for animals that are thermally adapted was expressed as 0.91% increase in maintenance energy requirement for each degree below 20°C to which the cattle have been adapted (NASEM, 2016).

Water Regulation

Water is an essential nutrient that must be provided for animals to ensure normal body function and maintain homeostasis. Animals experience thirst, which is activated by an increase in the concentration of electrolytes in body fluid and stimulates water intake and drinking behaviors (NASEM, 2016). Daily water intake (**DWI**) and drinking behavior can be affected by many factors, such as environmental conditions/weather patterns, housing, diet, age, stage of production, sex, and more. Quantifying DWI can be challenging due to the labor associated with manual collections. Due to the difficulty in collecting DWI, it is rarely reported in livestock research and reports, compared to feed intake.

Body fluid homeostasis and control is achieved through a complex balance of renal, adrenal, vascular, cardiac, brain, and endocrine influences (Samson, 2012). Furthermore, all of these systems converge at the kidney for regulation. Therefore, the primary site of action for most of the hormones involved with water regulation is the kidney (Samson, 2012). Hormones such as vasopressin, angiotensin II, and aldosterone act simultaneously to regulate water preservation during times of thirst. After thirst has been quenched and water needs have been met, action of these hormones will cease. There are different types of thirst and conditions that lead to different water intake mechanisms.

Types of Thirst

Thirst is defined as a motivation to seek and ingest water (Fitzsimons, 1998). There are two types of thirst: osmotic thirst and hypovolemia thirst. Typically, the concentration of sodium chloride in interstitial fluid is roughly 0.85-0.9%. Injecting a

neutral solution into the body that does not change that concentration will have no effect on interstitial fluid concentrations. However, if a hypertonic solution, where the concentration of sodium chloride exceeds 0.9% is injected, the concentration will increase in the interstitial fluid. The increased concentration around cells draws water out of those cells, inducing cellular dehydration. Cellular dehydration can be a potent stimulus for thirst. This is referred to as osmotic thirst and is common after eating high salt or high sugar foods (because excess glucose in interstitial fluid can have the same effect). This may also occur when an animal has decreased access to water, where water intake has decreased but access to sodium has not. In this scenario, the sodium in interstitial fluid would be increased because of a decrease in available fluid. Following, the individual would start to consume water.

Vasopressin activity in the kidney acts to conserve water from blood. Once more water is consumed than needed, the osmolality of plasma decreases. The reduction in plasma osmolality inhibits thirst and inhibits release of vasopressin from the posterior pituitary. Without the presence of vasopressin, kidney filtration returns to normal and water will be pulled from the blood plasma and sent to the bladder for elimination (Stockland, 2010).

A reduction in plasma volume is a powerful stimulus for thirst and referred to as hypovolemic thirst. This type of thirst can be activated by hemorrhage, excessive perspiration, or diarrhea (Fitzsimons, 1998; Stachenfeld, 2008). During episodes of hypovolemia, water and other solutes are lost without being pulled out of the cells themselves, unlike osmotic thirst. Rather than ingesting more water to quench thirst, like

for osmotic thirst, individuals experiencing hypovolemia need a replacement of water, sodium, and other solutes (Kalman and Lepeley, 2010).

Renal regulation

There are two main compartments of body fluid for animals: intracellular and extracellular. Approximately one third of body fluid is in the intracellular compartment. The remaining body fluid is in the extracellular compartment and further separated into two compartments: interstitial (between cells, 26%) and blood plasma (7%). The two compartment fluids are different in composition as well as primary location (Nelson, 2011). Typically, most of the body's potassium is located in the intracellular compartment and the extracellular fluid has higher concentrations of sodium and chloride ions. The differences are a result of differences in cell membrane and blood vessel walls.

Water is absorbed in the nephron of the kidney. The sections include: the proximal tubule, the loop of Henle (ascending and descending limbs), distal tubule, and the collecting duct. The filtrate enters into the nephron at high pressure, through the long convoluted tubule comprising several sections with specific functions. Water will then flow passively out of the descending limb into the surrounding tissue. After water outflow, sodium ions enter because there is a high concentration of sodium surrounding the tissue. The sodium concentration is highest at the bottom of the loop of Henle. As the filtrate is moved out of the loop and into the ascending limb the sodium is pumped out into surrounding tissue. The ascending limb is impermeable to water and thus the sodium leaves without the water. Therefore, the filtrate is dilute as it exits the ascending limb into the distal tubule. From the distal tubule water flows into the surrounding tissue and then

into the capillaries by osmosis. The waste from the filtrate flows into the collecting duct and will eventually move into the bladder to be eliminated. Water can be conserved in the distal tubule by increasing permeability via vasopressin (**VP**) to send more water to the circulation. Without VP the tubule becomes less permeable, resulting in diuresis.

Body water balance is one of the most important functions of the kidney. Because terrestrial animals faced desiccation, the kidneys evolved to reabsorb water in the glomerular filtrate. Under water restrictive conditions, the kidney can produce hypertonic urine, which is up to 8 times more concentrated than the osmolality of plasma (Klein, 2013). The proximal tubule can reabsorb more than 60% of filtered water during times of water conservation. Additionally, during times of water overload, the kidney can also produce hypotonic urine, as low as 1/3 the osmolality of plasma (Klein, 2013).

Specifically for ruminants, the rumen also plays an important role in water homeostasis during dehydration. Due to the large volume capacity, it can act as a water reservoir and can replenish water lost during prolonged dehydration to maintain normal blood volume (Jaber et al., 2013). The rumen also allows for large volumes of water intake, which can be temporarily sequestered in the rumen for slower absorption as to not upset homeostasis (Silanikove, 1994; Jaber et al., 2013). Thus, water restrictive conditions and rehydration will most likely affect ruminants differently than monogastric mammals.

Hormonal regulation of water balance

Vasopressin/Antidiuretic Hormone

The posterior pituitary gland contains glial elements (pituicytes), unmyelinated nerve fibers, and axon terminals of neurons whose cell bodies reside in the supraoptic and paraventricular hypothalamic nuclei (Samson, 2004). These areas secrete the two main hormones of the posterior pituitary, oxytocin and VP (also referred to as antidiuretic hormone, **ADH**). The primary action of VP is as an antidiuretic, which means that it conserves water. Vasopressin and ADH are structurally the same, but they typically act in different ways on different organs. Nonetheless, most use the two names interchangeably.

Vasopressin acts by binding to cell membrane receptors on the peritubular surface of the distal convoluted tubule and medullary collecting duct and activates adenlylyl cyclase. This stimulates cAMP, responsible for activating a protein kinase, initiating a phosphorylation cascade resulting in the input of an aquaporin in the luminal membrane. The aquaporin enhances the permeability of the cell to water (Samson, 2004). The increased water permeability allows back diffusion of solute-free water remaining in the urine after proximal tubule handling down the osmotic gradient from hypotonic urine to the hypertonic interstitium of the renal medulla (Samson, 2004). This results in an increase in urine osmolality, compared to plasma, and a decrease in urine flow.

Vasopressin has three main receptors, V_1, V_2, and V_3 (Samson, 2004). The V_1 receptor is mainly located on blood vessels and leads to vasoconstriction. The second is located on the renal collecting duct around the kidney and increases water permeability by addition of an aquaporin into the lumen membrane. The third is located on the anterior pituitary gland and acts to stimulate adrenocorticotropic release from that endocrine gland. The V_2 is the type most commonly active in relation to water regulation.

There are two types of stimuli that typically cause the release of VP from the posterior pituitary. One of the stimuli is intracellular dehydration of cerebral osmoreceptors. Osmoreceptors are located in two highly vascularized regions of the CNS: the vascular organ of the lamina terminalis and the subfornical organ. Although there is some degree of cell shrinking in all cells during dehydration, the osmoreceptors are the only cells that signal this condition to the paraventricular nucleus (**PVN**) and supraoptic nuclei (**SON**) in the hypothalamus, where VP release is ultimately stimulated.

There are 2 steps involved in biological signaling during water restriction or dehydration periods. First, a signal (even mild cellular dehydration) to release VP from the posterior pituitary can be sent to begin water conservation. If the dehydration continues even after conservation of water in the kidney, a second signal from the osmoreceptors will stimulate drinking behavior. The physiological mechanism is stimulated before the behavior in order to increase retained water once drinking behavior does increase, to avoid frequent drinking events. Following increased drinking behavior, VP causes the kidneys to retain more water and lowers blood osmolality. Decreasing plasma osmolality will inhibit drinking behavior and the animal will return to a water balanced state.

Another stimulus for VP release is reduced plasma volume. Decreases in blood volume are sensed by stretch receptors (baroreceptors) that are in the walls of the cardiac blood vessels. These receptors act on the PVN and SON to release VP, which will act as a vasoconstrictor to increase blood pressure. These actions are stimulated by the V_1 receptors. The same receptors will signal the brain through the vagus nerve to stimulate

thirst, and thus drinking behavior. Once plasma volume is restored, this system will be inhibited and the animal will return to a water balanced state.

Angiotensin and aldosterone

Another hormone that plays an important role in water regulation is angiotensin. Angiotensin is considered a parahormone because it is not secreted by an endocrine gland as true hormones are; paracrine cells in the blood secrete angiotensin. Angiotensin has two forms: I and II. Angiotensin II (**AII**) is more involved in water regulation and metabolism. Angiotensin II is formed in the circulation by the renin enzyme. The renin enzyme is produced in the kidney to convert angiotensinogen to angiotensin I. The lungs produce a converting enzyme (angiotensin converting enzyme) that cleaves two amino acids on angiotensin I to form AII (Ojeda and Griffin, 2004). Angiotensin II can also be formed in the brain and vascular cells. Production of AII is stimulated by decreased renal perfusion pressure or decreased renal sodium delivery to the macula densa (Samson, 2004). Angiotensin II's main actions are related to compensation during loss of fluid and electrolytes.

Angiotensin II has two receptor types: type 1 and type 2. Using receptor antagonist studies it was found that only type 1 receptors are involved in mediating water and sodium regulation (Fluharty and Sakai, 1995). Additionally, intracerebroventricular administration of A II receptor type 1 antisense oligodeoxynucleotides decreases drinking behavior in rats (Fluharty and Sakai, 1995).

Angiotensin II acts to constrict vascular smooth muscles and stimulates release of aldosterone from the zona glomerulosa in the adrenal gland (Ichikawa and Harris, 1991).

Aldosterone is another hormone that is imperative for maintaining fluid balance (Nelson, 2012). Aldosterone stimulates sodium pumping in the ascending limb of the Loop of Henle to stimulate sodium retention. Thus, the kidneys become saturated with sodium forcing water to be reabsorbed from the blood as it flows through the nephron, further reducing the amount of water directed to the bladder for excretion. Without aldosterone the kidney would send copious amounts of water and sodium to the bladder to be lost as urine, which would in turn cause massive behavioral changes in water consumption.

Effects on drinking behavior

The same hormones that regulate water balance in the body also stimulate and inhibit drinking behaviors by acting on the central processing systems. During cellular dehydration, VP affects drinking behavior by causing the kidneys to retain water, resulting in reduced blood osmolality, which will inhibit drinking behavior (Nelson, 2011). However, if dehydration persists, drinking behavior must be stimulated to increase water intake. Following consistent dehydration periods, VP, AII, and aldosterone will increase drinking behavior by acting directly and indirectly on the central nervous system. Aldosterone only affects drinking behavior indirectly by stimulating the kidneys to retain sodium, maintaining osmotic balance. Artificial doses of angiotensin stimulate drinking behavior (Nelson, 2011).

Lainez et al. (2004) investigated the differences in drinking behaviors across housing systems, time of year, and grouping. The average total amount of time cows spent drinking between summer and winter were not different, but the overall intake was significantly higher in the summer. Daily drinking patterns were different in summer and

winter, where drinking peaked around midday in the winter and summer peaked twice at 1000 and 1900 h. Thus drinking was associated with cooler times of day in the summer and warmer times of day in the winter.

Effects on feed intake

There is little research on the direct effects of water regulation hormones on feed intake, especially in ruminants. However, most research has shown that as water intake decreases, feed intake will follow the same trend. Parker et al. (2003) found that when animals were water deprived, their feed intake decreased up to 50%. Furthermore, the difference in feed intake increased the longer the sheep were water deprived compared to those with *ad libitum* access to water. Bond et al. (1976) found a 47% decrease in feed intake while steers were withheld from water. Utley et al. (1970) found that when steers were restricted to 80 or 60% of their normal water intake their feed intake decreased 0.3 and 1.4 kg/day, respectively. These results are typically explained in ruminants by the change in passage rate. A certain amount of water is required for normal passage rate in the digestive tract (Utley et al., 1970). Without the water to maintain passage rates, the rate will decrease causing a decrease in feed intake.

However, when vasopressin is injected into animals that are water deprived their feed intake tends to increase. Langhans et al. (1991) found that meal sizes were significantly larger after injecting vasopressin into animals that were water deprived compared to animals that had free access to water. These researchers speculated that the difference in meal size was caused by water deprivation affecting the mechanism of terminating meals. however, changes in plasma osmolality could have also been driving

the difference in meal size, as increased plasma osmolality typically suppresses feeding behavior.

Water Intake

Water intake was first reported by Ritzman (1924), who reported that water intake is a function of DMI. However, it was later determined that DMI alone could not predict water intake and temperature must also be included. Hicks et al. (1988) reported that DMI and temperature were the primary factors driving water intake. Arias and Mader (2011) reported that the primary factors to influence water intake were ambient temperature, minimum temperature, and THI. The authors also concluded that water intake is highest during summer months, most likely due to the cooling effects driving water intake.

Hicks et al. (1988) reported that average daily water intake of feedlot steers was 9.8 gallons (roughly 37 L) per head during the summer. The authors also reported that for each 1 degree increase in maximum temperature, water intake increased by 0.1 gallons. Ray (1989) reported average water intake of 32.1 L during the summer and 27.9 L in the winter. Parker et al. (2000) measured water intake on 50,000 feedlot steers in Texas and reported average water intake of 35.6 L per day. Brew et al. (2011) measured water intake of bulls, steers, and heifers individually in a pen setting and reported an average water intake of 29.9 L per head per day. These authors did not find an effect of temperature, but noted that temperature remained in the thermoneutral zone while measures were collected.

Hicks et al. (1988) reported that for each one lb increase in DMI (cracked corn high concentrate ration), water intake increased 0.3 gallons. Dietary salt also slightly decreased water intake (Hicks et al., 1988). However, Sexson et al. (2012) reported that DMI did not affect water intake in steers fed a steam-flaked corn ration; the authors also reported that body weight was related to water intake in feedlot steers. Brew et al. (2011) reported that there were no differences in water intake per kg of metabolic BW between heifers, steers, and bulls. Thus, diet and body weight may play a more complex role in water intake.

Association with water restriction

One of the challenges of reviewing water restriction literature is that there appears to be 2 main ways to restrict water. While some studies completely remove water access for restriction measures (simulating drought type conditions), others will limit water availability to a percent of normal or *ad libitum* water intake (simulating water scarcity situations; Steiger-Burgos et al., 2000). Because some small ruminant breeds (sheep and goats) are typically native to arid areas that experience such water challenges, most water restriction studies have been performed with small ruminants. Literature focusing on cattle in such conditions is limited.

During water restrictive conditions, VP is typically increased and feed intake decreases. Over a 72-hour water restriction period, feed intake significantly decreased in sheep (Parker et al., 2003). Meyer et al. (1989) reported that administering VP intravenously inhibits feed intake. The authors speculated that the difference in intake occurred due to reductions in meal size, not necessarily number of meals. Dairy cows that

were water restricted 25 and 50% of normal intake had a decreased feed intake during restriction that was driven by significantly decreased meal size, but increased meal frequency (Burgos et al., 2001). Thus, animals may still be eating, but having smaller more frequent meals.

After 24 and 72 hours of water restriction in goats, vasopressin was increased 2.5 and 10 fold, respectively; there was also an increase in plasma osmolality (Langhans et al., 1991). During water restriction the rumen fluid of goats had an increased osmolality, which might be the true driver of hypophagia in ruminants during water restriction. Prasetiyono et al. (2000) stated that hypophagia during water restriction is driven by increased plasma osmolality, decreased plasma volume and thirst level in goats. It is worth noting, however, that this study was conducted by completely withholding water rather than restricting access.

In sheep restricted to 60, 50, or 40% of normal water intake, cortisol and vasopressin significantly increased as water restriction increased. This difference also persisted over an extended period of time (2 weeks). Aldosterone tended to decrease as restriction level increased (Mengistu et al., 2016). During water restriction in pregnant dairy cows, cortisol was numerically, but not significantly decreased during 50% water restriction (Burgos et al., 2001). Future work should investigate how water restrictive conditions alter the endocrine water regulation and how to measure adaptability.

Complete blood count parameters are commonly used to assess dehydration and have been measured during water restrictive conditions. Hemoglobin initially decreased in sheep restricted to 80% of water intake, but increased in sheep with 60% water intake;

whereas PCV did not significantly increase until sheep were restricted to 40% water intake (Kumar et al., 2015). Water restriction in goats was shown to increase thirst level, plasma osmolality, and decrease plasma volume linearly as time without water increased (Prasetiyono et al., 2000). Although plasma osmolality was not significantly increased in water restricted cows, hematocrit was significantly increased from baseline to 50% water restrictive conditions (Burgos et al., 2001).

During water restriction in dairy cows sodium was significantly increased and while chloride was not significant, it numerically followed the same trend; potassium was not different during 50% water restriction (Burgos et al., 2001). During water restriction in sheep, urinary potassium and magnesium both decreased over a 72 hour period; plasma sodium and potassium significantly increased above baseline measures after a 72 hour water restriction (Parker et al., 2003).

Performance measures can also indicate how an animal is coping with a stressor. Body weight and feed intake are commonly affected by water restriction. Under 20 and 40% water restrictive conditions in sheep, body weight and ADG were significantly decreased compared to sheep with *ad libitum* water intake. Feed intake was also decreased in the 40% restricted ewes compared to control animals and tended to persist over time (Kumar et al., 2015). However, Brew et al. (2011) found that while water intake is correlated with feed intake and ADG, there was no relationship observed between water intake and gain:feed efficiency. Thus, the relationship between water intake and performance may be more complex and differences in body weight may be driven by differences in water weight rather than actual body or muscle mass.

Kumar et al. (2015) also found that RR and pulse rate were affected by water restriction, where restricted sheep had lower RR and PR in both the morning and afternoon compared to control sheep; however, there was no difference in rectal temperature between treatments, time of day, or over time. Similarly, Christopherson and Cosgrove (1999) reported no difference in rectal temperature or packed cell volume of sheep undergoing water restriction.

Conclusion

Water is an essential nutrient and may become a limiting resource for agriculture as climate change evolves, having widespread effects on livestock production. The current physiological response to water restriction is well defined in small ruminants adapted to arid environments, however, the response of *Bos taurus* beef cattle to prolonged water restriction is not well-defined. Additionally, limited research has evaluated behavioral responses to water restrictive conditions. Thus, the current research aims to address physiological and behavioral changes during extended water restriction in beef steers. Moreover, this work attempts to evaluate methods to identify cattle that may be more efficient under such conditions.

CHAPTER II

EFFECTS OF TEMPERAMENT ON ADAPTATION TO PROLONGED WATER RESTRICTION

INTRODUCTION

There has been an increased interest in cattle temperament due to its relationship to growth, immunological function, and stress responses (Voisinet et al., 1997; Burdick et al., 2011a; Bruno et al., 2017). It is generally accepted that "temperamental" cattle have a reactive stress response, a decreased immune response to health challenges, and less efficient growth (Petherick et al., 2002; Burdick Sanchez et al., 2014). Previously, temperament has been related to feed intake, average daily gain (**ADG**), and gain to feed efficiency (**G:F**) in cattle (Café et al., 2011; Bruno et al., 2017). However, water intake (**WI**) and drinking behaviors have not been evaluated in relation to temperament. Because WI is important for health and performance, relationships between temperament and WI merit further investigation.

The 2 most common measurement methods for cattle temperament are chute score (**CS;** Grandin, 1997) and exit velocity (**EV;** Burrow, 1997). Both measures are

indications of how cattle respond to humans or handling stress. Chute score measures how reactive an animal is when restrained (i.e. while held in a squeeze chute) and EV measures the speed of escape from restraint. Although these measures have been correlated with each other and previous work has combined the two measures (Cooke et al., 2009), other research has shown these measures may impact performance differently and should be treated as separate measures (Bruno et al., 2017).

Cattle with higher CS or faster EV typically have increased cortisol and epinephrine concentrations (Curley et al, 2006; Hulbert et al., 2011). Because these animals are believed to be more reactive or have a more excitable stress response (Burdick Sanchez et al., 2014), it is possible that differences in performance could be more pronounced during chronic stress. Limited water availability can cause significant stress (Mengistu et al., 2016; Benatallah et al., 2019) and this thus, water restriction was used to simulate a chronic stressor in this experiment.

The objective of this study was to investigate differences in performance, feed and WI, feeding and drinking behaviors, and growth efficiency between animals with differing temperaments during a baseline and chronic water restriction period. It is hypothesized that lower ranking (CSR1 and EVR1) steers will out perform the higher ranking steers under water restrictive conditions.

MATERIALS AND METHODS

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (ACUP #AG13-18).

Animals and treatments

Four feeding groups of mixed breed beef steers (*Bos taurus*) were used in a completely randomized design experiment. Group 1 (n =105; initial BW = 409 ± 27.6 kg) occurred from June to October 2016, group 2 (n = 123; initial BW = 341 ± 32.7 kg) occurred from January to May 2017, group 3 (n = 122; initial BW = 319 ± 32.7 kg) occurred from September 2017 to January 2018, and group 4 (n = 119; initial BW = 347 ± 33.6 kg) occurred from February to July 2018. Steers were blocked by initial BW into a light and heavy weight block (2 pens/block). Group 1 steers were procured from a private ranch. Steers from group 2 were procured from Oklahoma State University Field and Research Service Unit. Steers from groups 3 and 4 were procured from OSU Field and Research Service Unit (n = 91 and n = 91, respectively) and through multiple livestock markets (n = 31 and n = 26, respectively). All steers were housed at the Willard Sparks Beef Research Center in Stillwater, OK. A more detailed genetic breed classification is described in Ahlberg (2018a).

Within 24 h of arrival, cattle were weighed and ear tagged for individual identification and pen assignments. Steers were randomly assigned to pens in 2 weight blocks based on initial BW. Routine processing on the day of allocation (d -21) included viral and clostridial vaccinations (Titanium 5 PHM, Elanco, Greenfield, IN; Vision 7, Merck Animal Health, Madison, NJ), an injection of doramectin (Dectomax, Zoetis, Florham Park, NJ), a fenbendazole drench (Safe-Guard; Merck Animal Health), an antimicrobial metaphylaxis treatment of ceftiofur crystalline free acid (Excede; Zoetis) at a dose of 3.3 mL/100 kg, and application of RFID tags (Allflex, Irving, TX); Group 1 was vaccinated with Covexin 8 (Merck Animal Health), rather than Vision 7 due to the

owner's request. On d 0, steers were implanted with Compudose (25.7 mg estradiol; Elanco).

Steers were housed in 1 of four 31.9 × 11.3 m partly covered pens within a 3-sided part soil floor barn. Each pen had 11.3 × 9.1 m of shade. There were 2 pens per weight block. Each pen was equipped with 1 Insentec Roughage Intake Control (**RIC**) water bunk and 6 Insentec RIC feed bunks (Hokofarm Group, The Netherlands). The RIC software utilized by the system uses electronic ear tags to monitor animal presence at a feed or water bunk and calculates individual feed and WI by subtracting the final bunk weight from the start bunk weight for each individual's visit. The bunks also measure time and duration of each bunk visit. Additional specifications and information about the system can be found in Taylor (2016) and Allwardt et al. (2017). Each pen contained 26 to 33 steers, resulting in a 4.3-5.5 animal:feed bunk ratio.

