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BOVINE RESPIRATORY DISEASE: UNDERSTANDING HOW STRESS
MODULATES IMMUNE AND GROWTH PARAMETERS WHEN
CATTLE ARE CHALLENGED WITH RESPIRATORY
PATHOGENS (VIRAL AND BACTERIAL)

By

Shollie Falkenberg

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Animal Physiology
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

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Shollie Falkenberg
2011

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Candidate for Doctor of Philosophy Degree

Bovine respiratory disease (BRD) complex is a multi-factorial disease syndrome that results from various individual contributions and interactions of pathogen, host, and environmental/management factors. Despite the efforts in research, prevention and treatment, BRD remains a leading cause of economic loss in the cattle industry. While advances in therapeutics and new vaccines have been developed over the past 20 – 25 years, the incidence of respiratory disease does not appear to be on the decline, rather it is appears to be increasing. While bacterial and viral pathogens, and various stressors associated with BRD have been characterized, there are no animal models that can reproduce similar presentation of symptoms observed for BRD in the industry. Based on the etiology of BRD, a series of projects were designed to provide a better understanding of the individual and multiple contributions for the factors associated with the complex. It is believed that the viral pathogens or stressors can suppress immune defenses allowing opportunistic bacteria the ability to colonize and cause an infection. Therefore, trials

investigating the individual contribution that varying doses of infectious bovine rhinotracheitis virus and transportation stress have on cattle were conducted. A final project investigating the combination effect of the bacterial pathogen *M. haemolytica* and activation of the hypothalamic-pituitary-adrenal axis to elicit glucocorticoid release was evaluated. Ultimately, the research projects were designed to build upon each other to understand each component in the etiology of this disease.

DEDICATION

I dedicate this dissertation to my family; my parents, Leland and Janice Falkenberg; brother, Nyland Falkenberg; and my grandparents, James G. and Dorothy Coleman and Eric William and Sophie Falkenberg. My parents, for their unconditional love and support; my brother, for always listening and making me laugh; and my grandparents, for the inspiration to believe I could achieve whatever I wanted. My family has been instrumental in helping me achieve my goals and always supported me throughout all my endeavors. I would not be where I am today without them.

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When I began to write my acknowledgement section, I started reflecting on the time I have spent at Mississippi State and realized that I didn't completely appreciate every moment. While I have spent years and months compiling data that encompasses my dissertation, it is all the moments that aren't included in the following Chapters that have meant a great deal to me and make up the memories I will take away from my time here. You can get caught up in your: projects, classes, writing, analyzing data and just trying to get finished, that you lose focus on a lot of other important lessons. My undergraduate advisor (Dr. Howard Hesby) gave me some of the best advice when he told me; "never let classes get in the way of your education". I think there is a lot to what Dr. Hesby said after thinking back on my educational career both inside and outside the classroom.

First and foremost I would like to thank my committee for all of their help, advice, guidance and time. I believe I had a group of individuals that each challenged me to become a better scientist. My committee includes Dr's Ty Schmidt, Jeff Carroll, Jim Sartin, Rhonda Vann and Carla Huston. I would like to thank Dr. Schmidt for taking me on as his first graduate student and allowing me to learn along with him. Having the opportunity to be in his lab has been instrumental in preparing me for the next step in my career. I'd like to thank Dr. Carroll for all of his input, direction and conversation

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I greatly appreciate the help of all of these individuals and my family; they all have been instrumental in helping me accomplish my goals. Finally, I would like to thank God for blessing me with a great family and friends who have supported me during this journey; He will continue to be my source of strength.

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CHAPTER I

INTRODUCTION

The cattle industry can be categorized into four distinct sections. These sections are the cow/calf producer, the stocker or backgrounder, the feedlot, and the harvest facility. As cattle progress through these different sections of the industry not all cattle follow the same path through the production system. The immune and endocrine systems are activated/challenged due to a variety of stressors and pathogens. The potential stressors cattle can be exposed to include weaning, processing (vaccination, castration, etc.), relocation, feed and water deprivation (during relocation), environmental changes, interaction with unfamiliar cattle (commingling at the yard), as well as exposure to novel pathogens (pathogens not present within the home herd). Some of the stressors only occur once in a steer/heifer's life (weaning and castration), however, others could occur multiple times (relocation, processing, and comingling; depending upon the management of the cattle). Take relocation for example; cattle could be sold at local livestock auction to a background-lot. The cattle can spend 60 to 120 d at the backgrounding facility and upon reaching the desired weight, relocated to a feedlot. The final relocation beef cattle experience is transportation to the abattoir for harvest. During the life time of this one steer/heifer, it could have experienced the stress associated with processing (implants, vaccination, ear tag, and even ultra-sound) 3 to 4 times. Thus, a steer/heifer may

experience a wide range of unfamiliar experiences multiple times, experiences that can alter the endocrine and immune response to pathogenic challenges.

Passive, innate and acquired immune responses are all vital to the survival of the animal, but the first line of defense is the natural barriers. However, these systems can only prime the immune system when a particular pathogen associated molecular pattern (PAMP) is recognized for the pathogen (viral, bacterial, and parasitic). Introduction of novel, unfamiliar pathogens to the immune system result in a cascade of immune and endocrine responses to mount a response for specific for the pathogen encountered. For cattle that run the gauntlet of the cattle industry, each location introduces new pathogens ready to alter the animal's immune system. Couple this alteration to a steer/heifer that has just experienced a stressor and one is now facing the most devastating disease within the cattle industry, the Bovine Respiratory Disease (BRD) complex.

If there was a perfect storm in the cattle industry, it would be BRD. The BRD complex has a multi-factorial etiology. The etiology consists of weakening the immune system of cattle through production processes (relocation endocrine disruption, feed and water deprivation), relocation of cattle to new environments where the cattle are exposed to pathogens which the immune system is unfamiliar with, and the combination of these two factors allows opportunistic pathogens the opportunity to circumvent the animals immune defenses and colonize in the respiratory tract (Stress X Viral X Bacterial). When this perfect storm arrives it is detrimental to cattle production and profitability. In 1996, Smith reported an estimated economical impact of BRD to be well over \$600 million (Smith, 1996) annually to the beef industry. This estimated loss reported by Smith (1996) only accounts for the economic impact related to morbidity, treatment of morbid

cattle, and mortality (visual economic impact). The true cost of BRD is not fully known nor will it ever be fully accounted for in the cattle industry. This inability to account for the true economic impact is due to an inability to determine the impact of BRD on carcass weight (the most important variable in determining the value of a beef carcass) and carcass quality (USDA Quality Grade). Wittum et al. (1996) reported that on average, 42% of bovine lungs observed at the time of harvest showed indication of lung damage (lung lesions; low of 1% to a high of 75% of lungs exhibited damage) and Schmidt (2000) reported 23% of lungs observed during a carcass survey had visual lung tissue damage. Wittum et al. (1996) also reported that up to 70% of bovine lungs with tissue damage at the time of harvest were from cattle that had not been treated for respiratory disease. Gardner et al. (1999) reported that net return of cattle with lung lesions visible (average of simple lesions and lesions with active lymph nodes) at the time of harvest was \$46.91 less compared to cattle with no lung lesions. Schmidt (2000) reported an extrapolated economic impact of BRD on cattle observed in a survey of carcass and offal characteristics of fed cattle in the Texas Panhandle, cost the cattle industry \$11,173 in lost revenue on only 840 of 3630 (23% of lungs observed) head of cattle observed (based upon market average grid-based price of \$91.73). A rough extrapolation (survey was a snap-shot in time, not a True average) of the economic impact of BRD today using Schmidt (2000) data and current USDA Beef Carcass Price Equivalent Index (\$158.33; 24 Feb 2011) would be \$19,285 (a difference of \$8,112). Extrapolation of this figure to a single week's harvest (~615,000) and the cattle industry could be losing \$3,253,350/wk. While the BRD complex could potentially cost the cattle industry billions of dollars annually, the incidence rate and morbidity associated with

BRD have remained unchanged since the early 1990's even with the introduction of new antibiotics. Therefore, there needs to be a better understanding to help mitigate the losses associated with this devastating disease.

CHAPTER II

LITERATURE REVIEW

Factors associated with bovine respiratory disease

A variety of factors and agents have been associated with the bovine respiratory disease (BRD) complex. While pathogens associated with the BRD complex have proven to elicit immune responses, the effective exposure rate to elicit desired infectivity remains unknown for many of the pathogens. The complex interactions of viral and bacterial infections can potentiate the effects of one another and some have been reported to individually cause respiratory damage. Generally BRD is defined as the clinical manifestation of the interaction of a variety of factors that culminate into acute or chronic bronchopneumonia. Factors associated with BRD can be divided into infectious agents such as pathogenic viruses and bacteria, stressors, and host susceptibility. Host susceptibility refers to the individual ability of cattle to defend against infectious challenges. The susceptibility depends on the state of immune system (suppressed or competent), respiratory defenses, and the magnitude of endogenous and/or exogenous stress that the calf is exposed. The development of the infection is dependent on compromised immune defenses which allow pathogens the opportunity to cause disease.

Cattle afflicted with the BRD complex are commonly observed throughout the feedlot and stockering phase of beef production due to the presence of numerous

pathogens and stressors cattle can potentially encounter. The incidence of clinical BRD was reported as 14.4% by USDA-APHIS (2001), from a study of feedlots in 1999. While on average 14.4% of cattle in the feedlot were diagnosed with clinical symptoms of BRD, lung lesions at harvest were common with an observed prevalence ranging between 29.7 and 77% in feeder cattle (Wittum et al., 1996; Bryant et al., 1999; Thompson et al., 2006). Performance of feeder calves suffering from clinical and subclinical symptoms of BRD as they progress through the beef production chain is one of the major economical ramifications associated with the BRD complex. Gardner et al. (1999) reported that average daily gain (ADG) for healthy cattle, cattle with no lung lesions and non-active lymph nodes, and cattle with lung lesions and active lymph nodes at harvest were 1.57, 1.42, and 1.17 kg/d respectively. Bryant et al. (1999) reported on average cattle with lung lesions at harvest had a 0.03 kg/d reduction in gain. The reduction in gain during the feeding period observed by Bryant et al. (1999) for cattle with lung lesions coincided with lighter hot carcass weights (HCW) at harvest as well. Other research has reported similar findings, reporting a decrease in ADG for cattle diagnosed with BRD and went on to state ADG decreased further with each relapse with the disease (Schneider et al., 2009). Hutcheson and Cole (1986) reported that morbid cattle had an 11% reduction in feed intake when compared to non-morbid cattle. Reduction in feed intake and decrease in ADG for cattle affected by BRD resulted in major losses. The losses associated with reduction in feed and ADG have been implicated to be associated with the immune response mounted against the pathogen. As an animal's temperature rises, due to the febrile response of the immune insult, more metabolic energy is used for heat production at a time when the animal is consuming less energy. Ballou et al. (2008) suggested the

production of pro-inflammatory cytokines also repartition nutrients by increasing whole-body glucose utilization and increasing the catabolism of skeletal muscle and triacylglycerols.

The estimated economic impact from mortality and morbidity associated with BRD has been reported to range from \$480 million (Engelken, 1997) to \$750 million annually (Griffin, 1997). Cattle treated for BRD not only have medical costs associated with treatment, but morbid animals have decreased ADG, increased feed costs, decreased carcass weights and reduction in meat quality. Gardner et al. (1998) reported that healthy cattle with no lung lesions at harvest had a net return of \$20.03 more than cattle with lung lesions and non-active lymph nodes, and \$73.78 more than cattle with lung lesions and active lymph nodes. Gardner et al. (1998) went on to state that 75% of the decrease in profit was attributed to decreased weight gain and USDA quality grade; with the remaining 25% due to medicine costs associated with treatment. While the actual economical impact associated with BRD may never be known, the fact still remains that BRD is a devastating disease that cost producers and feeders millions of dollars annually. The incidence of BRD has not declined over the past years and remains one of the most significant diseases facing the beef industry. Since BRD is a complex disease with a multi-factorial etiology it is important to understand the individual contributions of each component associated with the disease. For the purpose of this review the individual contributions of the major pathogens both viral and bacterial and stressors that have been implicated to contribute to the onset of disease will be evaluated.

