THE EFFECTS OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN STRESS CHALLENGE DURATION OF BLOOD PARAMETERS AND PERFORMANCE IN GROWING BEEF STEERS

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ABSTRACT

The objective of this study was to determine the metabolic, hematologic, endocrine, and performance response of beef steers administered different durations of corticotropin releasing hormone (CRH) and vasopressin (VP) as an endocrine stress challenge. Steers (n =30; initial BW= 416 kg ± 19) were used in a randomized complete block design and assigned to 1 of 3 treatments: 1) Control intravenous jugular injection of 3 mL saline daily from d 0 to 3 (n = 10), 2) Acute 0.3 µg/kg of BW CRH (Bachem Ag, Bubendorf, Switzerland) and 1.0 µg/kg of BW arginine VP (Sigma-Aldrich, Co. St. Louis, MO) on d 0, followed by 3 mL of saline on d 1 to 3 (n = 10), or 3) Chronic 0.3 µg/kg of BW of CRH and 1.0 µg/kg of BW VP from d 0 to 3 (n = 10). Whole blood and serum samples were collected at h 0, 24, 48, 72, 144, and 336 for analysis of complete blood count and non-esterified fatty acids (NEFA). Additionally, rectal temperatures and serum samples were collected at h 0, 1, 2, 24, 25, 26, 48, 49, 50, 72, 73, 74, 144, and 336 after administration of CRH and VP for analysis of cortisol, insulin, and blood metabolites. There was a treatment × hour effect ($P = 0.003$) for total white blood cell count such that chronic had greater concentration than control but was not different from acute at 72 h. Conversely, monocyte concentration was less at h 144 for chronic cattle than both acute and control (treatment × hour interaction; $P \leq 0.01$). A treatment × hour interaction ($P = 0.03$) was observed for lymphocytes but no treatment differences existed within hour. Stress treatments did not affect ($P \geq 0.13$) red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, neutrophils, or neutrophil-to-lymphocyte-

ratio. Serum NEFA tended to be lower ($P = 0.09$) in control cattle compared to those that were stressed. Rectal temperature tended to be greater for both stress treatments relative to control (stress vs. no; $P = 0.06$). Cortisol was greater for cattle receiving the acute and chronic treatments than control at h 1 and greater for chronic than both acute and control at h 25, 26, 49, 50, 73, and 74 (treatment × hour; $P \leq 0.01$). Similarly, there was a treatment × hour interaction ($P \leq 0.01$) for serum glucose and serum insulin concentrations where cattle receiving the acute and chronic treatments had greater glucose at h 1 relative to control and cattle receiving chronic had greater glucose at h 25, 49, 50, 73, and 74 than acute and control. Serum insulin concentrations were greater in chronic and acute treatments than control at h 1 and greater for chronic compared to acute and control at h 25, 49, and 73 (treatment × hour $P \leq 0.01$). Serum phosphorus was lower for chronic and control vs. acute at h 25, less for chronic than acute at h 26 and 49, and less for chronic than acute and control at h 50, 73, and 74 (treatment × hour interaction; $P \leq 0.01$). A treatment × hour interaction ($P \leq 0.01$) was also observed for serum potassium (K) where acute and chronic had lower serum K at h 1 and 2 than control and chronic was less than both acute and control cattle at h 24, 25, 26, 49, 50, 73, and 74. Although a treatment × hour interaction ($P \leq 0.01$) was noted for blood urea nitrogen (BUN) concentrations, no differences existed within hour. Stress treatments did not affect ($P \geq 0.38$) final BW, ADG, DMI, and water intake from d 0 to 21. These results demonstrate that a CRH and VP challenge influences serum glucose, insulin, cortisol, electrolyte, and white blood cell concentrations. When comparing acute vs chronic treatments there were similar response on d 0 as both were administered CRH and VP, but from d 1 to 3 acute was different than chronic. These differences suggest that the chronic continued to

respond to the administration of CRH and VP on d 0 to 3. However, CRH and VP challenge demonstrated no effects on beef steer performance parameters of BW, ADG, and DMI suggesting that while there are changes in blood metabolites from the stress model used there was likely not a great enough response elicited to influence performance response

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The occurrence of stress has incited much debate over its definition and qualification in relation to cattle performance and well-being. Stress is a term that has taken on many definitions and has been difficult to define, yet has many negative consequences in the production and marketing of cattle. Stress has been termed as "the body's non-specific response to a demand placed on it" (Selye and Horava, 1953), "any external or internal challenge that disrupts the internal environment" (Sheridan et al., 1994), or "the sum of all biological reactions to physical, emotional, or mental stimuli that disturb an individual's homeostasis" (Pacak and Palkovits, 2001). Causes of stress can be characterized into 2 broad categories of either physical or psychological, and examples of these include hunger or disease and commingling or handling, respectively (Grandin, 1997). Physical stress caused from trauma is associated with evoking an immunological response cascade and psychological stress caused by surroundings is associated with a hormonal response cascade in cattle (Grandin, 1997). Exposure to these stressors can stimulate a biological response that may last for a few minutes up to weeks or months, which has been termed as either acute or chronic stress (Hughes et al., 2014). Acute stress occurs when the stressor duration is less than 24 h; such as a predator attack or human handling that is short term, but when stress persists for more than 24 h this is

termed as chronic stress. Chronic stress can occur when multiple acute stressors are compounded over time, an example of this is the receiving calf marketing process where a calf is transported from the cow calf operation to a sale barn to the feedlot and finally, processed at the feedlot into new commingled pens. However, both acute and chronic stress cause a cascade of events that can have negative impacts on animals' nutritional and immunological status after arrival to the feedlot, which can then affect dry matter intake (DMI), average daily gain (ADG), and gain to feed ratio (G:F). Furthermore, it not unusual for cattle to have limited access to feed and water during these points of the production system, thereby likely exposing them to both physical and physiological forms of stress, which may exacerbate the response and subsequent effects.

OVERVIEW OF THE STRESS RESPONSE

Stress is a stimuli that disrupts the bodies homeostasis, causing a biological cascade of events that may begin in the central nervous system and continue to the hypothalamus pituitary adrenal (HPA) axis (Minton, 1994). This cascade initiates when the central nervous system of the animal registers the stressor through the amygdala in the brain (Carroll and Forsberg, 2007). The amygdala then evokes the HPA axis at the base of the brain beginning with the hypothalamus to react (Carroll and Forsberg, 2007). Within the hypothalamus is the paraventricular nucleus, which is a group of neurons that can be stimulated to secrete corticotropin releasing hormone (CRH) and vasopressin (VP; Carroll and Forsberg, 2007). As CRH and VP is released, it is transported through the blood via the hypophyseal portal system to the anterior pituitary (Carroll and Forsberg, 2007). In the anterior pituitary, CRH and VP will bind to its target receptor to stimulate the release of adrenocorticotropin hormone (ACTH; Carroll and Forsberg, 2007). Once

ACTH is released, it will travel to the adrenal cortex of the adrenal glands to stimulate the release of glucocorticoids, such as cortisol, by binding to specific receptors within the adrenal cortex (Carroll and Forsberg, 2007). Cortisol will continue to be released until blood concentrations increase to a point of classic negative feedback on both the hypothalamus and anterior pituitary (Carroll and Forsberg, 2007). Once cortisol concentrations reach a maximum threshold, cortisol will inhibit the production of CRH, resulting in the inhibition of ACTH secretion and ending the increase in cortisol concentration (Bowen, 2019). This negative feedback loop allows for the body systems that have been stimulated (HPA axis) to return to homeostasis. As a way to allow for coping and adaptations over time, the body defense systems are designed for the ultimate survival of the organism (Carroll and Burdick Sanchez, 2014). Variation exists in the individual reaction to a given stressor and there are natural variations in stress response caused by perception of the stressor, duration of the stressor, temperament, breed, sex, and age of the animal (Hughes et al., 2014).

The majority of body cells (muscle cell, skin cells, nerve cells, etc.) express glucocorticoid receptors (GR) which allows cortisol to have diverse effects on the body and systems within the body (Oakley and Cidlowski, 2013). As cortisol is released in response to stressors, increased heart rate, blood pressure, blood glucose, respiration, and muscle tension is commonly observed. During the stress event, the body will temporarily shut down systems that are not needed to combat the stress such as metabolic and reproductive functions (Minton, 1994). These biological adaptations allow for the animal to react to, manage, and overcome the stressor.

Another group of hormones commonly associated with stress are the catecholamines: epinephrine and norepinephrine. Catecholamines are synthesized and secreted from chromaffin cells in the adrenal medulla of the adrenal glands, and are released in response to stimulation of the sympathetic nervous system (Bowen, 2019). Stressors stimulate the sympathetic nervous system via the hypothalamus of the brain to prepare the body for the flight-or-flight action. Receptors known as β– receptors are responsible for the mediation of vasodilation, increased heart rate and contraction of other organs, bronchodilation, and lipolysis that are associated with secretion of epinephrine, resulting in a 20 to 30% increase in metabolic rate (Kovacs and Ojeda, 2012). However, epinephrine and norepinephrine are difficult to measure experimentally due to a half-life of less than 1 min, which is why cortisol has been used more frequently in experimental measurements of stress.

POTENTIAL EFFECTS OF STRESS ON NUTRITION

Stress can interact with nutrition in 2 different ways: 1) produce or aggravate nutrient deficiencies, 2) nutrient deficiencies can prevent the animal from responding efficiently to the stressor or stressors (Cole et al., 1986). When cattle are exposed to stressors throughout the livestock marketing system, shifts in total body nutrient partitioning may negatively impact nutrient status and subsequently alter animal nutrient requirements. For example, as the body begins to react to stress and biological signaling cascades begin, there is a preferential shift in energy demand caused by changes in the hormones that regulate nutrient metabolism. These demands are met by either the body catabolizing energy stores such as adipose tissue and muscle cells or by nutritional supplementation. For example, cortisol acts as a lipolytic agent during both stress and

immune reactions, which could account for increased non-esterified fatty acid (NEFA) serum concentrations following a challenge event (Buntyn et al., 2016; Lippolis et al., 2017). Cortisol also promotes gluconeogenesis by stimulating the liver to convert fat and proteins into pyruvate, lactate, glycerol, and amino acids that are then converted into glucose for energy (Carroll and Forsberg, 2007). Consequently, greater serum glucose will likely cause increased serum insulin concentrations in an effort to increase glucose metabolism and uptake. For example, in a study where Brahman and Hereford steers were transported via truck over different time frames, greater serum concentrations of both glucose and insulin were observed immediately after transport (Browning and Browning, 2013), suggesting that nutrients are mobilized and used by the animal for energy supply to restore homeostasis.

In addition, research also indicates that cattle undergoing a stress and/or inflammatory event may catabolize body tissues to supply additional protein constituents needed during these times. During a stress incident the acute phase response (APR) is activated, which uses protein to synthesize acute-phase proteins, glucose pre-cursors, plasma proteins, antibodies, metabolic cofactors, and hormones (Li et al., 2007). Acute phase proteins that have been measured in cattle include haptoglobin, fibrinogen, and serum amyloid-A (Carter et al., 2002). Activation of the APR could potentiate a protein imbalance by altering nitrogen metabolism in favor of acute phase protein synthesis, particularly if protein intake is low. To combat this imbalance, the body will increase amino acid catabolism from tissues to support the shift in immune system caused by the stress event. Researchers have begun to consider the possibility of protein supplementation to alleviate this protein imbalance as protein deficiency has been

associated with immune suppression (Duff and Galyean, 2007). In a study where cattle were administered either LPS or no LPS and supplemented with ruminally degradable protein (RDP) or ruminally undegradable protein (RUP) a LPS × diet interaction was observed for nitrogen retention, suggesting that provision of 16% RUP may alleviate the tissue protein loss in LPS stressed cattle (Waggoner et al, 2009). In a series of studies Fluharty and Loerch (1996) designed to evaluate changes in differing crude protein (CP) concentrations, increasing CP above 12.5% resulted in greater ADG and DMI during the first 1 and 2 weeks after arrival, respectively. Over the entire 4 week trial, feed efficiency was improved in cattle fed 16% CP compared to 12.5% CP (Fluharty and Loerch, 1996), which again suggests that greater dietary CP is beneficial for cattle that may be experiencing stress and/or inflammation following feedlot arrival. However, more research is warranted to determine the optimal inclusion rate and degradability of CP in diets fed to stressed cattle.

When cattle are transported from ranch to sale barn and then to the feedlot, they are also deprived of both food and water which creates a physical stressor on these animals. As stressed cattle arrive at the feedlot their intake is low, averaging about 1.5% of body weight (BW) during the first two weeks, and at these low intakes it is difficult to meet nutrient requirements of the growing beef animal (Galyean and Duff, 1999). Upon arrival, stressed calves prefer diets with 72% concentrate during the first week, so for optimal eating patterns using higher-concentrate diets could be most beneficial (Lofgreen, 1983) as long as this is not offset by greater morbidity. Stressed calves consuming higher-concentrate diets had greater DMI in the first 2 weeks compared to stress calves consuming lower energy concentrate diets, but not for the remainder of the

28-d study (Fluharty and Loerch, 1996). This suggests that feeding higher concentrate diets to meet the altered energy demand associated with stress could compensate for lower DMI and enhance performance in stressed calves during the first 2 weeks in feedlot potentially allowing for improved performance the rest of the growing period.

RUMEN FERMENTATION

The enteric nervous system (ENS) regulates the supply of stress hormones in the gastrointestinal tract, which could alter both fermentation and digestibility via increasing cortisol and catecholamine concentrations (Verbrugghe et al, 2012). In ACTH challenged ewes, researchers concluded that salivary cortisol and serum cortisol concentrations were closely related, and therefore, swallowed saliva could be another route for cortisol to enter the rumen and intestinal tract (Yates et al., 2010). Introduction of stress hormones in the gut has the potential to alter the microbial populations of the rumen as well as the intestinal tract, and understanding these interactions can advance improvements in both animal health and production (Freestone and Lyte, 2009). Freestone et al., (1999) demonstrated that adding catecholamines to bacterial culture media increased the growth of *Streptococcus* bacteria. Freestone and Lyte (2010) have termed these interactions between host and microbes as "microbial endocrinology" and are using this platform to understand stress in relation to microbial populations and function in cattle. In serum-based culture media supplemented with catecholamines, a bacterial growth increase of 100 fold was observed compared to un-supplemented media (Lyte and Ernst, 1992; Freestone et al., 1999). These results suggest that even acute increases in catecholamines have the potential for lasting effects, as changes in microbial populations may influence

rumen fermentation and digestibility beyond the stress event. The specific factors that cause these changes in microbial populations have not been determined and research is still warranted in this area.