The first 21 d (d -21 to -1) after pen allocation were used as an acclimation period for steers to adjust to and learn to use the Insentec RIC system. Any animals that did not have consistent feed and water bunk visits, low feed or WI (i.e. not visiting bunks daily, daily feed intake < 4kg, daily WI < 10 kg, or combination of these conditions), or exhibited signs of anorexia (i.e. sunken around the hooks, gaunt appearance) by the end of the 21 d period were removed from the experiment. Following the 21 d acclimation period, baseline feed and WI were measured daily for 70 d (d 0 to 70; **BAS**) to establish an average daily feed and WI for each animal. Both feed and water were offered in *ad libitum* amounts during this period.

Using the 70 d of WI data, an average daily WI for the *ad libitum* period (**WIAL**) was calculated for each animal to determine quantities of water allowed for individuals during water restriction. Restriction levels were calculated by multiplying restriction level by the WIAL. Restriction levels decreased by 10% of the WIAL per wk such that animals spent 7 d at each restriction level before reaching 50% restriction (e.g. 7 d at 90% of WIAL, 7 d at 80% of WIAL, 7 d at 70% of WIAL, 7 d at 60% of WIAL). Upon reaching full restriction at 50% of WIAL, steers were maintained at 50% of WIAL for 42 d (d 99 to 140; **RST**). After the water restriction period was completed, steers were incrementally stepped back up to *ad libitum* WI over 6 d.

Diet

Each pen was fed a common growing diet throughout the study (Table 2.1). Feed was delivered 3 times daily at approximately 0700, 1030, and 1430 h. Feed was targeted to be offered *ad libitum*. Due to the increased challenge of feed calling for individual feed bunks, there were some days that bunks were empty before delivery of the first feeding. For this reason, rations were adjusted daily to provide *ad libitum* intake with minimal amounts of feed remaining in the bunks prior to the 0700 h feeding. These data were used to adjust feed amounts. Ingredient DM and diet DM were determined once weekly by drying samples for 48 h in a forced air oven (55˚C, Model 1327F, VWR Scientific Products, Cornelius, OR, USA).

Temperament measures

Two temperament measures were collected on each animal throughout the experiment: CS (Grandin, 1993) and EV (Burrow et al., 1989). Chute score was collected

by observing behavior in the chute for 10 s after the animal entered the chute, without squeeze applied or the head caught. Chute score was measured by a single trained observer. Chute score was measured on a 1 to 4 scoring scale, where a score of 1 was an animals that was calm or showed no movement, 2 was restless shifting by the animal, 3 represented an animal that was head throwing and squirming, and 4 indicated that the animal was violently shaking the chute.

Upon being released from the chute, exit time was measured over 1.5 m between two pairs of infrared sensors (FarmTek Inc., North Wylie, TX) and converted to EV (m/s). The first pair of sensors were placed 1.5 m in front of the opening of the chute and the second pair was placed an additional 1.5 m behind the first pair. Exit velocity and CS were collected on all weigh days (d 0, 14, 28, 42, 56, 70, 77, 84, 91, 98, 112, 126, and 140).

Steers were categorized into a CS rank (**CSR**) and an EV rank (**EVR**) based on their initial measure at d 0. The CSR was equal to their initial score on the 4-point scale. Only 2 animals had initial CS of 4, so those animals were assigned rank of 3; thus, there were 3 CSR (**CSR1**, **CSR2**, and **CSR3**). The EVR was broken into 3 scores, where **EVR1** steers had an initial EV 1 SD below the average, **EVR3** steers had an initial EV 1 SD above the average, and steers falling in between were ranked an **EVR2**.

Growth performance

Animals were not withheld from feed or water prior to weighing, but weights were obtained prior to the first daily feed delivery. Animal weights were recorded on d 0, 14, 28, 42, 56, 70, 77, 84, 91, 98, 112, 126, and 140. Performance measures were

calculated for 3 periods: BAS (d 0 to 70), RST (d 99 to 140), and d 99-112 (**RST1**), which was the first 2 wk of full water restriction. Reports have suggested that it can take 2 weeks for animals to adapt to water restrictive conditions (Mengistu et al., 2016). Thus, RST1 was also chosen to evaluate differences during the first 2 weeks of water restriction.

Average period feed intake and average period WI were calculated for each animal by summing daily intakes from the Insentec RIC system and dividing by the number of days in the period. The average period feed intake was multiplied by the average diet DM from the same period to determine average period DMI. Average period DMI and WI were also expressed as a percentage of BW by calculating daily BW and dividing DMI or WI by BW (**FI%BW** and **WI%BW**). Daily BW was calculated for each animal by regressing collected BW over 14 d increments. For example, daily BW from d 1-13 were regressed using BW collected on d 0 and 14. Daily number and duration of feed and water bunk visits were summed from individual event data from the Insentec RIC system.

Average daily gain was calculated for each animal as the total BW gain per period divided by the total number of days per period. Gain to feed ratio was calculated as body weight gain (kg) divided by DMI (kg) for each animal and calculated for each period.

Body condition score (**BCS**) was measured on each animal once during BAS and once during RST using the 9-point beef BCS scoring scale (Selk, 2017). Scores were collected by a single trained observer.

Medication protocol

Steers were examined for illness daily and treated if required. In order for an animal to qualify for treatment the animal must have displayed clinical signs (e.g. lethargy, emaciation, coughing, lameness, etc.) and had a rectal temperature exceeding 40°C. Although cattle used in this experiment would not typically be classified as high-risk, metaphylaxis treatment was performed at processing to ensure steers were healthy when entering the Insentec barn. Following metaphylaxis treatment, the treatment regimen for observed respiratory signs consisted of a single subcutaneous dose of florfenicol (Nuflor; Merck Animal Health) at a dose of 13.2 mL/100 kg for respiratory symptoms. If symptoms persisted after 5 d, the steer was retreated with florfenicol. The treatment regimen for conjunctivitis (pinkeye; indicated by tearing or photophobia) consisted of a single dose on 3 consecutive d of oxytetracycline (Biomycin; Boehringer Ingelheim, Ridgefield, CT) at a dose of 9.9 mL/100 kg. If symptoms persisted after 3 d, a single dose of tylosin (Tylan, Elanco) was administered at a dose of 8.8 mL/100 kg. Treatment of infectious pododermatitis (foot rot; indicated by limping, swelling, presence of cracked hoof, presence of rot) consisted of a single dose of oxytetracycline (Biomycin; Boehringer Ingelheim) at a dose of 9.9 mL/100 kg. If symptoms persisted after 4 d, a single dose of tulathromycin (Draxxin, Zoetis) was administered at a dose of 13.2 ml/100 kg. All animals were rechecked daily.

Statistical analysis

This experiment utilized a completely randomized block design with a longitudinal one-way treatment structure. The experimental unit was animal (n = 467). Initial measures of EV, CS, BW and final BW were analyzed using the GLM procedure of SAS 9.4 (SAS, Inc., Carey, NC, USA). Changes in EVR and CSR over the course of

the experiment were analyzed using the MIXED procedure of SAS. The model statement included EVR or CSR, day, and the interaction. Group was included as a random effect, where animal was specified as the subject and the covariance structure was unstructured. Orthogonal contrast matrices were calculated using the IML procedure (interactive matrix language) of SAS with the orpol function. Linear and quadratic effects were estimated using the lsmestimate statement of SAS for rank by day interactions.

All performance data were analyzed with the GLIMMIX procedure of SAS using animal as the experimental unit with 1 of 4 models. In the first and second models, the model statement included effects of EVR or CSR, period (BAS and RST), the interaction, and block. The third and fourth models included effects of EVR or CSR, period (RST and RST1), the interaction, and block. When not significant, block was removed from the model. Group and cattle source were included as random effects. Because breed and previous management were unknown for the cattle bought from the sale barn but known for the other 2 sources, source was included as a random effect to account for possible breed and management differences.

Main effects and interactions were considered significant at $P \leq 0.01$. Trends were considered at $0.01 \leq P \leq 0.05$. Ranking by period interactions were only significant for the CSR × period analysis of ADG.

RESULTS

There was a significant EVR by day interaction where each day was different from all other days for each ranking (Fig. 2.1; $P < 0.001$). Day effects of all EVR were characterized by a linear effect ($P < 0.001$). The day effect of EVR2 was characterized by

a quadratic effect ($P < 0.001$). There was also a significant CSR by day interaction where all measures were different from each other on all days (Fig. 2.2; $P < 0.001$). Time effects of CSR2 and CSR3 were characterized by linear and quadratic effects ($P < 0.0001$); time effect of CSR1 was characterized by a quadratic effect ($P < 0.0001$).

Initial measures of EVR and CSR were different among all ranks (Table 2.2; $P \leq 0.0001$). For both EVR and CSR there was no difference in initial BW ($P \geq 0.10$). For CSR, there was no difference in final BW ($P \geq 0.82$). Final BW was different between EVR ($P = 0.02$), where EVR1 steers had a 21.75 kg heavier final BW than EVR2 and EVR3.

Block did not affect FI%BW, ADG, G:F, WI%BW, water bunk visits, time at the water bunk, or time at the feed bunk, so it was removed from those models ($P \geq 0.33$). Body condition score, WI, and DMI were greater in the heavy block compared to the light block ($P \leq 0.01$). Visits to the feed bunk were increased in the light block compared to the heavy block ($P \leq 0.01$).

There were no significant interactions of EVR and period on any response variable in either model ($P \geq 0.13$). There were no differences in FI%BW, WI, WI%BW, ADG, G:F, feed or water bunk visits, or time at the feed or water bunk between EVR (Table 2.3; $P \geq 0.10$). Dry matter intake and BCS were higher in EVR1 and EVR2 than EVR3 steers ($P \leq 0.01$).

There was a CSR by period interaction for ADG (Fig. 2.3; $P < 0.01$), where CSR3 steers had the least ADG during BAS and CSR2 steers had greater ADG than CSR1 and CSR3 during RST ($P \leq 0.01$). There were no other CSR by period interactions for any

response variable in either model ($P \geq 0.07$). There were no significant effects of CSR on DMI, FI%BW, WI, WI%BW, feed or water bunk visits, or time at the feed or water bunk (Table 2.4; $P \geq 0.08$). Gain:feed tended to be greater in CSR1 and CSR2 compared to CSR3 ($P \leq 0.05$). Body condition score was higher in CSR1 and CSR2 than CSR3 ($P \leq 0.01$).

All performance response variables were significantly affected by period (Table 2.5; $P < 0.001$) when BAS was compared to RST. Dry matter intake, FI%BW, WI, WI%BW, ADG, G:F, and BCS were greater during BAS compared to RST ($P \leq 0.001$). Visits to the feed bunk and time at the feed bunk were greater during BAS compared to RST ($P \leq 0.01$). Water bunk visits and time at the water bunk were greater in RST compared to BAS ($P \leq 0.01$).

Comparing RST to RST1, WI, WI%BW, G:F, water bunk visits, and time at the water bunk were not different (Table 2.6; $P \geq 0.06$). Dry matter intake, FI%BW, ADG, feed bunk visits, and time at the feed bunk were all greater during RST1 compared to RST ($P \leq 0.01$).

DISCUSSION

Time effects

Over time, both EV and CS measures decreased across all ranks, which was expected as literature commonly reports habituation to handling (Burrow and Dillon, 1997; Café et al., 2011). Time effects were generally linear or quadratic in nature and the shapes of these curves were different between ranks in both EV and CS. Initially, EV increased from the first to second measurement in EVR1 and EVR2, similar to other

results (Petherick et al., 2002; Kilgour et al., 2006; Bruno et al., 2017). The decrease in EV in EVR3 steers from d 0 to 14 is also consistent with previous results (Bruno et al., 2017). Petherick et al. (2002) suggested this effect was due to initial increased fearfulness to handling, but in this study this increase in EV would have actually been the fourth time the cattle were handled (handling at arrival and processing) and the EV of EVR3 steers decreased on these days. Thus, it does not seem the difference is due to increased fearfulness, unless steers with a fast EV habituate more quickly than slower EV steers. The response may be due to a change in routine, since cattle were undisturbed for 21 d and then were weighed every 14 d.

Measures of EV in EVR1 and EVR3 had a subtle increase on d 77, which was the first measurement during water restriction and only a 7 d increment between weigh days, rather than 14 d. It seems likely that the difference in routine caused the small increase in EV, rather than the water restriction as the change in water availability was small (10% difference). It is also possible that the change was caused by a combination of a change in routine and water restriction. Although measures of EVR2 did not increase on this day, it is possible that the more extreme ranks are more sensitive to changes in routine, whereas the intermediate rank was unaffected by the change. Similarly, EV increased in EVR1 steers from d 98 to 112, during the full 50% water restriction. The increased velocity could be due to the water stress or another change in routine as weigh days go from 7 to 14 days apart over this interval.

Chute scores of all ranks also generally decreased over time, agreeing with previous results indicating that cattle habituate to human handling over time (Curley et al., 2006; Kilgour et al., 2006; King et al., 2006; Bruno et al., 2017). While CS in CSR2

and CSR3 decreased from d 0 to 14, CS increased in CSR1 between d 0 to 14, which corresponds with the differences seen in EVR1 (both low ranking). Although these ranks followed a similar trend, measures were only slightly correlated ($R^2 = 0.22$). Following d 14, scores only decreased until d 70 and then tended to be more constant thereafter. Both EV and CS were different between ranks on all days of the study, indicating that cattle only habituate to an extent and the ranks will continue to differ over long periods of time. These results have also been reported previously (Curley et al., 2006; Bruno, 2015). Most importantly, EV and CS continued to stay different between rankings, even during habituation.

Performance measures

The primary objective of this experiment was to evaluate differences in DMI, intake behavior, performance, and efficiency between EVR and CSR during BAS and RST. While it was hypothesized that there would be differences between EVR during a chronic stressor, there were no EVR by period interactions, indicating no differences between ranks during BAS or RST. It was hypothesized that EVR1 steers would perform better than higher ranking EV steers during water restriction because of the improved ability to cope with a stressor (Burdick et al., 2011b). This is partly due to the documented ability of those animals to adapt to acute stressors, and chronic stress is believed to be a culmination of many occurrences of acute stressors, also known as chronic intermittent stress (Ladewig, 2000). Numerous studies have reported that more temperamental cattle exhibit greater concentrations of cortisol, adrenocorticotropic hormone (ACTH), and epinephrine (Curley et al., 2006; Curley et al., 2008; Burdick et al., 2011b; Café et al., 2011b), linking temperament to a general endocrine stress

response. Differences in metabolism have also been reported in cattle with different temperaments (Burdick Sanchez et al., 2012; Burdick Sanchez et al., 2014), which could lead to differences in DMI, performance, and WI. These studies also demonstrated that cattle with different temperaments respond differently to an acute immunologic challenge (lipopolysaccharide challenge, **LPS**). Similarly, studies implementing water restriction have reported an increased cortisol response during water restriction (Mengistu et al., 2016; Benatallah et al., 2019), indicating a stress response during these conditions. Thus, it was believed that a similar difference in performance among temperament rankings reported during an acute stressor would be observed during water restriction, since both challenges stimulate a general endocrine stress response. However, there were no differences observed between EVR during water restriction. Differences may not have been observed because of the differences in secondary activated pathways following the stimulus. Additionally, previous differences in metabolism or endocrine measures were typically measured over a fairly short period and likely represent a response to a short-term stressor as opposed to a long-term stressor (Lockwood et al., 2017). Typically, studies that have measured responses of different temperaments have utilized an acute stressor, such as transportation, handling, LPS challenge, or regrouping. Lockwood et al. (2017) compared serum and hair cortisol as indicators of acute and chronic stress responses; the authors reported no difference in hair cortisol between cattle with different flight speeds, indicating no difference in stress response utilizing a chronic measure. Mengistu et al. (2016) restricted water up to 40% of normal for 1 or 2 wk and reported increases in cortisol in sheep, indicating a stress response. Benatallah et al. (2019) performed water restriction up to 75% with cattle and observed an increase in cortisol,

but only restricted water for 8 d. The increased cortisol during shorter water restriction periods may indicate a stress response before animals had the ability to adapt. Other results from this experiment (unpublished) suggest some degree of adaptation within the first 2-4 wk during water restriction, indicating that an adaptation response was initiated in order to respond to an external stressor. However, the stress may not have been as severe as previously assumed since the steers were able to adapt to such conditions and cope, with no apparent differences in coping ability. Thus, the water restriction was believed to be a chronic stressor, but cattle were able to acclimate to water restriction, regardless of EVR. These results indicate that there was not an apparent difference in adaptability between steers with different EVR, and that while performance, intake, and efficiency were altered by water restriction, measures were not different among cattle with differing EV.

While differences were not observed between EVR within periods, ADG was different between CSR during BAS and RST. During BAS, ADG was greater in CSR1 and CSR2, as expected. Previous literature has reported greater ADG in cattle with lower CS (Voisinet et al., 1997; Bates et al., 2014). However, that typical difference changed when steers underwent water restriction. During RST, ADG was greatest in CSR2, which was not expected. These results may indicate that the extreme CS steers (high and low ranking), may have more difficulty coping with water restriction. A high CSR may identify the "classic" idea of a reactive steer (i.e. fighting behavior, escape behavior, shaking the chute, etc.), due to the nature of the response of those animals to handling. As such, the more reactive steer is expected to have more trouble coping with the stressor. The low CSR can be misleading, as the calm response can be a true composed response

or it can be a “freezing” response, presented in reactive or stressed steers (Cooke et al., 2009; Bruno et al., 2017). The steers that exhibit the “freezing” response may perform well under normal conditions, but when exposed to an environmental stressor, such as water restriction, those steers may have more difficulty coping. In contrast, the intermediate steers (CSR2) were able to cope with the introduced water restriction and continued to have a greater ADG. Similarly, G:F and BCS were not different between CSR1 and CSR2 overall, but greater than CSR3 overall. These results suggest that steers with an intermediate CSR may perform best during long-term water restriction stress.

Water intake during RST was approximately 50% lower compared to BAS, indicating that the intended water restriction level was achieved. Typically as WI decreases, DMI also tends to decrease (Burgos et al., 2001; Parker et al., 2003; Alamar, 2005) because those measures are closely related. Thus, the decrease in DMI and FI%BW were both expected. The decrease in DMI resulted in reduced time at the bunk and visits to the bunk during RST, as expected. Langhans et al. (1991) reported that dehydration decreased DMI and speculated that the difference was due to smaller meal size. Burgos et al. (2001) also reported a decreased meal size under water restrictive conditions in dairy cows. Although this experiment did not measure meals per se, the results of those experiments agree with the results of this experiment (less time spent at and visits to the bunk). The dramatic decrease in DMI (25.6%) resulted in a extensive decrease in ADG (64.5%). The combination of both substantial changes from BAS to RST also led to a decreased G:F.

Dry mater intake, FI%BW, ADG, feed bunk visits, and time at the feed bunk were greater during RST1 compared to RST, most likely due to a delay in adaptation. The

decreased DMI and FI%BW were most likely driven by less water intake over time, driving less feed intake over time. As stated previously, with a decrease in DMI, it is not surprising that ADG also decreased. The difference in feeding behavior was also most likely driven by the decrease in DMI, which required less visits to the feed bunk and less time spent at the bunk. Thus, DMI and feeding behavior appear to change over time with prolonged water restriction.

The differences in drinking behavior are most likely related to implementation of water restriction with the Insentec RIC system. During BAS, the fill level of the water bunks was roughly 45 kg, meaning that cattle could consume large quantities of water per visit if desired. During RST, the fill level of the water bunks was programmed at roughly 7 kg so that steers could not drink in excess of the daily allotment. Because the volume was smaller, steers had to make more visits and usually spent more time at the bunk getting enough water per visit. If the fill level had been the same during both BAS and RST, visits would have been expected to decrease during RST since less water was allotted overall.

The difference in DMI, where EVR1 and EVR2 had greater DMI than EVR3, is similar to responses reported in previous research (Café et al., 2011; Llonch et al., 2018). Research commonly reports similar differences between CSR, however, there were no differences in DMI observed this experiment between CSR. Graham et al. (2001) proposed that differences in CS were not detected because the cattle were all generally docile and had experienced little management stress (no regrouping, backgrounded). Similarly, the majority of cattle used in this trial were preconditioned and did not experience regrouping. All cattle also had a minimum of 21 d prior to initiation of BAS

to acclimate to the new environment and feeding system. These lower stress conditions may be why differences were not observed in DMI between CSR. Although differences were observed in DMI between EVR, differences were not seen in FI%BW, indicating that true differences in intake are probably being driven by body weight rather than EVR. There were also no differences in feeding behavior between EVR, indicating that differences in DMI were not driven by differences in time spent at the bunk or daily bunk visits.

The relationship between performance and temperament measures (EV and CS) may vary in this study because of when measures were collected. In this experiment, rank was based on measures collected on d 0, but cattle had previously been through the chute 2 times at arrival and processing. Bruno et al. (2017) reported that EV changed from the first to second measure. In addition to more chute experience, the cattle also had 21 d to acclimate to their surroundings, including the Insentec RIC system that required human interaction at least twice a day in addition to daily pen walking. This period of acclimation could have caused the authors to miss earlier differences in performance or some temperament measures. Relationships between temperament and performance may also be more apparent in shorter measures (28 d increments). As discussed in Bruno et al. (2017), the timing of temperament measures can affect how those classifications relate to production measures (e.g. one vs. many measures, first vs. second measures). Consequently, the lack of usual reported differences in performance between CSR in this study may be due to the additional experience cattle had in the chute and the low-stress backgrounding.

The relationship between temperament and WI has not previously been reported. However, since there were no differences observed between CSR or EVR during BAS it seems that EV and CS are not related to WI or drinking behavior. Body condition was affected by EVR, where EVR0 steers had the highest BCS. Petherick et al. (2009) found that cattle that had "good handling" had a greater BCS than cattle that were handled poorly, which indicates that handling stress may affect body condition. Since EVR3 steers are believed to be more reactive to a stressor than EVR1 or EVR2, the differences reported are expected. However, the difference reported between BCS in this trial is small and probably not biological relevant.

In conclusion, it seems that there may be some differences in performance between CSR during periods of water restriction. Conversely, it appears that EVR does not impact intake or performance during a long-term water stressor. Additionally, traditionally reported differences in intake or performance between EVR were not observed, possibly due to docility of cattle or the experimental design, whereas, traditional differences were observed between CSR ranks. Lastly, CS and EV do not seem to be related to daily WI, measured in volume or as a percent of BW. It may be worthwhile to account for steer's chute score for management during water scarcity.

Table 2.1. Diet composition of common receiving cattle were offered for duration of experiment

Item	Amount (%)
Cracked corn	17
Sweet Bran™[1]	45
B-273[2]	6
Prairie hay	32

Item	Value
DM, %	73.3
TDN, %	74.8
Fat	4.2
Crude fiber	10.2
ADF	20.3
NDF	39.0
Crude protein, %	16.1

[1]Cargill, Inc. Blair, NE.

[2]Formulated to contain: 27.9% limestone, 0.9% MgO, 0.4% salt, 6.4% urea 41.0% corn grain, 21.7% wheat midds, 0.1% copper sulfate, 0.1% selenium premix, 0.6% zinc sulfate, 0.3% vitamin A, 0.08% vitamin E, 0.3% Rumensin-90, 0.2% Tylan-40.