Viral agents

More than twenty viruses have been associated with and observed to produce the respiratory disease complex in cattle (Dyer, 1981). The most common viruses involved in the BRD complex include bovine herpesvirus-1, infectious bovine rhinotracheitis virus (IBRV), bovine parainfluenza virus type-3 (PI-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV). Typically these viruses infect the upper respiratory tract, resulting in rhinitis, tracheitis, and bronchitis (Dyer, 1981). The viruses involved in the BRD complex generally do not cause major lung damage but rather predispose the lung to bacterial infections. Most viral agents, as single infectious agents, cause only mild to moderate pulmonary lesions, but the more severe lesions develop with dual infections of bacterial and viral agents (Dyer, 1981). Therefore, a major contribution of these agents is to cause suppression of the respiratory defenses.

Babiuk et al. (1996) suggested that in most cases the peak activity of immune cellular responses occurs at 7-10 d post-infection (post infection), and correlates with recovery from infection and before significant antibody is detected. Orr et al. (1990) inoculated calves with IBRV and reported a change in clinical symptoms and morbidity in the infected calves four days after infection; this was concurrent with the maximal nasal secretion of IBRV. Cattle infected with IBRV have increased rectal temperature (RT) and critical illness scores on days three through seven of the challenge period as well as having increased concentrations of acute phase proteins (APP) on 5-7 d (Schmidt et al., 2006). When cattle were infected with BRSV, tumor necrosis factor – α (TNF- α) was observed to be greater on 6 and 8 d post infection for infected calves (Rontved et al.,

2000). Similar results were reported by Grell et al. (2005) when BRSV was utilized to elicit an immune response, RT began to peak on the 4 d and mRNA expression for cytokines was detected as early as 1 d. Cattle experimentally infected with BVDV produced similar results as those infected with IBRV; onset of clinical symptoms observed approximately 7 d post infection coinciding with an increase in the cytokine interferon- γ (IFN- γ ; Ganheim et al., 2003). Ganheim et al. (2003) also observed an increase in APP approximately 8 d post infection following the increase in cytokine production. Results from viral challenge models suggest differences in clinical observations and detection of immune parameters starting approximately 5 to 7 d after cattle are infected and lasting for 2 to 3 d.

Bacterial agents

Pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma* spp., *Haemophilus somnus*, and *Chlamydia* spp. have been reported to infect the respiratory tract of cattle; however the exact role played by each agent is not fully understood. The majority of these bacterial agents are believed to be part of the normal flora present in the upper respiratory passages of healthy cattle. If these pathogens move into the lower cranial lobe after a primary virally-induced or stress-induced compromise of the respiratory defense mechanisms, the bacteria can overwhelm the immune defenses and cause infection. This is why BRD is so complex, one agent alone might only produce minor respiratory problems; however, when more than one pathological agent and stressor act on defense mechanisms of cattle this allows the opportunistic bacteria the ability to colonize and cause disease.

Several experiments have studied the immune response associated with *M. haemolytica* infections. Frank et al. (2003) reported that calves infected by intranasal inoculation of *M. haemolytica*, when nasal swabs were obtained 6 d post infection, colonization of the nasopharynx was evident in the majority of calves. Horadagoda et al. (1994) reported increased concentrations of tumor necrosis factor- α within 2 h of intra-tracheal inoculation with *M. haemolytica*. Horadagoda et al. (1994) also reported an increase in the APP serum amyloid A (SAA) observed 2 h after the inoculation; an increase in the concentration of haptoglobin was not observed. Whereas, Ganheim et al. (2003) reported an increase for the APPs haptoglobin and fibrinogen within 24 h and SAA on 1-2 d post infection. Burciaga-Robles et al. (2010) also reported an increase in RT within the first 24 h after calves were given an intra-tracheal inoculation with *M. haemolytica*. The increase in RT coincided with an increase in white blood cells and pro-inflammatory cytokines.

Stressors

Cattle are exposed to a variety of stressors during the production phases of the cattle industry. Stressors are those psychological or physical stimuli that evoke activation of the hypothalamic-pituitary-adrenal (HPA) axis to stimulate release of cortisol a potent glucocorticoid. Particularly, feeder cattle are exposed to a great deal of stress prior to and while located at a stocker or feedlot facility. These stressors include weaning, handling, and novel exposure to pathogens, facilities, and commingling with non-herd mates. Upon arrival at the facility, cattle are bombarded with another wave of stressors such as initial processing, environmental changes, and immunological challenges (bacterial

and/or viral). From the time a calf is weaned until arrival at the feedlot, the calf experiences several stressful events. Simultaneously, the calf is commingled and exposed to various bacterial and viral pathogens challenging the immune system which can also activate the stress axis. Many of the common production practices that cattle are exposed to on a regular basis have been implicated to act as a stimulus and evoke the stress response. Animals have evolved mechanisms that allow the ability to cope with exposure to a stimuli, however, some of these stimuli overwhelm the animal and the animal is unable to cope or acclimate and therefore elicit a stress response (Salak-Johnson and McGlone, 2007).

Activation of the HPA axis in response to a stimulus is a critical mechanism that gives living organisms the ability to maintain a homeostatic state. Cannon (1929) was the first to introduce the term “homeostasis” to describe “the coordinated physiological process which maintains most of the steady states in the organism”. The term stressor is used to quantify any stimulus that activates the HPA axis; regardless of magnitude of the response and what specific components are stimulated. The term “stress” generally revolves around mental strain, anguish, or anxiety; although a proper discussion of stress should include any and all factors responsible for the activation of the HPA axis and physiological consequences thereof. Hans Selye deserves much of the credit for introducing the term “stress” and for popularizing the concept of stress in the scientific and medical literature during the 20th century. Selye (1936) focused on the HPA axis as the key effector of the stress response. Selye (1936) identified the alarm reaction as a non-specific response that consisted of: enlargement of the adrenal gland; shrinkage of the thymus, spleen and lymph nodes; and ulceration of the gastric mucosa. Selye (1973)

redefined the alarm reaction as the “general adaptation syndrome” (GAS). The GAS was defined to have three successive phases: the alarm, resistance, and exhaustion stages. While the intensity of the stress response may vary, the neural and endocrine patterns would be similar among the three different stages. Characterizing and classifying different stressors is difficult due to perception, but a variety of stimuli have been considered and reported to activate the HPA axis. Weiner (1991) described stressors as selective pressures from the physiological and social environment that threaten or challenge an organism and elicit compensatory patterns in response to the stimuli. Chrous and Gold (1992) defined stress as a state of disharmony or threatened homeostasis, evoking physiological or behaviorally adaptive responses. Stressors can generally be divided into four main categories: 1) physical or chemical stressors; 2) psychological stressors that reflect a learned response to previously experienced adverse conditions; 3) social stressors reflecting disturbed interactions among individuals; and 4) stressors that challenge cardiovascular and metabolic homeostasis (Pacak et al., 1998).

The hypothalamic-sympathetic and the HPA system provide peripheral control of stress responses (Salak-Johnson and McGlone, 2007). The activation of the HPA axis consists of secretion of corticotrophin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus via the median eminence and hypothalamic-hypophyseal portal system (Gardner and Shoback, 2007). The cells of the anterior pituitary respond to CRH by synthesizing and secreting the proopiomelanocortin molecule that contains adrenocorticotropin hormone (ACTH) among other products (Gardner and Shoback, 2007). Pituitary derived ACTH circulates through the blood to the

adrenal cortex, where it acts on cells of the zona fasciculata to stimulate the secretion of cortisol (Gardner and Shoback, 2007).

Cortisol has been used as a common measure of stress in animal agriculture to determine the magnitude and duration of perceived stressors. One stressor that most, if not all, cattle encounter in a typical management system is transportation stress. Transportation stress for cattle in most studies is indicated by an increase in body temperature, increased heart and respiration rates, and activation of the HPA axis. Transportation stress has been reported to increase RT initially after loading, but RT returned to baseline values 2 h after the beginning of the transportation event (Burdick et al., 2011). While an increase in RT was observed during the transport period, cortisol concentrations did not differ between pre-and post-transport samples in this study (Burdick et al., 2011). Length of time cattle are subjected to transportation appears to have an impact on differences observed in circulation concentrations of cortisol when evaluating pre- and post-transport samples. While Burdick et al. (2011) and Cole et al. (1988) did not find differences in cortisol concentrations pre- and post-transport samples after an 8 h transport study. Other researchers have reported an increase in circulating glucocorticoids after transport (Crookshank et al., 1979; Locatelliu et al., 1987; Simensen et al., 1980; Kent and Ewbank, 1983). Grandin (1997) suggested that cortisol concentrations increase in response to handling, loading, unloading and during the first portion of the transport event. During repeated transports or long durations of transportation cortisol concentration may decrease as cattle acclimate to the process (Lay et al., 1996).

One method used to activate the HPA axis and simulate an acute stressor is to administer exogenous hormones to stimulate the response. Corticotropin releasing hormone, arginine vasopressin (AVP) and ACTH has been utilized as a replicable method to induce similar results to that of an acute stressor and to determine the sensitivity of the HPA axis. The benefit to injecting exogenous hormones in a known constant amount to each animal can cause stimulation of the HPA axis and does not rely of the perception of the animal, therefore producing a more consistent circulating hormone concentration within the animal. Lay et al. (1996) evaluated multiple doses of ACTH to mimic the physiological effects of a real stressor; determining that the greater dose of ACTH elicited the greater integrated cortisol response. However, peak plasma cortisol concentrations did not differ among the four doses of ACTH. Carroll et al. (2007) concluded that CRH and AVP elicited similar cortisol responses but when CRH and VP are given in tandem a more sustained cortisol response was observed. The stimulatory actions of CRH, AVP and ACTH are similar to that of naturally occurring stressors cattle experience while being processed through the various phases of production.

Transportation stress is an ever present concern due to the fact that at some point during the production system or during the life of livestock, animals are transported. Changes in physiological function have been reported pre-and post-transport; however, there is no data reporting the physiological changes observed during the transport period. While the individual contributions of stress and bacterial and viral pathogens have been reported, the synergistic effects and the relationship of combinations of multiple factors needs to be explored to fully understand the BRD complex. In an effort to better

understand the dynamics associated with each factor it is imperative to have an understanding of immune responses and interactions with other physiological processes.

Overview of the innate immune system

Defense mechanisms

The innate immune system is an initial defense mechanism to prevent the proliferation of pathogens once internalized. Infectious pathogens can be categorized into five groups: bacteria, virus, fungi, protozoa, and helminthes (Paul, 2008). Infectious agents can inhabit most all body compartments, but the two major classifications are typically either extra-or intracellular pathogens. The body has defense mechanisms to assist in prevention of infectious agents from gaining access to body compartments, colonizing and initiating an infection. The first line of defense includes physical barriers such as epithelia, mucosal secretions, tears, urine, and gastrointestinal acid (Paul, 2008). The physical barriers are backed up by a complement system and an antigen-nonspecific cellular component that is designed to elicit an immediate response following the recognition of an internalized pathogen (Carroll et al., 2008). Central dogma described the innate immune response as a non-specific response to the pathogen encountered. However it is now known that the innate response has specificity for pathogens encountered (Akira et al., 2006). It is imperative that the innate immune system has the ability to discriminate between self and non-self, and pathogen recognition can be accomplished by recognition of molecular structures that are unique to microorganisms and are not produced by the host (Akira et al., 2006). This distinction between self and

non-self has also led to the evaluation of differing responses between microorganisms, due in part to molecular structures produced by microorganisms. These structures, pathogen-associated molecular patterns (PAMPs), represent the main targets of the innate immune response. Recognition of PAMPs leads to the activation of immune responses (Akira et al., 2006). Recognition is accomplished by a developed set of nonclonal receptors that can recognize PAMPs and these receptors are germline-encoded pattern-recognition receptors (PRRs; Medzhitov and Janeway, 1997).