Changes in rumen microbial growth, species, and population may alter the rumen environment and fermentation characteristics such as ruminal pH, volatile fatty acids (VFA), and ammonia (NH_3) concentrations. In a study conducted by Galyean et al. (1981) where steers were not fasted or transported (CON), fasted and not transported (F), or fasted and transported (FT) via truck for 32 h, VFA concentrations were lower in FT steers compared to F steers. This could suggest that the added stress from truck transportation changed fermentation, rumen motility, and/or negatively impacted absorption and passage rate in the steers compared to fasting alone (Galyean et al., 1981). Ammonia concentrations followed similar patterns in F and FT steers (Galyean et al., 1981). In an in vitro study conducted by Samuelson et al., (2016), greater total VFA were observed when salivary cortisol was increased in dual-flow continuous culture fermenter systems from 0 to 3 ng/mL, but no difference in NH_3 between treatments was documented until after cortisol treatments were no longer applied (during recovery). Digestibility of organic matter (OM) and neutral detergent fiber (NDF) tended to be less for 9 ng/mL cortisol compared to 0, 3, or 6 ng/mL which researchers speculated could be caused by shifts in bacterial populations (Samuelson et al., 2016) although the rumen microbiome was not quantified. Therefore, it is possible that greater concentrations of stress hormones, such as cortisol, may cause changes in fermentation and digestibility in ruminants as a result of host microbiome interactions (Samuelson et al., 2016).

DIGESTION

The digestive system is highly regulated by the neuroendocrine system, with the main branches of stimulation occurring from the ENS and the gastroenteropancreatic (GEP) endocrine system. The ENS is a branch of the autonomic nervous system (ANS) which controls a variety of gut functions including water absorption, movement of electrolytes, and gut motility (Bravo, 2015). The ENS then branches into the parasympathetic (stimulates GIT secretion and contraction) and sympathetic (inhibits GIT secretions and motility) nervous systems (Tsuda et al., 2012). All of these systems could be impacted by changes caused by stressors perceived by the central and autonomic nervous systems. The main hormones that stimulate the GI tract include gastrin, cholecystokinin (CCK), glucagon-like peptide (GLP), and gastric inhibitory polypeptide (Hadley and Levine, 2006). Gastrin secretion is regulated by distention and the presence of acid and amino acids in the lumen of the stomach. When feed is consumed, the pH of the lumen will decrease due to secretion of hydrochloric acid to initiate the breakdown of feedstuffs (Hadley and Levine, 2006). Therefore, when cattle intakes are low, which is common in stressed, newly received cattle, gastrin secretion may decline and slow passage rate because the pH remains high and does not stimulate opening of the pyloric sphincter. Cholecystokinin is stimulated by the ENS as glucose, lipids, and proteins are sensed in the duodenum and may be involved in satiety signaling in the brain as well as digestion (Samuelson, 2018). Motilin is secreted into the lumen during the fasting state to empty the stomach and small intestine of the undigested portion of the previous meal, which is known as the migrating motor complex (Hadley and Levine, 2006). This process increases gastric emptying between meals to prepare the GI tract for the next meal.

Glucagon-like peptide (GLP) is present in two forms, GLP-1 and GLP-2. Fatty acids and proteins are known stimulants of GLP-1 and as GLP-1 is secreted, inhibition of gastric emptying and passage rate occurs. This regulation of appetite is thought to be mediated by GLP-1 receptors (Steinart et al., 2013). Gastric inhibitory peptide inhibits gastric secretions and motility and is stimulated by the presence of fat and glucose in the small intestine. Gastric inhibitory peptide has also been observed to increase the release of insulin in response to greater plasma glucose concentrations (Lewis, 1961).

In ruminants, the rumen is able to use feed that is unable to be digested by non-ruminant animals. The rumen uses microbial fermentation to break down this feed when microbes attach to incoming feed and release degradative enzymes. The rumen uses muscular pillars to contract and mix, sort, ruminate, and pass digesta. These contractions increase during feeding and can alter motility (Samuelson, 2018).

Hormones such as cortisol and insulin impact satiety and digestive tract function (Allen, 2009; Klasing and Korver 1997) and DMI is a physical regulator of rumen digestibility, motility, and rate of passage (Allen, 2009). Reduction in DMI is a typical observation in stressed animals and is a result of cross talk between cytokines and intake centers of the brain as well as reduction in gut motility (Allen, 2000; Klasing and Korver, 1997; Lippolis et al., 2017). Decreased DMI has been attributed to a reduction in ruminal contractions which decreases rumen motility, and passage rate of solids and liquids (Allen, 2000; Waggoner, 2009). In a study conducted by Lippolis et al., 2017, where 8 cannulated steers were infused with LPS, reduced DMI was observed on d 0 and 1, rumen liquid passage was reduced for 24 h after the challenge, and ruminal disappearance and degradability of DM was decreased for 48 h after the challenge in LPS steers

compared to steers administered saline. Greater cortisol concentrations were observed 2 to 16 h after the challenge and greater insulin and glucose concentrations were observed 2 h after the challenge followed by a decline in glucose from 4 to 12 h post- LPS administration. These results agree with work discussed previously in this review, which supports the hypothesis of the interactive effects on DMI, digestibility, motility, and passage rate in stressed cattle.

POTENTIAL EFFECTS OF STRESS ON THE IMMUNE SYSTEM

INNATE IMMUNE SYSTEM

There are two components to the immune system: innate immunity and adaptive immunity. Both components are needed for the immune system to provide adequate protection against infection, disease, or trauma. The innate immune system is a non-specific defense mechanism that will respond immediately once exposure to an antigen occurs and is considered to be the first line of defense against pathogens (Richeson, 2018). This front line of protection includes physical barriers (ex: skin, mucosal secretion, stomach acid) and complement and antigen-nonspecific cellular components. The cellular components of the innate immune system include neutrophils, monocytes, macrophages, dendritic cells, natural killer cells, basophils, and eosinophils that recognize pathogen-associated molecular patterns (PAMPs) to initiate pathogen killing mechanisms (Galyean and Duff, 1999). As the innate immune system responds to an invasion, cytokines will be released and initiate the APR (Mani et al., 2012). When the APR begins there will be fever, synthesis of acute phase proteins (APP) by the liver, increased white blood cells (WBC), and changes in behavior (Carroll and Forsberg, 2007). Once the release of proinflammatory cytokines becomes systemic, this will

stimulate the release of APP from the liver resulting in a febrile response and changes in liver metabolism. With this initial line of defense, the immune system is given time (over several days up to several weeks) to warrant a specific antibody response against pathogens via the adaptive immunity component.

Adaptive Immune System

Adaptive immunity is the component of the immune system that adapts and provides specific immune responses against pathogens for future encounters with previous pathogens by producing specific antibodies with immune memory (Richeson, 2018). If the immune system encounters the same pathogen again, then it has the ability to mount a faster and stronger response compared to the first-time exposure. There are two classifications to adaptive immunity: cell-mediated immunity and humoral immunity. Cell-mediated immunity involves the immune cells that fight against pathogen infected cells where humoral immunity involves the production of specific antibodies that fight against the pathogen. Both B and T lymphocytes (B and T Cells) are an important part of the adaptive immune response (Richeson, 2018). The B-cells produce antibodies that attach to the antigen and engulf the antigen to then present it to the T-cell. The T-cell can be divided into T-helper (T_{H1} and T_{H2}) cells (Richeson, 2018). These cells produce cytokines that stimulate B and T-cells to grow and divide to protect against future pathogen invasions. The T_{H1} cell produces proinflammatory cytokines (IFN-g and IL-2) which promote cellular immunity (immune response is carried out by cytotoxic cells) where T_{H2} cells produces anti-inflammatory cytokines (IL-4, IL-10, IL-13) to promote humoral immunity (immune response carried out by serum antibodies; Carroll and Forsberg, 2007).

Effects of Stress on Immune Systems

As knowledge of the detrimental effects of stress on the immune system has expanded, the terms immunoenhancing and immunosuppressive have been suggested as effects of stress on the immune system. Immunoenhancing effects can be described as the preparation of the immune system to respond to an invasion more appropriately, whereas, immunosuppression does not allow the immune system to fight invasions as necessary (Carroll and Burdick Sanchez, 2014). The immune system may be negatively affected due to dehydration, nutrition deficiencies, genetics, and stress (Carroll and Forsberg, 2007). Each system within the body serves a purpose and the purpose of the inflammatory response is to react to disturbances by attacking the source and allowing adaptation to unnormal conditions to ultimately regain functionality and homeostasis in the area of disturbance (Medzhitov, 2008). When the HPA axis is stimulated, some functions of the immune system are inhibited. The most common factor in change of the APR is stress (Hughes et al., 2014). This immune response is initiated by the innate immune system that is activated by cytokines (Lippolis et. al., 2017). The innate immune response is the body's first line of defense and response to invading pathogens and antigens. The APR is another early defense mechanism in response to trauma, infection, or inflammation and is activated by a systemic reaction of events (Hughes et. al, 2014).

An increase in serum cortisol concentration is used as an indicator of stress but is also associated with a decrease in the functionality of immune cells themselves (Minton, 1994). Throughout the body there are receptors to activate cell functionality, and during a stress response glucocorticoids may either stimulate or inhibit the innate immune response via GR receptors on immune cells. Continued increase in concentrations of

cortisol during chronic stress has been associated with preventing the release of pro-inflammatory cytokines, decreasing B-cell Ab production, and reducing inflammation (Hughes et. al, 2014). This can be either beneficial or detrimental to the animal. There are biological variations in response to stress from animal to animal due to breed, sex, and temperament. For example, temperamental cattle tend to have greater basal levels of cortisol compared to calm animals (Burdick Sanchez et al., 2016). Cortisol protects temperamental cattle by mitigating APR return to homeostasis. (Burdick Sanchez et al., 2016).

A primary APR indicator of stress is haptoglobin, which is a major hemoglobin-binding protein. By binding to free hemoglobin, it prevents the oxidative damage of tissue, which in turn limits the available iron for bacteria growth, creating a bacteriostatic environment (Carroll and Burdick Sanchez, 2014). In response to stress, there is typically an increase in haptoglobin and pro-inflammatory cytokines, but it is a more delayed and stable response compared to the innate immune response (Carroll and Burdick Sanchez, 2014). Cytokines such as TNF-α and IL-6 are a part of the acute inflammatory response and are important mediators of immune function by playing a role in tissue repair (Carroll and Forsberg, 2007). In cattle receiving an LPS challenge, an initial increase in both TNF-α and IL-6 followed by a gradual decline was observed (Carroll and Burdick Sanchez, 2014). Similar results have also been reported for IL-6 in calves receiving a CRH challenge (Carroll and Burdick Sanchez, 2014). However, not all stressors that activate the HPA axis reduce immune cell functions as research completed by Minton et al. (1990) using a single 6-h RIS challenge did not alter the lymphocyte blastogenic response to mitogens.

MODELS OF STRESS IN RESEARCH

In research there are multiple stress challenge models available, which contributes to variance among individual studies and difficulty interpreting the reported results as a whole. Some challenge models initiate physiological stress by using what happens naturally to the animal (such as transportation during the marketing system), while others directly alter the hormone signaling cascade by administering exogenous hormone injections (such as ACTH or CRH). These models can produce varied results in the animals stress response due to a more "controlled" verses natural approach, however, there are also other factors that may cause dissimilarities. For example, differences in research results can also be due to variation in breed, age, or sex of the animal, the type of model used, magnitude of the stress response, route of administration, environmental effects, and many other factors. It is important to understand each model and to acknowledge the difference in outcomes that may occur simply as a result of the variety in models used between research designs.

CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN

Corticotrophin releasing hormone and VP are released from the hypothalamus as part of the HPA axis and have a synergistic effect when secreted together, producing a greater response than when secreted alone (Kovacs and Ojeda, 2012). Therefore, CRH and VP have been used together to simulate a stress challenge in research and are stimulators of ACTH secretion from the pituitary gland. A common dose in cattle is 0.3 µg/kg of BW and 1.0 µg/kg of BW administered intravenously for CRH and VP, respectively (Carroll and Forsberg, 2007; Cooke et al., 2012; Falkenberg et al., 2013) There have also been studies using CRH only (0.5 µg/kg of BW), but given the

synergistic effect of CRH and VP it is likely these animals experience a different level of stress. Hulbert et. al, (2012) used CRH as a stress model with weaned Brahman calves and reported fluctuations in rectal temperature, increased cortisol, and changes in leukocyte populations 1 h after the challenge. When compared to truck transportation stress studies in calves, the CRH and VP model of stress results in a similar endocrine response, such as greater serum cortisol 1 h after the challenge (Falkenberg et al., 2013; Carroll and Forsberg, 2007).

Adrenocorticotropin Hormone

Adrenocorticotropic hormone (ACTH) is produced by the anterior pituitary when the HPA axis is stimulated by the hormones of the hypothalamus (CRH and VP). Once secreted, ACTH directly stimulates the release of glucocorticoids such as cortisol from the adrenal glands. Therefore, administration of ACTH is adjacent to the release in cortisol within the HPA axis compared to CRH and VP, which influences cortisol from higher up in the HPA axis cascade. A typical dose administered in stress challenge research conducted in both lambs and cattle is 80 IU of ACTH injected intravenously (Nockels et al., 1993). In a study completed in lambs that received ACTH, greater plasma cortisol concentrations were reported from h 1 through 4 post-injection than that observed in lambs receiving a saline injection only (Schroeder et al., 2018). In a study carried out by Nockels et al. (1993), they also observed greater serum cortisol for up to 8 h in crossbred steers after administration of 80 I.U. of ACTH. In a CRH and VP challenge study in heifers, serum cortisol increased 0.5 h after administration and did not return to near baseline concentrations until 5.5 h after the challenge (Buntyn et al., 2016).