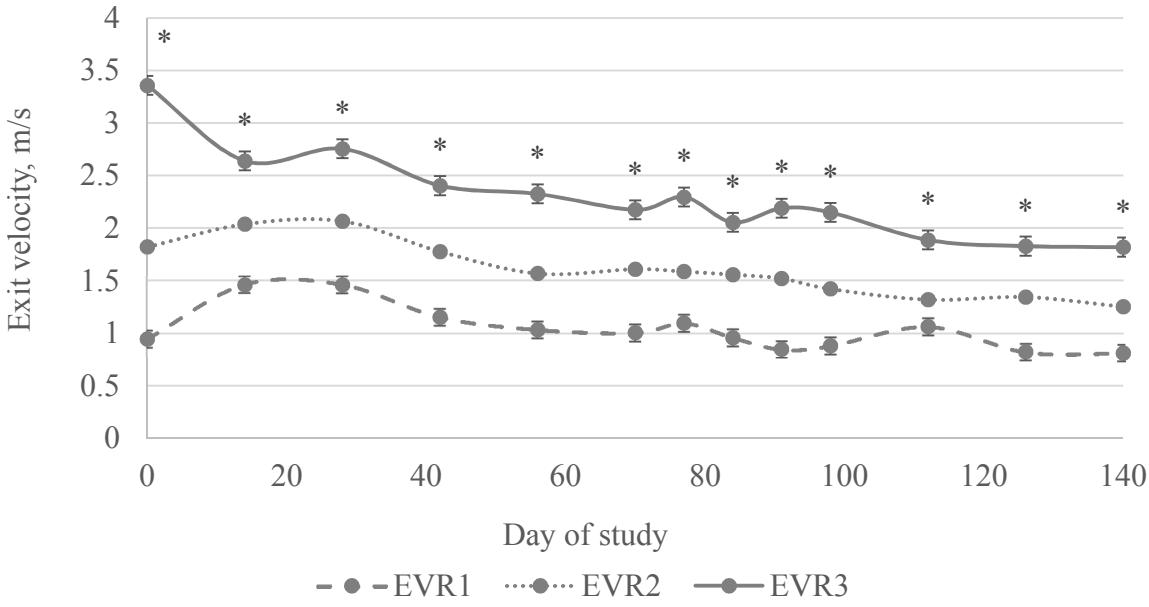

Figure 2.1. Least square means ± SD of exit velocity (**EV**) on each day of study by EVR. Ranking was based on initial measures collected on d 0, where EV 1 SD above the mean were ranked EVR3 (n = 69), 1 SD below the mean were ranked EVR1 (n = 56), and in between were ranked EVR2 (n = 342). There was a significant EVR by day interaction ($P < 0.0001$), where all measures were different from each other on every day of study, indicated by * ($P < 0.001$). Time effects of all ranks were characterized by a linear effect ($P < 0.0001$). Time effects of EVR2 were characterized by a quadratic effect ($P < 0.0001$).

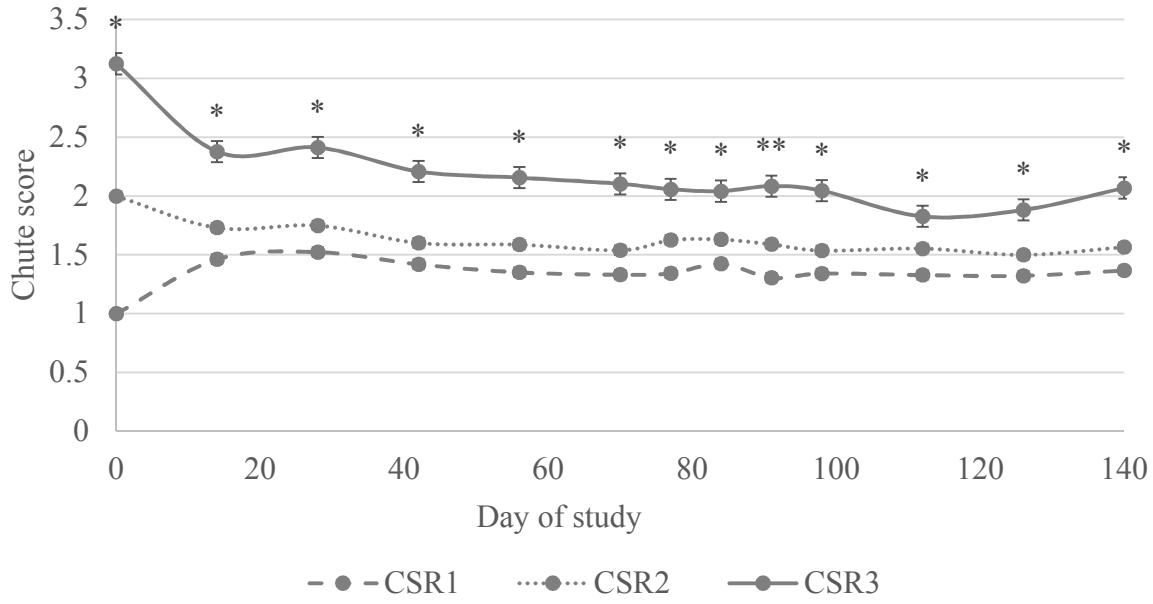

Figure 2.2. Least square means ± SD of chute score (**CS**) on each day of study by CSR. Ranking was based on initial measures collected on d 0: steers with a score of 1 (CSR1; n = 204), steers with a score of 2 (CSR2; n = 217), and steers with a score of 3 or 4 (CSR3; n = 46). There was a significant CSR by day interaction ($P < 0.0001$), where all measures were different from each other on every day of study, indicated by * ($P < 0.01$). Time effects for CSR2 and CSR3 were characterized by a linear effect ($P < 0.0001$). Time effects of all ranks were characterized by a quadratic effect ($P < 0.0001$).

Table 2.2. Sample sizes, initial temperament measures, initial body weight and final body weight

	EVR0[1]	EVR1[1]	EVR2[1]	SEM	*P*-value
n	56	342	69	-	-
Initial EV[1], m/s	0.94[a]	1.82[b]	3.41[c]	0.685	< 0.01
Initial BW, kg	364	352	346	49.4	0.10
Final BW, kg	532[x]	516[y]	505[y]	53.9	0.02

	CSR1[2]	CSR2[2]	CSR3[2]	SEM	*P*-value
n	204	217	46	-	-
Initial CS[2]	1.0[a]	2.0[b]	3.1[c]	0.34	< 0.01
Initial BW, kg	349	355	359	48.7	0.26
Final BW, kg	514	518	516	55.8	0.82

	Light block[3]	Heavy block[3]	SEM	*P*-value
n	231	236	-	-
Initial BW, kg	723	830	89.5	< 0.01
Final BW, kg	1087	1187	108.3	< 0.01

[1]Exit velocity was measured over 1.5 m. Ranking was based on initial measures collected on d 0, where EV 1 SD above the mean were ranked EVR2, 1 SD below the mean were ranked EVR0, and in between were ranked EVR1.

[2]Chute score was measured on a 1-4 scale. Ranking was based on initial measures collected on d 0: steers with a score of 1 (CSR1), steers with a score of 2 (CSR2), and steers with a score of 3 or 4 (CSR3).

[3]Cattle were randomly assigned to pens in 2 weight blocks (light and heavy). There were 2 pens/block.

[abc]Values with differing letters are significantly different at $P \leq 0.01$.

[xy]Values with differing letters tend to be different at $0.01 < P \leq 0.05$.

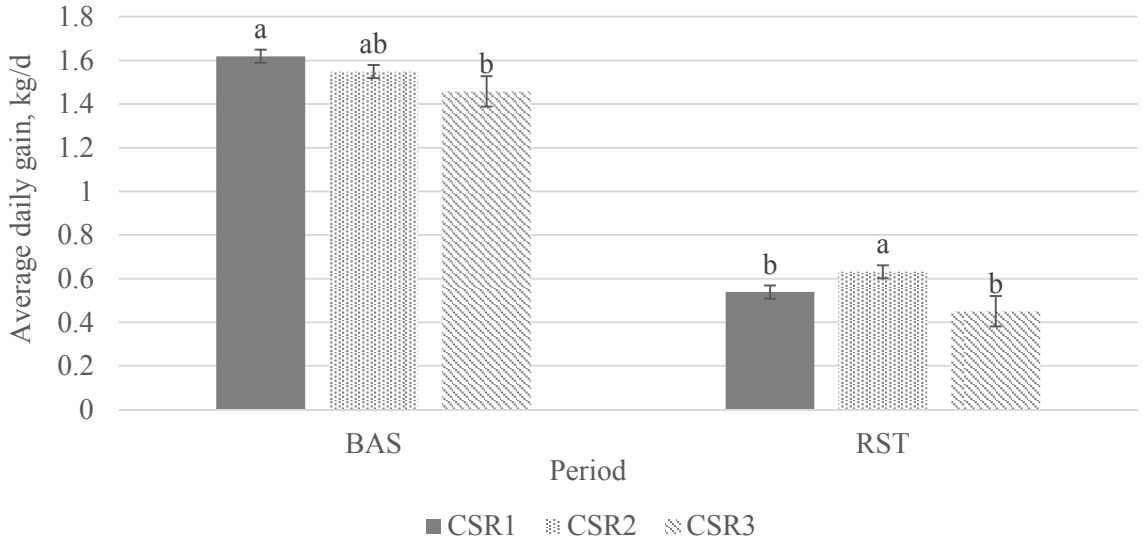

Figure 2.3. Least square means of average daily gain by CSR between experimental periods. Chute score was measured on a 1-4 scale. Ranking was based on initial measures collected on d 0: steers with a score of 1 (CSR1), steers with a score of 2 (CSR2), and steers with a score of 3 or 4 (CSR3). BAS refers to d 0-70 and RST refers to d 98-140. Values with differing letters are significantly different at $P \leq 0.01$.

Table 2.3. Least square means of intake, performance, efficiency, and feeding and drinking behavior by EVR[1]

	EVR0	EVR1	EVR2	SEM	*P*-value
DMI[2], kg/d	9.89[x]	9.82[x]	9.47[y]	0.118	0.02
FI%BW[2], %	3.03	3.04	2.99	0.033	0.36
WI[2], kg/d	27.6	28.2	27.0	0.65	0.21
WI%BW[2], %	6.24	6.42	6.25	0.124	0.29
ADG[2], kg	1.14	1.07	1.04	0.041	0.23
G:F[2]	0.111	0.103	0.106	0.0043	0.26
BCS[2]	7.1[a]	7.0[a]	6.8[b]	0.05	< 0.001
Feed bunk visits, count	45.7	46.0	44.0	1.21	0.33
Time at feed bunk, min/d	108.1	112.2	114.4	1.95	0.10
Water bunk visits, count	9.3	10.0	9.5	0.30	0.11
Time at water bunk, min/d	21.4	22.3	22.2	0.99	0.77

[1] Exit velocity was measured over 1.5 m. Ranking was based on initial measures collected on d 0, where 1 SD below the mean were ranked EVR0, EV 1 SD above the mean were ranked EVR2, and in between were ranked EVR1.

[2]Dry matter intake, DMI; DMI as a percent of body weight, FI%BW; water intake, WI; water intake as a percent of BW, WI%BW; average daily gain, ADG; gain:feed efficiency, G:F; body condition score, BCS.

[abc]Values with differing letters are significantly different at $P \leq 0.01$.

[xy]Values with differing letters tend to be different at $0.01 < P \leq 0.05$.

Table 2.4. Least square means of intake, performance, efficiency, and feeding and drinking behavior by CSR[1]

	CSR1	CSR2	CSR3	SEM	*P*-value
DMI[2], kg/d	9.83	9.76	9.55	0.145	0.21
FI%BW[2], %	3.06	3.03	2.96	0.041	0.08
WI[2], kg/d	27.5	28.1	29.1	0.79	0.15
WI%BW[2], %	6.29	6.41	6.53	0.152	0.28
ADG[2], kg	1.08[a]	1.09[a]	0.95[b]	0.050	< 0.01
G:F[2]	0.104[x]	0.107[x]	0.092[y]	0.0052	0.04
BCS[2]	7.0[a]	7.0[a]	6.8[b]	0.06	< 0.01
Feed bunk visits, count	45.5	46.3	43.2	1.47	0.16
Time at feed bunk, min/d	112.3	111.7	112.5	2.39	0.93
Water bunk visits, count	9.7	9.9	10.1	0.36	0.36
Time at water bunk, min/d	22.0	22.1	23.4	1.21	0.57

[1]Chute score was measured on a 1-4 scale. Ranking was based on initial measures collected on d 0: steers with a score of 1 (CSR1), steers with a score of 2 (CSR2), and steers with a score of 3 or 4 (CSR3).

[2]Dry matter intake, DMI; DMI as a percent of body weight, FI%BW; water intake, WI; water intake as a percent of BW, WI%BW; average daily gain, ADG; gain:feed efficiency, G:F; body condition score, BCS.

[abc]Values with differing letters are significantly different at $P \leq 0.01$.

[xy]Values with differing letters tend to be different at $0.01 < P \leq 0.05$.

Table 2.5. Least square means of intake, performance, efficiency, and feeding and drinking behavior by period[1]

	BAS	RST	SEM	*P*-value
DMI[2], kg/d	11.13	8.30	0.082	< 0.001
FI%BW[2], %	3.74	2.29	0.023	< 0.001
WI[2], kg/d	37.9	18.6	0.45	< 0.001
WI%BW[2], %	9.17	3.65	0.086	< 0.001
ADG[2], kg	1.54	0.54	0.028	< 0.001
G:F[2]	0.138	0.064	0.0030	< 0.001
BCS[2]	7.0	6.8	0.03	< 0.001
Feed bunk visits, count	46.3	43.7	0.75	< 0.001
Time at feed bunk, min/d	134.8	89.5	1.35	< 0.001
Water bunk visits, count	8.6	11.2	0.21	< 0.001
Time at water bunk, min/d	13.0	31.9	0.68	< 0.001

[1]BAS refers to the *ad libitum* water intake period from d 0-70 and RST refers to the full water restriction period where steers were maintained at 50% of average daily water intake from d 98-140.

[2]Dry matter intake, DMI; DMI as a percent of body weight, FI%BW; water intake, WI; water intake as a percent of BW, WI%BW; average daily gain, ADG; gain:feed efficiency, G:F; body condition score, BCS.

Table 2.6. Least square means of intake, performance, efficiency, and feeding and drinking behavior by period[1]

	RST	RST1	SEM	*P*-value
DMI[2], kg/d	8.30	8.75	0.092	< 0.001
FI%BW[2], %	2.29	2.44	0.021	< 0.001
WI[2], kg/d	18.6	18.9	0.270	0.49
WI%BW[2], %	3.65	3.78	0.048	0.05
ADG[2], kg	0.54	0.77	0.050	< 0.01
G:F[2]	0.064	0.080	0.0060	0.06
Feed bunk visits, count	38.5	40.2	0.62	0.05
Time at feed bunk, min/d	89.5	97.2	1.38	< 0.001
Water bunk visits, count	11.2	11.6	0.27	0.23
Time at water bunk, min/d	31.9	31.1	0.96	0.54

[1]RST refers to the full water restriction period where steers were maintained at 50% of average daily water intake from d 98-140 and RST1 refers to the first 2 weeks of full 50% RST from d 98-112.

[2]Dry matter intake, DMI; DMI as a percent of body weight, FI%BW; water intake, WI; water intake as a percent of BW, WI%BW; average daily gain, ADG; gain:feed efficiency, G:F.

CHAPTER III

EFFECTS OF PROLONGED WATER RESTRICTION ON INTAKE AND HEALTH OF FEEDLOT STEERS

INTRODUCTION

Climate change is expected to significantly impact future weather patterns, including increased ambient temperatures and decreased rainfall, leading to drought conditions and water insecurity (Timmermann et al., 1999). Water scarcity may also make it more difficult for livestock to perform during dry conditions or heat stress (Myers et al., 2017). These changes could have substantial negative impacts on animal agriculture, through decreased performance, increased health challenges, and animal welfare concerns. For these reasons, it is important to understand the physiological responses of livestock to water restriction. Benatallah et al. (2019) reported that dairy cows experiencing 25 and 50% water restrictive conditions had decreased DMI, increased respiration rates, and increased rectal temperature (**RT**). Kaliber et al. (2016) reported decreased plasma sodium (**Na**) and increased plasma potassium (**K**) in goats during water restrictive conditions. Casamassima et al. (2016) detected increases in red blood cell counts (**RBC**), hemoglobin (**HGB**), and hematocrit (**HCT**) in ewes during 80 and 60%

water restrictive conditions. Information regarding the physiological response of *Bos taurus* cattle to water restriction would be beneficial, especially as areas that rsise beef start to experience increased water insecurity.

There are also some limitations with current water restriction literature that is primarily focused on studies that utilize sheep or goats as the experimental model, whereas studies that utilize beef cattle are limited. While cattle are not traditionally considered a favorable livestock animal in arid areas, areas where cattle production is prominent in the U.S. may experience greater water insecurity in the future. Additionally, many studies either completely remove water for short periods of time or restrict water access for short periods, not addressing long-term effects of such conditions. Animals may be able to physiologically adapt to water restrictive conditions over long periods of time, which may be overlooked in shorter study periods. Finally, most of the literature has a limited sample size or does not collect individual animal data, though water intake is considerably variable between individuals. Long-term information on a large group of individual cattle in group pen settings is needed in the current water restriction literature.

The objective of this study was to evaluate the effects of long-term water restriction on feed and water intake, performance, and health. Steers were grouped by water efficiency for evaluation of performance and health.

MATERIALS AND METHODS

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (ACUP #AG13-18).

Animals and treatments

Four hundred and sixty-seven mixed breed beef steers were used in a randomized complete block design. Steers were fed in 4 feeding groups from 2016-2018. Group 1 (n = 105; initial BW = 409 ± 27.6 kg) occurred from June to October 2016, group 2 (n = 123; initial BW = 341 ± 32.7 kg) occurred from January to May 2017, group 3 (n = 122; initial BW = 319 ± 32.7 kg) occurred from September 2017 to January 2018, and group 4 (n = 117; initial BW = 347 ± 33.6 kg) occurred from February to July 2018. Cattle were procured from a private ranch (group 1), multiple livestock markets (groups 3 and 4; n = 31 and n = 26, respectively), or Oklahoma State University's Field and Research Service Unit (groups 2, 3, and 4; n = 123, n = 91 and n = 91, respectively). All groups were housed at the Willard Sparks Beef Research Center in Stillwater, OK. Steers were comprised of several breed combinations, but primarily British influenced. A more detailed breed breakdown can be found in Ahlberg (2018a).

Within 24 h of arrival, cattle were weighed and ear tagged for individual identification and pen assignments. Cattle were randomly assigned to pens within 2 weight blocks: a light and heavy. Blocking was based on initial BW. Routine processing on the day of allocation (d -21) included viral and bacterial vaccinations (Titanium 5 PHM, Elanco, Greenfield, IN; Vision 7, Merck Animal Health, Madison, NJ), an injection of doramectin (Dectomax, Zoetis, Florham Park, NJ), a fenbendazole drench (Safe-Guard; Merck Animal Health), and metaphylaxis treatment (Excede; Zoetis) at a dose of 3.3 mL/100 kg. Group 1 was vaccinated with Covexin 8 (Merck Animal Health), rather than Vision 7 per owner's request. On d 0, steers were implanted with Compudose (Elanco), an estradiol implant.

Steers were housed in one of 4 31.9 x 11.3 m partly covered pens within a 3-sided

partly concrete soil (6.5 x 11.3 m concrete) floor barn. There were 2 pens per weight block. Each pen had 11.3 x 9.1 m of shade. Each pen was equipped with 6 Insentec Roughage Intake Control (**RIC**) feed bunks and one Insentec water bunk (Hokofarm Group, The Netherlands) that utilize RFID tags. The RIC system measures feed and water intake by subtracting the bunk end weight from the bunk start weight following each animal's visit to the bunk. The Insentec RIC system allows for individual programming of feed and water access. Water was restricted by programming individual daily water allotments into the system. Once daily allotments were reached, steers were not able to access the water bunks. The Insentec program reset at 0000 h every night. Thus, each day was considered from 0000 to 2345 h and steers had that time to drink their daily allotment each day. More detailed information and specifications of the Insentec bunks can be found in Allwardt et al. (2017).

Summarized dates of each period and weather conditions for each group are reported in Table 3.1. Start and end dates indicate when each period started and ended for each group. Minimum and maximum values refer to daily average minimum and maximum ambient temperatures. Weather data were obtained from the Stillwater, OK station (3.5 km from WSBRC) of the Oklahoma Mesonet (http://www.mesonet.org). Average daily ambient temperatures during d 0-70 were 28.1, 9.7, 17.5, and 12.8°C for groups 1, 2, 3, and 4, respectively. Average ambient temperatures during RST were 21.6, 18.2, 1.8, and 27.2°C for groups 1, 2, 3, and 4, respectively.

The first 21 d following allocation to pens (d -21 to d -1) were used as an acclimation period for steers to acclimate and adjust to the Insentec RIC system for all feeding and drinking. Any steers that did not have consistent or adequate feed or water

intakes (i.e. not visiting daily, daily feed intake < 4 kg, daily water intake < 10kg, or any combination of these conditions) by the end of this period were removed from the trial. Following the 21 d acclimation, individual feed and water intakes were measured daily for 70 d to establish baseline feed and water intake. The 70 d period was the baseline (**BAS**) period. During this time steers had *ad libitum* access to feed and water.

Daily water intakes (**WI**) from the 70 d *ad libitum* period were used to calculate individual animal average daily water intakes (**ADWI**). Individual ADWI values were used to calculate water restriction amounts for each steer. The restriction value was calculated by multiplying the ADWI by the restriction level for each steer. Steers were stepped down 10% of ADWI every 7 d (e.g. 7 d at 90% of ADWI starting d 71, 7 d at 80% of ADWI starting d 78, 7 d at 70% of ADWI starting d 85, 7 d at 60% of ADWI starting d 92) until steers reached full water restriction at 50% of ADWI (d 99). Steers were maintained at 50% of ADWI for 42 d (**RST**). Following the 42 d, steers were stepped back up to *ad libitum* WI over 6 d.

A WI efficiency measure was calculated for each animal using WI and ADG measures collected during BAS. Water intake efficiency (**WIEFF**) was calculated as ADG (kg)/average daily water intake as a percent of BW (**WI%BW**), using the d 0 to 70 values. To evaluate differences between steers with different WIEFF, steers were categorized as low (**LWE**), medium (**MWE**), or high (**HWE**) WIEFF using k-means clustering in SAS 9.4 (SAS, Inc., Carey, NC, USA), where $k = 3$. This type of clustering partitions observations into k clusters such that each observation belongs to the cluster with the nearest mean.

Diets

All animals were fed a common growing diet throughout the trial. The diet was 17% cracked corn, 45% Sweet Bran™ (Cargill, Blair, NE), 32% prairie hay, and 6% dry supplement. Feed was delivered 3 times daily at approximately 0700, 1030, and 1430. Feed was targeted to be offered *ad* libitum, however, feed calling in individual feed bunks is more challenging due to increased intake variation compared to traditional cement style bunks. Thus, there were some days when feed bunks were empty prior to the first feed delivery. For this reason rations were adjusted daily to ensure *ad libitum* access with minimal feed refusals. Ingredient and diet DM were measured once weekly by drying samples for 48 h in a forced air oven (55˚C, Model 1327F, VWR Scientific Products, Cornelius, OR, USA).

Growth performance, rectal temperature, and feed intake

Animals were not withheld from feed or water prior to weighing. Weights were obtained prior to the first daily feeding. Animal weights were recorded on d 0, 14, 28, 42, 56, 70, 77, 84, 91, 98, 112, 126, and 140. Pens of steers were brought up to the barn in the same order on each day. Rectal temperature (**RT**) was also recorded on weigh days using a digital thermometer (GLA Agricultural Electronics, Obispo, CA) once the steer was restrained in the chute. Rectal temperatures are presented as daily average measures.

Performance and intake measures were collected in five periods. The first two periods are the BAS (d 0 to 70) and RST (d 98 to 140). In addition to these periods, the difference from BAS to RST is also presented (RST-BAS). There are also three periods presented within RST: d 98 to 112 (**RSTP1**), d 112 to 126 (**RSTP2**), and d 126 to 140

(**RSTP3**). Each of these three periods represent two week intervals during the full 50% restriction phase and were analyzed to examine differences in adaptation over time. Preliminary observations indicated that cattle showed some signs of adaptation depending on the week of water restriction. For this reason, the water restrictive condition was broken into the 3 two-week periods.

Daily feed intake and WI were calculated for each animal using data from the Insentec RIC system. The average feed intake was multiplied by a group's period average DM to calculate DMI. Daily DMI and ADWI were also expressed as a percentage of BW by calculating daily BW (**FI%BW** and **WI%BW**). Daily BW was calculated for each animal by regressing collected BW in 2 wk intervals using 14 d BW. For example, to calculate BW from d 1-13, BW collected on d 0 and 14 were regressed. Thus, a daily BW, FI%BW, and WI%BW were calculated for each animal.