Pathogen-associated molecular patterns (PAMPs)

While there is a large repertoire of PAMPs, the most recognized PAMPs include lipopolysaccharide (LPS) of gram-negative bacteria; lipoteichoic (LTA) of gram-positive bacteria; peptidoglycan; lipoproteins generated by palmylation of the N-terminal cysteines of many bacterial cell wall proteins; lipoarabinomannan and lipopeptides from mycobacteria; cytokine-phosphate-guanine (CpG) DNA; double-stranded RNA (dsRNA), that is produced by most viruses during the infective cycle; flaggellin and β -glucans and mannans isolated in fungal cell walls (Paul, 2008). All of these structures are produced by different types of microorganisms, but these structures are conserved to the microorganisms and not produced by the host. Therefore, PAMPs are generally involved in the basic metabolic functions of the microorganism and are considered molecular signatures since typically the organism cannot function or survive without the particular molecular pattern. Major pathogens of cattle, *Histophilus somni*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, bovine herpesvirus-1 (BHV-1; infectious bovine rhinotracheitis), PI-3, BRSV, and bovine viral diarrhea virus (BVDV),

all produce some type of PAMP that is recognized by epithelia, alveolar macrophages, and/or intravascular macrophages (Ackermann et al., 2010). Intravascular macrophages are located within the small capillaries of alveolar walls and attached to the underlying endothelial cells that are very active in the metabolic generation of inflammatory mediators (Ackermann et al., 2010). In the case of acute inflammation neutrophils will recognize PAMPs as well as cells associated with the adaptive immune responses, various dendritic cells, natural killer cells, alpha/beta and gamma/delta T cells, and B cells will all interact with microbial PAMPs to elicit an immune response (Ackermann et al., 2010). The lung expresses a wide variety of extracellular, cell surface, endosomal, and cytoplasmic receptors that recognize microbial PAMPs, termed pattern recognition receptors (PRRs).

Pattern-recognition receptors (PRRs)

The innate immune system uses a variety of PRRs to identify the different PAMPs associated with microorganisms to orchestrate a specific response to the type of structure it recognized or the infection that has been initiated. Different PRRs react with specific PAMPs, show distinct expression patterns, activate specific signaling pathways and lead to distinct responses (Akira et al., 2006). There are several classes of PRRs that the host uses to detect PAMPs that differ in function, expression, and location. There are three functional classes that PRRs can be classified as; PRRs that signal the presence of infection, PRRs that are phagocytic/endocytic, as well as secreted PRRs (Paul, 2008).

There are various detection methods that PRRs use to signal inflammation and can be either intracellularly or on the cell surface. The end result and implications after

activation of the signaling cascade leads to the induction of genes involved in the initiation of the inflammatory response (Akira et al., 2006). Receptors in this class are Toll-like receptors (TLRs), Dectin-1, NOD proteins, RIG-I and MDA-5 protein. Akira et al. (2006) described the various signaling pathways this class of PRRs typically use as: nuclear factor-kB (NF-kB), interferon regulatory factors (IRFs), Jun N-terminal kinase (JNK) and p38 MAP kinase; these are key signaling pathways to induce expression of pro-inflammatory cytokine genes. Based on the genes that are activated by the PRRs signaling pathway, there are a variety of products induced by the PRRs that include; proteins and peptides, inflammatory cytokines and chemokines, and genes that control adaptive immune responses.

Receptors that are classified as phagocytic or endocytic receptors include the macrophage mannose receptor (MR) and macrophage receptor with collagenous structure (MARCO; Paul, 2008). These receptors are generally located on the surface of immune cells such as; macrophages, neutrophils, and dendritic cells (DCs; Paul, 2008). Receptors in this class recognize PAMPs on the surface of the pathogen and mediate the uptake into the phagocytes, at which point there is delivery to the lysosomal compartments to be killed or disposed (Paul, 2008). There are several effector mechanisms that are used by phagocytes to destroy microorganism that are present. After phagocytosis, macrophages and DCs process the pathogen-derived proteins so the major histocompatibility complex (MHC) molecules can present the proteins to the T cells for recognition and induction of the adaptive immune response (Paul, 2008).

The other functional class of receptors is secreted PRRs and are categorized into four major structural classes: collectins, pentraxins, lipid transferases, and

peptidoglycan-recognition proteins (PGRPs; Paul, 2008). This class of receptors can perform three different functions; activation of the complement system, opsonization of microbial cells to assist in facilitating phagocytosis, as well as function in some cases as accessory proteins for PAMP recognition by trans-membrane receptors like TLRs (Paul, 2008). These secreted PRRs can be secreted by macrophages and epithelial cells into tissue fluids, but most are generally secreted into the serum by the liver. Many of the secreted products or proteins are known as acute phase proteins since production is usually increased during the acute phase response, which is a systemic inflammatory response induced by inflammatory cytokines such as IL-6, TNF- α , and IFN- γ .

Immune cells located in the respiratory track of cattle make up a portion of the lung defenses, and express extracellular PRRs including LPS binding protein, mannan-binding lectins such as ficolin and collectins, and also C-reactive protein and serum amyloid protein (Ackermann et al., 2010). The most recognized cell surface PRR on respiratory epithelia include TLRs, which are transmembrane receptors that have outer leucine-rich repeats (LRR) and cytoplasmic TIR/interleukin-1 (IL-1) receptor homology domains that transmit signals to the nucleus once the outer LRR is activated by PAMPs (Ackermann et al., 2010). Recognition through TLRs activates the NF- κ B or the interferon regulatory factors (IRF) -3 and -7 (Akira et al., 2006). Cattle have ten TLRs that have specific and sometimes overlapping PAMP affinities. The TLRs repertoire in cattle includes; TLR1 has affinity for triacyl lipopeptide of mycobacteria, TLR 2 has affinity for peptidoglycans of gram-positive organisms and lipoabinomannan of mycobacteria and zymosan of fungi, TLR 3 has affinity for dsRNA, TLR 4 has affinity for LPS; TLR 5 has affinity for flagellin; TLR 6 has affinity for diacyl lipopeptides of

mycoplasma; TLR 7 and 8 have affinity for single-stranded RNA (ssRNA); TLR 9 has affinity for CpG; and TLR 10 is not fully assessed (Ackermann et al., 2010). Depending on the pathogen encountered and receptor activated dictates the signaling cascade that ensues. Several of the TLRs signal through the MyD88 leading to NF-kappa B activity and inflammatory reactions, whereas other TLRs are MyD88 independent (signaling through TRIF/TRAF) and induce IRF-3 and -7 and type I interferon genes.

Bacterial pathogens isolated from the bovine lung during respiratory outbreaks include *Histophilus somni*, *Mannheimia haemolytica* and *Pasteurella multocida* which contain an LPS component that interacts with TLR 4 (Ackerman et al., 2010). Bovine respiratory viruses; BHV-1, PI-3, BRSV, and BVDV can infect the lung epithelia cells causing activation of TLR 3, 7, 8, and 9 signaling cascades (Ackermann et a., 2010).

Cells of the innate immune system

The PRRs of the innate immune system are expressed on a variety of cell types. Cellular component of the innate immune system are; neutrophils, monocytes, macrophages, DCs, natural killer cells, basophils, mast cells, eosinophils and surface epithelial cells (Carroll et al., 2008). The majority of these cells are derived from pluripotent hematopoietic stem cells that originate in bone marrow (Marsh and Kendall, 1996). Depending on the signals received by the pluripotent hematopoietic stem cells, the fate of the immature cells are determined. The immune cells are specialized to function at different stages of the immune response or infection to deal with the variety of pathogens the host encounters. These cells are responsible for rapid response host defense as well as immune surveillance (Marsh and Kendall, 1996). The innate immune

system also possesses a wide variety of antimicrobial effector mechanisms that vary in inducibility, site of expression, mechanism of action and activity against different types of pathogens. There are five major classes of antimicrobial effectors: enzymes that hydrolyze components of microbial cell walls, antimicrobial proteins and peptides that disrupt integrity of microbial cell walls, microbicidal serine proteases, proteins that sequester iron and zinc, and enzymes that generate toxic oxygen and nitrogen derivatives (Marsh and Kendall, 1996). It is in this way, that the innate immune can guarantee that the host can mount the appropriate immune response for the pathogen it has encountered, as well as ensure the appropriate magnitude. The innate defense provides several important elements when considering host protection. One of the most important components of the innate defense are cells of the innate system eliminating disease challenges without stimulating an extensive acquired immune response. So in essence the innate immune system can provide a local solution to what otherwise could be a systemic problem; as well as being efficient in resolving an infection before it overwhelms the immune defenses. In the case that acquired immune response is needed, the innate defenses facilitate stimulation of the acquired or adaptive immune response and the majority of the regulatory influence of the innate defense is regulated or generated by the production of the specific metabolites and cytokines.

Inflammatory response

It is important to understand the role of the different classes of receptors used to recognize and initiate the appropriate immune response as previously discussed. Upon recognition by a receptor a signaling cascade is initiated and generally followed by

induction of a pathogen associated pro-inflammatory response can be characterized by secretion of several of cytokines. The inflammatory response is characterized by different phases of immune responsiveness to an immune insult; the initial event involves the recognition of the pathogen leading to the production and/or release of inflammatory mediators that attract migratory cells to the site. Upon recognizing and binding of the mediator, phagocytic cells adhere to the endothelial cells and migrate from the bloodstream via increasing concentrations of chemotactic stimuli to the site of infection (Paul, 2008). When a sufficient amount of inflammatory mediators are encountered near the source of the signals, the immune cells involved are fully activated to ingest and destroy the agent(s) involved in infecting the host (Paul, 2008). These mediators can also release enzymes and toxic oxygen products into the external milieu to aid in the immune response associated with the infection. Generally acute inflammation caused by the release of these inflammatory mediators can be characterized by five cardinal signs: redness, increased tissue temperature (heat), swelling, pain and loss of function at the infected area (Tizzard, 2004). Redness and increased temperature are due to increased blood flow to the inflamed area; swelling is due to the accumulation of fluid; pain is due to the release of chemicals that stimulate nerve endings; and loss of function is caused by the combination of these factors. Under normal physiological conditions, inflammation subsides after the pathogen is eliminated, but in pathologic conditions the inflammatory response can become chronic and lead to tissue destruction (Tizzard, 2004). With acute inflammatory responses, endothelial cells become activated by the inflammatory mediators, open gaps, and allow the passage of serum factors into the alveolar lumens of the lung. The fluid contains dilute microbial agents and toxins, as well as containing

numerous molecules with protective immune functions such as complement and acute phase proteins.

Pro-inflammatory cytokines

The immune cells produce a variety of cytokines during an immune insult, and these cytokines can vary in functionality and overlap each other in other cases.

Cytokines are inflammatory molecules that act in an autocrine, paracrine or endocrine fashion and are pleomorphic (Webster Marketon and Glaser, 2008). The interleukins (IL) are a subfamily of cytokines that are generally produced by leukocytes. Other cytokines that can be produced include tumor necrosis factor- α (TNF- α) and the interferons (IFN). This review is a general overview of some cytokines that can be produced and the actions associated with the release of these cytokines; this is not a comprehensive list of all cytokines or mediators released during an inflammatory response. It is important to understand the implications after cytokines are produced and how cytokines regulate an immune response.

Tumor necrosis factor-alpha

Tumor necrosis factor- α is a cytokine produced from a subset of lymphocytes, T helper (Th) cell 1, involved in cell-mediated immunity and autoimmunity. This cytokine is generally one of the first produced during the inflammatory response, and is an important step in the development of both the inflammatory and immune responses due to its subsequent induction and feedback effects (Marsh and Kendall, 1996). It is produced by macrophages, a direct result of a PAMP binding to the receptor in the

immune cell. One of the main roles of TNF- α is to protect against infections by promoting macrophage activity and apoptosis to eliminate invading pathogens. Tumor necrosis factor- α can also function as an autocrine factor for macrophage differentiation and is responsible for stimulation of IL-1, as well as the indirect induction of IL-6 and acute phase proteins. Tumor necrosis factor- α has been reported to directly regulate neutrophil activation by mediating the adherence of polymorphonuclear (PMN) cells to endothelial cells and increasing the neutrophil respiratory burst which produces oxygen free radicals (Marsh and Kendall, 1996).

Interleukin-6

Interleukin-6 is an important regulator/trigger of the acute-phase response and is involved in the autoimmune response. Interleukin-6 has been reported to regulate hematopoiesis, inhibit antigen presentation and proliferation by macrophages, as well as stimulate the hypothalamic-pituitary-adrenal axis (Marsh and Kendall, 1996).