LIPOPOLYSACCHARIDE

Bacterial lipopolysaccharides (LPS) are components of many gram-negative bacterial pathogens that stimulate an endotoxin stress and inflammation response. Doses of LPS used in research vary and examples used are 0.2, 1.0, 1.5 µg/kg of BW and can be administered either subcutaneously or intravenously which could alter the severity of the response (Bernhard et. al., 2012; Löest, 2018; Waggoner, 2009). In a study described by Waggoner et al. (2009), growing calves administered 1.5 µg/kg of BW of LPS had greater rectal temperatures for 6 h after LPS administration than calves receiving no LPS. Additionally, serum cortisol increased from less than 20 ng/mL before LPS administration at h 0 to between 60 to 80 ng/mL at h 2 and did not return to baseline values within 12 h of LPS treatment (Waggoner et al., 2009). Löest et al. (2018) observed similar results when steers were administered 1.0 to 1.5 µg/kg of BW LPS as there were greater serum cortisol concentrations than control steers from 4 to 24 h after receiving LPS. Lipopolysaccharide initiates an acute pro-inflammatory immune response when dosed in cattle and may be responsible for a decrease in animal performance such as decreased DMI and ADG (Hulbert et al., 2013). As the LPS challenge stimulates both an inflammatory and stress response it could elicit a greater response in beef cattle compared to other models.

DEXAMETHASONE

Dexamethasone is a synthetic glucocorticoid commonly used to treat inflammation, but has also been used as a model for stress in beef cattle. Research suggests that glucocorticoids negatively influence immunity by altering immune cell function (Burton et al., 2005). Richeson et al. (2016) conducted a study in steers using

none, acute (0.5 mg/kg of BW on d 0), or chronic (0.5 mg/kg of BW on d -3 through 0) dexamethasone administration to determine immune status following administration of a respiratory vaccine. Richeson et al., (2016), observed that control had greater serum haptoglobin concentrations than chronic. Researchers also observed that the chronic treatment had greater bovine viral diarrhea virus (BVDV) titers than the acute and control treatments, suggesting that the chronic treatment caused immunosuppression that allowed enhanced modified live virus (MLV) replication (Richeson et al., 2016). In a study conducted by Seiver et al., (2019), where cattle were administered 40 mg of dexamethasone on d 0 through 2 greater white blood cell, neutrophil, and monocyte counts and a decline in lymphocyte count were observed which authors attributed to demonstrate leukocytosis and immunosuppression. This model does not seem to be as commonly used in research due to researchers wanting to elicit a more specific stress response but has been used in research evaluating health status.

Natural Stress

Natural stress models can also be used in research to mimic what occurs as a result of environmental and management stressors that may have negative consequences on animal well-being and performance in cattle (Cooke et al., 2012). Environmental stressors can include temperature, wind, rain, and snow whereas management stressors can include cattle handling, comingling, weaning, vaccination, etc. (Cooke et al., 2012). Additionally, transportation has been an area of research interest in recent years as this is a major component of the cattle marketing process and has been shown to elicit a stress response in livestock. For example, steers transported 344 km had greater blood concentrations of cortisol, glucose, and leukocytes than cattle maintained in holding pens

for the same length of time (Browning and Leite-Browning, 2013). Because they are herd animals, livestock (but sheep in particular) become stressed when isolated from one another. Therefore, in sheep, researchers have commonly used restraint and isolation stress (RIS) as a stress challenge model. In a study completed by Minton et al. (1992), lambs exposed to RIS for 6 h for 3 consecutive days had greater plasma ACTH and cortisol concentrations on d 1 and 3 of the challenge compared to lambs that received no treatment or cortisol. However, there are also negative implications to using natural stress models as these challenges are harder to control, which could compromise data outcomes.

There are many factors that can play a role in which stress model is chosen by a researcher including availability and the magnitude of stress desired. Minton (1994), mentions that injection of hormones does not include the full involvement of the central nervous system in cognition, integration, and homeostatic responses to a stressor. Carroll et al. (2014) noticed a more rapid increase in vaginal temperature when heifers were given 0.3 µg/kg of BW of CRH and 1.0 µg/kg of BW of VP compared to heifers given LPS, yet the magnitude of temperature increase was greater in LPS compared to the CRH and VP challenge. When comparing similar parameters between studies, it is important to note what stress model is being used in the study as well as the dose. Being aware of these slight differences allows for further understanding of why results may vary from study to study. For example, when relocating heifers, Falkenberg et. al. (2012) observed a rectal temperature of 39˚C and an increase in cortisol from 24 to 44 ng/mL after 120 min of truck relocation. Hulbert et. al. (2015) observed the same 39˚C rectal temperature and an increase in cortisol from 20 to 40 ng/mL 1 h after a CRH challenge. In an LPS challenge, Waggoner et. al. (2009) reported the same 39˚C rectal temperature and an

increase in cortisol from 60 to 80 ng/mL 2 h after infusion of 1.5 µg/kg of BW of LPS, but also observed an increase in serum glucose concentrations from 90 to 120 mg/dL. Dunlap et. al. (1981) also reported that cortisol concentrations in postpartum beef cows increased from 60 to 80 ng/mL in postpartum beef cows 1 h after administration of 200 IU ACTH. Comparing these research studies highlights there is a different stress model being used, blood samples were collected at different times post stress, and different variables were measured, all of which possibly impacted the reported outcomes.

EFFECTS OF STRESS ON PERFORMANCE AND HEALTH IN THE FEEDLOT

Cattle have the ability to cope with stressful situations over time as an evolutionary design for survival (Dragos, 2010). Multiple stressors that cattle experience during transition from the cow-calf operation to the feedlot can create a compounding effect that leads to chronic stress circumstances. Recent studies suggest that stress, and more specifically chronic stress has negative impacts on health, well-being, and productivity of animals. (Hughes et al., 2014). As mentioned previously, there is also evidence that stress decreases DMI which will inadvertently decrease overall animal performance including ADG and F:G via nutrient deficiency or reduction (Duff and Galyean, 2007; Galyean and Hubbert, 1995).

Stress is also associated with greater body temperature and heart rate as a result of changes in concentrations of stress hormones and the immune response. Catecholamines are known to increase heart rate, vasodilation, and bronchodilation by binding β-receptors on effector cells (Kovacs and Ojeda, 2012). The immune response induces the synthesis of cytokines causing inflammation, and as a coping strategy cattle decrease metabolic heat production by reducing DMI to alleviate an increase in core body temperature

(Carroll and Forsberg, 2007). These changes in body temperature and heart rate as well as others may leave the body in a deficient state by increasing metabolic needs while DMI is low. To compensate for changes in energy metabolism the animal may adjust activities such as increased sleep, and decreases in foraging and social behavior to conserve energy (Carroll and Forsberg, 2007). When in a deficient state and introduced to an immune challenge, the animal may not have the ability to overcome the challenge which has the potential to increase the rate of both morbidity and mortality (Carroll and Forsberg, 2007).

EFFECTS OF ACUTE VS. CHRONIC STRESS

As emerging research is conducted there has been speculation that acute stress may prime the immune system, whereas chronic stress and prolonged exposure to stress hormones can shift immune cells in favor of an immunosuppressive event (Carroll and Forsberg, 2007). Acute stress is associated with a duration of less than 24 h and the hormones produced are thought to prime the immune system for a possible pathogen invasion that could lead to infection. In contrast, for an animal affected by prolonged, chronic stress lasting more than 24 h, hormones shift immune system function from a preparation stage to a suppressive stage (Hughes et al, 2014).

The immunopriming effects of acute stress are caused by the bodies first response to an increase in glucocorticoids that will potentiate effects such as proliferation and activation of effector cells. The immune cells will start the initial release of cytokines which in turn stimulates the continued release of glucocorticoids. With increased cortisol, the cells will then self-regulate to prevent over stimulation and possible self-destruction, as well as immune cell resistance to future cortisol stimulation (Elenkov et al., 2002). In

rats subjected to delayed type hypersensitivity, acute stress administered for 2 h increased leukocyte proliferation whereas, chronic stress administered for 5 hours suppressed proliferation and relocation (Dhabhar and Mcewen, 1997).

When immune cells become tolerant to cortisol, they may lack the ability to respond properly to continued stress (chronic stress) and possibly develop an excess response mechanism (Derijk and Sternberg, 1994). Cortisol can also inhibit gene expression within immune cells by passive diffusion into cells that then bind to GR receptors, which can alter genes within the nucleus of the cell. Cells affected by gene expression include lymphocytes, macrophages, and granulocytes which makes them more susceptible to changes during stress (Rabin, 1999). As the stress response initially is stimulatory to the immune system for preparation, after continual stimulation the effects become inhibitory as the production of cytokines, antibodies, and other immune cells declines.

For example, as an animal experiences continued stressors it prolongs the secretion of glucocorticoids (cortisol) that stimulates the cells of the immune system to secrete cytokines, which in turn stimulates the secretion of more glucocorticoids. If this cycle continues, there is a possibility of heightened alert in immune cells that could lead to self-attack, so to protect itself the body will down-regulate the cells after continual glucocorticoid stimulation (Carroll and Forsberg, 2007). The negative feedback mechanism of the HPA axis in response to elevated concentrations of glucocorticoids and subsequent CRH, VP, and ACTH secretion allows for regulation of the response. Another way chronic glucocorticoid exposure disrupts immune cells is by inhibiting gene expression by activating the GR receptors in the immune cell. As glucocorticoids

increase and bind the higher affinity mineralocorticoid receptor (MR), the glucocorticoid will then occupy the GR receptors on lymphocytes, macrophages, and granulocytes invoking proliferation, cytokine secretion, antibody secretion, and cytolytic activity (Madden, 1995; Scheinman, 1995). Chronic exposure to glucocorticoids has negative effects on the thymus as well by causing pre-B and pre-T cell apoptosis which will reduce the formation of lymphocytes and cause atrophy of the thymus gland itself (Fraker, 2004). In a dexamethasone study, authors concluded that both acute and chronic stress suppressed the APR, but were unclear if the model was parallel with what might be observed in field conditions (Hughes et al., 2017). Current research is limited on the effects of duration of naturally occurring and exogenous hormone stress outside of transit stress models on the nutritional status and digestion in cattle and additional work needs to be done comparing acute vs. chronic stress challenges on these parameters.

CONCLUSIONS FROM THE LITERATURE

Stress is a complex phenomenon that affects many integrated systems including the central nervous, neuroendocrine, digestive, and the immune systems. As stress occurs throughout the beef production system, it is important for producers to understand these stressors to help mitigate the potentially negative impacts on both animal health and nutrition. Though these behavioral and metabolic changes are important for the animal's survival, the overall negative impact may limit the nutrients available for other important functions such as growth and performance. As researchers continue to understand the full effects of stress on livestock production the differentiation between acute and chronic stress requires further emphasis in research.

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CHAPTER II

IMPACTS OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN STRESS CHALLENGE DURATION ON COMPLETE BLOOD COUNT AND SERUM METABOLITES OF GROWING BEEF STEERS

ABSTRACT

Corticotropin releasing hormone (CRH) and vasopressin (VP) administration in cattle induces an endocrine stress challenge, which may impact immune and nutritional status. The objective of this study was to evaluate stress challenge duration on metabolic, hematologic, endocrine, and performance parameters in growing beef steers. Thirty steers (416 ± 19 kg of body weight) were used in a randomized complete block design with 2 blocks and 3 treatment groups. Steers were housed individually for 14-d adaptation, a 6-d collection period, and a 15-d post-challenge observation period, for a total of 35-d. Treatments were intravenous injection of: 1) Control (saline on d 0 through 3; n = 10); 2) Acute (CRH and VP injection on d 0, saline on d 1 through 3;n = 10); or 3) Chronic (CRH and VP injection on d 0 through 3; n = 10) at h 0, 24, 48, and 72. Rectal temperatures and serum samples were collected at h 0, 1, 2, 24, 25, 26, 48, 49, 50, 72, 73, 74, 144, and 336 respective to treatment injection for analysis of cortisol, insulin, and blood metabolites. Additionally, whole blood and serum were collected at h 0, 24, 48, 72, 144, and 336 for analysis of complete blood count, and non-esterified fatty acids (NEFA). Rectal temperature tended to be greater for both stress treatments relative to the control (stress vs. no $P = 0.06$). Cortisol was greater for cattle receiving the acute and chronic

treatments than control at h 1 and greater for chronic than both acute and control at h 25, 26, 49, 50, 73, and 74 (treatment × hour $P \leq 0.01$). Similarly, there was a treatment × hour interaction ($P \leq 0.01$) for serum glucose and serum insulin concentrations where cattle receiving the acute and chronic treatments had greater glucose at h 1 relative to control and cattle receiving chronic had greater glucose at h 25, 49, 50, 73, and 74 than acute and control. Serum insulin concentrations were greater in chronic and acute treatments than control at h 1 and greater for chronic compared to acute and control at h 25, 49, and 73 (treatment × hour $P \leq 0.01$). Serum phosphorus was lower for chronic and control than acute at h 25, lower for chronic than acute at h 26 and 49, and lower for chronic than acute and control at h 50, 73, and 74 (treatment × hour interaction $P \leq 0.01$). A treatment × hour interaction ($P \leq 0.01$) was also observed for serum potassium (K) where acute and chronic had lower K at h 1 and 2 than control and chronic was less than both acute and control cattle at h 24, 25, 26, 49, 50, 73, and 74. There was a treatment × hour interaction ($P = 0.003$) for total white blood cell count such that chronic had greater concentration than control but was not different from acute at 72 h. Conversely, monocyte concentration was less at h 144 for chronic cattle than both acute and control (treatment × hour interaction $P \leq 0.01$). Stress treatments did not affect ($P \geq 0.13$) red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, neutrophils, or neutrophil-to-lymphocyte-ratio. Serum NEFA tended to be lower ($P = 0.09$) in controlled cattle compared to those that were stressed. These results demonstrate that a CRH and VP challenge influences serum glucose, insulin, cortisol, electrolyte, and white blood cell concentrations.