Average daily gain (**ADG**) was calculated for each animal as the total BW gain in the period divided by the total number of days in the period. Gain to feed ratio (**G:F**) was calculated as body weight gain divided by DMI for each animal and calculated for each period.

Medication protocol and morbidity measures

A metaphylaxis treatment was administered at processing to ensure a low morbidity rate when steers were moved into the Insentec barn. Steers were examined for signs of sickness and dehydration daily and treated if required. Only 1 animal exhibited signs of dehydration that were severe enough to require intervention. In order for an animal to qualify for treatment, the animal must have displayed clinical signs (e.g.

lethargy, emaciation, coughing, lameness, etc.) and have a RT exceeding 40°C. Following metaphylaxis treatment, the treatment regimen for observed respiratory symptoms consisted of a single subcutaneous administration of Florfenicol (Nuflor; Merck Animal Health) at a dose of 13.2 mL/100 kg. No animals were treated more than once. The treatment regimen for conjunctivitis (pinkeye; indicated by tearing or photophobia) consisted of 2 to 3 subcutaneous doses of oxytetracycline (Biomycin; Boehringer Ingelheim, Ridgefield, CT) on consecutive days at a dose of 9.9 mL/100 kg. If symptoms persisted after 3 d, tylosin (Tylan; Elanco) was administered at a dose of 8.8 mL/100 kg. The treatment regimen for infectious pododermatitis (foot rot; indicated by limping, swelling, presence of cracked hoof, presence of rot) consisted of one subcutaneous dose of oxytetracycline (Biomycin; Boehringer Ingelheim) administered at a dose of 9.9 mL/100 kg. If symptoms persisted after 3 d, steers were treated with a single dose of tulathromycin (Draxxin; Zoetis) at a dose of 2.4 mL/100 kg. Animals were observed daily. Third treatments only occurred for treatment of conjunctivitis in group 3 because animals failed to respond to the second treatment.

Prevalence of disease was used as a measure of morbidity. Morbidity was broken into 6 categories: treated for respiratory disease, treated for infectious pododermatitis, animals that displayed symptoms of respiratory disease but were not treated (RT below 40°C), treated for pinkeye, and total treated. Criteria used to diagnose and treat each category are previously described above.

Blood collection

Blood was collected via the jugular vein into 1 purple top EDTA and 1 red top vaccutainer tube (Becton Dickinson, Franklin Lakes, NJ) on d 70, 84, 98, 112, 126, and 140. Steers were not withheld from feed and water before blood collection. Blood sampling occurred between 0300 and 0800 h, depending on the weigh day. Red top vaccutainer tubes were centrifuged at 3,000 × g for 15 minutes. Serum was immediately collected and stored in duplicate in a -80°C freezer (model #UXF70086D63, Thermo Fisher, Waltham, MA) for future analysis. Purple top vaccutainers of whole blood were analyzed for complete blood counts using an Idexx Procyte Hematology Analyzer (IDEXX Laboratories, Westbrook, ME). Following blood collection, tubes were kept on ice until serum collection or CBC analysis. Samples were analyzed within 8 h of blood collection. The Procyte Hematology Analyzer measures blood parameters by laser flow cytometry and optical fluorescence. Data collected from the Procyte analyzer included incidence of left shift in white blood cells (**WBC**), WBC, RBC, WBC differentials, HGB, HCT, mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), reticulocytes, platelets, and neutrophil:lymphocyte ratio (**N:L**).

One serum tube was thawed and analyzed for Na, K, and Cl using a Carelyte Electrolyte Analyzer (Diamond Diagnostics, Holliston, MA). The Carelyte Electrolyte Analyzer measures electrolytes using the ion selective electrode measurement principle. Briefly, an ion exchanging membrane reacts to an electrical change initiated by the unknown sample causing a change in the membrane potential. A galvanic measuring chain in the electrode measures the difference between the potential on either side of the membrane to report electrolytes.

The 20 steers with the highest WIEFF from the HWE group and 20 steers from the LWE group with the lowest WIEFF were chosen for analysis of serum vasopressin and aldosterone in order to determine differences in renal water regulation. Serum samples from days 70, 98, 126, and 140 were used for analysis. Vasopressin was measured using a competitive ELISA kit following manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY). Samples were prepared using a solid-phase extraction protocol and thus did not require additional dilution. Aldosterone was measured using a competitive ELISA kit (Cayman Chemical, Ann Arbor, MI) that utilized an aldosterone-acetlycholinesterase (**ACHe**) conjugate. Samples were diluted to a 1:1 ratio for analysis. All experimental samples were analyzed in duplicate and standards were analyzed in triplicate.

Statistical Analysis

The experiment utilized a completely randomized block design and the experimental unit in this trial was animal (n = 467). Performance and efficiency measures (DMI, ADWI, ADG, G:F, FI%BW, WI%BW) were analyzed using the MIXED procedure of SAS 9.4. All time periods were analyzed individually, but the model was the same for all analysis of response variables. Periods were kept separate by utilizing a by statement in the SAS analysis, where data was sorted by time period (BAS, RST, change, RST1, RST2, and RST3). For all response variables the model statement included effects of WIEFF and block. Group and source were included as random effects and the subject was animal within group. Because some cattle were purchased from sale barns, the specific breed composition and previous management of those animals is mostly unknown and could vary for each steer. However, the breed composition and

previous management for steers obtained from OSU and the private ranch are consistent for all steers within a group. Additionally, sources of cattle were mixed for groups 2 to 4. Thus, source was included as a random variable to account for genetic and environmental differences. Differences in means were analyzed with a pdiff statement allowing for a Tukey adjustment.

Morbidity data are expressed as the percent of animals that met the description of each disease category out of the total. Morbidity was analyzed using the GLIMMIX procedure of SAS where the model included effects of WIEFF and block. Group and source were included as random effects. Differences in means were analyzed with a pdiff statement allowing for a Tukey adjustment.

Whole blood CBC data and serum electrolytes (Na, K, Cl) were analyzed using the GLIMMIX procedure of SAS where the model included effects of day, WIEFF, the interaction, and block. Group and source were included as random effects. The subject was specified as individual within group and differences in means were analyzed with a pdiff statement allowing for a Tukey adjustment. A slice option was also included for differences between day and WIEFF. Aldosterone and vasopressin were analyzed using the GLIMMIX procedure of SAS where the model included effects of day, WIEFF, and block. Because all samples collected on a given day were not measured on 1 plate for vasopressin, plate was included as a random effect. However, all aldosterone samples collected on the same day were analyzed on 1 plate. There were complications with the plate measuring aldosterone on d 98 so those results were removed from analysis. Rectal temperature data were analyzed using the GLIMMIX procedure of SAS where the model included day, WIEFF, the interaction, block, and order of entry. Because weigh days took

anywhere from 2-7 hours, order of entry was included. Group and source were included as random effects. The subject was specified as individual within group and differences in means were analyzed with a pdiff statement allowing for a Tukey adjustment. A slice option was also included for differences between day and WIEFF.

Main effects and interactions were considered significant at $P \leq 0.05$. Trends were considered at $0.05 \leq P \leq 0.10$.

RESULTS

Performance measures

While initial BW during BAS was not different among WIEFF groups, final BW during BAS, initial BW during RST, and final BW during RST were greater in the HWE than MWE and LWE (Table 3.2; $P \leq 0.001$). During both BAS and RST, WI was lower in HWE and MWE than LWE (Table 3.3; $P \leq 0.001$). Change in WI followed the same pattern ($P \leq 0.001$). The same relationships were observed for WI%BW. During both BAS and RST, WI%BW was least for HWE, intermediate for MWE, and greatest in LWE ($P \leq 0.001$). Medium water efficiency and HWE steers had greater DMI than LWE during BAS ($P \leq 0.001$), but there was no difference during RST or for DMI change ($P \geq 0.44$). Dry matter intake decreased from BAS to RST (11.27 to 10.13 kg/d; $P < 0.001$). There was not a difference in FI%BW during BAS ($P \geq 0.10$). During RST, FI%BW was lowest in HWE compared to MWE and LWE ($P \leq 0.001$). The change in FI%BW was greatest in HWE and MWE compared to LWE ($P \leq 0.001$). Feed intake as a percent of BW decreased from BAS to RST (3.76 to 2.29% BW; $P \leq 0.001$). During BAS, HWE steers had a greater ADG than MWE, and MWE had a greater ADG than LWE (Table

3.3; $P \leq 0.001$). Change in ADG was greater in HWE and MWE than LWE ($P \leq 0.001$). During BAS, G:F was greater in HWE, intermediate in MWE, and lowest in LWE ($P \leq 0.001$). There were no differences in G:F during RST ($P \geq 0.35$). Change in G:F was greater for HWE and MWE than LWE ($P \leq 0.001$).

During RSTP1, RSTP2, and RSTP3 WI was lower in HWE and MWE compared to LWE (Table 3.4; $P \leq 0.001$). Similarly, WI%BW was lowest in HWE, intermediate in MWE, and lowest in LWE during RSTP1, RSTP2, and RSTP3 ($P \leq 0.001$). There was no difference in DMI during RSTP1, RSTP2, and RSTP3 ($P \geq 0.13$). During RSTP1, RSTP2, and FI%BW during RSTP3 was lesser in HWE compared to MWE and LWE ($P \leq 0.001$). During RSTP1, ADG was greater in HWE and MWE compared to LWE ($P \leq 0.001$). During RSTP2, there was a trend for increased ADG in LWE compared to MWE and HWE ($P \leq 0.10$). There was no difference in ADG during RSTP3 ($P \geq 0.62$). There was a trend for G:F to be greater in HWE and MWE compared to LWE during RSTP1 ($P \leq 0.10$). There was no difference in G:F during RSTP2 and RSTP3 ($P \geq 0.17$).

Water intake was greater in the heavy block compared to the light block during BAS (38.93 compared to 35.97 kg/d; $P < 0.001$) and RST (19.13 compared to 17.65 kg/d; $P < 0.001$). During BAS, WI%BW was greater in the light block than the heavy block (9.27% compared to 8.92%; $P < 0.05$). Feed intake as a percent of BW was greater in the light block compared to the heavy block during BAS (3.82% compared to 3.71%; $P < 0.01$). Dry matter intake was greater in the heavy block compared to light during both BAS (11.77 compared to 10.77 kg/d $P < 0.001$) and RST (10.69 compared to 9.56 kg/d; $P < 0.001$). Average daily gain tended to be greater in the light block during BAS (1.59 compared to 1.54; $P < 0.10$). Gain:feed was greater in the light block compared to the

heavy block during BAS (0.147 compared to 0.129; $P < 0.001$) There was no block difference in ADG during RST ($P \geq 0.91$). There was no block difference in WI%BW, FI%BW, or G:F during RST ($P \geq 0.28$).

Rectal temperature and morbidity

Rectal temperatures were affected by both WIEFF (Figure 3.1; $P < 0.001$) and day (Figure 3.2; $P < 0.001$). Low WIEFF steers had a greater RT than HWE and MWE (Figure 3.1; $P \leq 0.001$). Time effects of RT were characterized by a linear (Figure 3.2; $P < 0.001$) and quadratic effect ($P < 0.001$). Rectal temperatures increased from d 0 to d 14, decreasing thereafter. Rectal temperature was lowest on d 140. Chute order affected temperature with a linear (Figure 3.3; $P < 0.001$) and quadratic decline ($P < 0.001$).

The percentage of animals in each category of treated for respiratory disease was lower in HWE compared to MWE and LWE (Table 3.5; $P < 0.01$). Percentage of animals in each category of pulled but not treated and total antimicrobial treatments were lower in HWE compared to LWE, but not different from MWE ($P \leq 0.02$). There was no difference in morbidity for animals treated for infectious pododermatitis or conjunctivitis among WIEFF groups ($P \geq 0.24$). The majority of all morbidity treatments (all categories) occurred during BAS.

Blood Parameters

Blood parameters were not affected by a WIEFF by day interaction ($P \geq 0.13$). Red blood cell counts and HGB increased from d 70 to d 84, where they remained elevated until returning to levels similar to d 70 on d 126 and 140 (Table 3.6; $P \leq 0.05$). Hematocrit increased from d 70 until d 112 ($P \leq 0.05$) and then decreased on d 126 and

140 ($P \leq 0.05$) to a level greater than d 70. Mean corpuscular volume steadily increased from d 70 to 140 ($P \leq 0.05$). Reticulocytes were not different from d 70 to 98 through d 140, but were lowest at d 84 ($P \leq 0.05$). Platelets and neutrophils were greater on d 70 and 84 than d 98, 112, 126, and 140 ($P \leq 0.01$). Lymphocytes were lowest on d 70, increased on d 84 and greatest on d 140. Lymphocytes were not different on days 98, 112, or 126 ($P \leq 0.01$). Monocytes were greater on d 70 than d 84 to 140 ($P \leq 0.01$). Neutrophil to Lymphocyte ratio was greatest on d 70, decreased on d 84, and was lowest from d 98 to 140 ($P \leq 0.01$). Presence of a left shift was lowest on d 70, decreasing until d 112, and not different from d 112 to 140 ($P \leq 0.01$). Mean corpuscular hemoglobin, WBC, and basophils were not different among days ($P \geq 0.89$).

Red blood cell counts were lower in MWE compared to LWE (Table 3.7; $P \leq 0.01$). Hemoglobin was lower for the MWE than both other groups ($P \leq 0.01$). Hematocrit and MCV were greatest in HWE compared to LWE and MWE ($P \leq 0.01$). Platelets were greater in LWE than MWE and HWE ($P \leq 0.05$). There were no differences in MCH, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, eosinophils, neutrophil:lymphocyte, or presence of a left shift between WIEFF groups ($P \geq 0.11$).

Vasopressin was not affected by day or WIEFF (Tables 3.6, 3.7; $P \geq 0.10$) overall. Aldosterone was greater on d 70 and 126 than d 140 (Table 3.6; $P \leq 0.001$). Aldosterone was not different between LWE and HWE steers ($P = 0.62$).

Day by WIEFF interactions were observed for serum Na, K, and Cl measures (Table 3.8; $P \leq 0.02$). On d 70, LWE steers had greater Na than MWE, but not different

than HWE steers ($P \leq 0.05$). Serum Na also increased until d 98, decreased on d 112 and 126, and increased again on d 140. Serum Cl had the same pattern of change by day. On d 98 K was greater in LWE than MWE and HWE steers ($P \leq 0.05$). Serum K also increased until d 112, and decreased thereafter.

DISCUSSION

This experiment is distinctive compared to other water restriction research in the literature for a few reasons. Much of the research with water restriction has been done using sheep or goat models (Li et al., 2000; Parker et al., 2003; Alamer, 2005; Kaliber et al., 2015). Most literature that examines the effects of water restriction in cattle utilizes a short period of restriction or uses dairy cows as a model (9 d; Burgos et al., 2001; 8 d; Benatallah et al., 2019). Additionally, feed efficiency is a commonly measured production trait, but water use efficiency has not been reported to the author's knowledge. As cattle become exposed to water restrictive conditions, the response and ability to adapt will be imperative for producers. This work provides information regarding the response of beef cattle to such condition, over a long period of time. Other research trials have investigated water restriction by housing animals individually (Casamassima et al., 2016; Mengistu et al., 2016; Kaliber et al., 2016), which can affect feed and water intake, behavior, and health. This trial was able to restrict water intake individually within a pen setting, enabling more natural behaviors to persist while still collecting data on individual animals. Lastly, the sample size of previous work has generally been small (10 animals or less per group or treatment) due primarily to the demands of creating an artificial water restrictive environment for individually housed animals (Burgos et al., 2001; Casamassima et al., 2008; Benatallah et al., 2019).

However, due to the considerable inter-animal variation in *ad libitum* water intake (CV = 29.9%; Ahlberg et al., 2018b), a larger sample size is necessary to evaluate water use efficiency and the physiological response of cattle to limited water conditions. This study had a large sample size (n = 467) with sampling periods spanning throughout the year and all seasons.

Since the goal of this experiment was to restrict cattle's water intake, it is important to ensure that the targeted restriction level was achieved. The average WI during RST as a percent of average WI during BAS was 47, 47, 49, and 57% for groups 1, 2, 3, and 4, respectively, making the average restriction for all groups 50%. Thus, the targeted restriction level was achieved. One shortcoming of using WI from BAS to determine water allowance during RST is the difference in time of year between BAS and RST. For example, BAS could be measured during thermoneutral conditions whereas RST occurs during heat stress. Thus, true average WI may be underestimated and restriction may be more severe than 50%. Alternatively, if BAS occurred during heat stress or hotter environmental conditions and RST occurred during thermoneutral or colder environmental conditions, steers may be restricted but not as severe as intended. It is worth mentioning that steers never consumed less than the allotted amount during RST, indicating all cattle were restricted. However, the relative severity of the restriction may have varied, again, based on timing of BAS collection and timing of RST.

Dry matter intake decreased by 1.14 kg/d from BAS to RST, which is consistent with previous reports (Burgos et al., 2001; Parker et al., 2003; Koknaroglu et al., 2008; Benatallah et al., 2019), where DMI decreased during water restriction. Since WI is highly correlated with DMI, decreased WI should result in a decrease in DMI. Since there

was not a significant difference in DMI change between WIEFF groups, it does not appear that one WIEFF category responded better or worse to water restrictive conditions. Although during BAS, MWE had a greater FI%BW than both LWE and HWE, while during RST the LWE and MWE had a greater FI%BW than HWE WIEFF. Although all groups were restricted based on their own BAS measures, the LWE had the smallest decrease in FI%BW, which could be related to physiological water demands. Since those steers required and drank more water, their FI%BW may be less affected than the other WIEFF groups since their overall WI is greater. The decreased FI%BW in the HWE steers could also be driving the decreased WI in those steers, since less feed intake could also require less WI. The increased WI and WI%BW in LWE were expected due to WIEFF assignments and how water restriction levels were calculated.

Although no differences were observed in initial BW, final BW during BAS and BW during RST were greater in HWE than LWE and MWE steers. Since there was not a difference in ADG during RST, this difference was probably driven by the difference observed during BAS. The change in ADG was greater in MWE and HWE steers than LWE, probably driven by greater ADG in those groups during BAS. It seems that performance was more severely impacted in the HWE steers, compared to the LWE steers that had a less severe decrease in performance. The difference in ADG and G:F during RST1 in HWE and MWE steers indicate that these steers adapted more quickly to water restriction. Alternatively, LWE had a greater ADG and G:F during the second 2 wk period, presenting signs of adaptation after some time. While all animals appear to adapt, the period required for adaptation may vary. These differences in adaptive performance may be important depending on the length of water restriction cattle may experience.

In this experiment, RT decreased from the BAS to RST (39.5 to 39.3°C). Casamasima et al. (2016) restricted water in sheep for 28 d at either 80 or 60% of average WI and did not observe a difference in RT. However, other authors have observed an increase in RT during spring and summer while animals were being water restricted (Alamer and Al-Hozab, 2004; Alamer, 2005; Ghanem et al., 2008; Benatallah et al., 2019). Results of this trial may differ from previous work because of the species used and effects of environmental conditions. Other restriction research has typically used sheep or goats, and often breeds that are adapted to hot or arid environments. The response of those animals may vary from cattle that have not been selected to withstand such conditions. Other studies investigating effects of water restriction on RT have measured RT during cool or hot periods and over shorter periods of time (d compared to wk). This trial collected RT over a 140 d period, 7 or 14 d apart, that spanned all times of year and environmental conditions. As shown in Table 3.1, there was also a large range of average minimum daily ambient temperatures between periods and groups. The increase in RT on d 14 seems to be driven by groups 1 and 3, where 84 and 60% of the steers had a RT ≥ 40.0°C, respectively. Additionally, although differences in this trial are significant, all total daily average RT were within the normal range for cattle, overall (Porter et al., 2011).

Rectal temperatures were also generally greater in the LWE than the MWE and HWE groups. This could be an indication of a greater metabolic rate in LWE. A greater metabolic rate would also be in agreement with the performance results as LWE steers had a decreased ADG and decreased final BW compared to MWE and HWE. Although statistically significant, RT was always within the normal biological range for cattle

(Porter et al., 2011). Since there were also no observed differences in morbidity between the groupings, differences in RT are likely not related to clinical health. Order of chute entry also affected RT, where RT decreased with increasing chute entry order. This could be the result of animal handling on collection days. Cattle were all brought to holding pens at the same time before handling on collection days. Steers that entered the chute earlier had a shorter interval between handlings (interval between being moved out of pens and restrained in the chute). Cattle brought up and taken to the chute later in the order had anywhere from 30 min to 3 h in the holding pens between handlings. During that time steers were observed to be lying or standing still, allowing the animals to relax and possibly allowing time for RT to decrease.

The incidence of respiratory illness in this experiment was exceptionally low at approximately 1% of all animals. These results are not necessarily surprising as all animals received metaphylaxis treatment during processing. Metaphylaxis, or treatment of an entire group of cattle with a U.S. FDA approved antimicrobial with the intent of controlling disease, is commonly used for high-risk cattle upon arrival to reduce respiratory illness (Samuel and Richeson, 2015). Although cattle used in this experiment were not classified as high-risk, metaphylaxis was performed to ensure a low morbidity rate, especially as cattle entered the Insentec barn. Because acclimating to the Insentec feeding system can cause stress, metaphylaxis was administered to decrease morbidity and other possible stressors during the acclimation period. In addition to the metaphylaxis treatment each animal received upon arrival, cattle had been weaned and completed a backgrounding program. The greater incidence of conjunctivitis compared to other treatment categories was driven by group 3, which succumbed to a severe breakout of

conjunctivitis, where roughly 75% of the herd was treated for conjunctivitis and 48% of those cattle that were treated were treated multiple times.

The LWE steers had greater incidences of respiratory illness, exhibiting clinical signs of illness, and total overall antimicrobial treatments. Steers in the low category may have been more susceptible to illness or may have displayed more clinical signs than other steers. As herd animals, cattle typically attempt to hide signs of illness. The LWE steers may have had difficulty hiding symptoms of illness. Moreover, it is not surprising that both BRD treatments and pulled but not treated were greater in the LWE, since these measures are correlated. The increased morbidity in the LWE category may have also affected performance to some extent, if cattle in that category were experiencing more subclinical illness. The steers that were displaying symptoms of illness but did not have a fever may have been experiencing subclinical illness. It is worth noting that the majority of all treatments, including conjunctivitis, occurred during the BAS period. There were also no serious illnesses, complications, or mortalities during water restriction. These results suggest that a 50% water restriction in healthy feedlot cattle throughout the year does not cause serious illness or mortality.

Over time and with increasing water restriction, serum Na and Cl exhibited slight increases. This was expected, as Na will typically increase during dehydration in order to increase water in extracellular fluid. Some authors have speculated that hypernatremia is due to increased Na retention in the rumen and the kidneys to increase water retention during water restriction (Jaber et al., 2004; Burgos et al., 2001). Numerous studies have reported an increase in Na and decrease in K with increasing water restriction levels (Parker et al., 2003; Casamassima et al., 2008; Kaliber et al., 2015). Casamassima et al.

(2016) restricted water to 60 and 80% of average WI in sheep and reported an increase in both plasma Na and Cl over time and with increasing water restriction. Alamer (2005) completely restricted water for 4 d and observed increased plasma Na in sheep. The increase in K over time and with increasing water restriction in this trial was not expected as K usually decreases with increasing water restriction. Ayoub and Saleh (1998) also reported increases in K in camels and goats, but during a shorter period of water restriction. Potassium results from this trial may differ from others (Parker et al., 2003; Kaliber et al., 2015) due to the length and extent of water restriction. The normal ranges reported for the specific machine used for cattle in this experiment were 135-148, 4-5.8, and 96-109 for Na, K, and Cl, respectively. Thus, all measures reported in this study remained within normal limits for cattle.