Interleukin-6 is also vital in acute and/or chronic inflammation at the site of infection and for regulation of sickness behavior and fever.

Interferon-gamma

Interferon- γ was originally described on the basis of its antiviral activity, but it is now been established that IFN- γ has important pleiotropic regulatory effects on the immune and inflammatory processes (Paul, 2008). Interferon- γ is produced by T-lymphocytes as well as Th1 CD4 subpopulations (Paul, 2008), but the major source of IFN- γ is the natural killer (NK) cells that produce IFN- γ in response to mitogens or to

microbial products in the presence of TNF- α (Marsh and Kendall, 1996). The production of IFN- γ is regulated by the interaction of a variety of cytokines, as well as IFN- γ regulating other cytokines through a negative feedback mechanism (Marsh and Kendall, 1996). The effects of IFN- γ include up-regulation of TNF- α production by macrophages and the induction of adhesion molecules tetonic for inflammatory cell extravasion (Paul, 2008). One of IFN- γ most significant roles with respect to rapid host defenses is the ability to activate macrophages for a variety of functions including, cytotoxicity and the generation of reactive oxygen and nitrogen intermediates.

Macrophages, neutrophils, and NK cells constitute a diminutive group of immune cells whose function is to aid in the early defense of the host from pathogens. Cells involved in the innate immune response provide critical homeostatic regulation within tissues and contribute to priming the acquired immune response by antigen presentation and processing. Macrophages, neutrophils and NK cells release cytokines and other metabolites that can act on a variety of cellular targets; this can also influence other physiological systems including endocrine systems and the growth axis. The immune and neuroendocrine system influence each other through a series of mediators such as cytokines, neurotransmitters and hormones as a method to assist in the regulation of homeostasis (Matarese and La Cava, 2004). Cytokines are mediators and communicate between different systems in the body, but the majority of cytokines function to communicate with each other to influence the production of and respond to signals from other cytokines (Matarese and La Cava, 2004). Since cytokines can alter production of other endocrine hormones it is imperative to understand the role of cytokines secreted during an immune response can have on these other endocrine systems. While these

cytokines still have crucial roles in regulating the immune response it is imperative that the immune response stays within critical set-points to eliminate the chance of over-responding and overwhelming the body. If the proper immune response is not mounted there can be implications on other systems like the HPA and somatotrophic axis.

Stress and the innate immune system

Cytokines, peptide hormones and neurotransmitters, as well the receptors are endogenous to the brain, endocrine and immune systems. It is the commonality between the immune and neuroendocrine systems that allow for a similar chemical language to be utilized for communication. The cross-talk between these systems suggests an immunoregulatory role for the brain and a sensory function for the immune system, which in turn assists in maintaining homeostasis of the body. The interplay between the immune and endocrine system is generally associated with pronounced effects of stress on the immune system. The HPA axis plays a key role in the stress response; and both internal and/or external stimulants generalized as “stressors” can activate the HPA axis. The pro-inflammatory cytokines as previously discussed are chemical messengers that can also stimulate the HPA axis when the body is experiencing stress or an infection. Another HPA/cytokine interaction that warrants further examination is the regulation of cytokine gene transcription by alterations in NF- κ B. The two main neuroendocrine pathways generally activated during a stress response that regulate the immune system are the HPA axis and the sympathetic nervous system (SNS). Activation of the HPA axis generally results in the release to glucocorticoids (GCs), and release of catecholamines;

epinephrine and norepinephrine from the SNS. However, there are other neuroendocrine factors that are released following a stressor that can also regulate the immune system.

Stress pathways: HPA and SNS

Upon stimulation of the HPA axis by a stressor, CRH is secreted from the paraventricular nucleus (PVN) of the hypothalamus. In some cases AVP is released from the supraoptic and paraventricular nucleus of the hypothalamus (Tsigos and Chrousos, 2002). Secretion of CRH and AVP can act synergistically to stimulate the anterior pituitary to secrete adrenocorticotropin hormone (ACTH), as well AVP can stimulate cells in the posterior pituitary to produce antidiuretic hormone (ADH) into the systemic circulation (Tsigos and Chrousos, 2002). In turn, ACTH acts on the zona fasciculata and reticularis cells in the adrenal glands to synthesize and secrete GCs (Webster Marketon and Glaser, 2008). Glucocorticoids are a class of hormones derived from cholesterol. Cortisol, the GC of primary concern in cattle, functions to: stimulate gluconeogenesis, proteolysis of muscle tissues, and lipolysis of adipose tissues (Sherwood, 1997). These metabolic shifts ensure that the body has adequate energy available to address the perceived stressor. Activation of the SNS results in secretion of acetyl choline from the pre-ganglionic sympathetic fibers in the adrenal medulla. This induces release of the catecholamines epinephrine and norepinephrine. Catecholamines are a class of hormones derived from tyrosine (Sherwood, 1997).

Glucocorticoids modulate immune responses

Stress can affect various aspects of the immune system. Stress can reduce NK cell activity, reduce the number of lymphocytes, decrease the ratio of helper to suppressor T cells, decrease antibody production, reactivate latent viruses, and modulate cytokine production (Webster Marketon and Glaser, 2008). While all the effects of stress on immune parameters are important, for the purpose of this review, the focus will be on the impact and interaction of cytokines and activation of the HPA axis.

Anti-inflammatory properties of glucocorticoids

Glucocorticoids have been used therapeutically for a variety of inflammatory and immune diseases (De Bosscher et al., 2000), and it is generally accepted that the anti-inflammatory and immunomodulatory actions are usually due to inhibition of the activity of transcription factors. Some transcription factors that have been identified include activator protein-1 (AP-1) and NF- κ B that are involved in activation of pro-inflammatory genes that produce the cytokines. Glucocorticoids act to suppress the production and effects of humoral factors involved in the inflammatory response. Glucocorticoids can, inhibit leukocyte migration to sites of inflammation, interfere with the functions of endothelial cells, and alter leukocyte and fibroblast function (Cato and Wade, 1996). Due to GCs hormonal and lipophilic nature, GCs pass freely through the cell membrane; at the level of gene expression GCs exert effects by binding the glucocorticoid receptor (GR; De Bosscher et al., 2000). The GR is a transcriptional factor that once bound can regulate several genes either positively or negatively. The GR belongs to a super family of thyroid/steroid nuclear hormone receptors and functionally is composed of three

domains: a N-terminal constitutive action domain (Tau-1 or Af-1); a central binding domain; and a C-terminal ligand binding domain (De Bosscher et al., 2000). When the GR is exposed to its ligand and binding occurs, a conformational change ensues causing the release of the GR thus allowing its translocation to the nucleus (De Bosscher et al., 2000).

The transcription factor NF- κ B activates genes coding for cytokines, cytokine receptors, chemotactic proteins and adhesion molecules. Activation of NF- κ B is accomplished by signals or agents such as cytokines, stress, bacterial or viral infections. There is a cascade of events that trigger activation of NF- κ B but there a variety of phosphorylation events, regulatory and transcriptional steps, and interactions that occur in order to activate the NF- κ B-dependant target genes. Therefore, NF- κ B has been implicated in controlling inflammatory processes and a number of studies have reported the inhibitory effects of GCs on the transcriptional factors NF- κ B, AP-1 or cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) to try and explain the repressive actions of GCs on immune target cells. Glucocorticoids can sequester NF- κ B in the cytoplasm to exert actions on the target cells. The proposed mechanism of action GCs use alter NF- κ B activity is to induce an increase in the synthesis of I κ B- α , a precursor to activation of NF- κ B, which in turn caused retention of NF- κ B with the cytoplasm (Auphan et al., 1995). These researchers suggested that the mechanism of action was gene repression of GCs and was hypothesized to occur by stopping the NF- κ B from interacting with its response element and allowing gene transcription. Another method described by investigators is the protein-protein interaction model (McEwan et al., 1997). There are several mechanistic models to

explain the inhibitory protein-protein interactions between the GR and NF- κ B. The transcriptional inhibition by the GR could be a result of the masking of transactivating domains, induction of post-translational modifications, conformational changes within the transcription initiation complex or competition for coactivators (Dumont et al., 1998). The last method described involves the coactivator proteins that interact and potentiate the activating capacity of the nuclear receptors. Coactivator proteins mediate the transcriptional activity, not only of nuclear receptors, but also of other transcriptional factors typically by acetyltransferase functions (Glass et al., 1997).

It is generally assumed the immunomodulatory actions of GCs on immune target genes occur on the transcription factors or CREB. While the exact mechanisms are not clearly understood the three methods provide an explanation for the negative cross-talk between NF- κ B and GR. There are numerous studies that have examined the relationship between stressors and cytokine production; GCs have been reported to depress cytokine synthesis in the brain and oppose the actions of cytokines on the blood brain barrier (Turnbull and Rivier, 1999). Researchers have evaluated different perceived stressors and how these stressors can modulate the immune parameters of interest. Examination stress was associated with a decrease in IL-2 receptor mRNA concentrations and protein expression in peripheral blood leukocytes compared to baseline concentrations (Glaser et al., 1999). Examination stress was also associated with a decrease in the production of IFN- γ by peripheral blood leukocytes stimulated with concanavalin A (Con A) or phytohaemagglutinin (PHA) in medical students as compared to IFN- γ production one month prior to exams (Glaser et al., 1986). Production of IL-6 and TNF- α at wound sites was less in couples experiencing marital stress, but TNF- α concentrations were greater in

more-hostile couples the morning after a conflict (Kiecolt-Glaser et al., 2005). These studies assist in implicating that stress can impact cytokine production, but since there are a variety of stressors and can vary in magnitude, duration, and timing; all of these factors can affect the impact a stressor can or will have on an immune response or more specifically cytokine production.

Pro-inflammatory properties of glucocorticoids

Glucocorticoids are generally considered anti-inflammatory, but there is emerging evidence that under some conditions GCs sensitize central pro-inflammatory responses (Sorrells and Sapolsky, 2007). While it has been demonstrated that large doses of synthetic glucocorticoids have immunosuppressive actions (Behrend, E. N. and R. J. Kemppainen, 1997), recent research suggests that the concentration and timing are important factors when determining the effects on immune parameters instead of generalizing GCs as immunosuppressive. The activity of NF- κ B and expression of NF- κ B target genes increase following acute stress in PBMC (Bierhaus et al., 2003). Moreover, peripheral and central nervous system concentrations of pro-inflammatory cytokines were increased in rats following an acute stressor (O'Connor et al., 2003). Acute stress or decreased concentrations of GCs has been reported to enhance neutrophil proliferation and survival (Liles et al., 1995) as opposed to chronic stress where the same effects are not revealed. Acute stress models have also been observed to enhance peripheral inflammation by GC-enhanced leukocyte migration to the area of localized inflammation (Dhabhar and McEwen, 1999). Not only does the magnitude and duration of the stressor have a significant impact on the outcome of the immune challenge but the

timing also plays a critical role. When administered prior to an immune challenge, GC's have been reported to augment the subsequent cytokine response. Treatment with GCs 12 h prior to an LPS and IFN- γ stimulation elicited an increase in NF- κ B signaling and an increase production in IL-6, TNF- α and nitrite in cells from murine macrophages (Smyth et al., 2004). Similarly, humans exposed to GCs 12 h prior to an LPS stimulation were observed to have increased concentrations of TNF- α and IL-6 (Barber et al., 1993). Johnson et al. (2002) also observed an increase in TNF- α , IL-6 and IL-1 β in the rat when given a GC challenge 24 h prior to LPS. In each of these challenges, GCs had either been removed from the media or concentrations had returned to baseline at the time of exposure to the immune or LPS challenge. Therefore, these effects are not due to the concentration of GCs at the time of the challenge, instead GCs appear to be preparative effect of the subsequent inflammatory response. Frank et al., (2010) evaluated whether acute administration of exogenous GCs could reproduce the stress-induced sensitization of neuroinflammatory responses using different timing relationships between GC administration and LPS challenge. These researchers reported that GCs potentiate both the peripheral (liver) and central (hippocampus) pro-inflammatory response to a peripheral LPS challenge if GCs are administered prior (2 and 24 h) to the LPS challenge. In contrast, (Frank et al., 2010) also reported that when GCs were administered after (1 h) the peripheral LPS challenge, GCs suppress the pro-inflammatory response to LPS in both liver and hippocampus.