INTRODUCTION

Stress involves a complex interaction between the hypothalamic pituitary adrenal (HPA) axis, central nervous system, and the sympathetic nervous system that interact with many other biological systems, such as the immune system. Stress induced changes in the neurological and endocrine systems may alter immune status and/or immune response of the animal by mitigating changes in immune cell population and functionality (Hughes et al., 2015). As economic loss due to morbidity and mortality caused by disease, such as bovine respiratory disease, increases researchers have suggested there is an association between stress, nutrition, and animal health (Galyean et al., 1999). In addition to elucidating the complex interactions between stress, the immune system, and nutritional status, there is importance in researching methods of reducing stress as this may improve livestock performance reduce death loss.

The length of time that animals are undergoing stress may have different impacts on the bodies systems and are categorized as either acute or chronic stress. Acute stress is only endured for a short length of time (less than 24 h) and stimulates the animal's flight-or-fight response, whereas chronic stress is experienced for a longer length of time (more than 24 h) and stimulates a prolonged response that is more likely to impact the animal long term. Glucocorticoids, specifically cortisol in cattle, are released during the stress response and elicit biological effects such as metabolic shifts, alterations in growth, regulation of the stress response, and influences on immune function (Carroll and Forsberg, 2007).

Many studies have examined the effects of acute stress, but there is little work on the comparison of acute vs. chronic stress models. The objective of the current study was

to determine the effect of an acute vs. chronic stress challenge on serum metabolites, complete blood count, and performance of beef cattle administered an injection of CRH along with VP. Our hypothesis was that increasing the duration of time cattle are stressed (i.e. chronically stressed cattle) would result in more negative effects as evidenced by differential changes in the biological markers of nutritional and immunological status.

MATERIALS AND METHODS

This study was conducted from March 2018 to June 2018, during 2 separate 35-d periods, at the West Texas A&M University Research Feedlot in Canyon, TX. All experimental procedures followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching and were approved by the Institutional Animal Care and Use Committee at West Texas A&M University (IACUC #02-02-18).

ANIMALS AND TREATMENTS

Thirty crossbred Angus steers from the West Texas A&M University Nance Ranch were received for this study. Before initiation of the study, steers were trained with halters to be acclimated to human contact, restraint, and isolation. The 35-d observation period consisted of 14-d for adaptation, 6-d for collections, and 15-d for post-challenge observation. On d -15 and -14 steers (n=30; initial BW= 416 ± 20 kg) were weighed and stratified by body weight (BW) into 2 blocks due to limited availability of individual feeding pens. Within each block, cattle were randomly assigned to 1 of 3 treatments injected intravenously in the jugular vein: 1) Control (saline (3 mL) daily from d 0 to 3: n = 10), 2) Acute 0.3 µg/kg of BW CRH (Bachem Ag, Bubendorf, Switzerland) and 1.0 µg/kg of BW arginine VP (Sigma-Aldrich, Co. St. Louis, MO) on d 0, followed by saline (3 mL) on d 1 to 3: n = 10), or 3) Chronic 0.3 µg/kg of BW of CRH and (1.0 µg/kg of

BW) VP from d 0 to 3; n = 10). Therefore, this study was a randomized complete block design consisting of 30 animals in 2 blocks with 3 treatments (10 animals per treatment). Individual animal served as the experimental unit. Throughout the study, steers were individually penned, fed, and watered.

BLOOD COLLECTION AND ANALYSIS

Blood was collected via jugular venipuncture into a 4 mL evacuated tube containing EDTA (BD Vacutainer K2EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) at h 0, 24, 48, 72, 144, and 336 respective to initial administration of treatments on d 0. After collection, samples were transported to the West Texas A&M University Animal Health Laboratory and immediately analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME). This analysis was used to evaluate complete blood counts including red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), white blood cell count (WBC), and concentrations of neutrophils (NEU), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO), neutrophil percent (NEU%), lymphocyte percent (LYMPH%), monocyte percent (MONO%), eosinophil percent (EOS%), and basophil percent (BASO%) as well as neutrophil to lymphocyte ratio.

Blood was also collected via jugular venipuncture into a 10 mL evacuated tube containing no additive (BD Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) at h 0, 1, 2, 24, 25, 26, 48, 49, 50, 72, 73, 74, 144, and 336 respective to the initial administration of treatments. Samples were placed into an insulated cooler with ice packs upon sampling, transported to the West Texas A&M University Animal Health

Laboratory, and centrifuged at 2,000 × *g* for 20 min at 20˚C. Once centrifugation was complete, serum was extracted and aliquoted into 3 labeled microtubes that were stored at -20˚C until further analysis. Before analysis, serum was thawed overnight in a laboratory refrigerator at 4 ˚C. An automated chemistry analyzer (Vet Scan VS2, Abaxis, Union City, CA) was used to measure serum chemistry variables including glucose, blood urea nitrogen (BUN), calcium (Ca), creatine, albumin, phosphorus (P), sodium (Na), potassium (K), chloride (Cl), and carbon dioxide (CO_2) concentrations.

Serum insulin was analyzed on the same set of samples using a commercially available bovine-specific insulin enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Alpco Diagnostics, 80-INSBO-EO1, Salem, NH). The minimum detectible concentration was 0.1 ng/mL of insulin and the intra- and interassay coefficients of variation were 6.58 and 4.99%, respectively. Serum NEFA concentrations were analyzed using a commercially available enzymatic assay kit (HR Series NEFA HR (2); Wako Diagnositics, Richmond, VA) as described by Buntyn et al. (2016). Serum cortisol concentrations were analyzed by the endocrinology laboratory at New Mexico State University in Las Cruces, NM using a radioimmunoassay (RIA) as described by Yates et al., (2010). The intra- and interassay coefficients of variation were 2.9 and 5.6%, respectively. Rectal temperature was recorded at h 0, 1, 2, 24, 25, 26, 48, 49, 50, 72, 73, 74, 144, and 336 using a digital thermometer (GLA Agriculture Electronics, San Luis Obispo, CA).

PERFORMANCE

Cattle were fed equal parts of a common receiving diet (Table 2.1) twice daily at approximately 1.75% of BW from d -14 to 7. Animals were limit-fed during the

collection period to minimize feed refusals and mimic the low DMI typically observed for newly received calves. From d 7 to 21, feed delivery was increased by 0.227 kg per day on a DM basis if all feed was consumed by the animal in an attempt to achieve ad libitum intake during the post-challenge observation period and determine if performance impacts existed after the stress challenge. Animal BW was obtained on d -14, 0 and 21 to determine initial, treatment, and final BW and all weights were recorded on 2 consecutive days to account for gut fill differences and minimize variation. Average daily gain (ADG) was determined for d 0 to 21 by taking the final BW minus the BW on d 0 then dividing by 21 d. Dry matter intake was determined by calculating dry matter of the feed offered to the animal from d 0 to 21. Water intake was determined using a digital display water meter (Fill-Rite 820, Fort Wayne, IN), fitted with a water hose on one end and an open and close water valve on the opposite end, to deliver and measure amount of water added to the water trough each day.

STATISTICAL ANALYSES

All variables were analyzed using SAS 9.3 (SAS Inst. Inc., Cary, NC). Animal was used as the experimental unit in a randomized complete block design experiment. Thirty steers were blocked by weight (15 lightest steers in block 1; 15 heaviest steers in block 2). There were 10 steers per treatment. The MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) with repeated measures was used to analyze CBC variables, serum metabolites, and serum chemistry. Akaike information criterion was used to determine the best fit covariance structure to be used for each dependent variable. The UNIVARIATE procedure was used to test normality. Variables that were determined to be non-normal were $\log_2$-transformed to achieve improved normality. The model for

these variables included the effects of treatment, hour, treatment × hour, and a contrast statement was used to evaluate the contrast of stress compared to no stress treatments. Statistical significance was established if the *P*-value was ≤ 0.05 and a tendency was reported if the *P*-value was between 0.06 and 0.10.

RESULTS AND DISCUSSION

COMPLETE BLOOD COUNT

Concentrations of RBC, HGB, HCT, MCV, MCHC and MCH were not different ($P \geq 0.13$) among treatments. There was a treatment × hour interaction for RDW (Fig 2.1; $P < 0.01$); and evaluation of the within hour means revealed there were no relevant time points that were different among treatments. A treatment × hour interaction was observed for PLT ($P = 0.02$; Fig 2.2) where PLT count was less in acute cattle than control at h 144 but not different than chronic. These results are not consistent with those reported by Hughes et al. (2015) where DEX administration did not affect PLT. In contrast, in a CRH and VP challenge conducted by Buntyn et al. (2016), an increase in PLT was observed immediately after administration. There was a treatment × hour interaction for WBC ($P \leq 0.01$; Fig 2.3), such that WBC was greater for cattle receiving the chronic treatment than control 72 h after the initial CRH and VP challenge but did not differ from acute. Greater WBC could be an indication of activation of the immune system response following the administration of CRH and VP or stress-associated effects on leukocyte translocation. Hughes et al., (2015) conducted a similar study where 0.5 mg/kg of BW of dexamethasone (DEX) was administered on d 0 (acute), d -3 through 0 (chronic), or no DEX (control) and observed an increase in WBC in chronic steers compared to control through d 1. In a CRH and VP challenge using the same dose as the current study, greater

WBC was observed from 2 to 8 h after administration of the challenge (Buntyn et al., 2016). Increased WBC have also been reported in natural stress challenge models. When transporting Brahman and Hereford steers for 4 h, greater total WBC count was observed compared to penned cattle (Browning and Leite-Browning, 2013). The delayed increase in WBC reported in the present study may have occurred because of the time needed for the immune system to mount a response post-challenge. However, there is a possibility that we might have observed more pronounced differences in WBC in the current study if blood samples for CBC were collected more frequently relative to challenge administration. While there was a treatment × hour interaction for LYMPH ($P = 0.04$; Fig 2.4.), evaluation of the within hour means revealed there were no relevant time points that were different among treatments. This is in contrast to Buntyn et al., (2016) where LYMPH increased 2 h post CRH and VP challenge and remained increased through h 8 of the study and Hughes et al., (2015) where LYMPH decreased in chronic steers immediately after DEX administration compared to both acute and control treatments. However, in cattle transported for 4 h there was a decrease in LYMPH count that returned to basal concentrations the day after transport (Browning and Leite-Browning, 2013) which could explain the lack of differences observed in the current study when samples were collected 24 h after the challenge. There was also a treatment × hour interaction for MONO ($P \leq 0.01$; Fig 2.5) counts where MONO was less in chronic steers compared to acute and control steers at h 144. The decrease in MONO from h 72 to 144 follows a pattern similar to WBC count, which likely occurred because MONO are a component of the measurement of overall WBC. There was a treatment × hour interaction for EOS ($P \leq 0.01$; Fig 2.6) where EOS were greater for acute at h 24 compared to

control but were not different for chronic. There was a treatment × time interaction for EOS% ($P = 0.01$; Fig 2.7) where acute was greater compared to control at h 48, which is expected due to the similar trend observed for EOS. However, the differences found between treatments are likely a result of random variation as evidenced by differences in concentrations for acute cattle at h 0 than chronic and control. Data reported by Buntyn et al., (2016) suggests that immune cell populations and response are altered by administration of CRH and VP, which agrees with the response of circulating immune cells in the current study. Remaining CBC variables such as NEU, BASO, NEU%, LYMPH%, MONO%, BASO%, and neutrophil to lymphocyte ratio were not different ($P \leq 0.20$; Table 2.2) among treatments. Administration of CRH and VP has been reported to decrease neutrophil counts (Buntyn et al., 2016) whereas administration of DEX (Anderson et al., 1999; Hughes et al., 2015) and transportation of cattle (Browning and Leite-Browning, 2013) has resulted in increased neutrophil counts in previous research.

BLOOD CHEMISTRY

A treatment × hour interaction ($P \leq 0.01$) was observed for cortisol (Fig 2.8) where cattle receiving the chronic and acute treatments had greater cortisol concentrations at h 1 compared to control, and chronically challenged cattle had greater serum cortisol concentrations than those receiving either acute or control at h 25, 26, 49, 50, 73, and 74. Greater cortisol concentrations suggest that the HPA axis was stimulated after administration of the CRH and VP stress challenge used in this study. Interestingly, the serum cortisol concentration of cattle receiving the chronic treatment continued to peak near concentrations of 40 ng/mL within 1 to 2 h of CRH and VP administration for all 4 d of the stress challenge, and suggests that the duration of challenge application did

not impact the magnitude of response observed over time. Injection of CRH and VP has been reported to increase serum cortisol concentrations in cattle from h 0.5 through 5.5 (Buntyn et al., 2016) and h 0.5 through 4 (Hulbert et al., 2012) after administration. Furthermore, infusion of 1.5 μg/kg of BW LPS increased serum cortisol from h 0 to 4, and did not return to baseline concentrations within 8 h in steers (Waggoner et al., 2009; Löest et al., 2018). In heifers transported for 4 h via truck, serum cortisol increased from 2 h after initiation of transport until the end of the experimental period (Falkenberg et al., 2012). It is possible that in the current study, sustained increases in serum cortisol might have been observed if sampling had occurred for longer than h 2 after the challenge. However, the return to baseline evidenced by samples collected before administration of the challenge each day (h 24, 28, and 72) suggests that cortisol concentrations were regulated by the negative feedback mechanism of the HPA axis and returned to homeostasis. Serum glucose concentrations had a treatment × time interaction ($P \leq 0.01$; Fig 2.9) where concentrations were greater for cattle administered acute and chronic treatments at h 1 compared to control cattle and were greater for chronic cattle at h 24, 25, 49, 73, and 74 compared to cattle administered either the acute or control treatments. Chronic cattle also had greater glucose at h 50 compared to control, but did not differ from acute. The results observed in the present study agree with previous research conducted by Browning and Leite-Browning (2013) where serum glucose in heifers increased immediately after transport. In steers administered 1.5 μg/kg of BW of LPS, serum glucose increased from h 0 to 2 followed by a decrease from h 2 to 4 that remained less than no-LPS steers until the end of the study at h 12 (Löest et al., 2018). Cortisol is a glucocorticoid that stimulates gluconeogenesis in the liver by mobilizing fat and protein

into glycerol and amino acids that are then converted to glucose (Buntyn et al., 2016; Carroll and Burdick Sanchez 2014). The initial increase and later decline to baseline in serum glucose concentrations observed for the acutely challenged cattle suggests that cattle that are stressed respond quickly and can then regulate systems to achieve homeostasis once relieved of the stressor. Similarly to the results reported for cortisol, the response observed for serum glucose concentrations in chronically treated cattle suggests continued application of a stress challenge did not impact the occurrence or magnitude of glucose mobilization as the cattle responded comparably each day of the challenge.