In this experiment, RBC, HGB, and HCT increased with increasing water restriction levels until d 112, consistent with other studies. Casamassima et al. (2016) reported increased RBC, HGB, and HCT with increasing severity and length of water restriction in sheep. Hamadeh et al. (2006) also reported increased RBC during water restriction. Both authors attributed the increase to decreased plasma volume, rather than an actual increase in RBC count. Similar results with HGB have been reported in other water restriction literature, utilizing sheep (Ghanem, 2005; Hamadeh et al., 2006). The increase in HCT indicates that animals did experience some level of dehydration during water resriction. These results are also consistent with previous reports using sheep (Alamer, 2005; Ghanem et al., 2008). While previous research has shown an increase in RBC and HGB until d 28, measures collected in this study increased until d 112 (42 d of restriction, including step down period of 28 d) when levels start to decrease to levels

collected during BAS. Thus, it seems that over time, cattle in this study were able to adapt to decreased water availability and possibly increase their plasma volume, leading to normal values of RBC, HGB, and HCT. The MWE steers had a lower RBC and HGB than the LWE and HWE steers, which could be indicative of decreased plasma volume in LWE and HWE. Additionally, the HWE steers had a greater HCT than the MWE, also indicating a lower plasma volume. However, these differences between WIEFF categories were minor. Nonetheless, all measures collected on all days were within the normal ranges for cattle (Porter et al., 2011). Igbokwe and Ajuzieogu (1991) observed a decrease in MCV during early water restriction and attributed the difference to smaller erythrocyte size. The drop in MCV during early restriction in this trial may have been related to erythrocyte size due to water loss. Although differences were observed in RET and PLT, the cause of these differences is unknown and may warrant further research, but both remained within normal diagnostic ranges.

Differences were not observed in WBC between WIEFF groups or between days. After a 72 h water deprivation in camels WBC were increased, but WBC decreased during water restriction in goats (Ayoub and Saleh, 1998). Igbokwe and Ajuzieogu (1991) observed a decrease in WBC, neutrophil, lymphocyte, and eosinophil counts during prolonged water restriction. It was hypothesized that the WBC counts in this trial would be indicative of a stress response due to the water restriction acting as a chronic stressor. In such a scenario, it would be expected that neutrophils would increase, lymphocytes would decrease, and the N:L ratio would increase (Davis et al., 2008). However, the opposite occurred in this experiment, where the neutrophils decreased, lymphocytes increased, and the N:L decreased. The cause of this opposite relationship is

unknown, but does indicate that the cattle may not have been as stressed as expected. It is also possible that cattle had significant time to adapt to such conditions and any signs of a stress response indicated by WBC changes were missed. Blood was only collected every 14 d, and following 7 d of water restriction. For example, after 7 d at 80% of normal intake, the steers could have adapted and physiological differences may have been missed. Overall, the CBC results suggest cattle are resilient and have the ability to physiologically adapt to water restrictive conditions in a feedlot setting.

There was no difference in vasopressin between WIEFF groups or days of the study, although there was a numeric trend for an increase with increasing water restriction and then a slight decrease after 6 wk of 50% water restriction. Mengistu et al. (2016) reported that vasopressin increased with increasing water restriction in water restricted sheep. These authors also observed a similar trend where vasopressin increased until 50% of normal intake, and started to decrease at 40% restriction (40% of normal intake). Similarly, Kaliber et al. (2016) reported increased vasopressin with increasing water restriction in goats. El-Nouty et al. (1980) reported an increase in vasopressin in cattle during dehydration, but cattle in that study were also exposed to heat stress. Vasopressin was expected to increase during water restriction, due to its known activity in water retention. Vasopressin results of this trial may be indicative of an adaptive response to the water restriction, as it appears that plasma volume returns to normal while vasopressin is decreasing. Aldosterone was not different between WIEFF groups, but did decrease with extended water restriction. El-Nouty et al. (1980) reported a decrease in aldosterone in cattle during dehydration and heat stress. Mengistu et al. (2016) did not report a difference in aldosterone between sheep at different levels of water restriction.

Although a difference was detected during water restriction, the change in aldosterone did not match differences in serum Na as expected due to the Na retentive action of aldosterone (El-Nouty et al., 1980). The decrease in aldosterone may also indicate the ability of a steer to adapt to water restrictive conditions, as steers appear to be more able to adapt to such severe conditions after 6 wk of water restriction.

Results of this trial provide further information regarding the physiological response and possible adaptation of beef cattle to extended water restriction. These results suggest that beef cattle may be able to adapt to 50% water restriction over a 6-week period. Results also suggest that there may be some individual differences in adaptability such that some cattle may be better or more quickly able to adapt to water restriction. Because of the more rapid adaptation to these conditions, MWE or HWE steers may be better suited to areas with risk of drought conditions or areas vulnerable to water scarcity because of the ability to quickly adapt.

Table 3.1. Average ambient temperature, relative humidity, and temperature humidity index[1] by group and period[2]

	Group 1		Group 2		Group 3		Group 4	
	BAS	RES	BAS	RES	BAS	RES	BAS	RES
Start date	6/3/2016	9/9/2016	1/10/2017	4/18/2017	9/7/2017	12/14/2017	2/27/2018	6/5/2018
End date	8/11/2016	10/20/2016	3/20/2017	5/29/2017	11/15/2017	1/24/2018	5/7/2018	7/16/2018
Ambient temperature, ˚C								
Mean	28.1	21.6	9.7	18.2	17.5	1.8	12.8	27.2
SD	2.52	4.68	6.27	4.55	6.59	6.79	6.06	4.31
Minimum	21.6	10.9	-1.4	8.5	4.2	-11.7	1.1	20.6
Maximum	31.9	30.2	25.3	26.5	29.3	15.5	24.5	30.3
Relative humidity, %								
Mean	68.6	70.8	63.0	72.3	69.7	63.3	61.3	71.0
SD	8.41	9.69	16.25	10.24	11.20	14.15	13.94	6.74
Minimum	52.3	52.0	23.5	55.9	46.9	36.0	34.2	55.1
Maximum	89.5	90.1	99.9	98.0	96.5	92.7	91.8	89.9
Temperature humidity index								
Mean	78.1	68.7	51.0	63.5	62.6	40.3	55.5	77.1
SD	3.11	7.03	9.20	6.92	9.74	9.18	8.48	3.10
Minimum	68.3	52.0	31.0	48.2	44.5	22.0	40.1	68.5
Maximum	82.3	80.1	71.9	76.5	79.6	59.5	73.1	80.9

[1]Daily mean, standard deviation, minimum, and maximum values for each response.

[2]Access to water was *ad libitum* during d 0-70 (BAS) and 50% of normal on d 98-140 (RST).

Table 3.2. Least square means of body weight at the beginning and end of BAS[1] and RST[1] by WIEFF[2] grouping

	LWE	MWE	HWE	SEM	*P*-value
n	152	183	132	-	-
BAS WIEFF	0.132[c]	0.183[b]	0.222[a]	0.051	0.04
RST WIEFF	0.147	0.150	0.182	0.166	0.16
Initial BAS BW, kg	356	347	356	50.4	0.16
Final BAS BW, kg	447[a]	462[b]	482[c]	47.9	< 0.01
Initial RST BW, kg	479[a]	490[a]	509[b]	49.9	< 0.01
Final RST BW, kg	504[a]	513[a]	534[b]	53.5	< 0.01

[1]Access to water was *ad libitum* during d 0-70 (BAS) and 50% of normal on d 98-140 (RST).

[2]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

[abc]Values with different letters differ at $P < 0.001$.

Table 3.3. Least square means of performance[1] and efficiency within each water allowance period[2] by WIEFF[3] grouping

	LWE	MWE	HWE	SEM	*P*-value
WI, kg/d					
BAS	41.3[b]	36.5[a]	34.6[a]	0.82	< 0.01
RST	20.1[b]	17.9[a]	17.2[a]	0.37	< 0.01
Change	-21.2[b]	-18.5[a]	-17.5[a]	0.49	< 0.01
WI%BW, %					
BAS	10.24[c]	8.90[b]	8.16[a]	0.152	< 0.01
RST	4.04[c]	3.53[b]	3.26[a]	0.06	< 0.01
Change	-6.20[c]	-5.36[b]	-4.90[a]	0.112	< 0.01
DMI, kg/d					
BAS	10.82[a]	11.40[b]	11.61[b]	0.116	< 0.01
RST	9.86	10.17	10.37	0.213	0.22
Change	-0.96	-1.23	-1.24	0.193	0.44
FI%BW, %					
BAS	3.73	3.82	3.74	0.039	0.10
RST	2.36[b]	2.32[b]	2.21[a]	0.027	< 0.01
Change	-1.37[a]	-1.50[b]	-1.53[b]	0.039	< 0.01
ADG, kg/d					
BAS	1.29[a]	1.60[b]	1.80[c]	0.024	< 0.01
RST	0.63	0.56	0.61	0.051	0.54
Change	-0.66[a]	-1.04[b]	-1.19[b]	0.063	< 0.01
G:F					
BAS	0.119[a]	0.141[b]	0.156[c]	0.0016	< 0.01
RST	0.066	0.056	0.06	0.0055	0.35
Change	-0.053[a]	-0.085[b]	-0.096[b]	0.0061	< 0.01

[1]Water intake, WI; water intake as a percent of BW, WI%BW; dry matter intake, DMI; DMI as a percent of BW, FI%BW; Average daily gain, ADG; gain to feed efficiency, G:F.

[2]Access to water was *ad libitum* during d 0-70 (BAS) and 50% of normal on d 98-140 (RST).

[3]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

[abc]Values with different letters differ at $P < 0.01$.

Table 3.4. Least square means of performance[1] and efficiency within each restriction period[2] of the trial by WIEFF grouping[3]

	LWE	MWE	HWE	SEM	*P*-value
WI, kg/d					
RSTP1	20.5^{b}	18.1^{a}	17.4^{a}	0.39	< 0.01
RSTP2	19.9^{b}	17.8^{a}	17.1^{a}	0.39	< 0.01
RSTP3	19.9^{b}	17.9^{a}	17.0^{a}	0.36	< 0.01
WI%BW, %					
RSTP1	4.21^{c}	3.64^{b}	3.40^{a}	0.070	< 0.01
RSTP2	3.99^{c}	3.50^{b}	3.21^{a}	0.063	< 0.01
RSTP3	3.92^{c}	3.46^{b}	3.17^{a}	0.053	< 0.01
DMI, kg/d					
RSTP1	8.59	8.96	8.78	0.147	0.14
RSTP2	8.41	8.58	8.48	0.126	0.53
RSTP3	8.34	8.42	8.41	0.124	0.87
FI%BW, %					
RSTP1	2.49^{b}	2.48^{b}	2.36^{a}	0.033	< 0.01
RSTP2	2.38^{b}	2.35^{b}	2.21^{a}	0.030	< 0.01
RSTP3	2.26^{b}	2.18^{b}	2.09^{a}	0.032	< 0.01
ADG, kg/d					
RSTP1	0.67^{a}	0.95^{b}	0.95^{b}	0.093	0.03
RSTP2	1.15^{x}	0.82^{y}	0.83^{y}	0.122	0.06
RSTP3	-0.10	-0.11	0.00	0.092	0.63
G:F					
RSTP1	0.070^{y}	0.098^{x}	0.104^{x}	0.0112	0.06
RSTP2	0.143	0.11	0.108	0.0157	0.18
RSTP3	-0.026	-0.024	-0.005	0.0117	0.36

[1]Water intake, WI; water intake as a percent of BW, WI%BW; dry matter intake, DMI; DMI as a percent of BW, FI%BW; Average daily gain, ADG; gain to feed efficiency, G:F.

[2]3 two-week periods during restriction: d 98 to 112 (RSTP1), d 112 to 126 (RSTP2), and d 126 to 140 (RSTP3).

[3]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

[a,b,c]Values with different letters differ at $P < 0.05$; [x,y]Values with different letters differ at $P < 0.10$.

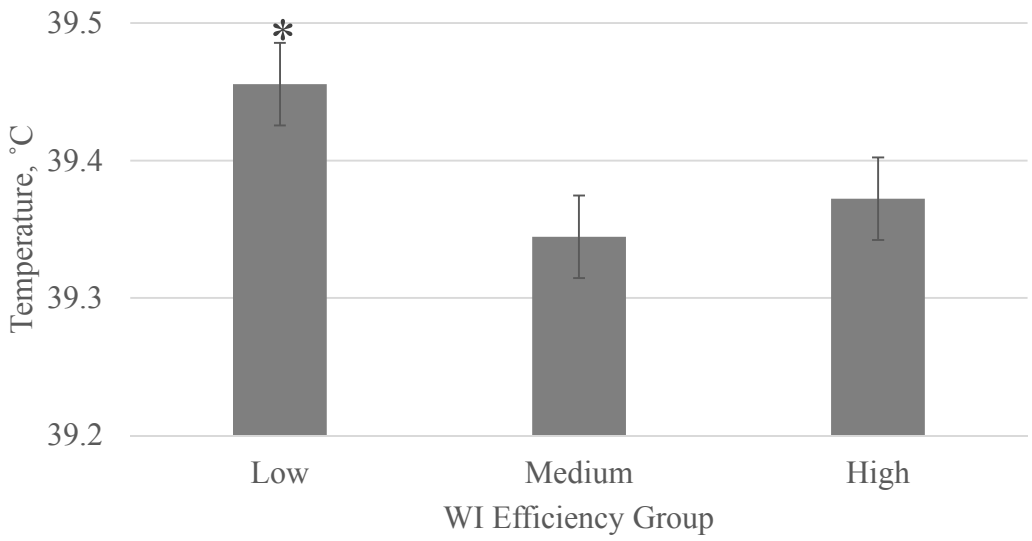

Figure 3.1. LS means ± SEM for rectal temperature (˚C) between water intake efficiency groups. Water intake efficiency (WIEFF) was measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF. Temperatures were higher in LWE than MWE and HWE steers, indicated by * ($P < 0.0001$).

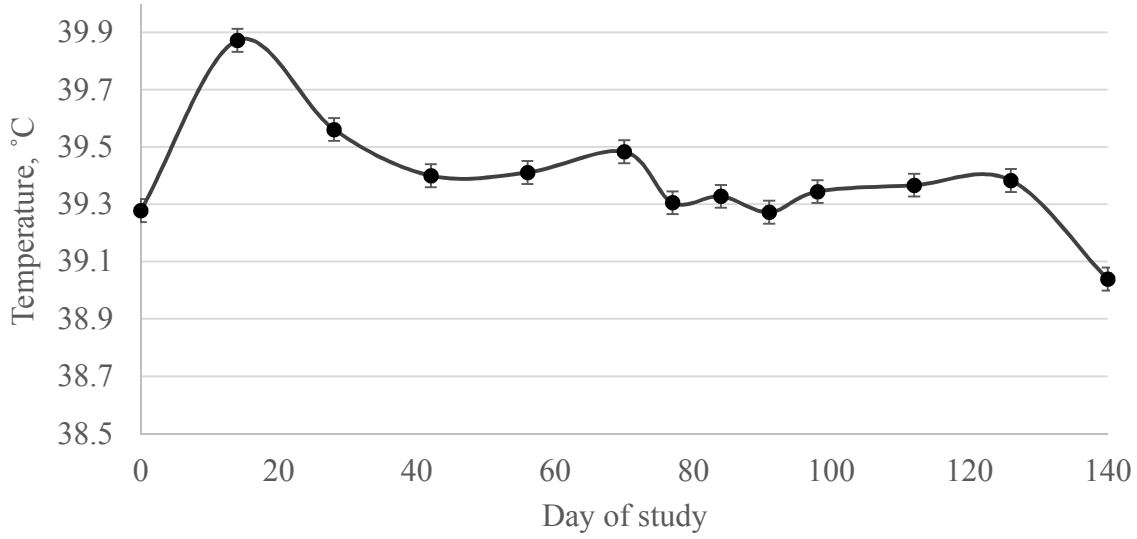

Figure 3.2. LS means ± SEM of rectal temperature (˚C) throughout the study. There was a significant effect of day on rectal temperatures ($P < 0.0001$). Day effects were characterized by linear ($P < 0.0001$) and quadratic effects ($P < 0.0001$).

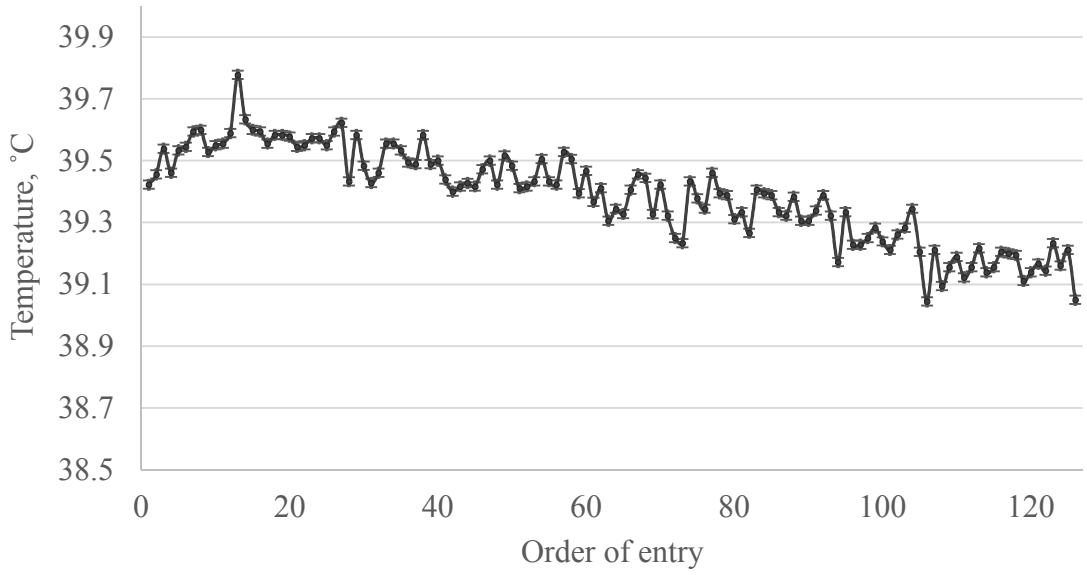

Figure 3.3. LS means ± SEM of rectal temperature (˚C) by order of entry of steers into the squeeze chute. Order of entry effects were characterized by linear ($P < 0.0001$) and quadratic effects ($P < 0.0001$).

Table 3.5. Least square means of animals[1] within each morbidity category[2] by WIEFF grouping[3]

	LWE	MWE	HWE	SEM	P-value
Treated for respiratory disease, %	3.08[a]	0.00[b]	0.00[b]	0.954	0.02
Treated for infectious pododermatitis, %	5.86	4.62	1.63	2.367	0.26
Treated for conjunctivitis, %	20.78	17.81	14.88	17.350	0.24
Pulled, but not treated, %	5.39[a]	1.30[ab]	0.00[b]	1.406	0.01
Total, %	35.16[a]	24.11[ab]	16.64[b]	19.460	< 0.01

[1]Presented means represent average percent of animals treated out of the total number of animals within that group.

[2]Treated once for respiratory disease; treated at least once for infectious pododermatitis (foot rot); displayed symptoms of respiratory illness and were pulled from the pen, but did not have a fever and were not treated; treated at least once for conjunctivitis (pinkeye); total antimicrobial treatments.

[3]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

Table 3.6. Least square means of CBC blood parameters[1] by day of study[2]

	70	84	98	112[1]	126[1]	140[1]	SEM	P-value
RBC, M/ uL	8.15[a]	8.35[b]	8.35[b]	8.35[b]	8.09[a]	8.08[a]	0.049	< 0.001
HGB, g/dL	13.0[a]	13.3[b]	13.3[b]	13.4[b]	13.0[a]	13.1[a]	0.06	< 0.001
HCT, %	36.2[a]	37.4[bc]	37.9[bc]	38.2[c]	37.1[b]	37.5[bc]	0.18	< 0.001
MCV, fL	44.8[a]	45.1[a]	45.7[b]	45.9[b]	46.1[bc]	46.8[c]	0.17	< 0.001
MCH, pg	16.2	16.1	16.1	16.2	16.2	16.4	0.08	0.14
RET, x 10^3/uL	3.89[c]	2.50[a]	3.34[b]	3.55[bc]	3.51[bc]	3.46[bc]	0.119	< 0.001
PLT, x 10^3/uL	273[b]	277[b]	246[a]	232[a]	225[a]	218[a]	6.0	< 0.001
WBC, x 10^3/uL	11.9	12.2	12.1	12.0	11.8	12.3	0.12	0.11
NEUT, x 10^3/uL	3.26[b]	2.99[b]	2.65[a]	2.57[a]	2.35[a]	2.59[a]	0.072	< 0.001
LYMPH, x 10^3/uL	6.82[a]	7.45[b]	7.78[bc]	7.69[bc]	7.72[bc]	7.88[c]	0.098	< 0.001
MONO, x 10^3/uL	1.66[b]	1.50[a]	1.44[a]	1.48[a]	1.48[a]	1.46[a]	0.032	< 0.001
EO, x 10^3/uL	0.22	0.23	0.25	0.28	0.26	0.32	0.005	< 0.001
BASO, x 10^3/uL	0.002	0.002	0.002	0.002	0.002	0.003	0.0003	0.35
Neutrophil:Lymphocyte	0.528[c]	0.458[b]	0.391[a]	0.374[a]	0.343[a]	0.366[a]	0.0119	< 0.001
Presence of Left shift	0.260[a]	0.343[ab]	0.379[b]	0.422[b]	0.366[b]	0.393[b]	0.0223	< 0.001
Vasopressin[3], pg/ml	15.34	-	20.23	-	21.48	19.42	9.63	0.10
Aldosterone[3], pg/ml	110.6[b]	-	-	-	117.5[b]	50.6[a]	8.08	< 0.001

[1]Red blood cells, RBC; hemoglobin, HGB; hematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; reticulocytes, RET; platelets, PLT; white blood cells, WBC; neutrophils, NEUT; lymphocytes, LYMPH; monocytes, MONO; eosinophils, EO; basophils, BASO.

[2]Access to water was *ad libitum* on d 70 (BAS), 80% of normal on d 84, 60% of normal on d 98, 50% of normal on d 112, 126, and 140.

[3]missing values represents days when measures were not evaluated.

[a,b,c]Values with different letters differ at $P < 0.05$.

Table 3.7. Least square means of complete blood count parameters[1] by WIEFF grouping[2]

	LWE	MWE	HWE	SEM	P-value
RBC, M/ uL	8.28[b]	8.15[a]	8.26[ab]	0.038	0.01
HGB, g/dL	13.3[b]	13.1[a]	13.3[b]	0.04	< 0.001
HCT, %	37.3[a]	36.9[a]	37.9[b]	0.14	< 0.001
MCV, fL	45.4[a]	45.7[a]	46.2[b]	0.14	< 0.001
MCH, pg	16.2	16.2	16.2	0.07	0.89
RET, x 10^3/uL	3.45	3.23	3.45	0.088	0.08
PLT, x 10^3/uL	263[b]	242[a]	230[a]	4.6	< 0.001
WBC, x 10^3/uL	12.13	12.05	11.99	0.091	0.60
NEUT, x 10^3/uL	2.70	2.72	2.78	0.056	0.55
LYMPH, x 10^3/uL	7.67	7.54	7.47	0.077	0.14
MONO, x 10^3/uL	1.51	1.53	1.47	0.024	0.22
EO, x 10^3/uL	0.25	0.26	0.27	0.010	0.11
BASO, x 10^3/uL	0.002	0.002	0.002	0.0002	0.64
Neutrophil:Lymphocyte	0.405	0.404	0.420	0.0092	0.35
Presence of Left shift	0.372	0.348	0.363	0.0170	0.34
Vasopressin, pg/ml	18.5	-	19.7	9.47	0.30
Aldosterone, pg/ml	97.2	-	88.6	6.50	0.36

[1]Red blood cells, RBC; hemoglobin, HGB; hematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; reticulocytes, RET; platelets, PLT; white blood cells, WBC; neutrophils, NEUT; lymphocytes, LYMPH; monocytes, MONO; eosinophils, EO; basophils, BASO.

[2]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

[a,b,c]Values with different letters differ at $P < 0.05$.