It is still unclear what governs when GCs are pro- or anti-inflammatory, but GC concentration, time course, magnitude and the activation state of the immune system are all significant factors contributing to the outcome. There are several trends that emerge

among the varying GC effects on inflammation such as: decreased-doses or concentrations, acute-exposure, and GC presence prior to inflammation all tend to have pro-inflammatory properties or augment it; whereas high-doses or concentrations, chronic-exposure and GC presence after inflammatory process tend to have anti-inflammatory properties.

The bi-directional communication between the immune and neuroendocrine systems plays a critical role in modulating or regulating the adequate response of the HPA axis to stimulatory factors produced by immune cells. Therefore, not only can GCs have pro- and anti-inflammatory properties on immune components, but there is a feedback system between components of the immune system and the HPA axis. As mentioned previously the common signals that are shared between the two systems are cytokines, and within the HPA axis there are a variety of areas where cytokines can exert actions and augment a response. When understanding the organization of the HPA axis, the PVN of the hypothalamus is the primary CNS source of corticotrophin-releasing hormone (CRH), which is the major regulator of ACTH that in turn stimulates the release of GCs. Researchers have evaluated feedback mechanisms along and within the HPA axis to better understand where cytokines regulate the activity. There are a variety of areas of interest among researchers that have been investigated and hypothesized as a possible regulatory process along the HPA axis. The hypophysiotropic region of the PVN receives diverse inputs from other nuclei within the hypothalamus, as well as other regions of the CNS including areas within and outside blood-brain barrier (Turnbull and Rivier, 1995). Neurons project to the external zone of the median eminence and release CRH into the hypophysial portal circulation. Then, CRH stimulates synthesis and

secretion of ACTH and other proopiomelanocortin (POMC)-derived peptides. The release of ACTH is a potent inducer of the secretion of GCs from the adrenal cortex, and these steroids inhibit the synthesis and secretion of CRH within the hypothalamus and POMC-derived peptides in the pituitary. The CRH neurons within the PVN can also produce a number of additional peptides, most notably AVP which can interact with CRH to stimulate ACTH secretion. Therefore, there are multiple regulatory steps at which the activity of the HPA axis may be modulated, and all the process mentioned above have been proposed as sites at which cytokines can also regulate HPA activity.

HPA activation by immune components

Receptors for a variety of the cytokines have been identified within the HPA axis. Studies using the rat brain have reported widespread binding of both IL-1 α and β in the hypothalamus (Turnbull and Rivier, 1995). Yabuuchi et al. (1994) observed similar results and reported receptors were present within the hypothalamic regions such as the ventromedial hypothalamus and arcuate nucleus, as well as other regions in the brain relevant to the regulation of HPA activity. Earlier research (Cunningham et al., 1992) reported receptors to be concentrated in the hippocampus, but were not detected in the hypothalamus. There are also IL-6 binding sites within the bovine hypothalamus (Cornfield and Sills, 1991), and the IL-6 receptor mRNA has been observed within the preoptic, dorso-medial, and ventro-medial areas of the rat hypothalamus (Turnbull and Rivier, 1995). Receptors have also been observed in anterior pituitary glands of rodents for IL-6 and TNF- α (Velkeniers et al., 1994). The presence of receptors for cytokines

within tissues associated with the HPA axis provides the potential for cytokines to modulate HPA activity and neuroendocrine-immune responses.

Given the abundance of cytokine receptors within the HPA axis, it is plausible that cytokines have profound effects on HPA activity. Immunoneutralization of CRH has been demonstrated to inhibit activation of the rat HPA axis by intravenous IL-6 and TNF- α (Sapolsky et al., 1987), which supports the concept that these cytokines initiate ACTH secretion by stimulating the secretion of CRH. Increases in IL-6 mRNA in the PVN (Lee and Rivier, 1994) as well as TNF- α rapidly stimulate CRF secretion from the rat median eminence (Sapolsky et al., 1987). The combination of data provides evidence that ACTH secretion induced by cytokines can be mediated by CRH. Rivest et al. (1992) administered IL-1 β directly in the cerebroventricular and observed a rapid increase in ACTH as compared to intravenous administration. These researchers suggested IL-1 injected centrally activated the HPA axis by acting on the cell bodies of the hypophysiotropic neurons within the PVN, whereas IL-1 administered systemically acts elsewhere within the HPA axis. Since increased secretion of CRH appears to be a necessary step in elevating ACTH secretion following both systemic and centrally administered cytokines, it seems likely that cytokines act at the hypophysiotropic nerve terminals in the median eminence. *In vitro* studies provide further evidence that cytokines can stimulate the hypothalamus to release CRH. Incubation with IL-6 consistently produces a rapid (within 20 min) increase in CRH release from hypothalamic explants (Spinedi et al., 1992). The effect of cytokines on pituitary ACTH secretion generally requires long incubation time (2-8 h) to observe stimulatory effects of IL-6 and TNF- α on pituitary ACTH secretion (Turnbull and Rivier, 1995). The abundance of

cytokine receptors within the adrenal glands, IL-6 and TNF- α have been reported to induce GC secretion of adrenal cell cultures (Mazzocchi et al., 1993). Generally the stimulatory effects of cytokines on adrenal GC secretion *in vitro* have been observed only after 24 h of incubation, suggesting that GC secretion by the adrenal occurs only after prolonged exposure to cytokines.

The HPA system communicates bi-directionally with the neuro-immune-endocrine system. The neuro-immune-endocrine interface is mediated by cytokines acting in an autocrine, paracrine, or endocrine fashion and by doing so can regulate pituitary development, cell proliferation, hormone secretion and feedback control of the HPA axis (Haddad et al., 2002). The products that transmit information from the immune compartment to the CNS or HPA axis act as immunotransmitters and function as immunoregulatory neuroendocrine inputs. The importance of systemically and locally produced cytokines interacting with other systems in the body and modulating responses may explain some of the effects observed in growth and pathophysiology of diseases.

Growth and innate immune response

Hammond (1944) described the concept of prioritization of nutrients to different tissues to ultimately ensure the survival of the animal. Elsasser et al. (2008) expanded on this subject and inferred that to better understand the concept of redirecting metabolism during the pro-inflammatory state, one could assume surviving an immune insult takes priority over other biological needs, especially growth and tissue accretion in a young animal. Generally at the onset of the pro-inflammatory response, several of the first responder cytokines are purely catabolic in nature, promoting a physiological state of

readiness to retrieve needed energy substrates from storage depots like fat and in some instances muscle (Elsasser et al., 2008). These first responders are subsequently downregulated and reversed back towards an anabolic state and function as the later arriving anti-inflammatory cytokines and acute phase proteins reestablish normal function in the body. The initial responses are generally catabolic for two main reasons: 1) when fever is present the caloric demand can increase as much as 30% for each 1°C increase in core body temperature (Baracos et al., 1987) and 2) if the increase in temperature is accompanied, as it usually is, by decrease in feed intake that occurs during pro-inflammatory stress, the needed calories are channeled away from other resources and processes in a prioritized manner that is proportional to the severity of the response initialized due to the immune insult (Elsasser et al., 2008).

Growth hormone (GH)

A pituitary hormone, growth hormone (GH), has been reported to exhibit a variety of effects on the cells and organs associated with immune function. Classically, GH is defined as a peptide hormone that is synthesized and secreted primarily by the somatotrophic cells in the anterior pituitary (Webster Marketon and Glaser, 2008). The production of GH is pulsatile, and is controlled by hypothalamic hormones such as GH-releasing hormone (GHRH), hypothalamic GH release-inhibiting factor and somatostatin (SS), as well as other metabolic hormones such as leptin and ghrelin (Webster Marketon and Glaser, 2008). Upon stimulation, GHRH is generally secreted from the arcuate nucleus of the hypothalamus which then exerts its action on the somatotrophic cells of the anterior pituitary to release GH (O'Connor et al., 2008). Growth hormone receptors are

located in a variety of tissues, but are primarily associated with the stimulation of liver cells to produce IGF-1 a hormone that has anabolic properties. Moreover, GH has been reported to mediate the proliferation of a number of cell types including chondrocytes, fibroblasts, adipocytes, myoblasts and lymphocytes (O'Connor et al., 2008). Many of the effects observed due to GH appear to be mediated either directly or indirectly by IGF-1. Circulating concentrations of GH are greatest in the immediate neonatal period, decreasing during childhood but increasing again during puberty (O'Connor et al., 2008).

Insulin-like growth factor-I (IGF-I)

The IGFs can display pleiotropic properties, including the ability to promote cellular proliferation, differentiation, and metabolism or hypertrophy (McCusker et al., 2006). In other words, IGFs can play a role in nutrient transport, energy storage, gene transcription, and protein synthesis with these actions being common for both IGF-I and II. These actions are common to both IGF-I and IGF-II and all responses are mediated by either the type 1 IGF receptor (IGF-1R) or the insulin receptor, which has a similar structure but differ in ligand preferences (O'Connor et al., 2008). This means that IGF-I, IGF-II and insulin can have the same effects on cells with differing sensitivities that are proportionate to the affinity for the receptors and also depends on the abundance of the type of receptor that is available on the target cell (McCusker et al., 2006). With the vast presence of receptors and ligands, it should not be surprising that the IGF system in combination with GH, accounts for the majority of post natal growth (83%) in mice (Lupu et al., 2001). It should also be noted that when comparing large breeds versus

small breeds of dogs that a single polymorphism in the IGF-I gene is common to all small breeds but absent in large breeds (Sutter et al., 2007).

Secretion of IGF-I and therefore the serum concentrations that are measured are sensitive to nutritional and endocrine control. A variety of studies have reported that when IGF-I concentrations in serum are elevated (~100 ng/mL) that these concentrations are positively correlated to growth rate (McCuster et al., 2006). While serum concentrations have been correlated with increased growth rate, it should be noted that local concentrations of IGF-I rather than circulating IGF-I (which is generally derived from the liver), is the primary causative factor impacting growth (O'Connor et al., 2008). Another important regulatory factor associated with and unique to IGF-I and IGF-II, but absent from insulin, is a group of greater affinity IGF binding proteins (IGFBPs) that are present in extracellular fluids bind both IGF-I and IGF-II (O'Connor et al., 2008). The IGFBPs are responsible for maintaining a large circulating pool of IGF-I and IGF-II with less than 5% "free/active" hormone (Yakar et al., 2005). Therefore, approximately a 20-fold greater total hormone concentration is needed to cause the same biological action than what would be required or needed if IGFBPs were absent.

Growth modulation by cytokines

Both the immune and endocrine systems constantly survey the body and its environment to coordinate adaptive mechanisms that ensure homeostasis is maintained. The ability of the body to adapt is necessary to maintain optimal health and well being because it allows the body to fend off pathogenic insults, divert metabolic resources during times of nutritional insufficiency, and cope with physical and psychological

stressors. When young animals are in a state of “good” overall health, the metabolic actions of GH and IGF-1 are largely shifted towards anabolic processes or “growth”. Although in coordination with the immune system when signals associated with the pro-inflammatory cascade are recognized, the anabolic properties can be constrained and as the signals or severity of the immune challenge progress these molecules can become more catabolic in nature. Therefore, cytokine and hormone secretion is a dynamic process that results in an ever-changing cellular microenvironment. Cells are equipped with both hormone and PAMP receptors that interpret this interactive network of signals in order to respond in a physiologically appropriate fashion (O’Connor et al., 2008).