Insulin followed similar trends to that of cortisol and glucose and a treatment × time interaction ($P \leq 0.01$; Fig 2.10) was observed where chronic and acute challenged animals had greater concentrations of insulin 1 h after the challenge compared to control, and chronic challenged animals had greater insulin than acute or control at 25, 49, 73, and 74 h. Infusion of 1.5 µg/kg of BW LPS in steers resulted in an increase in serum insulin from h 2 to 4 after administration followed by a subsequent decline from h 4 to 6, likely in response to the increase in serum glucose that was also observed 2 h after the challenge (Waggoner et al., 2009). Greater serum insulin was also reported 2 h after administration of CRH and VP to heifers (Buntyn et al., 2016). Insulin potentiates glucose use from either food that is consumed or from stores in the body for energy (Kovacs and Ojeda, 2012). The increase in serum glucose for chronic in the present study would stimulate the secretion of insulin to increase the uptake of glucose and return concentrations to baseline levels. In the current study, a tendency ($P = 0.09$; Table 2.2) for NEFA concentrations to be greater was observed for cattle receiving stress vs. control, which agrees with previous research where cattle transported for 24 h had a greater NEFA concentration than non-

transported cattle on d 1 after transport (Marques et al., 2012). Lippolis et al., (2017) also reported greater serum NEFA concentrations from h 6 to 48 after infusion of 0.5 µg/kg of BW of LPS, thus signaling lipolysis was caused by greater circulating glucocorticoids and initiation of the inflammatory response.

There was a treatment × hour interaction for BUN ($P \leq 0.01$; Fig 2.11); however, no relevant treatment differences were observed within the time periods serum was sampled. In the current study the lack of treatment differences reported for BUN is supported by only a tendency occurring in the NEFA results of the experiment, suggesting that the energy demand could have been mostly been met by glucose and amino acid catabolism was not needed. A treatment × hour interaction was observed for Ca ($P \leq 0.01$; Fig 2.12) where concentrations were least at h 1 and 26 in acutely challenged cattle compared to control, but not different from chronic at h 1 and less for chronic at h 73. In dairy heifers, a decrease in Ca was observed within 30 min of injection of 250 IU of ACTH and continued to decrease throughout the 24-h collection period (Wegner and Stott, 1972). However, in transported steers, Ca was not greatly influenced by transport treatments (Galyean et al., 1981). In the current study the decrease in Ca may be due to increased renal secretion of electrolytes. A treatment × hour tendency ($P = 0.08$; Table 2.3) acute was greater than control at h 1 and 48, but did not differ from chronic. This disagrees with the 7% decrease in plasma albumin observed by Buckham et al. (2008) 24 h after transportation of young bulls. Phosphorus (treatment × time interaction; $P \leq 0.01$; Fig 2.13) was less for chronic cattle than acute on h 25, 26, 50, 73, and 74 and was less for chronic than control at h 49, 50, 73, and 74. Cattle transported for 32 h had less P compared to non-transported cattle which agrees with findings in the

current study (Galyean et al., 1981). A treatment × time interaction was also observed for Na ($P \leq 0.01$; Fig 2.14) where steers administered the acute treatments had less Na compared to control at h 1 but did not differ from chronic, and less than chronic and control at h 2. Chronic steers also had greater Na concentrations at 48 h compared to control, lower concentrations than both acute and control at h 49, and lower concentrations compared to acute at h 73. Potassium ($P \leq 0.01$; Fig 2.15) was less for both acute and chronic at h 1, 2 and 24 compared to control, and less at h 25, 26, 49, 50, 73, and 74 in chronic compared to the acute and control groups. This agrees with results reported by Wegner and Stott (1972), who observed a relationship between Na and K, where Na increased and K decreased 30 min after ACTH administration. The decline in K and increase in Na in the current study indicate that these minerals respond to cortisol and likely the subsequent aldosterone effect. Aldosterone causes renal stimulation and excretion of K and absorption of Na (Wegner and Stott, 1972). Insulin also potentiates the uptake of K from urinary excretion to modulate the renin-angiotensin-aldosterone system (Ferrannini et al., 1992). The renin-angiotensin-aldosterone system affects glucose tolerance by regulating plasma potassium levels to stimulate the release of insulin (Ferrannini et al., 1992). In a study in sheep infused with cortisol, researchers observed an increase in urine output and a decrease in urinary Na (Parker et al., 2003) following infusion. Parker et al., (2003) attributed the changes in urinary output to cortisol inducing diuresis to prevent the over vasodilation from VP. In the current study, a diuretic effect of cortisol could explain the shifts in electrolytes and minerals, however urine output was not quantified. A treatment × hour tendency (P = 0.09; Table 2.3) was observed for Cl where chronic had the greatest concentration followed by acute then control. At h 144

and 336 chronic had greater Cl concentrations than control, but did not differ from acute. Heifers administered CRH had increased Cl within 1 h of treatment administration that remained increased until h 8 and never returned to pre-treatment concentrations (Wegner and Stott, 1972). In contrast, research conducted in steers administered cortisol did not report an increase in Cl until 20 h after treatment but concentrations remained increased until h 100 (Parker et al., 2004). A treatment × time interaction ($P \leq 0.01$; Fig 2.16) was also observed for total CO_2 which was lower in cattle administered the acute treatments at h 1, 26, and 49 compared to control. Carbon dioxide concentrations were also lower for chronic cattle compared to control cattle at h 1 but lower for chronic than both control and acute at h 336. The results observed for this variable are likely not of biological importance and the interaction that occurred could be due to random variance among animals within the sample subset rather than treatment differences.

RECTAL TEMPERATURE

There was no treatment × day interaction or treatment effect for rectal temperature, but there was a tendency ($P = 0.07$) for cattle receiving one of the stress treatments to have greater rectal temperatures compared to control cattle. This agrees with previous research where rectal temperature was increased from h 0 to 2 following infusion of LPS in steers and remained elevated for 12 after administration (Löest et al., 2017; Waggoner et al., 2009). In relocated heifers, rectal temperature increased 60 min into the relocation event and gradually declined from 90 through 210 min (Falkenberg et al., 2012). In contrast, newly weaned calves transported for 12 h had no increases in rectal temperature during or following transit (Crookshank et al., 1979). Heifers challenged using CRH and VP exhibited increases in vaginal temperature within 25 min

of administration (Buntyn et al., 2016). In the current study a “spot” rectal temperature was used as measurement instead of continuous measurement which may have influenced results. Previously, researchers have used body temperature as a physiological indicator of both stress and immune challenge (Carroll and Burdick Sanchez, 2014), but this measurement can vary due to environmental factors, breed, and sex of the animal.

PERFORMANCE

An additional objective of this experiment was to determine if a CRH and VP challenge affects performance in cattle. Therefore, in the present study cattle performance was monitored in an effort to determine if acute changes in the blood parameters measured translated to longer-term biological growth effects in the animal. No differences ($P \geq 0.38$) were observed between treatments for final BW, ADG, DMI, and water intake (Table 2.4). In transported steers, no difference in BW was observed throughout a 12-d period and it was concluded there were no residual effects of transit on BW (Browning and Leit-Browning, 2013). Alternatively, feeder cattle exposed to 24 h of transit stress had reduced ADG compared to control cattle, but overall BW was not impacted at the end of the 28-d period, and no effects for DMI were detected between treatments. Less DMI was observed in steers receiving infusion of LPS on d 0 and 1, but no differences were found from d 2 to 6 (Lippolis et al., 2017). Changes in DMI during the initial phase of the collection period may have been caused by increased concentrations of hormones and metabolites that modulate DMI via the CNS shortly after challenge application (Lippolis et al., 2017). Because of the shorter experimental time frames in most intensive studies it is challenging to compare previous research with the current study as cattle performance was observed over the entire experimental period (d 0

to 21). During intensive stress studies, performance is not commonly reported due to the short time animals are on study, as well as the natural variation between animals when individual animal is used as the experimental unit. These studies also use individual tie stalls where the animal is fitted with numerous devices that may alter normal body functions in all treatments including the control groups. While the cattle in the chronic treatment were challenged repeatedly, the blood variables previously reported suggest that animals were able to return to baseline within 24 h after the CRH and VP challenge each day. Therefore, these acute changes in blood chemistry might be less likely to impact long-term biological effects such as the mobilization of nutrients that could impact performance. While there were no differences between treatments, cattle in the control group had a numerically increased ADG and final BW compared to both acute and chronic groups. There is a possibility that the stress model used did not elicit a strong enough response to create a detectible difference in performance long-term.

CONCLUSIONS

This data suggests that a stress challenge of CRH and VP altered some of the immune cell populations, specifically WBC and MONO in chronically challenged cattle. In this experiment, CRH and VP administration increased serum cortisol concentrations which can be attributed to stimulation of the HPA axis. The cortisol, glucose, and insulin responses observed in the cattle receiving the chronic treatment suggests that cattle continued to respond to the recurrent challenge on d 0 through 3, but the effect was static over time. The decline in cortisol 2 h after administration of the challenge is likely due to the half-life of cortisol being only 120 min and in the absence of additional stress, cortisol rapidly returned to baseline. Increased serum glucose concentrations suggest that the

CRH and VP stress challenge altered glucose metabolism. Insulin increases mimic those observed for glucose and are expected as insulin is released in response to glucose to signal the liver, muscle, and fat cells to increase uptake of glucose to be used for energy.

Decreased electrolyte concentrations in the serum of stress challenged cattle were not expected, but could be a result of increased urination, although this was not measured in the present study. No differences in final BW, ADG, and DMI suggest that even chronically stressed cattle may be able to overcome changes in hormones and blood metabolites over time. Further research is warranted in comparing acute vs. chronic stress to understand the full effects of chronic stress in comparison to acute stress on the immune status of cattle as there are a multitude of other variables such as cytokines and APP that need to be researched and were not included in this study.

TABLE 2.1. DIET COMPOSITION

ITEM	AMOUNT
INGREDIENT % OF DM	
Corn grain, flaked	13.9
Sweetbran	56.4
Corn stover	19.05
Cane molasses	7.30
Corn oil	0.0
Supplement[1]	3.34
NUTRIENT ANALYSIS[2]	
DM, %	67.8
CP, % of DM	16.5
NEm, mcal/kg	1.78
NEg mcal/kg	1.17

[1]SUPPLEMENT CONTAINED 68.75% CALCIUM CARBONATE, 19.15% SALT, 5.25% POTASSIUM CHLORIDE, 2.66% MAGNESE SULFATE, 0.3% COPPER SULFATE, 0.27% SODIUM SELENITE, 0.3% VITAMIN A, 0.12% VITAMIN E, AND 34 G/T MONENSIN (ELANCO ANIMAL HELATH, INDIANAPOLIS, IN).

[2] NUTRIENT ANALYSIS WAS CONDUCTED AT SERVITECH IN AMARILLO, TX

TABLE 2.2. OVERALL EFFECTS OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN ADMINISTRATION ON HEMATOLOGY AND SERUM NON-ESTERIFIED FATTY ACID CONCENTRATION OF BEEF CATTLE SAMPLED ON HOUR 0, 24, 48, 72, 144, AND 336.

	TREATMENT[2]				*P*-VALUE		
ITEM[1]	Control	Acute	Chronic	SEM	TRT	TRT × Hour	Stress vs. Control
Red Blood Cells, M/µL	9.20	9.10	9.48	0.23	0.29	0.78	0.64
Hemoglobin, g/dL	13.1	13.3	13.5	0.29	0.57	0.65	0.31
Hematocrit, %	39.6	40.7	41.7	0.81	0.20	0.66	0.12
Mean corpuscular volume, fL	43.2	44.7	44.0	0.76	0.36	0.09	0.21
Mean corpuscular hemoglobin, pg	14.3	14.7	14.2	0.26	0.35	0.44	0.58
Mean corpuscular hemoglobin concentration, g/dL	33.1	32.8	32.3	0.29	0.13	0.59	0.10
Reticular Disc Width	35.7	35.6	35.9	1.04	0.97	< 0.01	0.96
Reticulocyte Count, K/µL	2.47	2.62	2.89	0.45	0.59	0.43	0.43
Platelet , K/µL	315	276	294	39.74	0.45	0.02	0.27
White Blood Count, K/µL	12.2	12.6	12.5	0.57	0.82	< 0.01	0.54
Neutrophil, K/µL	2.73	2.83	3.09	0.31	0.71	0.31	0.56
Lymphocyte, K/µL	7.59	7.66	7.41	0.60	0.95	0.04	0.94
Monocyte, K/µL	1.49	1.60	1.56	0.19	0.79	< 0.01	0.53
Eosinophil, K/µL	0.34	0.54	0.48	0.09	0.24	0.03	0.10
Basophil, K/µL	< 0.01	< 0.01	< 0.01	< 0.01	0.20	0.68	0.56
Neutrophil, %	22.4	22.6	24.9	2.44	0.69	0.22	0.62
Lymphocyte, %	52.5	60.1	59.1	3.84	0.56	0.18	0.31
Monocyte, %	12.3	12.9	12.1	2.20	0.69	0.10	0.78
Eosinophil, %	2.84	4.32	3.89	0.65	0.23	0.03	0.10
Basophil, %	0.008	0.007	0.018	0.01	0.21	0.30	0.45
Neut:Lymph[3]	0.389	0.417	0.461	0.06	0.64	0.34	0.45
Non-Esterified Fatty Acid, ng/mL	0.17	0.21	0.20	0.02	0.23	0.28	0.09

[1] COMPLETE BLOOD COUNT VARIABLES ANALYZED USING A HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX, WESTBROOK, ME).

[2] CON= CONTROL, 2 ML SALINE ADMINISTERED I.V. ON H 0, 24, 48, 72;
ACUTE= ACUTE, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 2 ML SALINE ADMINISTERED I.V. ON H 24, 48, 72;
CHRONIC= CHRONIC, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 24, 48, AND 72.