Table 3.8. Average electrolyte concentrations within each WIEFF grouping[1] by day of study[2]

	LWE	MWE	HWE	SEM	Day	WIEFF	Day*WIEFF
Na, mmol/L					< 0.01	0.06	0.02
d 70	147[b]	145[a]	146[ab]	0.3			
d 84	146	145	145	0.3			
d 98	148	148	149	0.4			
d 112	147	146	147	0.4			
d 126	145	146	146	0.3			
d 140	148	148	149	0.3			
K, mmol/L					< 0.01	0.05	< 0.01
d 70	4.67	4.68	4.68	0.030			
d 84	4.65	4.68	4.67	0.030			
d 98	4.84[b]	4.70[a]	4.70[ab]	0.032			
d 112	4.73	4.63	4.71	0.030			
d 126	4.74	4.75	4.83	0.031			
d 140	4.75	4.74	4.81	0.030			
Cl, mmol/L					< 0.01	0.12	0.02
d 70	105	105	105	0.3			
d 84	106	106	106	0.3			
d 98	107	107	107	0.3			
d 112	105	105	106	0.3			
d 126	106	106	106	0.3			
d 140	108	108	109	0.3			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. The 3 categories were high (HWE), medium (MWE), and low (LWE) WIEFF.

[2]Access to water was *ad libitum* on d 70, 80% of normal on d 84, 60% of normal on d 98, and 50% of normal on d 112, 126, and 140.

[a,b,c]Values with different letters differ at $P < 0.05$.

CHAPTER IV

EFFECTS OF PROLONGED WATER RESTRICTION ON THE BEHAVIOR OF FEEDLOT STEERS

INTRODUCTION

Continuing climate change is expected to impact water availability globally, which can have devastating impacts on animal agriculture through decreased animal performance and profitability (Rosenzweig et al., 2001; Beach et al., 2010). Water availability is expected to decrease in areas with a high prevalence of cow/calf operations and cattle feedlots in the U.S., such as the Midwest and Southern Great Plains (EPA, 2014). Such conditions can cause significant stress to cattle and understanding their behavioral responses will be imperative for effective management as well as for selection of easier adapting cattle. Changes in water availability may lead to changes in behavior as the animal attempts to adapt to water restrictive conditions (Adams et al., 1998).

Lack of water availability can be a significant stressor for livestock and can lead to behavioral or physiological modifications as the animal attempts to cope (Kaliber et al., 2016; Benatallah et al., 2019). Since behavioral changes are the first level of

adaptation to an environmental stressor, the behavioral changes of cattle during water restrictive conditions are important to understand. However, most water restriction literature does not include behavioral measures for 2 possible reasons. First, measuring feeding, drinking, and animal behavior can be labor and time intensive, which can limit the amount of data that can be collected. However, new animal behavior monitoring technologies allow for more and efficient behavioral data collection. Second, pen behavior and social interactions may be a limitation in some designs since animals are often penned individually and have little to no contact with other animals (Casamassima et al., 2016; Mengistu et al., 2016).

Using new feeding technologies, water restrictive conditions can be implemented in a pen setting, allowing for social interactions among animals. Another benefit of newer feeding systems is the detailed record of feeding behavior. Newer technologies may offer information regarding competition at the bunk without requiring as much time and labor that was previously required by manual observation. Huzzey et al. (2014) reported that consecutive visits 26 s apart could be used to measure competition in dairy cows, where there were 2 cows per feed bunk. Due to the increased number of animals in pens and per feed bunk, it was believed in this experiment that bunk visits of 10 s or less could be measured to indicate competition at the bunk.

Additionally, it is worthwhile to know whether or not some animals can adapt to water restrictive conditions. Animals that can effectively adapt may be better suited for areas vulnerable to water scarcity, whereas animals that do not adapt as well may be better suited for more water secure areas. Since feed efficiency is commonly used as a

measure of animal performance, these trials aim to utilize water efficiency as a measure of production during water restrictive conditions and assess differences in adaptability.

The objective of this study was to examine behavioral changes in feedlot steers with differing water efficiency levels undergoing long-term water restriction conditions.

MATERIALS AND METHODS

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (ACUP #AG13-18).

Animals and treatments

Crossbred beef steers (n = 467) were used in a replicated completely randomized block design from October 2016- July 2018. Cattle were fed in 4 feeding groups: group 1 (n = 105; initial BW = 409 ± 27.6 kg), group 2 (n = 123 initial BW = 341 ± 32.7 kg), group 3 (n = 122; initial BW = 319 ± 32.7 kg), and group 4 (n = 117; initial BW = 347 ± 33.6 kg). Steers in group 1 were obtained from a private ranch. Group 2 steers were obtained from the Oklahoma State University Field and Research Service Units Angus herd. Steers in groups 3 and 4 were obtained from an order buyer through multiple livestock markets (n= 31 and 26, respectively) and the OSU herd (n = 91 and 91, respectively). Steers were housed at the Willard Sparks Beef Research Center in Stillwater, OK. A detailed breed analysis is described in Ahlberg (2018a).

Within 24 h of arrival, cattle were weighed and ear tagged for individual identification and pen assignments. Steers were randomly allocated into pens in 2 weight blocks by initial BW (light and heavy). Routine processing on the day of pen allocation

(d -21) included viral and clostridial vaccinations (Titanium 5 PHM, Elanco, Greenfield, IN; Vision 7, Merck Animal Health, Madison, NJ), an injection of doramectin (Dectomax, Zoetis, Florham Park, NJ), a fenbendazole drench (Safe-Guard; Merck Animal Health), and an antimicrobial metaphylaxis treatment of ceftiofur crystalline free acid (Excede; Zoetis) at a dose of 3.3 mL/100 kg. Group 1 was vaccinated with Covexin 8 (Merck Animal Health), rather than Vision 7 due to requirement of cattle owner. On d 0, steers were implanted with Compudose (Elanco), an estradiol implant.

Steers were housed in 1 of four 31.9 × 11.3 m partly covered pens within a 3-sided part soil surfaced barn (6.5 x 11.3 m concrete). Each pen had 11.3 × 9.1 m of shade. There were 2 pens per weight block. Each pen was equipped with 6 Insentec Roughage Intake Control (**RIC**) feed bunks and 1 Insentec water bunk. The system was programmed to record the starting weight and time of each individual animal bunk visit as well as the end weight and time of each visit. Feed and water intake (**WI**) were calculated by subtracting the end bunk weight from the start weight of each bunk visit. Total time (s) spent at the bunk was also recorded for each visit. Additional specifications and information about the RIC system were reported in Allwardt et al. (2017).

Following allocation, steers were allowed 21 d to acclimate to pen conditions and the Insentec RIC system. Any animals that did not have consistent feed and water bunk visits (not visiting each bunk daily), low feed or WI (daily feed intake < 4kg, WI < 10kg, or a combination), or exhibited signs of anorexia (gaunt appearance) were removed from the experiment.

Baseline feed and water intakes were measured for 70 d (**BAS**) following the 21 d acclimation starting on d 0. During BAS cattle had *ad libitum* access to feed and water. An average daily water intake (**ADWI**) was calculated for each individual animal based on intakes during BAS. The ADWI was used to calculate restriction water quantities for each individual animal. Restriction water quantities were calculated by multiplying the percent restriction by the ADWI for each animal. Following BAS, water allowance was decreased 10% every 7 d over 28 d until 50% water restriction was obtained. Thus, cattle had 7 d at 90% of ADWI starting on d 71, 7 d at 80% of ADWI starting on d 78, 7 d at 70% of ADWI starting on d 85, and 7 d at 60% of ADWI starting on d 92. Once at 50% of ADWI (starting on d 99), cattle were maintained at this restriction level for 42 d (**RST**). The Insentec system reset every night between 2345 h and 2400 h. Thus, a day was considered between 0000 h and 2345 h and steers had that amount of time to drink their daily water allowance. During the step down period and RST, feed was available *ad libitum*. Following RST, steers were stepped back up to *ad libitum* water allowance over 6 d.

Cattle were categorized into 3 water efficiency categories: High (**HWE**), Medium (**MWE**), and Low (**LWE**) by water use efficiency. Water use efficiency (**WIEFF**) was measured as average daily gain (**ADG**) divided by average water intake as a percent of body weight (ADG/WI%BW) during BAS. Cattle were grouped into the 3 categories using k-means clustering where $k = 3$. This clustering partitions observations into clusters based on the observation's distance from the mean. This method created unequal categories ($n = 152$ in LWE, $n = 183$ in MWE, $n = 132$ in HWE). Cattle were categorized

this way to evaluate differences between animals with differing abilities to efficiently utilize water.

Diets

All steers were fed the same growing ration throughout the experiment. The ration consisted of 17% cracked corn, 45% Sweet Bran™ (Cargill, Blair, NE), 32% grass hay, and 6% supplement. The diet had an average DM of 75% across all experiments. Feed was delivered 3 times a d at approximately 0700 h, 1030 h, and 1430 h. Feed was targeted to be offered *ad libitum*. However, since feed calling was more challenging in individual feed bunks, there were some days that feed bunks were empty before the first feeding. For this reason, feed delivery amount was adjusted daily. Gates on the bunks were locked in the "closed" position during feeding to prevent animals from accessing feed during feed delivery. Pens were always fed in the same order, feeding pen 1 first and pen 4 last. Bunks were cleaned daily between 0600 h and 0700 h. Diets were prepared and adjusted daily to ensure *ad libitum* intake with minimal feed remaining prior to the next feeding. Ingredient and diet DM were measured once weekly by drying samples for 48 h in a forced air oven (55˚C, Model 1327F, VWR Scientific Products, Cornelius, OR, USA).

Behavioral measures

Steers were individually identified using a combination of colored tape and 3 marker locations on each steer's body to differentiate individuals on video recordings for behavioral measures. Marking tape (Brady Corporation, Milwaukee, WI) was either red, yellow, or blue in color and attached (Livestock tag cement, Akron, OH) to 1 or more on

the shoulder, rib, and rump of each steer. Tape was initially applied on d 56 to both sides of the steer. Tape was reapplied to each side on each following weigh day on d 70, 77, 84, 91, 98, 112, 126, and 140 as necessary.

Cattle were monitored by 8 fixed network video cameras (Axis Communications, Chelmsford, MA), with 1 monitoring the inside (shaded) and 1 monitoring the outside (unshaded) of each pen. Video recordings were used for behavioral observations. Video footage was recorded using MediaRecorder (Noldus, Wageningen, The Netherlands), compressed and stored using four 24 tb WD MyCloud external hard drives (Western Digital, San Jose, CA), and was viewed using VLC Media Player (VideoLan, Paris, France). Daily video files were stored in three 6-hour videos per camera (i.e. twenty-four 6-hour video files were stored per d) from 0400-0959 h, 1000-1559 h, and 1600-2159 h. Cameras were turned on at d 56 and remained on and recording until the end of the experiment for each group.

Cameras were programmed to begin recording at 0400 h each morning and to stop at 2200 h each night. Because feeding and drinking patterns could be impacted by water restriction, the entire 18-hour period was chosen for behavioral observation of each group. Due to the labor intensive nature of logging these observations, 6 d were chosen for behavioral observation for each group: 2 consecutive d during BAS (**BBM**; d 58 and 59), 2 consecutive d in early RES (**RBM1**; d 114 and 115), and 2 consecutive d in late RES (**RBM2**; d 128 and 129). This totaled to 24 d of observation (6 d for 4 groups). For consistency, 1 individual conducted all behavioral analysis utilizing a continuous behavioral sampling method.

Animals were observed for agonistic interactions and were scored based on dyad interactions. Specific behaviors observed are listed in Table 4.1. Each time an animal engaged in a social interaction, the animal ID of the steer initiating the interaction was recorded. The initiator animal was defined as being the steer that performed the butting or displacement, initiated the fighting, initiated the mounting, or initiated a threatening behavior and caused a change in posture of the steer receiving said behavior. The location of behavior was also recorded. Possible locations were the feed bunk, the water bunk, or other area in the pen (Table 4.2).

Locomotor measures were measured on a subset of steers in each pen. Five animals in each pen (20 animals/group) were fitted with an IceTag pedometer (IceRobotics, Edinburgh, UK) on d 56. IceTags were placed on the right hind leg just above the fetlock joint. Steers were selected so that the average BW of the 5 steers in the pen would match the average BW of the entire pen. Locomotor behavior was measured from d 56-140 and recorded every 15 min. Data were downloaded from the IceTags every 2 wk. Steers were checked daily to ensure pedometers were attached and replaced if lost. IceTags recorded time spent standing, time spent lying, lying bouts, number of steps, and a motion index. Step counts were measured every time the steer lifted its leg, based on the amount of force used. Motion index indicates overall activity of the animals, calculated on all 3 planes of the pedometers.

Medication protocol and morbidity measures

Steers were examined for signs of sickness and dehydration daily and treated if required. Only 1 animal (group 4) exhibited signs of dehydration that warranted

intervention. In order for an animal to qualify for antimicrobial treatment, the animal must have displayed clinical signs (e.g. lethargy, emaciation, coughing, lameness, etc.) and had a rectal temperature exceeding 40°C. Following metaphylaxis treatment, the treatment regimen for observed respiratory symptoms consisted of a single subcutaneous administration of florfenicol (Nuflor; Merck Animal Health) at a dose of 13.2 mL/100 kg for respiratory symptoms. If symptoms persisted after 5 d, a second treatment of florfenciol was administered. The treatment regimen for conjunctivitis (pinkeye; indicated by tearing or photophobia) consisted of 2 to 3 subcutaneous doses of oxytetracycline (Biomycin; Boehringer Ingelheim, Ingelheim am Rhein, Germany) at a dose of 9.9 mL/100 kg on consecutive d. If symptoms persisted, tylosin (Tylan; Elanco) was administered at a dose of 8.8 mL/100 kg. The treatment regimen for infectious pododermatitis (foot rot; indicated by lameness, swelling, presence of cracked hoof, presence of rot) consisted of 1 subcutaneous dose of oxytetracycline (Boehringer Ingelheim) at a dose of 9.9 mL/100 kg. If symptoms persisted after 3 d, steers were treated with a single dose of tulathromycin (Draxxin; Zoetis) at a dose of 13.2 mL/100 kg. All animals were rechecked daily. Overall morbidity was low. Two steers wearing pedometers (1 in group 3 and 1 in group 4) were removed from trial. The steer in group 3 was removed due to a non-related joint infection in the proximal interphalangeal joint and pedometer data were excluded. The steer in group 4 was excluded due to signs of dehydration (presence of bladder stones) and pedometer data were also excluded. There were no mortalities during the experimental period, except 1 steer in group 4 that died due to complications associated with thymic lymphoma.

Statistical analysis

Analysis was broken into 5 periods of each d. The 5 periods were: 0000-0359 h (**PER1**), 0400-0959 h (**PER2**), 1000-1559 h (**PER3**), 1600-2159 h (**PER4**), and 2200-2359 h (**PER5**). These periods were chosen to evaluate how behavior changed depending on the time of d and water allowance throughout the trial. One feed delivery occurred during PER2 (0730 h) and 2 feed deliveries occurred during PER3 (1030 and 1430 h). Only PER2, PER3, and PER4 were examined for behavioral analysis using video files because the cameras were not recording during PER1 and PER5. Feeding, drinking, and locomotor behavior were evaluated during all periods.

Using the data provided by the Insentec RIC system, detailed feeding behavior can be measured. Time at the feed and water bunks as well as number of visits to each were determined. Recorded visits were also broken into visits of 0 seconds, visits less than 10 seconds, and total daily visits. The RIC system also records the specific time of each visit, so bunk visits were analyzed by the 5 periods.

The experiment utilized a complete randomized block design and the experimental unit was animal. Response variables within each period were analyzed separately. Feeding and drinking behavior were analyzed using the GLIMMIX procedure of SAS where the model included effects of WIEFF, water allowance (**WA**; BAS or RST), the interaction, and block. Group was included as a random effect. Frequency of agonistic interactions was analyzed using the GLIMMIX procedure of SAS (SAS 9.4, Carey, NC) where the model included behavior, WIEFF, WA (BBM, RBM1, and RBM2), and the 2-way interactions. Location of behaviors was analyzed using the GLIMMIX procedure of SAS where the model included WA (BBM, RBM1, and RBM2), WIEFF, and the interaction. Locomotor behavior was analyzed using the GLIMMIX

procedure of SAS where the model included effects of WIEFF, WA (BAS or RST), the interaction, and block. Group was included as a random effect.

Significance was declared when $P \leq 0.05$ and a trend was defined when $0.05 < P \leq 0.10$.

RESULTS

There were no WA, WIEFF, WA by WIEFF interactions, or block differences for visits of 0 seconds to the feed or water bunk during any period (Table 4.3 and 4.4; $P \geq 0.12$). Visits less than 10 s at the feed bunk were all affected by a WA by WIEFF interaction (Table 4.5; $P \leq 0.05$). Daily visits less than 10 s to the feed bunk decreased in all WIEFF from BAS to RST and were lowest in HWE steers ($P \leq 0.05$). Visits less than 10 s to the feed bunk increased from BAS to RST in all WIEFF steers during PER1, but decreased for all during PER3, PER4, and PER5 ($P < 0.05$). During PER2, visits to the feed bunk less than 10 s did not change from BAS to RST for HWE and MWE steers ($P \geq 0.05$), but decreased for LWE steers ($P \leq 0.05$). During BAS there was not a difference in visits less than 10 s to the feed bunk between WIEFF during PER1 ($P \geq 0.05$). During PER2 and PER3 of BAS, visits less than 10 s to the feed bunk were greater in LWE, followed by MWE, and HWE ($P \leq 0.05$). During PER4 and PER5 of BAS, visits less than 10 s to the feed bunk were greatest for LWE compared to MWE and HWE ($P < 0.05$). During PER1 and PER2 of RST visits less than 10 s were greater in LWE and MWE than HWE ($P < 0.05$). During PER3, PER4, and PER5 of RST, visits less than 10 s to the feed bunk were not different between WIEFF ($P \geq 0.05$). During PER1 visits less

than 10 s were greater for the heavy block, but during PER2, PER3, and PER4 visits were greater in the light block ($P < 0.05$). There was not a block effect during PER5 ($P = 0.41$)

Daily visits to the feed bunk were affected by a WA by WIEFF interaction in all periods (Table 4.6; $P \leq 0.05$) except PER4 ($P = 0.28$). Total daily visits to the feed bunk decreased from BAS to RST and were lowest in HWE steers ($P \leq 0.05$). Daily visits to the feed bunk increased from BAS to RST during PER1 for all WIEFF ($P \leq 0.05$), but decreased during PER3, PER4, and PER5 for all WIEFF ($P \leq 0.05$). During PER2 daily feed visits decreased from BAS to RST for MWE and HWE, but increased in LWE ($P \leq 0.05$). During PER1 of BAS, feed bunk visits were greater in LWE and MWE than HWE ($P \leq 0.05$). During PER3 of BAS, feed bunk visits were greatest in LWE, followed by MWE and HWE ($P \leq 0.05$). During PER5 of BAS, feed bunk visits were greater in LWE than MWE and HWE ($P \leq 0.05$). Feed bunk visits were not different between WIEFF during PER2 and PER4 of BAS ($P \geq 0.10$). During PER1 and PER5 of RST, feed bunk visits were greater in LWE than MWE and HWE ($P \leq 0.05$). During PER2 and PER3 of RST, feed bunk visits were greatest in LWE, followed by MWE, and HWE ($P \leq 0.05$). During PER4, daily feed bunk visits were greater during BAS than RST (18.1 and 9.71, respectively) and greater for LWE than HWE and MWE (14.8 compared to 13.5 and 13.5, respectively; $P \leq 0.01$). There was a block effect on daily feed bunk visits in PER2, PER3, PER4, and PER5, where visits were greater in the light block, except during PER5 ($P \leq 0.01$). There was no difference between block during PER1 ($P = 0.92$).

Total daily duration at the feed bunk was decreased from BAS to RST and greatest in MWE steers (Table 4.3; $P \leq 0.05$). A WA by WIEFF interaction was observed during PER2 and PER3. During PER2 and PER3 time at the feed bunk was greater

during BAS than RST (Table 4.7; $P \leq 0.05$). During PER2, time at the bunk was lowest in LWE during BAS, but greatest in LWE and MWE during RST ($P \leq 0.05$). During PER3, time at the feed bunk was greatest in MWE during BAS and greater in MWE and HWE during RST ($P \leq 0.05$). During PER1 time at the bunk was decreased from BAS to RST in all WIEFF ($P = 0.07$). During PER3, PER4, and PER5, time at the feed bunk was greater in the heavy block ($P \leq 0.05$), but not different during PER1 and PER2 ($P \geq 0.70$). Time at the feed bunk was not affected by a WIEFF by WA interaction during PER4 or PER5 (Table 4.7; $P \geq 0.23$). During both periods, time at the feed bunk was greater during BAS than RST ($P \leq 0.05$).

Visits to the water bunk that were less than 10 s were not different between WIEFF and WA during PER1, PER2, PER4, PER5, or total daily (Table 4.8; $P \geq 0.11$). During PER3, visits less than 10 s to water bunks were greater during RST than BAS in LWE steers ($P < 0.01$) but not different in HWE and MWE ($P \geq 0.10$). During PER4, visits less than 10 s to the water bunk were greater in HWE than MWE and LWE ($P \leq 0.05$). During PER1, PER2, and PER4, visits less than 10 s to the water bunk increased from BAS to RST ($P \leq 0.01$). There was not a block effect on visits less than 10 s to the water bunk ($P \geq 0.11$). There were no WA, WIEFF, WA by WIEFF, or block differences in visits of 0 seconds to the water bunk during any period (Table 4.7; $P \geq 0.12$).

Average daily visits to the water bunk increased from BAS to RST and were lowest in HWE steers ($P \leq 0.05$). Daily visits to the water bunk increased from BAS to RST for all WIEFF during PER1 and PER2 ($P \leq 0.05$). During PER3, daily water visits deceased from BAS to RST in HWE and MWE, but increased in LWE ($P \leq 0.05$). During PER4, visits decreased from BAS to RST in all WIEFF ($P \leq 0.05$). Daily visits to the

water bunk were not different between WIEFF during PER1 of BAS ($P \geq 0.05$). Daily water bunk visits were greatest in LWE, followed by MWE and HWE during PER2 and PER3 of BAS ($P \leq 0.05$). During PER4 of BAS, water bunk visits were greater in LWE than MWE and HWE ($P \leq 0.05$). During PER1 of RST, water bunk visits were greater in HWE than MWE and LWE ($P \leq 0.05$). During PER2 of RST, water bunk visits were greatest in LWE, followed by MWE, and HWE ($P \leq 0.05$). During PER3 and PER4 of RST, water bunk visits were greater in LWE than MWE and HWE ($P \leq 0.05$). During PER1, PER2, and PER3, water bunk visits were greater in the heavy block ($P \leq 0.05$), and there were no differences in water bunk visits during PER4 and PER5 ($P \geq 0.10$). Daily visits to the water bunk were not affected by a WA by WIEFF interaction during PER5 (Table 4.9; $P \geq 0.53$).

Daily time spent at the water bunk increased from BAS to RST; duration was lowest in HWE during BAS and LWE during RST ($P \leq 0.05$). During PER2, PER3, and PER4 time at the water bunk was greater during RST than BAS ($P \leq 0.05$). During PER1, time at the water bunk increased from BAS to RST in all WIEFF groups ($P \leq 0.05$), and during RST time at the water bunk was greatest in HWE steers compared to MWE and LWE ($P \leq 0.05$). Time at the water bunk was not different between WIEFF during PER1 or PER5 of BAS ($P \geq 0.05$). During RST, time at the water bunk was greatest in HWE during PER1, but lowest in HWE during PER5 ($P \leq 0.05$). Time at the water bunk was greater in the heavy block during PER2, but greater in the light block during PER3 and PER4 ($P \leq 0.05$). Block did not affect time at the water bunk during PER1 and PER5 ($P \geq 0.20$). There was not a WA by WIEFF interaction or WIEFF effect in time at the water bunk during PER2, PER3, or PER4 (Table 4.10; $P \geq 0.29$).