Within a single cell, integration of these signals received by receptors on the cell is called intracellular crosstalk. This is possible because the cellular signaling machinery is shared among distinct ligand-activated pathways (O’Connor et al., 2008). Intracellular enzymes and substrates are often affected by entirely unrelated ligands, and this can lead to signal amplification. Alternatively, activation of one pathway may interfere with signal transduction of another signal or subsequent pathway. This type of receptor crosstalk may result in cellular resistance to a given ligand. Due to the diversity of signals that can be transmitted in a cell, cellular responses can vary tremendously depending on a variety of factors. Some of these factors include; the relative ligand concentration, whether the ligand originates from the immune or endocrine system, the number of immune and hormone receptors on the cell, and signaling kinetics following activation of each receptor (O’Connor et al., 2008). Depending on the severity of the signals received and the body’s perception of the immune insult, this can manifest into an inability for tissues to respond to GH signals. It has been suggested the part of the inability of GH to relay

signals and repress GH actions are driven through the effects of pro-inflammatory cytokines such as TNF- α (Elsasser et al., 2008). The resistance of tissues to GH is further confounded when voluntary food intake is decreased and there is an uncoupling of the somatotrophic axis as described by Thissen et al. (1999). In order the better understand these processes, it would be valuable to understand how these pro-inflammatory cytokines can exert actions and impact the growth axis.

GH and IGF-I resistance and signaling by cytokines

Growth hormone resistance and somatotrophic axis uncoupling has been attributed to multiple actions of pro-inflammatory cytokines like TNF- α and IL-1 and downstream effectors on both the regulation of GH release from the pituitary, which consequently consists of inhibition of GH release stimulating properties of GHRH and thyrotropin releasing hormone (TRH), as well as direct actions at peripheral target cells such as JAK-STAT (signal transducer and activators of transcription) signaling in hepatocytes (Elsasser et al., 2001 and Daniel et al., 2002). The direct effects on target cells include decreased GH receptor (GHR) content, altered activity of STAT proteins, increased activity of suppressors of cytokine signaling (SOCS) proteins by TNF- α , and a generalized decrease in IGF-I message transcription (Elsasser et al., 2008). These researchers went on to suggest that the metabolic actions of IGF-I are not redirected, not only in the terms of IGF-I message transcription and tissue plasma concentrations, but rather also through the redistribution of IGF-I to tissues that are affected by the pro-inflammatory patterns of IGFBPs. Elsasser et al. (2007) demonstrated when using well-fed rapidly growing cattle and administering an LPS challenge, the pattern of change in

IGFBP-2 and -3 concentrations were differentially affected over time whereas the plasma concentration of IGF-I progressively declined over the 24 h period following the LPS challenge. Elssasser et al. (2007) went on to report that while the plasma IGFBP-3 (the major bulk carrier protein for IGF-I in plasma) content declined through 8 h after LPS and then rebounded to the normal range by 24 h, that the IGFBP-2 (IGF carrier that facilitates transendothelial IGF-I transfer) concentration was significantly increased at 4 h post-LPS and remained elevated even at 24 h. Based on this data and other research (Elsasser et al., 1995) indicates an intricate balance between the IGFBP gene transcription and translation and the increased production and release of specific binding proteins under the control of pro-inflammatory cytokines like TNF- α modulate the actions of IGF-I.

Another factor that contributes or impacts the GH signaling cascade is observed in the activity of several signal transduction elements in the GH and GHR cascade. When considering the contribution of GH to metabolism it is generally considered an anabolic agent whereas pro-inflammatory cytokines, namely TNF- α , are considered catabolic. Data suggests that localization to caveolar membrane domains elicit a more dynamic interaction between these contrasting effector molecules. These caveolae regions serve as the signal integrators which functionally redirect cellular metabolic responses during the proinflammatory response. The JAK-2 and AKT are major signal transduction elements through which both GH and TNF- α signaling occur (Barsacchi et al., 2003). Activation of the JAK-2 is accomplished by the catalyzed phosphorylation of specific tyrosine residues and this phosphorylation facilitates the dimerization of one of the STAT proteins and contributes to its translocation to the nucleus where it exerts its effect as a

gene transcription initiating factor (Elsasser et al., 2008). Other research supporting this claim evaluated the effect of a parasitic disease model in cattle, and concluded that plasma IGF-I concentrations were significantly reduced by infection with *Sarcocystis cruzi* and *Eimeria bovis* (Elsasser et al., 1995). Elsasser et al. (1995) went on to suggest the reduction in plasma concentrations of IGF-I in the cattle affected with *S. cruzi* were accompanied by reduced hepatic IGF-I mRNA content, but no changes were observed in GHR binding or GHR mRNA. When using an endotoxin model to examine the release of GH, the GH release in response to an injection of LPS is reduced in cattle (Elsasser et al., 1992) and this response is paralleled by a reduction in IGF-I (Elsasser et al., 2000). Daniel et al. (2002) while reviewing the literature suggested that the increase in cortisol observed during an endotoxin challenge may have a role in the reduced GH release.

The nitration of JAK-2 and the associated decrease in phosphor-STATb translocation to the nucleus were consistent with much of the GH resistance associated with endotoxemia (Elsasser et al., 2008). Elsasser et al. (2007b) described that after 7 h post immune challenge, membrane caveolin-1 content decreased and nitration of JAK-2 increased as did the phosphorylation activation of endothelial nitric oxide synthase (eNOS). Research from this lab (Elsasser et al., 2007b) also wanted to understand the consequences of intracellular generation of nitrated JAK-2 might have on IGF-I production in hepatocytes. Liver biopsy tissue was obtained from calves prior to and 7 h post LPS infection and hepatocytes had relatively greater concentrations of IGF-I mRNA in states of good health and nutrition, but within 7 h after the LPS challenge there was a 4.5-fold increase in cellular JAK-2 nitration associated with a 63% decrease in IGF-I mRNA concentration (Elsasser et al., 2007c). The inability of GH signals to be further

transmitted to the nucleus was linked to the increased generation of nitric oxide (Elsasser et al., 2008). When a glucocorticoid receptor agonist was utilized, the reduction in IGF-I was not as apparent as what was observed with the endotoxin model (Li et al., 1997).

Anabolic agents such as GH have also been proposed to improve the overall health and well-being of the animal by increasing anabolic actions to counteract the catabolic actions associated with disease states and modulate immune responses. Sartin et al. (1998) suggested that treatment with GH during an immune response improves protein anabolism, enhance nitrogen retention, improve protein synthesis, reduces urea generation, and improved wound healing. Elsasser et al. (1994) gave a decreased dose (0.2 microgram/kg, iv) endotoxin bolus and demonstrated that the use of GH decreased the TNF response to the endotoxin challenge and decreased membrane content of TNF receptors. When calves were implanted with an anabolic steroid approximately 3 weeks prior to being infected with *E. bovis*, the implanted calves had an increase in BW, fewer days of fever and diarrhea, and an improvement in the acute phase response (Heath et al., 1997). Conversely, when a catabolic parasitic model was used to induce disease, administration of GH to counter the catabolic effects of disease, there were no GH-induced improvements and the administration of GH appeared to have detrimental effects (Elsasser et al., 1995).

Conclusions

The HPA system communicates bi-directionally with the neuro-immune-endocrine system. This is of special importance when evaluating diseases such as BRD

that have a multifactorial etiology. The neuro-immune-endocrine interface is mediated by cytokines acting in an autocrine or paracrine or endocrine fashion which modulates pituitary hormone secretion and feedback control of the HPA axis. Information will be transmitted from the immune compartments to the CNS acting as immunotransmitters and function as immunomodulatory neuroendocrine products. Not only do immune products modulate CNS responses, but secretory products from the HPA axis can be immunomodulatory. Glucocorticoids as well as growth factors have been implicated to regulate immune responses. The challenge when studying the bi-directional communication between these systems is directly related to understanding the multiple factors that can influence both the immune system and HPA axis such as: timing, magnitude, duration, nutritional status and overall health. The interaction between the immune system and HPA axis is a revolving axis meaning that activation of one system can have consequences for the other. Therefore, when trying to understand and build models to study BRD understanding of this complicated and integrated system that BRD uses to infect the host must be considered.

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CHAPTER III
EVALUATION OF ENDOCRINE AND IMMUNE DISRUPTION OF STEERS
CHALLENGED INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

A manuscript to be submitted to *The Journal of Animal Science*

Abstract

Twelve crossbred steers (228.82 ± 22.15 kg BW) were used to evaluate the endocrine response of steers administered an immune challenge utilizing infectious bovine rhinotracheitis virus (IBRV). Steers were randomly assigned to a Control (CON) or an IBRV challenged group. Viral challenged steers received an intra-nasal dose of IBRV (8.0 TCID₅₀/0.3 mL solution; 4 ml total volume; 2ml/nostril) and CON steers received an intra-nasal dose of saline (2 ml/nostril). Prior to the challenge (-1 d) steers were fitted with a rectal probe and a blood sample was obtained via jugular venipuncture. On 0 d, steers were moved to their respective isolated paddocks. The first 48 h post-infection, blood was collected via single jugular venipuncture. At 72 h post-infection steers were fitted with indwelling jugular catheters and placed into individual stanchions. Blood samples were intensively collected on 4-8 d post-infection. Serum was analyzed for cortisol (CORT), interleukin (IL)-6, interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), growth hormone (GH), and insulin-like growth factor-1 (IGF-1). On 2 d of the

trial IBRV steers had increased rectal temperature as compared to CON steers ($P < 0.05$). Rectal temperature was greatest on 4 d, and returned to baseline on 6 d in the IBRV steers. The response patterns for cortisol, IFN- γ and GH all followed a similar pattern for IBRV steers increasing on approximately 2 d, largest increase on 4 d, and subsiding by 6 d. While there was a difference ($P < 0.05$) in GH concentrations between the IBRV and CON steers, IGF-1 concentrations did not differ ($P > 0.05$) between the 2 groups. Collectively the data revealed the alterations in the somatotrophic axis were not associated with a large increase in circulating concentrations of pro-inflammatory cytokines. Results suggest that the low dose of the virus used in the present study, while sufficient to elicit a febrile response, was not pathogenic enough to elicit a robust immune response. This could help explain the lack of a detectable cytokine response.

Introduction

One of the most economically devastating disease the beef industry faces is bovine respiratory disease (BRD). It has been reported that the economic impact of BRD from mortality and morbidity ranges from \$480 million (Engelken, 1997) to \$624 million annually (Smith, 1996). The BRD complex is the clinical manifestation of a complex interaction of a variety of factors that culminate into acute or chronic bronchopneumonia. Factors associated with the BRD complex are; infectious agents (viral and bacterial), stressors, and host susceptibility making it a difficult disease to diagnose and treat. Each of these factors or agents can potentiate the effects of others and this can lead to overwhelming the body's defense mechanisms.

There are a variety of viral pathogens associated with the BRD complex which have been implicated to cause suppression of the immune system and allow opportunistic bacteria the ability to overwhelm the host defense. Researchers (Schmidt et al., 2006; Babiuk et al., 1996; Orr et al., 1990; Rontved et al., 2000; Grell et al., 2005) have evaluated clinical and immunological parameters associated with viral challenges, but there is little information examining the impact the immune response to IBRV has on endocrine growth parameters. Previous research (Schmidt et al., 2006; Babiuk et al., 1996; Orr et al., 1990; Rontved et al., 2000; Grell et al., 2005) suggest viral challenge models observe differences in clinical observations and detection of immune parameters starting approximately 5 to 7 days after cattle are infected and last for 2 to 3 days.

Researchers (Carroll, 2008; Spurlock, 1997 and Elsasser et al., 2008) have suggested immune challenge models can cause alteration in the somatotrophic axis and support the evidence there is bidirectional communication between the endocrine and immune system. Based on the decrease in growth parameters associated with cattle diagnosed to have symptoms associated with the BRD complex (Gardner et al., 1999), it would suggest that pathogens implicated to cause respiratory problems and associated with the BRD complex could potentially impact the somatotrophic axis. Daniels et al. (2002) reported general response mechanisms during a disease challenge to include; immune components to be activated, endocrine response will favor catabolic reactions rather than anabolic, nutrients are repartitioned to fuel the increase in energy demands, and less essential functions are suspended. Disease challenge models have been reported to mediate the actions of endocrine responses namely those associated with the somatotrophic axis. Previous research reported plasma IGF-1 concentrations were

reduced by infection with *Sarcocystis cruzi* or *Eimeria bovis* but no differences were detected with GH concentrations (Elsasser et al., 1995). Elsasser et al. (1995) went on to report that even after resolution of feed intake and acute phase proteins had declined, there was still a reduction in IGF-1. Therefore, the objective of this study was to identify the pro-inflammatory response following IBRV exposure and to evaluate the impact on endocrine growth parameters.