[3] NEUT:LYMPH = NEUTROPHIL TO LYMPHOCYTE RATIO.

TABLE 2.3. OVERALL EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN ADMINISTRATION ON SERUM CHEMISTRY AND RECTAL TEMPERATURE OF BEEF CATTLE SAMPLED ON HOUR 0, 1, 2, 24, 25, 26, 48, 49, 50, 72, 73, 74, 144, AND 336.

	TREATMENT[2]				*P*-VALUE		
ITEM[1]	Control	Acute	Chronic	SEM	TRT	TRT × Hour	Stress vs. Control
Cortisol, ng/mL	8.02	8.00	20.9	1.71	<0.01	<0.01	<0.01
Glucose, mg/dL	83.9	84.3	93.4	2.01	<0.01	<0.01	<0.01
Insulin, mg/dL	1.32	1.45	1.83	0.15	0.06	<0.01	0.09
Blood Urea Nitrogen, mg/dL	12.5	13.1	12.6	3.03	0.89	<0.01	0.80
Calcium, mg/dL	10.5	10.4	10.3	0.09	0.41	<0.01	0.20
Creatine, mg/dL	1.07	1.06	1.07	0.04	0.96	0.46	0.90
Albumin, g/dL	3.02	3.09	3.07	0.10	0.48	0.08	0.26
Phosphorus, mmol/L	8.75	8.72	8.43	0.17	0.18	<0.01	0.30
Sodium, mmol/L	143	143	143	0.28	0.98	<0.01	0.84
Potassium, mmol/L	4.96	4.81	4.55	0.17	<0.01	<0.01	<0.01
Chloride, mmol/L	96.4	96.6	96.8	0.33	0.67	0.09	0.46
Blood Carbon Dioxide, mmol/L	27.6	27.1	27.2	0.27	0.38	<0.01	0.18
Rectal Temperature, °F	101.91	102.09	102.04	0.17	0.18	0.98	0.07

[1] BLOOD CHEMISTRY VARIABLES ANALYZED FROM VETSCAN VS2 CHEMISTRY, ELECTROLYTE, IMMUNOASSAY AND BLOOD GAS ANALYZER.

[2] CON= CONTROL, 2 ML SALINE ADMINISTERED I.V. ON H 0, 24, 48, 72;
ACUTE= ACUTE, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 2 ML SALINE ADMINISTERED I.V. ON H 24, 48, 72;
CHRONIC= CHRONIC, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 24, 48, AND 72.

TABLE 2.4. OVERALL EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN ADMINISTRATION ON PERFORMANCE OF BEEF CATTLE.

	TREATMENT[1]				*P*-VALUE	
ITEM	Control	Acute	Chronic	SEM	TRT	Stress vs. Control
Steers	10	10	10			
Initial BW, kg	416	416	416	21.28	0.99	0.99
Treatment BW, kg	441	439	440	24.35	0.98	0.86
Final BW, kg	477	471	475	19.57	0.77	0.58
ADG, kg						
Day 0 to 21	1.74	1.53	1.67	0.25	0.38	0.31
DMI, kg/d						
Day 0 to 21	9.10	9.11	9.09	0.24	0.54	0.95
Gain:Feed, kg						
Day 0 to 21	0.192	0.169	0.184	0.03	0.39	0.31
Water Intake, L/d						
Day 0 to 21	49.8	49.6	49.7	8.48	0.99	0.93

[1] CON= CONTROL, 2 ML SALINE ADMINISTERED I.V. ON H 0, 24, 48, 72.
ACUTE= ACUTE, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 2 ML SALINE ADMINISTERED I.V. ON H 24, 48, 72.
CHRONIC= CHRONIC, 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP I.V. ON H 0, 24, 48, AND 72.

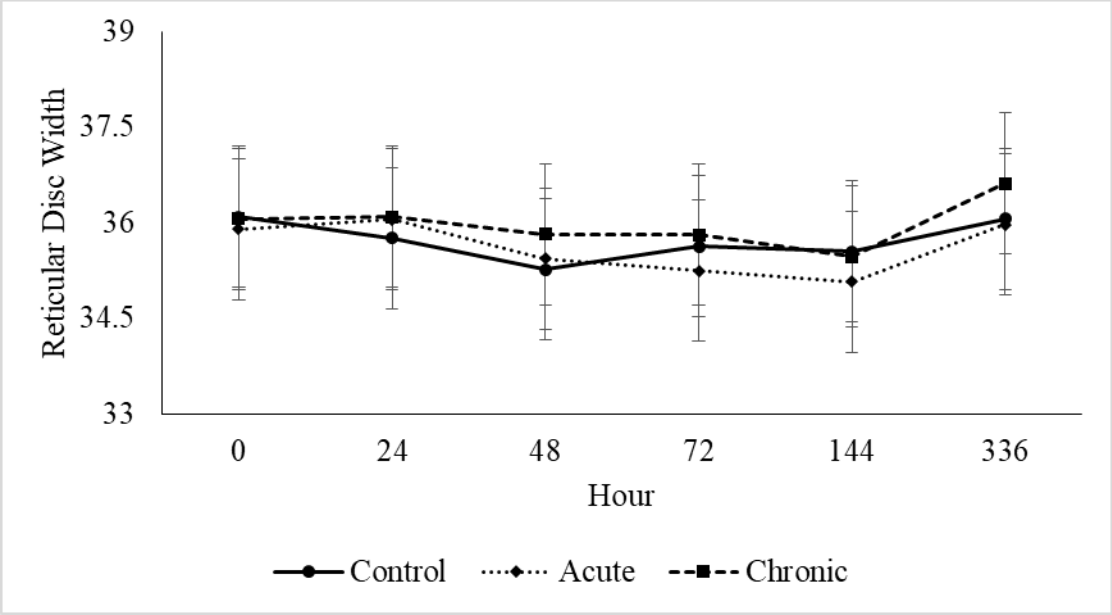

FIGURE 2.1. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON RETICULAR DISC WIDTH (RDW) IN WHOLE BLOOD OF BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, AND WESTBROOK, ME). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.97$), AND HOUR ($P \leq 0.01$). A, B, C MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

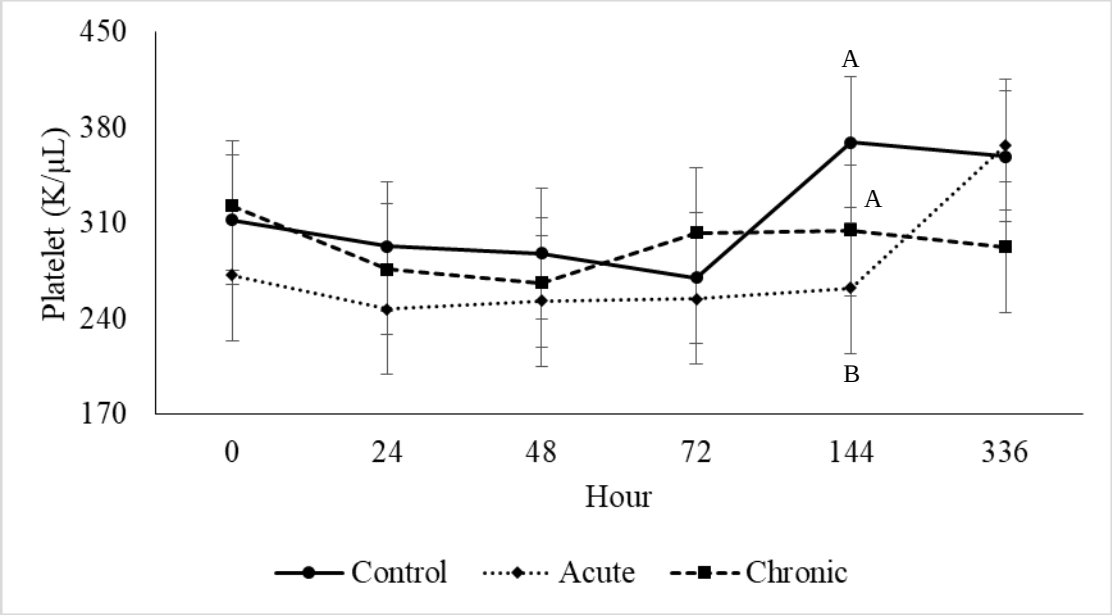

FIGURE 2.2. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON PLATELET (PLT) COUNT IN WHOLE BLOOD AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, AND WESTBROOK, ME). EFFECT OF TREATMENT × DAY ($P = 0.02$), TREATMENT ($P = 0.44$), AND HOUR ($P \leq 0.01$). A, B, C MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

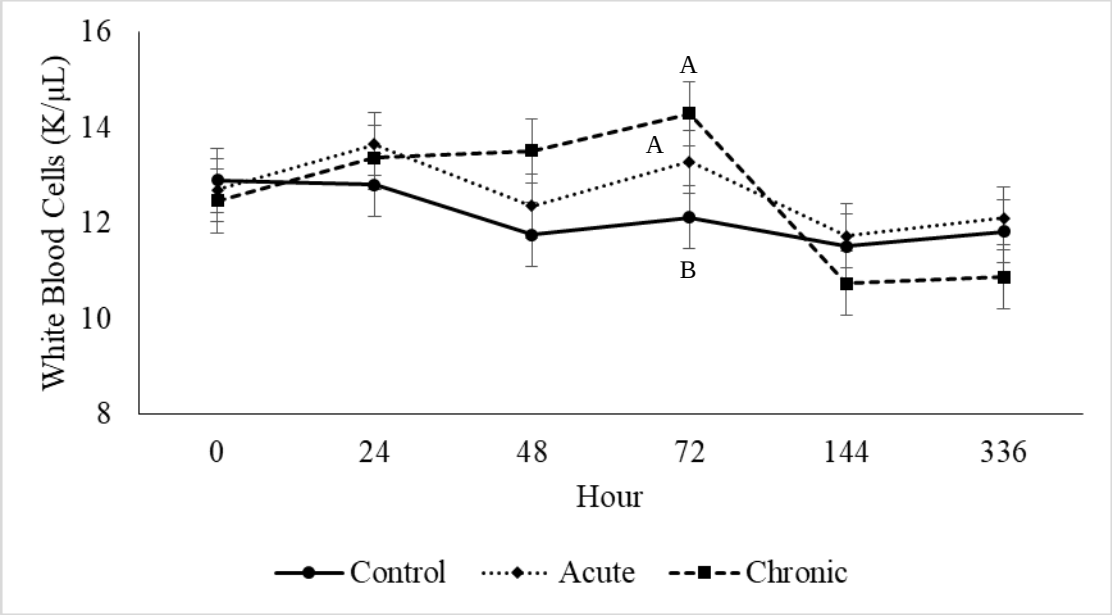

FIGURE 2.3. EFFECT OF DURATION OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN ADMINISTRATION ON WHITE BLOOD CELL (WBC) CONCENTRATION IN WHOLE BLOOD OF BEEF CATTLE. ON D 0, CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, AND WESTBROOK, ME) TO DETERMINE COMPLETE BLOOD COUNT. EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.93$), AND HOUR ($P \leq 0.01$). A, B, C MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

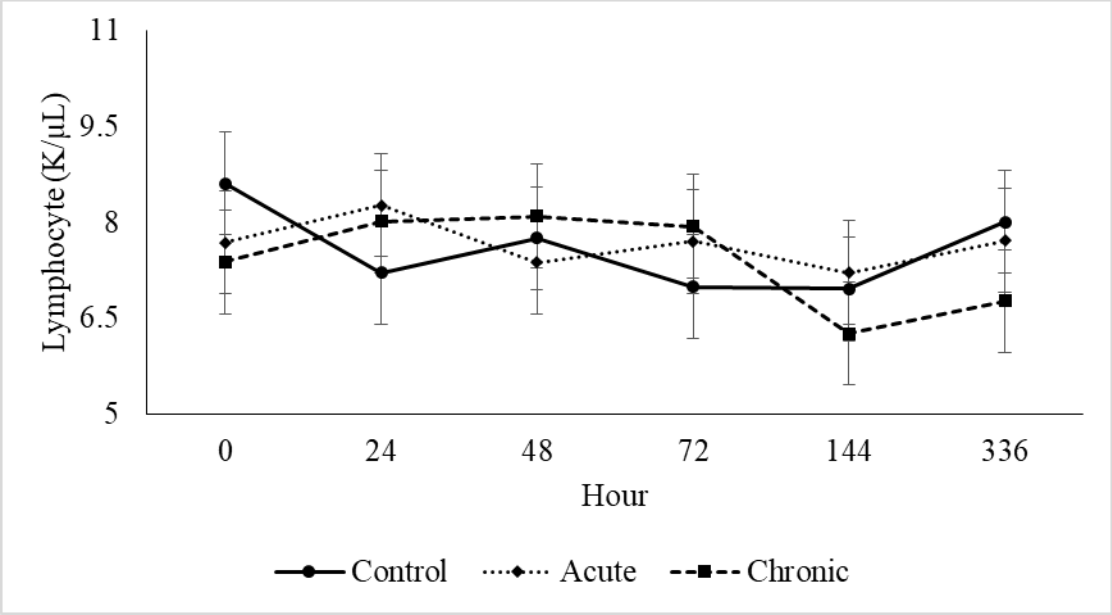

FIGURE 2.4. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON LYMPHOCYTE (LYMP) COUNT IN WHOLE BLOOD OF BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, WESTBROOK, ME). EFFECT OF TREATMENT × DAY ($P = 0.03$), TREATMENT ($P = 0.95$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