There were no behavior by WIEFF interactions in frequency of behaviors in any period ($P \geq 0.97$). There was not a behavior by WA interaction in frequency of behavior during PER2 (Fig. 4.1; $P = 0.18$). During PER2, observations at the feed bunk initially decreased from BBM to RBM1, but increased from RBM1 to RBM2; whereas observations at the water bunk increased from BBM to RBM1 and again to RBM2 (Fig. 4.2; $P \leq 0.05$). During PER3 and PER4, displacements were greater during BBM compared to RBM1 and RBM2 (Figs. 4.3 and 4.5, respectively; $P \leq 0.01$), but butting, fighting, and mounting were not different ($P \geq 0.11$). During PER3, observations at the feed bunk decreased and behaviors at the water bunk increased from BBM to RBM1 and again to RBM2 (Fig. 4.4; $P \leq 0.05$). During PER4, observations at the feed bunk decreased from BBM to RBM1 and again to RBM2, but increased at the water bunk from BBM to RBM1 and RBM2 (Fig. 4.6; $P \leq 0.05$).

Daily standing time was not affected by a WA by WIEFF interaction ($P > 0.15$). Daily standing time increased from BAS to RST and was overall lowest in HWE compared to MWE and LWE steers ($P \leq 0.01$). During PER2 standing time was greater during RST, whereas during PER4 standing time was greater during BAS ($P \leq 0.05$). During PER1 and PER5, total standing time increased from BAS to RST ($P \leq 0.01$), whereas standing time decreased during PER3 ($P \leq 0.05$). During PER1 and PER5, the HWE had the least standing time during BAS, but the greatest during RST. During PER3, MWE had the greatest standing time during BAS, but LWE had the greatest during RST ($P \leq 0.05$). Standing time was greater in the light block during PER1, PER4, and PER5 ($P \leq 0.05$), but not different during PER2 and PER3 ($P \geq 0.20$). There was not a WA by WIEFF interaction in total standing time during PER2 or PER4 (Table 4.11; $P \geq 0.14$).

Daily lying bouts increased from BAS to RST and were greatest in HWE ($P \leq 0.05$). There was not a WA by WIEFF effect on lying bouts during PER1 and PER5, but bouts were decreased from BAS to RST during both periods (Table 4.12; $P \leq 0.01$). During PER3 and PER4 lying bouts were not different between WIEFF, but bouts were lower in MWE during RST during both periods ($P \leq 0.05$). Lying bouts were greater in the light block during PER1, PER2, and PER3 ($P \leq 0.05$), but not different during PER4 and PER5 ($P \geq 0.13$).

Daily step counts increased in all WIEFF groups fro BAS to RST ($P \leq 0.05$). Step counts also increased throughout the d, peaking during PER3, and then decreased in PER4 and PER5. During PER1 step counts were lowest in HWE during BAS, but lowest in LWE during RST (Table 4.13; $P \leq 0.05$). During PER2, PER3, and PER4, HWE had the lowest step counts in both BAS and RST ($P \leq 0.05$). During PER5, there was a WA by WIEFF interaction ($P \leq 0.01$) where there was no difference due to WIEFF during BAS, but during RST step counts increased and LWE had the lowest step count. Step counts were greater in the light block in all periods ($P \leq 0.05$).

Daily motion index increased in all WIEFF groups fro BAS to RST ($P \leq 0.05$). Motion index increased throughout the d, peaking during PER4, and sharply decreased into PER5 (Table 4.14). During PER1, PER2, and PER5 motion index increased from BAS to RST in all WIEFF ($P \leq 0.05$). During PER3 and PER4 motion index decreased from BAS to RST in all WIEFF ($P \leq 0.05$). During PER2, PER3, and PER4 motion index was lowest in HWE compared to LWE ($P \leq 0.05$), whereas, LWE had the lowest motion index during PER1 and PER5 ($P \leq 0.05$). Motion index was greater in the light block in all periods ($P \leq 0.05$).

DISCUSSION

The desired water restriction level of 50% was achieved. Complete water intake results are presented earlier (ch III). As WI decreased, DMI also decreased, which was expected as DMI and WI are highly correlated (Burgos et al., 2001; Parker et al., 2003; Benatallah et al., 2019). Since DMI decreased during RST, it is not surprising that daily duration at the feed bunk decreased during RST as well. The increased time spent at the water bunk during RST was related to the Insentec RIC system and the methodology used to restrict water intake. Insentec water bunks can be programmed to hold various amounts of water. During BAS, the bunks were programmed to hold 40-47 kg of water at one time. This amount was slowly decreased towards the end of BAS, and during RST bunks were programmed to hold only 5-7 kg of water at one time to prevent cattle from drinking in excess of the daily allotment. Thus, while steers were allotted less total water per d, steers had to make more visits to the bunk, and the lower water level resulted in animals spending more time at the water bunk to drink total daily water allowance by design.

Nonetheless, the change in both water and feed bunk behavior depended on the period of day, and the Insentec system may have artificially altered the feeding behavior patterns as well due to when the next day's water allowance began. The Insentec system was programmed to reset every night between 2345-2400 h. Therefore, daily water allowance was reset at that time and steers could begin drinking again at 0000 h. The water restriction step-down period (when water allowance was decreased 10% every 7 d) lasted 28 d, so steers could acclimate to the new levels of restriction. This period likely allowed for cattle to become accustomed to when the system reset. In addition to ample time to learn, once the first animal visited the bunk and finished drinking, the water bunk

would fill back up with water, making loud water running sounds that could be heard by other steers. As a result, many of the steers probably became accustomed when the bunks reset within the water restriction step down period by hearing the bunks refilling and observing other steers drinking. This likely explains the magnitude of increase in water bunk visits from BAS to RST during PER1. Many steers drank the entire daily allotment during PER1 during restriction.

Visits of 0 seconds were included in the analysis to examine differences in repetitive, or frustration, behavior. Frustration in animal behavior has been defined as "interference with an occurrence of an instigated goal-response at its proper time in the behavior sequence" (Scott, 1948). When a steer attempted to obtain feed or water from a bunk, the steer would present the RFID tag to the reader above the gate. Once the RIC system detected and read the RFID tag in the left ear, the bunk gate would lower (open position) so the steer could access feed or water. Thus, a visit of 0 seconds could indicate 1 of 2 scenarios. The first scenario being that the visit was extremely short because the steer was immediately displaced by another steer, which commonly occurs at the feed bunk following feed delivery. This scenario is more common for visits between 0 and 10 s, not necessarily only 0 s, as the bunk can still go down but the animal is displaced quickly. The second scenario occurs when the animal has attempted to access the bunk, but the bunk gate did not lower. This scenario would happen to the water bunks once steers had drank the total daily allotment. Visual observation of steers during RST indicated that steers would attempt to access the water bunk numerous times over several minutes if the gate did not lower matching the definition of frustration behavior. Thus, visits of 0 s at the water bunk were expected to increase during RST, especially during

PER1 and PER2. However, these measures did not differ between BAS and RST. There was a trend for these visits to increase at the feed bunk for MWE and LWE during PER1, which may be related to the increased bunk visits during PER1 and driven by scenario 1.

Since steers were drinking early in the morning, steers also tended to visit the feed bunks early in the morning, which is most likely why feed bunk visits increased during PER1 of RST. DeVries et al. (2008) reported the greatest incidence of feeding of dairy heifers using Insentec feeding bins occurred immediately following each feed delivery (2 feed deliveries per day). A similar pattern was reported in Schwartzkopf-Genswein et al. (2003), where feeding activity of feedlot cattle peaked in the morning and early evening, still following feed delivery times. However, time at the feed bunk and visits to the feed bunk did not peak until PER3 and PER4 during BAS, though feed deliveries were performed once during PER2 and twice during PER3. The results of the current experiment may differ from previous experiments because of the degree of competition in pens, the length of time period measured, or because of the increased feed delivery frequency. Hosseinkhan et al. (2008) reported that competition did not affect DMI or feeding time, but did decrease meals per day and length of each meal. Nevertheless, feeding behavior continued to differ during RST, where time at the feed bunk and visits increased during PER2, remained consistent throughout the day, and abruptly decreased during PER5. Although, this change in daily pattern was probably driven by the accompanied decreased DMI, resulting from decreased water allowance. The results of this experiment indicate that changes in water availability can alter daily feeding behavior patterns.

Ten second visits to the bunks were presented as an indication of competition and displacements. Because these visits likely represent quick bunk displacements during times of high bunk competition, these visits were hypothesized to be greater during BAS and decrease during RST. Similarly, these 10 s visits were expected to be most frequent during PER2 of BAS and then shift during RST to be most frequent during PER1, since cattle had *ad libitum* access to feed. While these visits did increase during PER1 of RST, 10 s visits were not greatest during PER2 of BAS. It was believed that these visits would be greatest during PER2 because the first feed delivery of the day occurred during PER2 and typically attracted most all animals to the feed bunks. Previous literature has reported that the greatest frequency of displacement and interactions occur following feed delivery (DeVries et al., 2004; DeVries et al., 2008; Keyserlingk and Weary, 2010). It should be noted, previous literature delivered feed twice daily, rather than 3 deliveries in the current experiment. Deliveries were also more evenly spaced (8 h intervals), whereas deliveries in the current experiment were spaced by 3.5 and 4 h. Additionally, visits less than 10 seconds were fairly consistent throughout the day during BAS. This may be because bunk competition was considerably high due to the increased steer:bunk ratio (4-6 steers per feed bunk), resulting in more continuous competition throughout the day as opposed to spikes in competition that follow feed delivery times (0730, 1030, and 1430). DeVries et al. (2008) reported a decrease in DMI, time spent at the bunk, and feeding rate in cows in a competitive feeding setting (2 cows per RIC bunk) compared to a noncompetitive setting (1 cow per RIC bunk), stating that small increases in competition can impact feeding behavior. In the current experiment, there was an average of 4-6 steers per feed bunk (depending on the feeding group) and up to 33 steers per water bunk. Thus, the

competition for both feed and water was probably greater than assumed leading to significant alterations in feeding behavior. Typically, as competition for a resource is increased, agonistic interactions will also increase (Craig 1986), which could explain why both feed and water bunk visits less than 10 s persisted throughout the d, as bunk competition remained high.

The increase in step counts, motion index, and numerical increase in displacements during PER2 between BAS and RST indicate increased activity and feed and water bunk attendance. While displacements peaked in PER3 during BAS (the period with 2 feed deliveries) during RBM1 and RBM2 displacements peaked during PER2. The increase in displacements during PER2 coincides with the change in feeding and drinking behavior as well as increased activity measures during earlier morning hours. Kaliber et al. (2015) reported that duration of walking decreased with intensifying water restriction, whereas duration of standing and lying both increased. The authors suggested that the decrease in daily walking duration was for a more economical use of energy. The authors also attributed increased standing time to a thermoregulatory response, to conserve water and energy. Activity and social interactions can both be energetically costly, especially if bunk displacements are unsuccessful and do not result in gaining access to feed or water. During periods of decreased DMI and water allowance, energy and water conservation may be especially important. Thus, it is possible that the shift in social interactions and pen activity to earlier hours was to conserve energy and water throughout the day since feed and water intake were decreased.

Changes in pen behavior are important in order to effectively manage cattle, especially during water scarcity. While motion index and step counts peaked at PER4

(early evening) during BAS, the peak for both measures occurred during PER2, early to mid-morning of RST. These results indicate that changes in water availability altered daily activity patterns of cattle when water allowance was restricted. These shifts in daily activity are similar to responses seen in desert animals that alter foraging and locomotor activity to coincide with cooler morning temperatures (Cain et al., 2006). However, the change in behavior patterns could also be due to the timing of the water availability reset. Future research in water restriction utilizing a similar design should attempt to evaluate whether behavioral differences are due to actual restriction or an artifact of the restriction system.

Previous research has related feed efficiency or performance measures (ADG, G:F, residual feed intake) to behavioral measures in order to predict and understand differences in performance (Hickman et al., 2002; McGee et al., 2014). Water efficiency may also be useful in identifying more efficient animals or predicting future performance, especially as water becomes a limiting resource in animal agriculture. Pen behavior during a baseline period may also be useful in identifying water efficient animals. In the current experiment, HWE steers appeared to have behavioral differences similar to differences reported elsewhere. Hickman et al. (2002) reported that steers classified as "high ADG steers" spent less time at the feed bunk and that high feed efficiency steers had the most variable feed intake. Kayser and Hill (2013) reported that duration of time an animal's head was in the bunk and feeding rate were significantly correlated to DMI. McGee et al. (2014) reported that feeding behaviors were strong predictors of DMI during both growing and finishing phases. Llonch et al. (2018) reported that animals with a low residual feed intake had increased activity measures.

Generally, HWE steers had the least activity and spent the least amount of time standing throughout the d, except late at night during RST. In addition to less activity, HWE steers also had less visits the feed bunks during 0400 to 2200 h and spent less time at the feed bunk during PER2. However, visits to and time spent at the feed bunk were greater early in the morning and not different late at night. These results suggest that the HWE steers were more efficient in altering daily behavioral patterns to conserve energy during water restriction. The HWE steers may have adapted daily behavior patterns to be most active at night and early morning to avoid additional thermal stress, similar to behaviors observed in desert animals. Even during BAS, HWE steers had less overall activity and visits to the bunk. This inherent difference may have made these steers better able to adapt to water restriction. The results of the current experiment match previously reported differences using alternate animal efficiency models (Hickman et al., 2002) and offer a new method to identify cattle that can perform successfully under water restrictive conditions.

Although typical agonistic interactions were measured in this trial, no dominance measures or inferences were conducted, as opposed to other studies that measured similar behavior (Mackay et al., 2013) with similar systems. However, it does not seem that traditional measures of dominance, specifically displacement at the bunk, are best suited for this type of feeding system. Using all feeding groups, it was determined that if a steer's head was in the bunk it could not identify whether a person or another steer was attempting to displace without additional vocalization. Thus, it seems that the steers were not able to identify who was attempting to displace them in any scenario; while the steer attempting to displace knew the other steer's identity, the steer that was the recipient of

the behavior did not. Since the ability to identify other individuals is a necessary aspect of a dominance hierarchy (Price, 2008), it seems that bunk displacements are not an appropriate measure of dominance when feeding with an Insentec RIC system.

Other traditional measures of agonistic behavior, such as butting, fighting, and bulling were also measured, but occurred infrequently. It should be noted that at the time video recording was initiated, the groups of cattle should have been socially stable since they had been in the same pens without any additional comingling for 77 d. Even for such large groups, this should be ample time to establish a stable social hierarchy with minimal agonistic interactions. Other agonistic interactions (fighting, bulling, butting) were expected to increase with RST since a resource was being limited, however there were no differences in any of these behaviors over time. Although water levels were restricted, each steer still had abundant time to drink the daily allotment, which may be why differences in behavior were not observed. Other studies where food is used as a limited resource to increase competition are designed so that food is truly a limited resource. That is where no additional food is available following consumption. While water was restricted for each individual, it was not a true limited resource since animals still had access to their allocation until it was consumed completely during the day.

It seems that prolonged water restriction altered daily behavioral patterns in feedlot steers, shifting behavior to the early morning and increasing overall activity and social interactions. The extent that actual water restriction versus the design of the Insentec system played in the behavioral pattern change warrants further investigation. The degree of bunk competition in this experiment may have also affected feeding and drinking behavior. Thus, the relationship between water allowance and stocking rate may

also merit future investigation. Results of this experiment also indicate that HWE steers may be able to more effectively adapt to water restriction due to the observed decrease in activity and social interactions, ultimately conserving energy and water. In conclusion, it appears that cattle were able to adapt to water restriction behaviorally and that pen activity during a baseline period may be used to predict water efficiency in the feedlot.

Table 4.1. Ethogram of observed social behaviors

Social Behavior	Definition
Butting	A steer uses head contact, to the body of the recipient steer, resulting in 1 step or more
Displacement	A steer uses his jowl, neck, and (or) shoulders to insert himself between 2 other animals or between an animal and an inanimate object such that the recipient steer(s) takes at least 1 step away
Threatening	A steer exhibits a posture whereby his nose and/or horns are presented in the direction of the recipient steer or by exhibiting a forceful swing of the head towards the recipient steer. There is no physical contact that occurs between steers and these postures or movements are followed by avoidance of the recipient steer
Fighting	A steer uses physical contact to push the recipient steer. The recipient steer uses physical contact to push back at the instigating steer
Bulling	One steer mounts another steer with both front legs off the ground, from any angle, and touches the reactor steer

Table 4.2. Definition of possible locations recorded during behavioral observations

Location	Definition
Feed bunk	Social interaction occurred within 1 steer's body length of feed bunk
Water bunk	Social interaction occurred within 1 steer's body length of water bunk
Pen	Social interaction occurred more than 1 steer's body length away from feed and water bunks

Table 4.3. Daily feed bunk behavior by WIEFF[1] and water allowance[2]

				P-value		
	BAS	RST	SEM	WA	WIEFF	WA*WIEFF
0 s visits, frequency/d						
HWE	1.06	1.17	0.033	< 0.001	0.18	0.22
MWE	1.07	1.25	0.032			
LWE	1.06	1.23	0.035			
Visits less than 10 s, frequency/d						
HWE	1.76[c]	1.71[d]	0.168	< 0.001	< 0.001	< 0.001
MWE	1.85[b]	1.85[b]	0.168			
LWE	2.16[a]	1.84[b]	0.168			
Bunk visits, frequency/d						
HWE	13.81[b]	8.68[e]	1.078	< 0.001	< 0.001	< 0.001
MWE	13.94[b]	9.08[d]	1.078			
LWE	14.69[a]	10.26[c]	1.078			
Duration at the bunk, min/d						
HWE	32.8[a]	22.2[d]	0.41	< 0.001	< 0.001	< 0.01
MWE	33.3[a]	22.7[c]	0.35			
LWE	31.5[b]	22.4[c]	0.38			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[abcd]Values with different letters differ at $P < 0.05$.

Table 4.4. Frequency of 0 second visits to the feed bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	1.28	1.26	0.168	0.06	0.93	0.20
	MWE	1.21	1.40	0.147			
	LWE	1.09	1.46	0.153			
PER2							
	HWE	1.05	1.04	0.031	0.12	0.18	0.12
	MWE	1.04	1.13	0.026			
	LWE	1.04	1.06	0.026			
PER3							
	HWE	1.07	1.09	0.042	0.20	0.64	0.64
	MWE	1.08	1.09	0.040			
	LWE	1.08	1.15	0.041			
PER4							
	HWE	1.03	1.02	0.025	0.21	0.67	0.70
	MWE	1.05	1.03	0.027			
	LWE	1.06	1.01	0.027			
PER5							
	HWE	1.14	1.02	0.105	0.63	0.76	0.47
	MWE	1.00	1.07	0.083			
	LWE	1.03	0.99	0.099			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

Table 4.5. Frequency of visits to the feed bunk less than 10 s by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	1.62^{c}	2.12^{b}	0.117	< 0.001	0.09	0.02
	MWE	1.58^{c}	2.35^{a}	0.109			
	LWE	1.69^{c}	2.27^{a}	0.112			
PER2							
	HWE	1.67^{d}	1.70^{cd}	0.172	0.48	< 0.001	< 0.001
	MWE	1.78^{bc}	1.84^{b}	0.171			
	LWE	2.00^{a}	1.86^{bc}	0.171			
PER3							
	HWE	1.78^{c}	1.53^{d}	0.214	< 0.001	< 0.001	< 0.001
	MWE	1.92^{b}	1.60^{d}	0.212			
	LWE	2.33^{a}	1.72^{d}	0.213			
PER4							
	HWE	1.85^{b}	1.42^{c}	0.237	< 0.001	< 0.001	< 0.001
	MWE	1.92^{b}	1.42^{c}	0.235			
	LWE	2.31^{a}	1.51^{c}	0.236			
PER5							
	HWE	1.42^{b}	1.21^{c}	0.070	< 0.001	0.03	0.02
	MWE	1.41^{b}	1.32^{bc}	0.067			
	LWE	1.61^{a}	1.27^{c}	0.072			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcd]Values with different letters differ at $P < 0.05$.

Table 4.6. Daily visits to the feed bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	6.51^{d}	8.49^{b}	0.502	< 0.001	< 0.001	< 0.01
	MWE	6.95^{c}	8.38^{b}	0.501			
	LWE	7.24^{c}	8.79^{a}	0.502			
PER2							
	HWE	11.29^{b}	10.13^{c}	1.069	< 0.001	< 0.001	< 0.001
	MWE	11.55^{b}	11.30^{b}	1.068			
	LWE	11.75^{b}	12.12^{a}	1.069			
PER3							
	HWE	17.46^{c}	8.68^{f}	1.603	< 0.001	< 0.001	< 0.001
	MWE	17.97^{b}	9.08^{e}	1.602			
	LWE	19.03^{a}	11.25^{d}	1.602			
PER4							
	HWE	17.71	9.29	1.195	< 0.001	< 0.001	0.28
	MWE	17.62	9.33	1.193			
	LWE	18.87	10.77	1.194			
PER5							
	HWE	5.56^{b}	3.98^{d}	0.410	< 0.001	< 0.001	< 0.01
	MWE	5.72^{b}	3.85^{d}	0.408			
	LWE	6.48^{a}	4.33^{c}	0.410			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.7. Duration (min) of daily visits to the feed bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	20.9	18.5	0.52	< 0.001	0.12	0.07
	MWE	22.6	18.4	0.43			
	LWE	21.8	18.4	0.47			
PER2							
	HWE	27.3[a]	22.8[e]	0.60	< 0.001	< 0.01	< 0.001
	MWE	27.5[a]	24.9[c]	0.51			
	LWE	25.3[b]	24.9[d]	0.56			
PER3							
	HWE	48.4[b]	25.6[e]	0.83	< 0.001	< 0.001	< 0.01
	MWE	49.2[a]	25.8[e]	0.71			
	LWE	45.0[c]	25.2[d]	0.77			
PER4							
	HWE	47.7	26.3	0.68	< 0.001	0.02	0.36
	MWE	47.1	25.8	0.58			
	LWE	45.6	25.5	0.63			
PER5							
	HWE	17.8	10.3	0.37	< 0.001	0.30	0.23
	MWE	18.9	10.3	0.31			
	LWE	18.7	10.3	0.35			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.8. Daily water bunk behavior by WIEFF[1] and water allowance[2]

				P-value		
	BAS	RST	SEM	WA	WIEFF	WA*WIEFF
0 s visits, frequency/d						
HWE	1.05	1.04	0.026	0.04	0.83	0.47
MWE	1.05	1.02	0.026			
LWE	1.08	1.01	0.027			
Visits less than 10 s, frequency/d						
HWE	1.29	1.56	0.034	< 0.001	< 0.01	0.18
MWE	1.26	1.59	0.032			
LWE	1.24	1.53	0.033			
Bunk visits, frequency/d						
HWE	2.50^{f}	3.64^{b}	0.201	< 0.001	< 0.01	< 0.001
MWE	2.59^{e}	3.55^{c}	0.200			
LWE	2.74^{d}	3.85^{a}	0.200			
Duration at the bunk, min/d						
HWE	3.2^{z}	10.6^{x}	0.34	< 0.001	0.86	0.07
MWE	3.3^{z}	10.3^{x}	0.29			
LWE	3.6^{y}	9.9^{w}	0.32			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[abcde]Values with different letters differ at $P < 0.05$.

[wxyz]Values with different letters differ at $P < 0.10$.

Table 4.9. Visits of 0 second to the water bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	1.01	1.00	0.048	0.80	0.74	0.68
	MWE	1.00	1.03	0.030			
	LWE	1.00	1.00	0.030			
PER2							
	HWE	1.13	1.09	0.060	0.31	0.15	0.85
	MWE	1.02	1.00	0.057			
	LWE	1.07	1.00	0.068			
PER3							
	HWE	1.03	1.01	0.074	0.72	0.67	0.93
	MWE	1.04	1.02	0.081			
	LWE	1.06	1.06	0.045			
PER4							
	HWE	1.06	1.11	0.117	0.58	0.91	0.61
	MWE	1.07	1.00	0.177			
	LWE	1.15	1.00	0.159			
PER5[4]							
	HWE	1.02	0.93	0.275	0.89	0.73	-
	MWE	1.26	-	0.314			
	LWE	1.14	-	0.184			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[4]There were no 0 s visits by MWE or LWE steers during PER5 of RST.