Material and methods

The experiment was conducted at the Leveck Animal Research Facility at Mississippi State University (MSU). The Animal Care and Use Committee at MSU approved the experimental protocol (08-023) for this study.

Thirty days prior to weaning twelve crossbred steers (207.89 ± 15.45 kg BW) were identified at the Prairie Research Facility in Prairie, MS prior to receiving vaccinations for *Mannheimia haemolytica* and a modified-live vaccine (MLV) for IBRV, parainfluenza virus (PI-3), bovine respiratory syncytial virus (BRSV) and bovine viral diarrhea virus (BVDV). At weaning a blood sample was obtained to ensure all twelve steers were seronegative for IBRV, after which the steers were transported to the Leveck Animal Research Facility at MSU. Steers were allowed an adjustment period of 30 days upon arrival at the Leveck Animal Research Facility and allowed free access to hay and water while being supplemented with a 35% cotton gin mote, 35% hay, and 30% corn gluten supplementation diet to obtain a 2.2 kg/d gain. During the adjustment period the steers were brought up four times a week and intensively handled to ensure the steers were accustomed to being haltered and restrained in the metabolism crates utilized during

the trial period. Twenty-four hours prior to the challenge steers were processed through the working facilities and fit with a rectal temperature monitoring device as described by Reuter et al. (2010) that recorded temperature at one minute intervals. A weight was recorded and a blood sample was also obtained via a single venipuncture of the jugular vein. Upon exiting the working facility steers were sorted into their respective treatment groups and moved to isolated paddocks where supplementation was provided and free access to water.

On 0 d (0 h) prior to the steers receiving treatment challenges, and on 1 d, 24 h post infection, and 2 d (48 h post infection) a blood sample was collected via a single venipuncture of the jugular vein. Each day the CON steers were brought up to the working facility first to prevent cross contamination between the IBRV and CON steers. Steers were individually restrained in the chute and with the head positioned so that the jugular vein was exposed and the blood sample could be obtained. After the blood samples were obtained for each treatment group, steers were moved back into isolated paddock and offered the supplementation and allowed free access to water. Blood samples were allowed to clot for 30 minutes at 21°C and then centrifuged at 3,000 x g for 30 minutes (4°C) and serum was separated. Serum was collected and transferred into 15 mL microcentrifuge tubes^c for storage and frozen (-80°C) for later analysis.

On 0 d, after the blood sample had been obtained, the IBRV steers received a 4 mL dose of IBRV (2 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution) and CON steers received a 4 mL placebo dose of saline (0.9 w/v NaCl) solution (2 mL/nostril). All doses for each steer were placed in pre-labeled 15 ml conical tubes that were capped and kept at room temperature prior administering the solution to each steer. Viral and placebo doses

were administered using a glass and metal atomizer attached to a compressor-nebulizer method as described by Schmidt et al. (2006). Each steers head was restrained so that the atomizing device could be inserted into the nasal cavity and the solution could be administered. After the atomizing device was in place the opposing nostril was covered to help facilitate adequate dispersal into the nasal cavity. Upon inhalation the cut-off valve was closed, which resulted in emission of the pre-determined dose of a consistent mist into the nasal cavity of each animal within seconds. The same procedure was repeated in the opposing nostril for each animal. The atomizing device was sterilized between administration of viral and placebo doses by flushing the atomizer with 70% isopropyl alcohol.

On 3 d (72 h post infection), all steers were fitted with an indwelling jugular vein catheter for serial blood collection. Catheters consisted of approximately 150 mm of PTFE tubing (6417-41 18TW; Cole-Palmer; o.d. = 1.66 mm) which was inserted into the jugular vein using a 14-gauge x 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). Catheters and needles were sterilized in Nolvasan[®] solution before use. The catheter was maintained in place using tag cement and a 5.1 cm wide porous surgical tape and fitted with extensions made of sterile plastic tubing (Tygon S-50 HL; VWR Scientific; i.d. = 1.59 mm; o.d. = 3.18 mm) for collection of blood samples without disturbing the steers. After catheters were inserted and functioning, steers were moved into individual metabolism stanchions. At 80 h (after all catheters were inserted and steers were in the stanchions) blood was collected every 4 hours. Blood samples were collected every hour for 64 h (96 – 160 h); after which samples were collected every 4 h (164 – 208 h), and every 12 hours (220 – 364 h), and finally every 24 hours until the

completion of the trial (676 h). When a blood sample was obtained, approximately 5 ml of waste sample was pulled from the catheter to ensure a clean blood sample was collected. After the 5 ml of waste was pulled, the S-Monovet[®] (Sarstedt, Inc.) collection tube was connected to the catheter and a 14 mL sample was obtained from each steer. After the sample was obtained the catheter was flushed with 5 mL of saline followed by 3 mL of heparinized saline to serve as a lock. After the first 12 h blood sample was obtained (220 h) the catheters were removed and steers were removed from the stanchions and returned to isolation paddocks. Blood samples were handled as previously described. Once the steers were returned to the respective isolated paddocks, steers were offered the supplementation and had free access to water. All steers were monitored each day to ensure recovery from the intensive sampling and monitor any clinical signs of illness.

Serum concentrations of the pro-inflammatory cytokines, TNF- α , IL-6 and IFN- γ were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines. For all cytokines, the intra-assay variation was less than 10 %, and the inter-assay variation was less than 10 %.

Serum samples were analyzed for GH and IGF-1 as previously described (Elsasser et al., 1989). For the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined at 150 pg bGH/assay tube with 50% binding of tracer achieved at 1,800 pg/tube. Increasing volumes of plasma displaced tracer counts in a fashion parallel to that of the standard curve. Recovery of nonlabeled

bGH averaged 97% for 300, 600 and 1,200 pg added to 200 microliters serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$.

Concentrations of IGF-I were determined following acidification for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual sample was diluted and neutralized with a 1:80 dilution with assay buffer. Anti human/bovine IGF-1 primary serum was procured from GroPep (Adelaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of nonlabeled IGF-1 added to plasma before acidification averaged 95% with intra- and interassay coefficients of variation $\leq 10\%$.

Statistical analysis

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS as a completely random design; and the model included sampling time, treatment, and sample time x treatment. Sample treatment x time was used as the error term to test whole plot effects. Rectal temperature was initially recorded at 1-minute intervals, but subsequently averaged over 24-hour intervals to facilitate comparisons to other immune and physiological parameters. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pairwise differences among

least squares means at various sample times were evaluated with the PDIF option of SAS.

Area under the curve was calculated for variables of interest by $[(\text{Time 1} + \text{Time 2}) * (\text{time between Time 1 and Time 2})] / 2$ to determine the ng or pg*h/ml concentration of each hormone over a given time period. Only when variables of interest were significant (F-test with $P < 0.05$) was the area under the curve calculated. Results from these calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

Results

At the conclusion of the trial, all CON cattle were seronegative for titers of IBRV; cross-contamination had not occurred during the trial. All IBRV steers exhibited a titer (1:8) for IBRV when tested at the conclusion of the trial period.

There was a treatment x time interaction ($P < 0.01$) for rectal temperature (RT, Figure 1). No differences ($P > 0.05$) in initial RT (-24 to 48 h) were observed between the two treatment groups. Difference in RT between the CON and IBRV steers appeared at 72 h post infection. The difference in RT between the IBRV steers was observed from 72 to 120 h post infection ($P < 0.05$) as compared to the CON steers. At 144 h temperature in the IBRV steers returned back to RT values observed prior to the challenge at which point there was no differences between CON and IBRV. The greatest RT response for the IBRV steers was observed 72 post infection and the CON steers also had an observed increase in RT at this time point. Although, the IBRV steers had a greater RT temperature at 72 h, part of the increase in RT at 72 h could be attributed to

the catheterization procedure, handling and acclimation to the metabolic stanchions for both CON and IBRV steers.

There was a treatment x time interaction ($P < 0.01$) for IFN- γ . Steers in the IBRV treatment group had increased ($P < 0.05$) circulating concentration of IFN- γ starting approximately 90 h post infection, with peak concentration observed at 96 h post infection (Figure 2). The IBRV steers had greater ($P < 0.05$) IFN- γ concentrations from 94 through 112 h post infection when compared to the CON steers. At approximately 152 h post infection concentration on IFN- γ were similar to values observed prior to the challenge in the IBRV steers. There was an increase ($P < 0.05$) in IFN- γ production in the CON steers at 136 h post infection, this is similar to the peak observed in the IBRV infected steers at 96 h post infection. The peak concentration observed at 136 h post infection in the CON steers was greater ($P < 0.05$) when compared to the IBRV steers. At approximately 152 h post infection, both treatment groups had concentrations of IFN- γ that were similar to values observed prior to the challenge. As mentioned previously, the increase in IFN- γ was not due to exposure of IBRV since the CON steers were still sero-negative for IBRV. The greatest differences and peaks observed at specific time points post infection for IFN- γ was during h 96-120 h (d 4-5) post infection which was deemed to be the critical immune response period. Therefore, when the total concentration of IFN- γ produced during h 96 -120 post infection was calculated (area under the curve, $\text{pg}^*\text{h}/\text{ml}$), the IBRV steers had greater ($P = 0.05$; Table 1) total production of IFN- γ .

No treatment x time interaction was detected for IL-6 ($P > 0.05$), evaluation of pairwise comparisons between the treatment groups at specific time points detected

notable differences (Figure 3). Circulating concentrations of IL-6 were relatively undetectable throughout the study, but a slight increase for the IBRV steers was observed at 136 h post infection. When the increase concentration (136 h post infection) of IL-6 was observed in the IBRV steers, this was greater ($P < 0.05$) than the concentration in the CON steers. By 152 h post infection both CON and IBRV steers had IL-6 concentrations similar to those detected prior to the IBRV challenge.

A treatment x time interaction was not observed for TNF- α ($P > 0.05$); evaluation of pairwise comparisons between the treatment groups at specific time points detected notable differences (Figure 4). Circulating concentrations of TNF- α for both treatment groups were similar throughout the first 120 h post infection. No differences were detected between the IBRV and CON cattle until 136 h post infection at which time point there was an increase ($P < 0.05$) in TNF- α for the IBRV steers. At approximately 152 h post infection, both CON and IBRV steers had detectable concentrations of TNF- α , but no differences were observed between the two treatment groups. At 184 h post infection both CON and IBRV steers had similar concentrations detected prior to the challenge.

A significant treatment x time interaction ($P < 0.01$) was observed for cortisol (Figure 5). There were no differences in initial cortisol concentrations between the treatment groups. The differences observed in cortisol concentrations between the CON and IBRV steers were observed at 94, 96, 120 and 122 h post infection with the CON steers having increased cortisol values at these specific time points. While the CON steers had increased concentrations of cortisol at these time points, both treatment groups had similar cortisol response patterns. Starting at 72 h post infection both treatment groups had an increase in circulating cortisol. The greatest ($P < 0.05$) concentration of

cortisol for CON steers was observed at approximately 96 h post infection or 24 h after being moving into the metabolic stanchions. The steers (CON and IBRV) were not removed from the metabolic stanchions till 220 h post infection, but cortisol concentrations returned to values similar to that observed prior to going into the stanchions at 140 h post infection. It appeared that both treatment groups had an adjustment period after being moved into the metabolic stanchions as indicated by the increases in cortisol concentrations for both the CON and IBRV steers, but returned to baseline concentrations prior to being removed from the stanchions. While differences were observed at specific time points post infection for CORT, when the total concentration of cortisol produced from h 96 -120 post infection was calculated (area under the curve, ng*h/ml), there were no differences ($P > 0.05$; Table 1) between the CON and IBRV steers during the critical immune response period.

Treatment x time interaction was detected for GH ($P = 0.03$). The circulating concentration of GH did not differ ($P > 0.05$) between the CON and IBRV steers till 124 h post infection at which an increase was observed for the CON steers (Figure 6). Another increased concentration of GH was observed for the CON steers at 148 h post infection ($P = 0.003$); no other differences were observed till 156 h post infection. Steers in the CON treatment group had greater ($P < 0.05$) GH circulating concentrations from 156 – 184 h post infection compared to the steers in the IBRV treatment group. Starting at 196 h post infection and continuing throughout the remainder of the trial, there were no differences between the CON and IBRV steers in terms of GH concentrations. While differences were observed at specific time points post infection for GH, when the total concentration of GH produced from 96 -120 h post infection was calculated (area under

the curve, ng*h/ml), there were no differences ($P > 0.05$; Table 1) between the CON and IBRV steers during the critical immune response period. When GH concentration as a function of ng*h/ml was calculated for time periods 120-144 (d 5-6) and 144-192 (d 6-8) h post infection after the critical immune response period, the CON steers had greater ($P < 0.05$) total production of GH during these time periods.