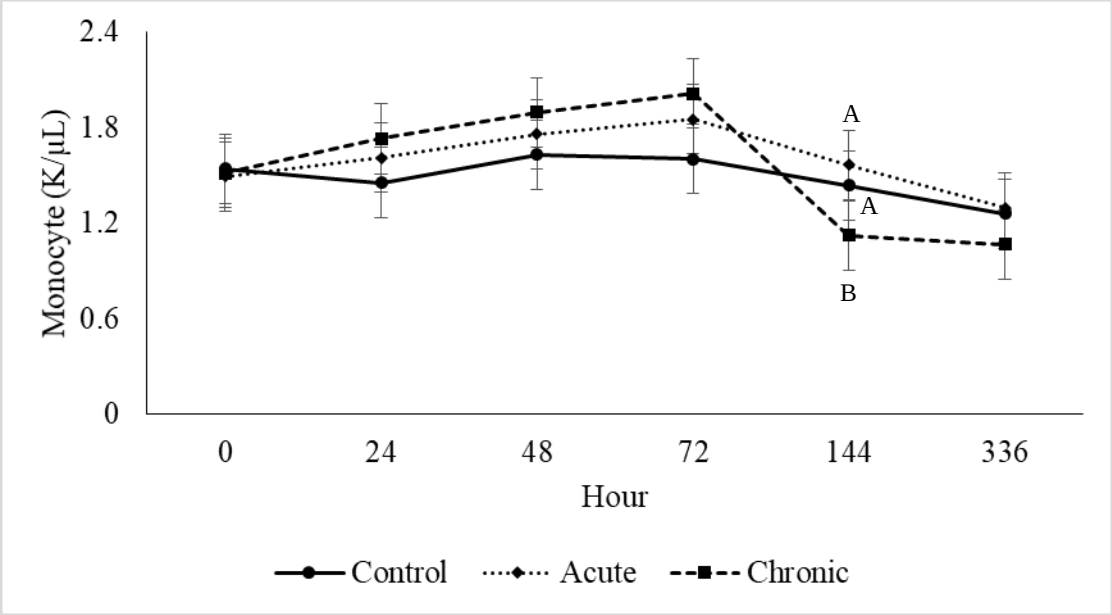

FIGURE 2.5. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON MONOCYTE (MONO) COUNT IN WHOLE BLOOD OF BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, WESTBROOK, ME). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.84$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

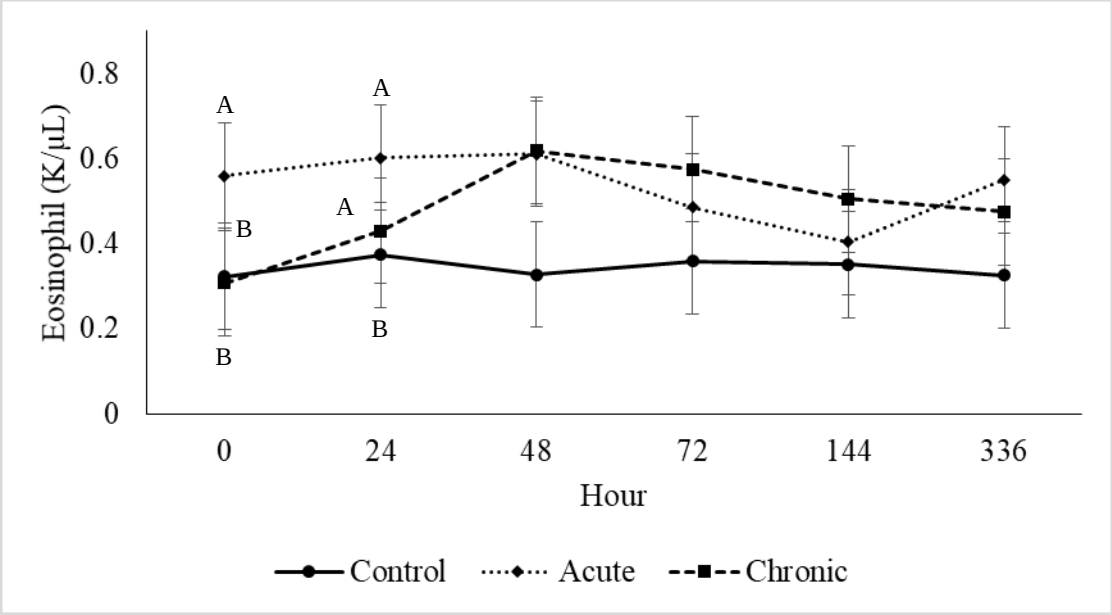

FIGURE 2.6. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON EOSINOPHIL (EOS) COUNT IN WHOLE BLOOD OF BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, WESTBROOK, ME). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.46$), AND HOUR ($P \leq 0.05$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

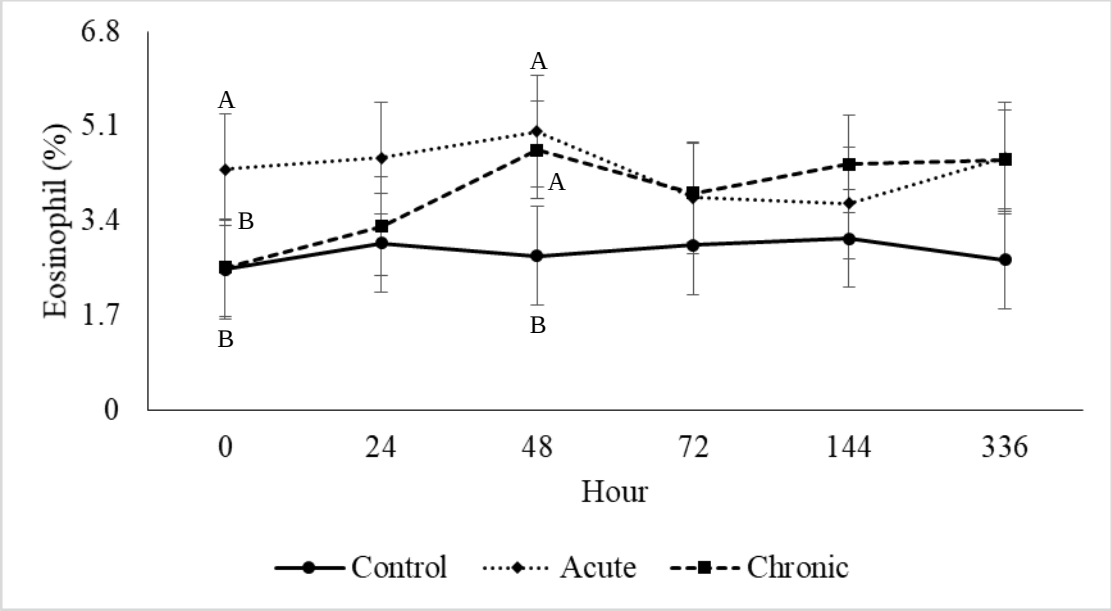

FIGURE 2.7. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON PERCENTAGE OF EOSINOPHILS (PEREOS) IN WHOLE BLOOD OF BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING AN AUTOMATED HEMATOLOGY ANALYZER (IDEXX, PROCYTE DX HEMATOLOGY ANALYZER, WESTBROOK, ME) EFFECT OF TREATMENT × DAY ($P = 0.02$), TREATMENT ($P = 0.51$), AND HOUR ($P = 0.10$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

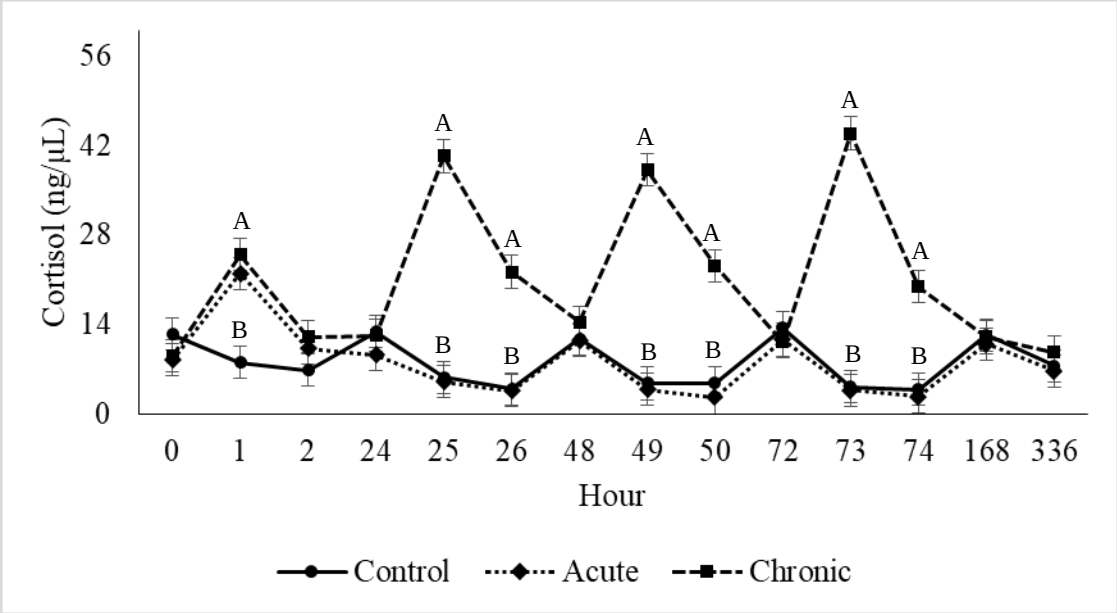

FIGURE 2.8. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM CORTISOL CONCENTRATION AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A RIA FOR CORTISOL CONCENTRATION ANALYSIS AS DESCRIBES BY YATES (2010). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P \leq 0.01$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

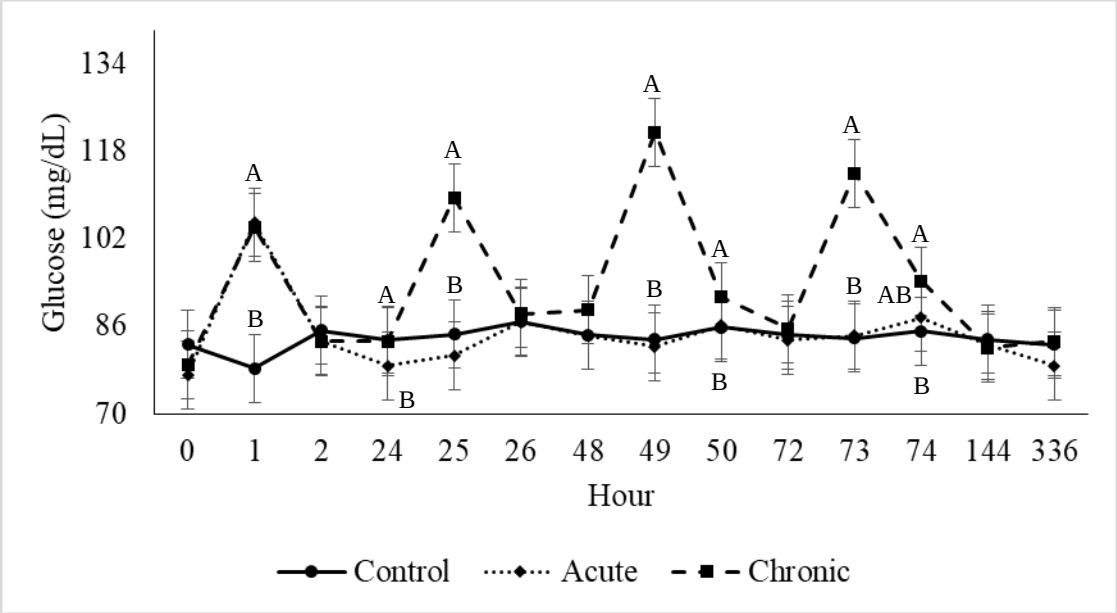

FIGURE 2.9. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM GLUCOSE CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF STERILE SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P \leq 0.01$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

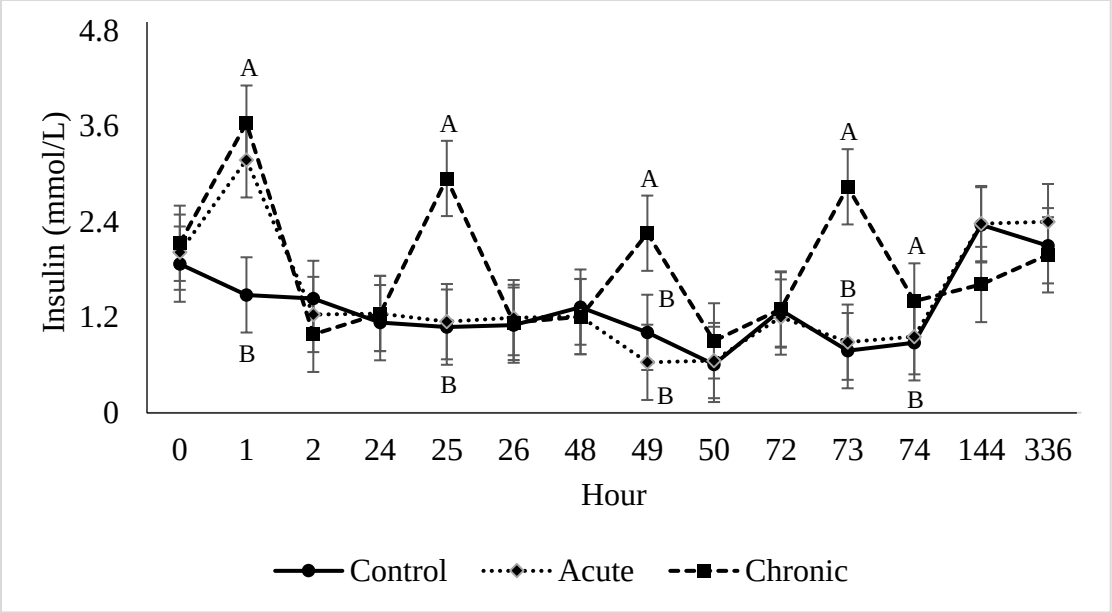

FIGURE 2.10. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM INSULIN CONCENTRATION AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BOVINE SPECIFIC INSULIN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) KIT ACCORDING TO THE MANUFACTURER'S INSTRUCTIONS (ALPCO DIAGNOSTICS, 80-INSBO-EO1, SALEM, NH). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.06$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