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.10. Visits less than 10 seconds to the water bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	1.29	1.70	0.142	< 0.001	0.73	0.61
	MWE	1.27	1.77	0.134			
	LWE	1.23	1.74	0.137			
PER2							
	HWE	1.22	1.38	0.036	< 0.001	< 0.01	0.13
	MWE	1.19	1.33	0.034			
	LWE	1.18	1.26	0.035			
PER3							
	HWE	1.32[b]	1.32[b]	0.044	0.02	0.23	< 0.01
	MWE	1.30[b]	1.29[b]	0.039			
	LWE	1.26[b]	1.42[a]	0.036			
PER4							
	HWE	1.33	1.46	0.047	0.04	< 0.01	0.11
	MWE	1.29	1.28	0.044			
	LWE	1.27	1.32	0.047			
PER5							
	HWE	1.17	1.15	0.050	0.71	0.50	0.71
	MWE	1.14	1.18	0.053			
	LWE	1.19	1.22	0.057			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.11. Daily visits to the water bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	1.61[c]	5.59[a]	0.294	< 0.001	< 0.001	< 0.001
	MWE	1.65[c]	5.12[b]	0.294			
	LWE	1.62[c]	4.96[b]	0.294			
PER2							
	HWE	1.96[f]	3.90[a]	0.202	< 0.001	< 0.001	< 0.001
	MWE	2.04[e]	4.02[b]	0.201			
	LWE	2.11[d]	4.43[c]	0.202			
PER3							
	HWE	3.07[d]	2.78[e]	0.252	< 0.001	< 0.001	< 0.001
	MWE	3.25[c]	2.87[e]	0.251			
	LWE	3.48[b]	3.76[a]	0.251			
PER4							
	HWE	2.88[b]	2.55[c]	0.252	< 0.001	< 0.001	0.04
	MWE	2.96[b]	2.49[c]	0.251			
	LWE	3.17[a]	2.79[b]	0.252			
PER5							
	HWE	1.55	1.75	0.052	< 0.001	0.02	0.53
	MWE	1.51	1.73	0.050			
	LWE	1.56	1.83	0.053			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.12. Duration (min) of daily visits to the water bunk by WIEFF[1] and water allowance[2] throughout the day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	2.2[d]	10.1[a]	0.41	< 0.001	< 0.001	< 0.001
	MWE	2.3[d]	8.6[b]	0.34			
	LWE	2.5[d]	7.3[c]	0.37			
PER2							
	HWE	2.8	11.3	0.73	< 0.001	0.39	0.29
	MWE	2.9	9.8	0.62			
	LWE	2.9	9.9	0.68			
PER3							
	HWE	4.7	11.2	1.01	< 0.001	0.37	0.95
	MWE	4.9	11.9	0.86			
	LWE	5.7	12.3	0.94			
PER4							
	HWE	3.9	11.6	0.93	< 0.001	0.89	0.32
	MWE	4.0	12.0	0.79			
	LWE	4.6	10.8	0.87			
PER5							
	HWE	2.2[c]	6.5[b]	0.30	< 0.001	0.05	0.05
	MWE	2.2[c]	7.8[a]	0.25			
	LWE	2.2[c]	7.4[a]	0.28			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

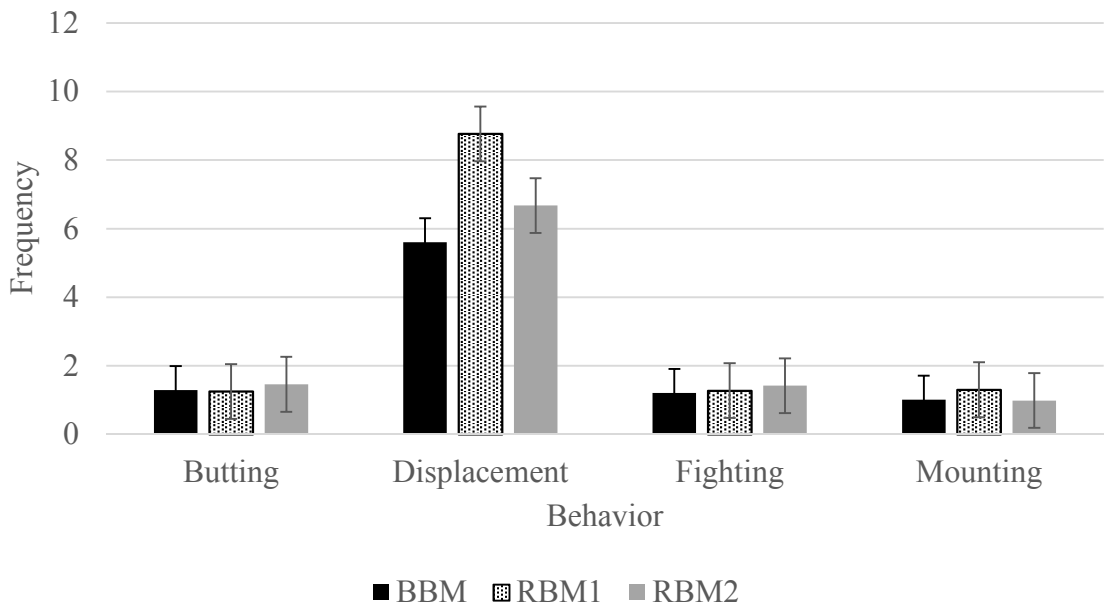

Figure 4.1. Frequency of social interactions between water allowance (WA) during PER2. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 0400 to 0959 h (PER2). A behavior by water allowance interaction was not observed ($P \geq 0.18$).

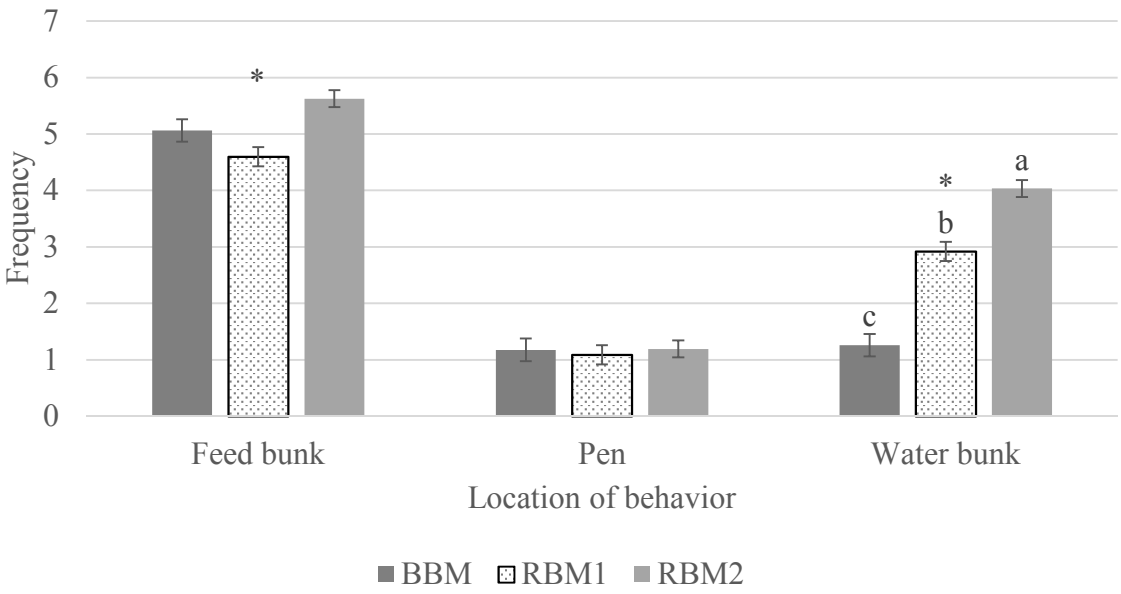

Figure 4.2. Frequency of location of social interactions between water allowance (WA) during PER2. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 0400 to 0959 h (PER2). Values with different letters differ at $P < 0.05$. * indicates a significant behavior by water allowance interaction ($P \leq 0.001$).

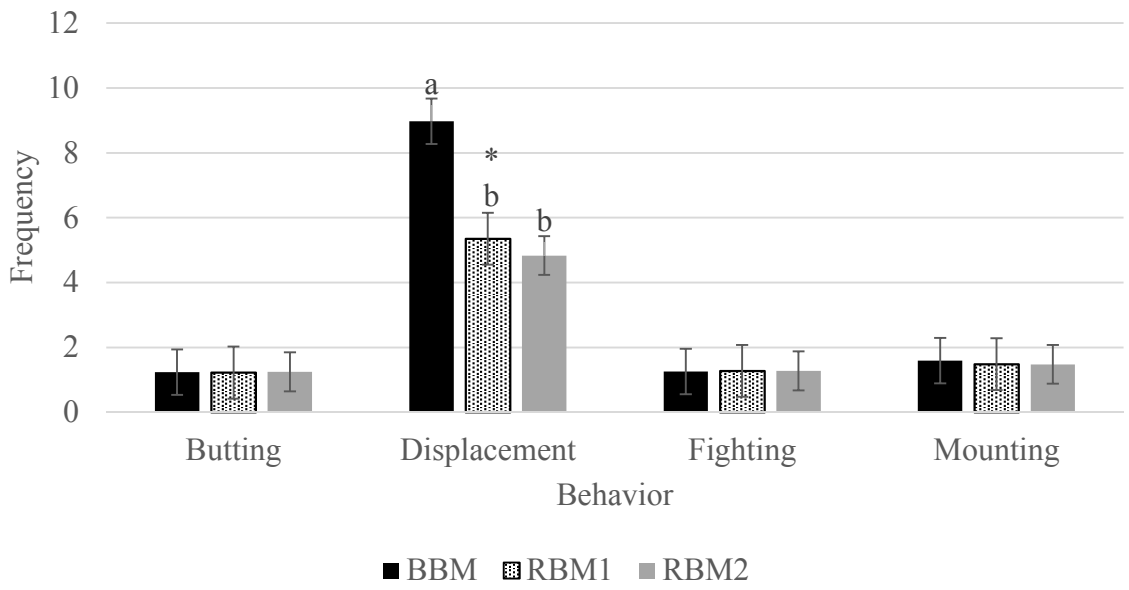

Figure 4.3. Frequency of social interactions between water allowance (WA) during PER3. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 1000 to 1559 h (PER3). Values with different letters differ at $P < 0.05$. * indicates a significant behavior by water allowance interaction ($P \leq 0.001$).

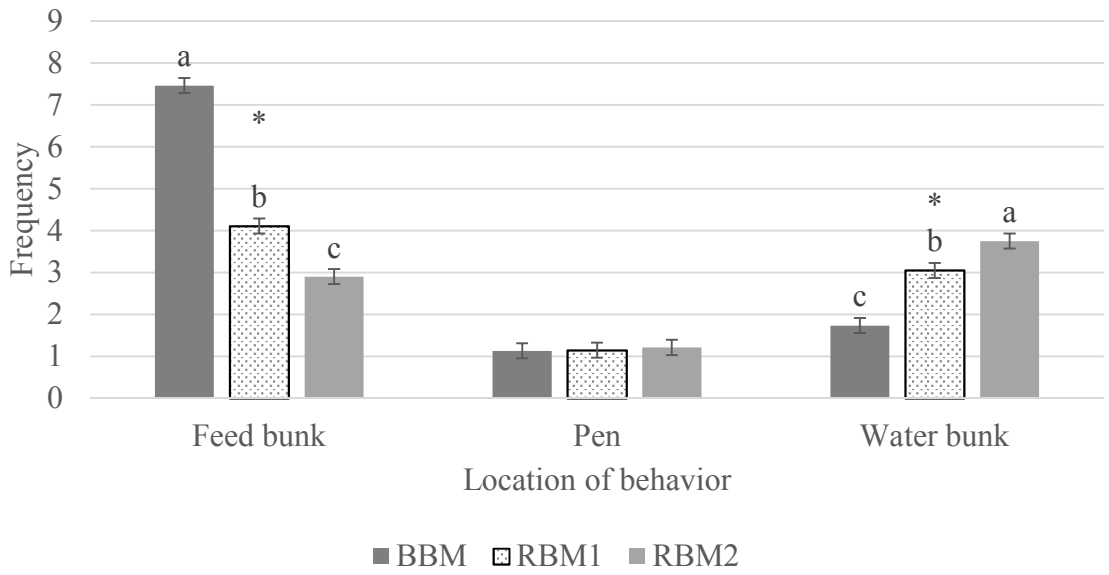

Figure 4.4. Frequency of location of social interactions between water allowance (WA) during PER3. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 1000 to 1559 h (PER3). Values with different letters differ at $P < 0.05$. * indicates a significant behavior by water allowance interaction ($P \leq 0.001$).

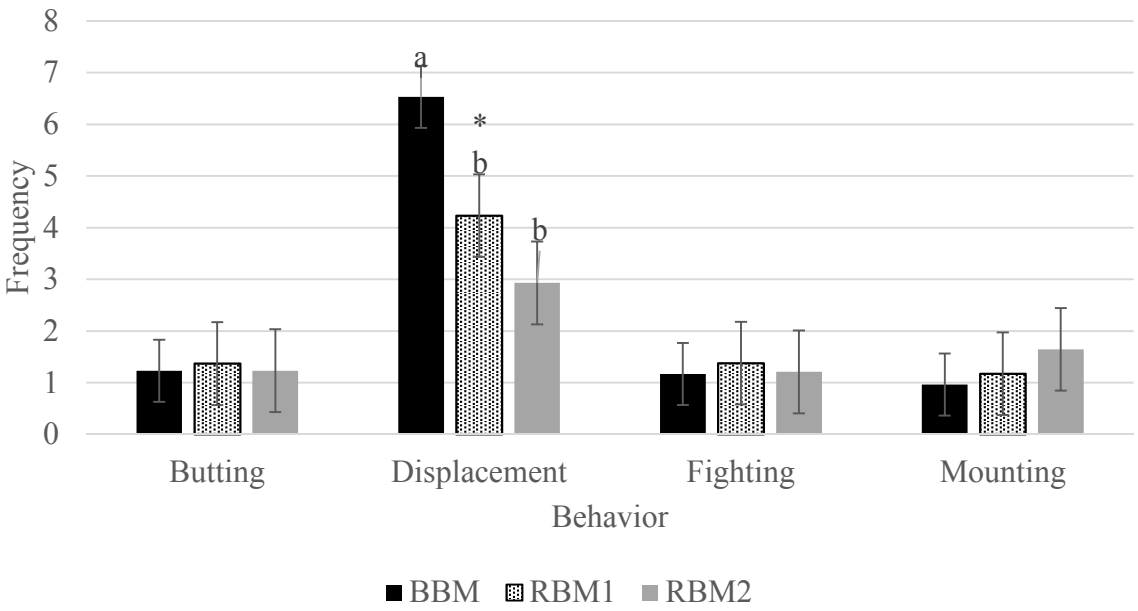

Figure 4.5. Frequency of social interactions between water allowance during PER4. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 1600 to 2159 h (PER4). Values with different letters differ at $P < 0.05$. * indicates a significant behavior by water allowance interaction ($P \leq 0.001$).

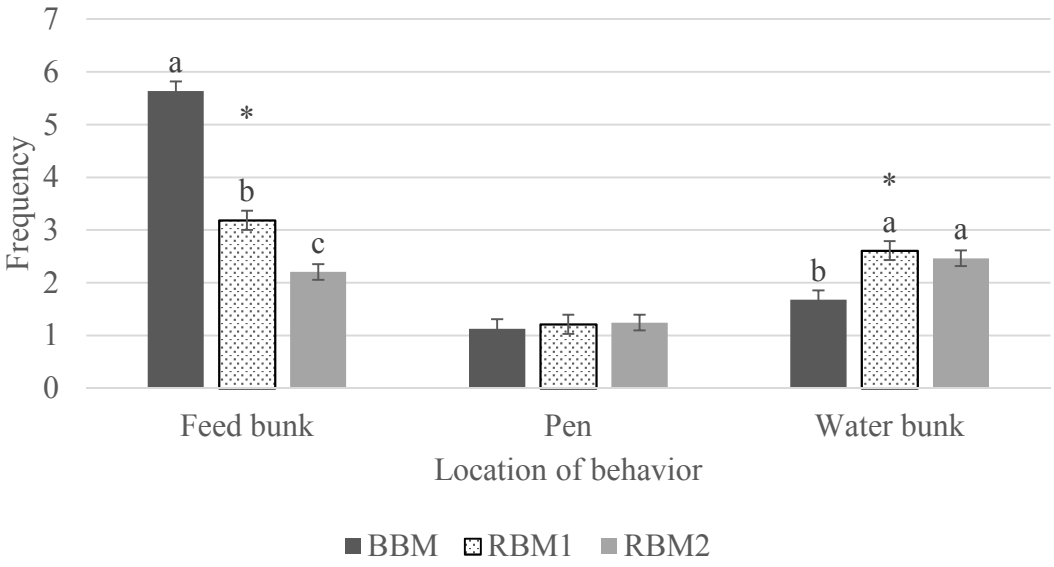

Figure 4.6. Frequency of location of social interactions between water allowance (WA) during PER4. Water allowance was *ad libitum* during BBM (d 58 and 59) and 50% of average during RBM1 (d 114 and 115) and RBM2 (d 128 and 129). Data presented represents social observations measured during 1600 to 2159 h (PER4). Values with different letters differ at $P < 0.05$. * indicates a significant behavior by water allowance interaction ($P \leq 0.001$).

Table 4.13. Daily locomotor behavior by WIEFF[1] and water allowance[2]

				P-value		
	BAS	RST	SEM	WA	WIEFF	WA*WIEFF
Standing time, min/d						
HWE	591.6	645.6	17.44	< 0.001	< 0.001	0.15
MWE	631.0	666.8	17.11			
LWE	621.3	660.7	17.43			
Number of lying bouts, frequency/d						
HWE	12.36^{ab}	12.80^{a}	0.783	< 0.001	< 0.001	< 0.01
MWE	11.81^{c}	11.94^{c}	0.775			
LWE	11.48^{c}	12.60^{b}	0.783			
Number of steps/d						
HWE	854.4^{c}	1066.7^{a}	64.39	< 0.001	< 0.001	< 0.01
MWE	1012.1^{b}	1107.4^{a}	62.98			
LWE	973.8^{b}	1148.9^{a}	64.38			
Motion index						
HWE	3835^{e}	4560^{bc}	269.7	< 0.001	< 0.001	0.02
MWE	4464^{cd}	4763^{ac}	262.0			
LWE	4237de	4970^{a}	269.7			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.14. Total standing time (min) by WIEFF[1] and water allowance[2] by day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	29.7[d]	105.6[a]	4.22	< 0.001	< 0.01	< 0.01
	MWE	38.0[c]	109.0[a]	3.96			
	LWE	36.3[cd]	98.0[b]	4.22			
PER2							
	HWE	142.8	161.9	5.48	< 0.001	< 0.001	0.43
	MWE	151.5	166.2	5.30			
	LWE	152.1	171.1	5.48			
PER3							
	HWE	218.6[b]	181.0[e]	5.23	< 0.001	< 0.001	< 0.01
	MWE	231.2[a]	192.9[d]	4.94			
	LWE	225.0[ab]	202.2[c]	5.23			
PER4							
	HWE	168.9	147.6	10.88	< 0.001	0.18	0.14
	MWE	174.6	147.6	10.80			
	LWE	168.7	147.9	10.88			
PER5							
	HWE	19.6[d]	38.3[a]	2.55	< 0.001	0.33	< 0.001
	MWE	21.1[cd]	38.9[a]	2.39			
	LWE	24.8[c]	31.6[b]	2.55			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.15. Total lying bouts (number of bouts) by WIEFF[1] and water allowance[2] by day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	2.16	1.96	0.078	< 0.01	< 0.001	0.14
	MWE	1.90	1.85	0.071			
	LWE	1.89	1.85	0.078			
PER2							
	HWE	3.04^{bc}	3.24^{a}	0.194	< 0.001	< 0.001	< 0.001
	MWE	2.79^{d}	2.95^{c}	0.190			
	LWE	2.57^{e}	3.17^{b}	0.194			
PER3							
	HWE	3.69^{c}	4.25^{a}	0.382	< 0.001	0.52	0.03
	MWE	3.73^{c}	4.05^{b}	0.379			
	LWE	3.60^{c}	4.30^{a}	0.382			
PER4							
	HWE	2.72^{a}	2.65^{ab}	0.106	< 0.001	0.05	< 0.01
	MWE	2.73^{a}	2.41^{c}	0.100			
	LWE	2.64^{ab}	2.62^{b}	0.106			
PER5							
	HWE	1.11	0.93	0.044	< 0.001	< 0.01	0.66
	MWE	0.99	0.85	0.039			
	LWE	1.05	0.87	0.044			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.16. Total step counts (number of steps) by WIEFF[1] and water allowance[2] by day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	37.4[c]	234.5[a]	14.07	< 0.001	< 0.01	0.04
	MWE	52.3[c]	251.1[a]	13.28			
	LWE	46.3[c]	214.2[b]	14.07			
PER2							
	HWE	186.5[d]	263.4[ab]	21.13	< 0.001	< 0.001	0.04
	MWE	219.8[c]	266.4[b]	20.70			
PER3	LWE	225.1[c]	289.0[a]	21.13			
	HWE	263.3[bc]	232.5[d]	29.47	< 0.001	< 0.001	< 0.01
	MWE	306.0[a]	243.6[d]	29.10			
	LWE	299.5[ab]	280.9[c]	29.46			
PER4							
	HWE	311.5[b]	226.2[d]	16.11	< 0.001	< 0.001	< 0.001
	MWE	359.0[a]	232.2[d]	15.27			
	LWE	327.0[b]	258.9[c]	16.10			
PER5							
	HWE	23.6[c]	72.8[a]	6.78	< 0.001	0.02	< 0.001
	MWE	32.1[c]	68.4[a]	6.40			
	LWE	32.7[c]	50.1[b]	6.78			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$.

Table 4.17. Total motion index by WIEFF[1] and water allowance[2] by day[3]

					P - values		
		BAS	RST	SEM	WA	WIEFF	WA*WIEFF
PER1							
	HWE	196^{d}	971^{b}	58.8	< 0.001	< 0.01	0.07
	MWE	250^{d}	1059^{a}	55.2			
	LWE	226^{d}	906^{c}	58.9			
PER2							
	HWE	789^{d}	1098^{b}	87.7	< 0.001	< 0.001	0.04
	MWE	942^{c}	1119^{b}	85.6			
	LWE	950^{c}	1200^{a}	87.7			
PER3							
	HWE	1092^{cd}	1004^{d}	119.2	< 0.001	< 0.0001	< 0.01
	MWE	1290^{a}	1072^{d}	117.4			
	LWE	1261^{ab}	1211^{bc}	119.2			
PER4							
	HWE	1390^{b}	965^{d}	85.2	< 0.001	< 0.01	< 0.001
	MWE	1610^{a}	1002^{d}	81.2			
	LWE	1437^{b}	1124^{c}	85.2			
PER5							
	HWE	115^{c}	303^{a}	27.8	< 0.001	0.02	< 0.001
	MWE	144^{c}	287^{a}	26.2			
	LWE	149^{c}	212^{b}	27.8			

[1]Water intake efficiency (WIEFF) measured as ADG/water intake as a percent of body weight. Groups were assigned using k-means clustering where $k = 3$. Groups are categorized as either low (LWE), medium (MWE), or high (HWE) WIEFF.

[2]Access to water (WA) was *ad libitum* during d 0-70 (BAS) and 50% of average daily water intake during d 98-140 (RST).

[3]Periods throughout the day: 0000 to 0359 h (PER1), 0400 to 0959 h (PER2), 1000 to 1559 h (PER3), 1600 to 2159 h (PER4), and 2200 to 2359 h (PER5).

[abcde]Values with different letters differ at $P < 0.05$

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