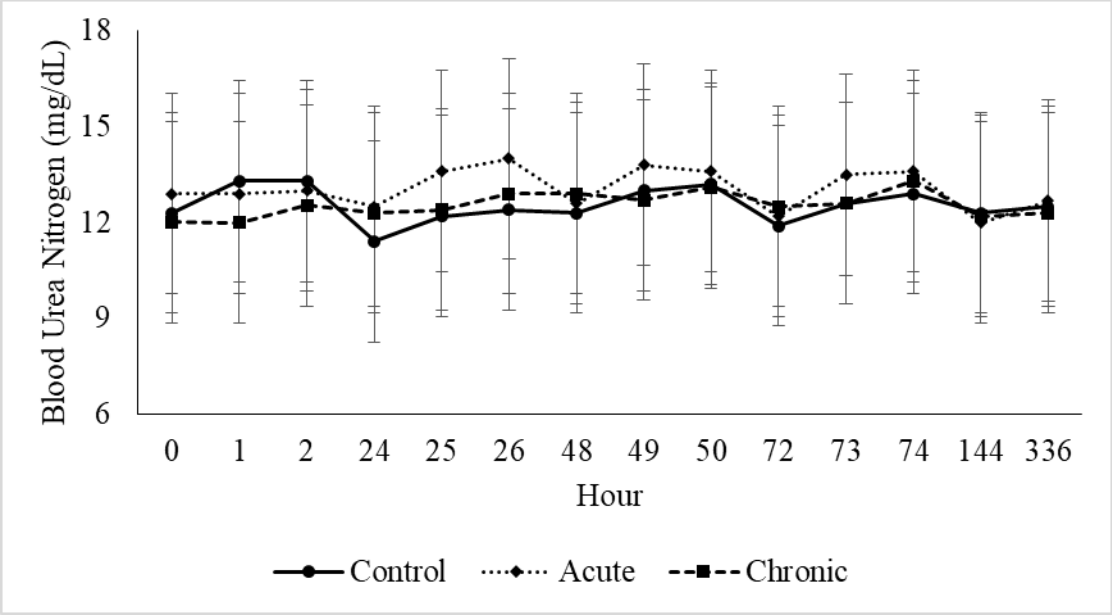

FIGURE 2.11. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM BLOOD UREA NITROGEN (BUN) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.89$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

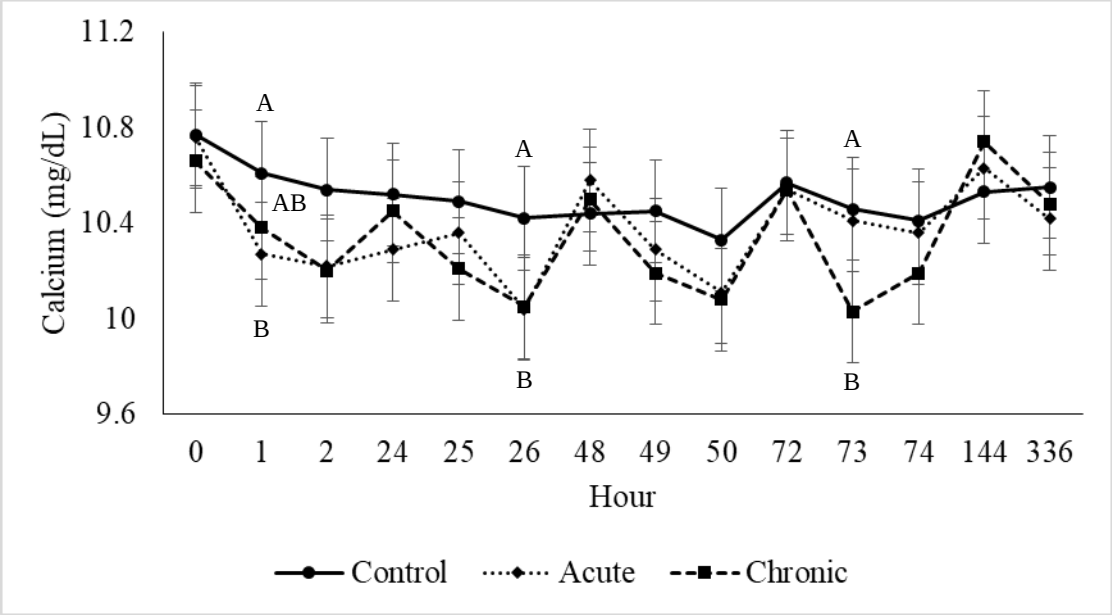

FIGURE 2.12. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM CALCIUM (CA) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.40$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

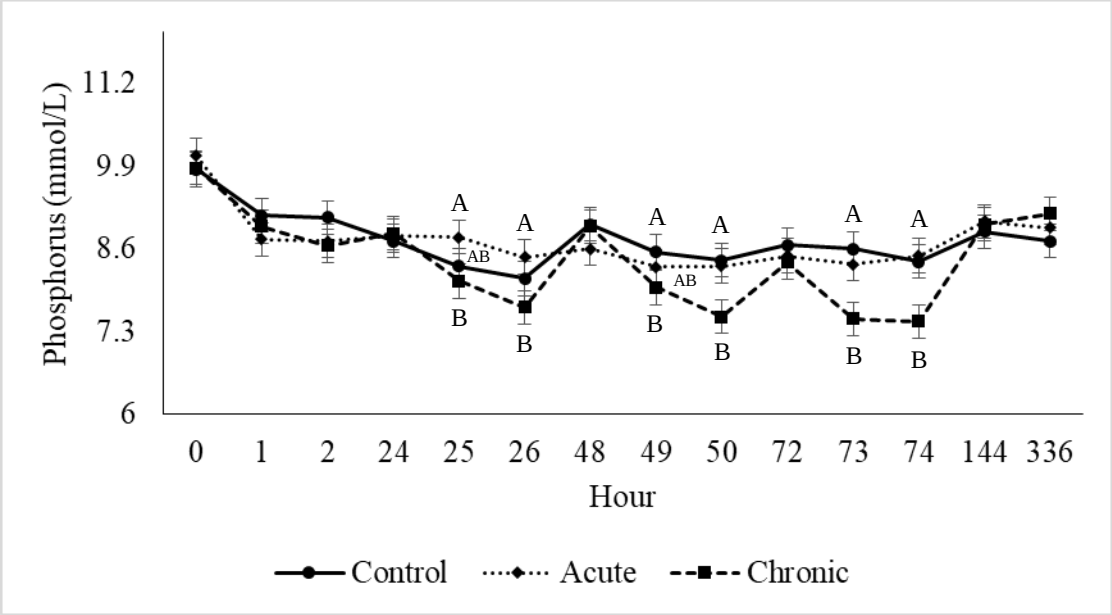

FIGURE 2.13. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM PHOSPHORUS (P) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.16$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

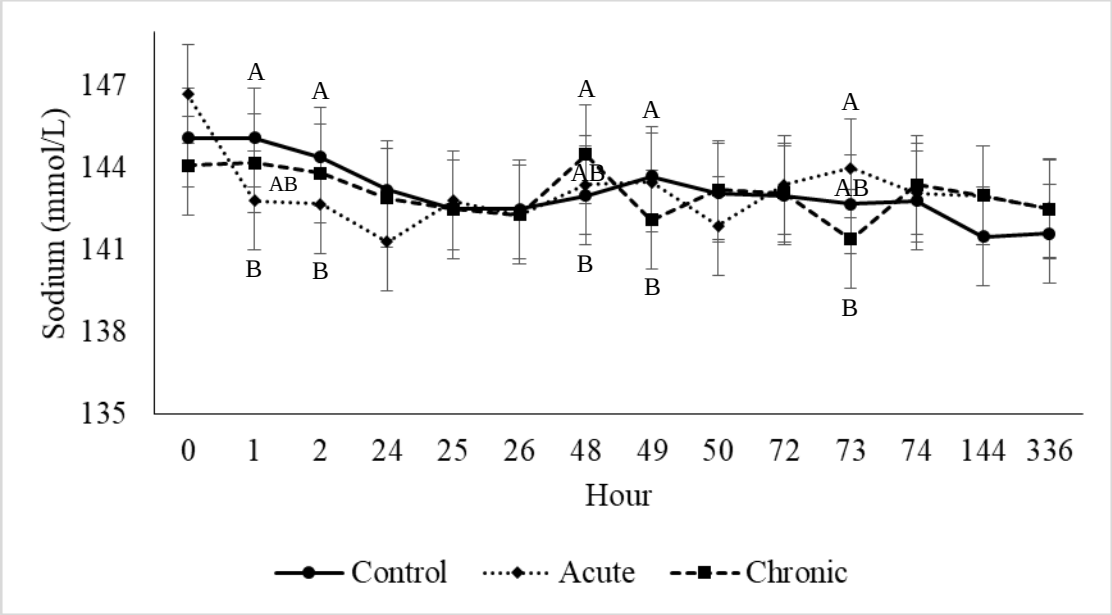

FIGURE 2.14. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM SODIUM (NA) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS. EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.97$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

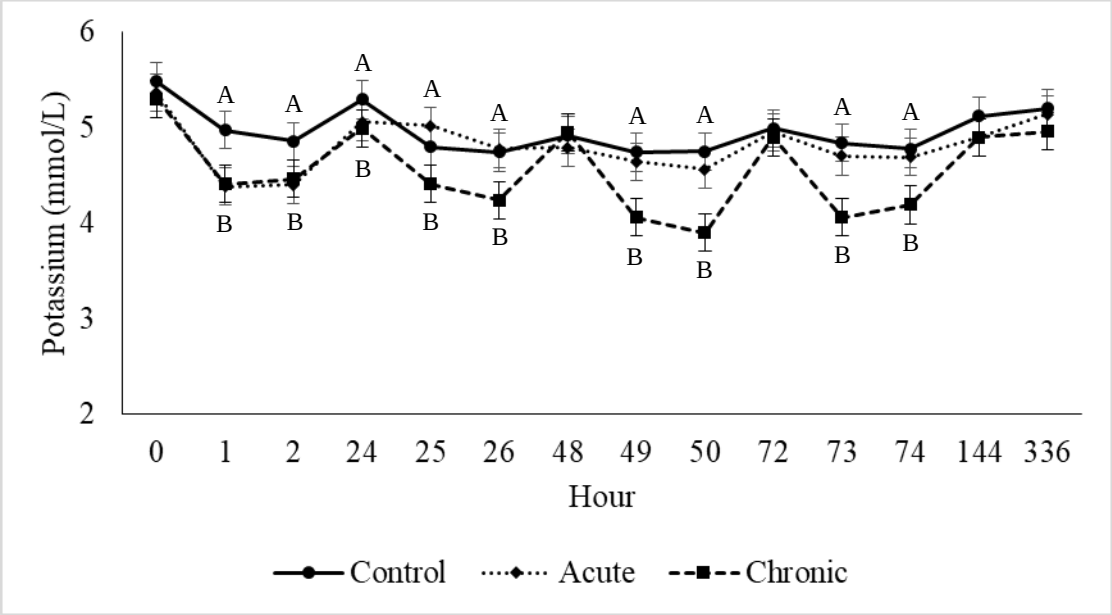

FIGURE 2.15. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON SERUM POTASSIUM (K) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P \leq 0.01$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

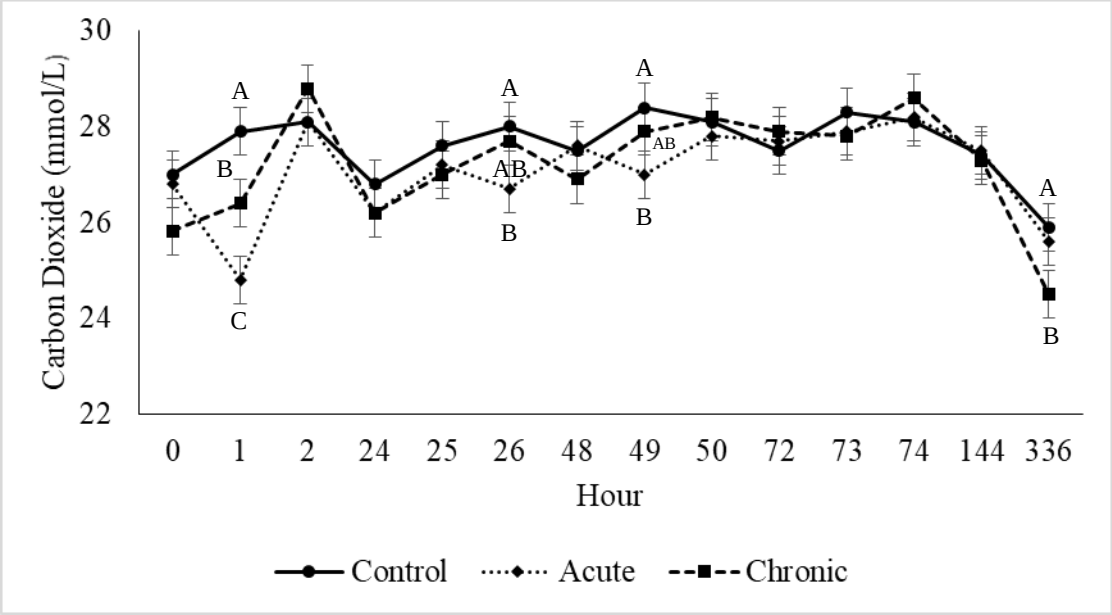

FIGURE 2.16. EFFECT OF CORTICOTROPIN RELEASING HORMONE AND VASOPRESSIN CHALLENGE DURATION ON TOTAL SERUM CARBON DIOXIDE (TCO_2) CONCENTRATION IN BLOOD SERUM AFTER ADMINISTRATION IN BEEF CATTLE. ON D 0 CONTROL CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND ACUTE AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 0. ON D 1, 2, AND 3 CONTROL AND ACUTE CATTLE WERE ADMINISTERED 3 ML OF SALINE INTRAVENOUSLY AND CHRONIC CATTLE WERE ADMINISTERED 0.3 µG/KG OF BW OF CRH AND 1.0 µG/KG OF BW OF VP INTRAVENOUSLY AT H 24, 48, AND 72. SAMPLES WERE ANALYZED USING A BLOOD CHEMISTRY ANALYZER (VETSCAN VS2, ABAXIS). EFFECT OF TREATMENT × DAY ($P \leq 0.01$), TREATMENT ($P = 0.37$), AND HOUR ($P \leq 0.01$). SUPERSCRIPTS OF A, B, AND C, USED TO REPRESENT MEANS WITHIN HOUR DIFFER ($P \leq 0.01$).

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