No treatment x time interaction was detected for IGF-I ($P > 0.05$), evaluation of pairwise comparisons between the treatment groups at specific time points detected notable differences (Figure 7). Circulating concentrations of IGF-I was greater ($P < 0.05$) for the CON steers at 48 h post infection. There were no differences in IGF-I concentrations between the CON and IBRV treatment groups for the remainder of the trial. Concentrations of IGF-I did decline rapidly at 96 h post infection for both CON and IBRV treatment groups. After the IGF-I concentration declined at 96 h post infection, concentrations remained relatively suppressed for the remainder of the trial period (300 h post infection).

Discussion

Cytokines are proteins produced by macrophages and other immune cells in response to an immune insult that bind to specific receptors on cells in an autocrine, paracrine, or endocrine fashion that can alter and affect the function of those cells (Paul, 2008). The pro-inflammatory cytokines include interleukin-1, 4, and 6, tumor necrosis factor- α , and interferon- γ (Paul, 2008). Pro-inflammatory cytokines induce the five cardinal signs of inflammation that are characterized by: redness, increased tissue temperature (heat), swelling, pain, and loss of function at the infected area (Tizzard,

2004). Redness and increased temperature are due to increased blood flow to the inflamed area; swelling is due to the accumulation of fluid; pain is due to the release of chemicals that stimulate nerve endings; and loss of function is due the combination of these factors. Under normal physiological conditions, inflammation subsides after the pathogen is eliminated, but in pathologic conditions the inflammatory response can become chronic and lead to tissue destruction (Tizzard, 2004). The febrile response helps deter viral and bacterial growth, because both bacteria and viruses grow optimally at temperatures that are less than that of the human body (Paul, 2008). Generally at the onset of the pro-inflammatory response, several of the first responder cytokines are purely catabolic in nature, promoting a physiological state of readiness to retrieve needed energy substrates from storage depots like fat and in some instances muscle (Elsasser et al., 2008). These first responders are subsequently down-regulated and reversed back towards anabolic state and function as the later arriving anti-inflammatory cytokines and acute phase proteins reestablish normal function in the body. The initial responses are generally catabolic for 2 main reasons (Elsasser et al., 2008): 1) when fever is present the caloric demand can increase as much as 30% for each 1°C increase in core body temperature (Baracos et al., 1987) and 2) if the increase in temperature is accompanied, as it usually is, by decrease in feed intake that occurs during pro-inflammatory stress, the needed calories are channeled away from other resources and processes in a prioritized manner that is proportional to the severity of the response initialized due to the immune insult. Hammond (1944) described the concept of prioritization of nutrients to different tissues to ultimately ensure the survival of the animal. Elsasser et al. (2008) expanded on this subject and inferred that to better understand the concept of redirecting metabolism

during the pro-inflammatory state, one could assume surviving an immune insult takes priority over other biological needs, especially growth and tissue accretion in a young animal. Immunological parameters evaluated in the current study would suggest that the IBRV steers had greater sustained rectal temperatures (3 d) as well as increased concentrations of IFN- γ from 96-120 min post infection in comparison to CON steers. Results from this study would suggest immunological responses associated with a febrile response remained greater in the IBRV steers for a prolonged period of time, and this could elicit alterations in the somatotrophic axis. The difference observed for IFN- γ over time rather than TNF- α and IL-6 for the IBRV steers in this study, are indicative of a viral immune challenge. Increases in IFN- γ have been described on the basis of its antiviral properties (Paul, 2008). Bovine respiratory viruses such as IBRV can infect the lung epithelia cells causing activation of Toll-like receptor (TLR) 3, 7, 8, and 9 initiating a signaling cascade geared towards viral activation of immune responses (Ackermann et al., 2010).

Growth hormone has been reported to mediate the proliferation of a number of cell types as well as exhibit a variety of effects on the cells and organs associated with immune function. Many of the effects observed due to GH are mediated either directly or indirectly by IGF-1. Therefore, the role of GH as a key anabolic hormone that is part of the somatotrophic axis has been evaluated as a potential mediator of growth during an immune challenge. The relationship between GH and IGF-I during an immune challenge has also been assessed to understand alterations in the somatotrophic axis. Fan et al. (1994) reported GH concentrations are reduced during endotoxin challenge and Peisen et al. (1995) concluded that the pulsatile response of GH is transiently suppressed during an

endotoxin challenge in rats. Similar results were reported for cattle during an immune challenge, and Elsasser et al. (1987) determined blood concentrations of GH were reduced during the immune challenge. The anabolic action of GH is accomplished in part by the activation of cells in the liver to produce IGF-I (Spurlock, 1997). Elsasser et al. (1987) also reported infected cattle to have decreased IGF-I concentrations and reduced growth rates. Spurlock (1997) suggested the reductions in circulating concentrations of IGF-I during the immune challenge likely reflect decreased synthesis in multiple tissues. Fan et al. (1996) reported uncoupling of the somatotrophic axis during an immunological stress, in which GH preceded the drop in IGF-I and GH returned to a normal range while IGF-I was still decreased after 24 h post challenge. While no differences in IGF-I were detected in the current study, there was a transient drop in IGF-I at 96 h that remained throughout the duration of the trial. The reduction observed in total GH concentration (ng*h/ml) during 5-8 d for the IBRV steers would suggest similar alterations observed during endotoxin challenges. Generally differences in GH during endotoxin challenges occur during the challenge period, but it would appear there was a delayed suppression of GH associated with the viral challenge. The delayed suppression in GH could be attributed the type of challenge given. Endotoxin challenges initiate a rapid immune response and are generally recognized and resolved quickly, whereas the viral challenge elicited a sustained response and no immune parameters associated with febrile responses were detected till 3 d. Another factor that could be implicated with mediating the GH response associated with the suppression of GH and IGF is the increase in cortisol associated with the steers being moved to metabolic stanchions at 72 h post infection. Cortisol concentrations were increased for both the CON and IBRV from 72-

150 h post infection, and the increase in cortisol was simultaneous with the increase in IFN- γ in the IBRV steers. The dual activation of the immune and HPA axis could attribute to the reduction in GH observed for the IBRV steers. The transient decline in IGF-I was concurrent with the steers being moved into the metabolic stanchions and increase in cortisol concentration, but neither the CON nor IBRV steers recovered to initial GH concentrations for the remainder of the trial.

Conclusion

While a pronounced clinical infection was not observed for the IBRV infected calves, results from this study would suggest IBRV can alter endocrine parameters associated with the somatotrophic axis. The simultaneous increase in cortisol during the immune challenge could have potentiated the alterations observed for the endocrine parameters. It is unclear if the combination increase in cortisol and IFN- γ elicited the change or if it was solely the increase in IFN- γ . Therefore, a better understanding of multiple interactions associated with stressors and immune challenges needs to be elucidated.

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Table 3.1 Mean circulating concentrations (pg or ng*h/ml) for interferon- γ , cortisol and growth hormone during time intervals post infection (post infection) for Control and infectious bovine rhinotracheitis virus (IBRV) treatment groups

Variable of Interest	Time interval	Treatment groups ^a		SEM	P-value
		Control ^b	IBRV ^c		
Interferon- γ (pg*h/ml)	96 – 120 h p.i.	610.90	2790.55	873.38	0.05
Cortisol (ng*h/ml)	96 – 120 h p.i.	2826.00	1810.83	624.36	0.18
Growth hormone (ng*h/ml)	96 – 120 h p.i.	197.36	165.51	23.71	0.31
Growth hormone (ng*h/ml)	120 – 144 h p.i.	1335.56	755.64	131.06	0.006
Growth hormone (ng*h/ml)	144 – 192 h p.i.	3883.12	1977.12	632.45	0.04

^aMeans within a row different between treatment groups if $P \leq 0.05$

^bControl = [4 mL dose of saline (0.9 w/v NaCl) solution (2 mL/nostril)]

^cIBRV = Infectious bovine rhinotracheitis virus, [4 mL dose of IBRV (2 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

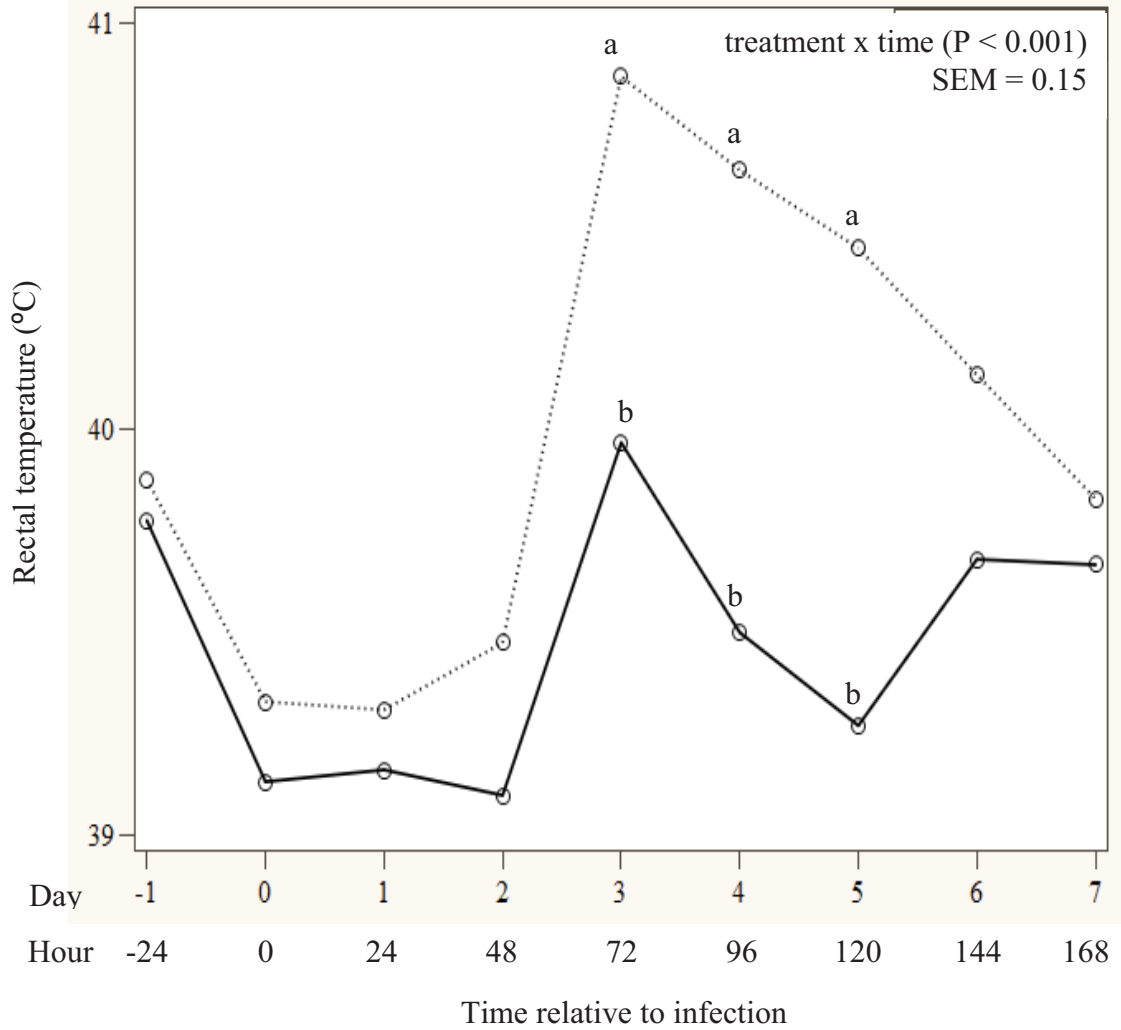


Figure 3.1 Mean rectal temperature (RT) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ··○··) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl)

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

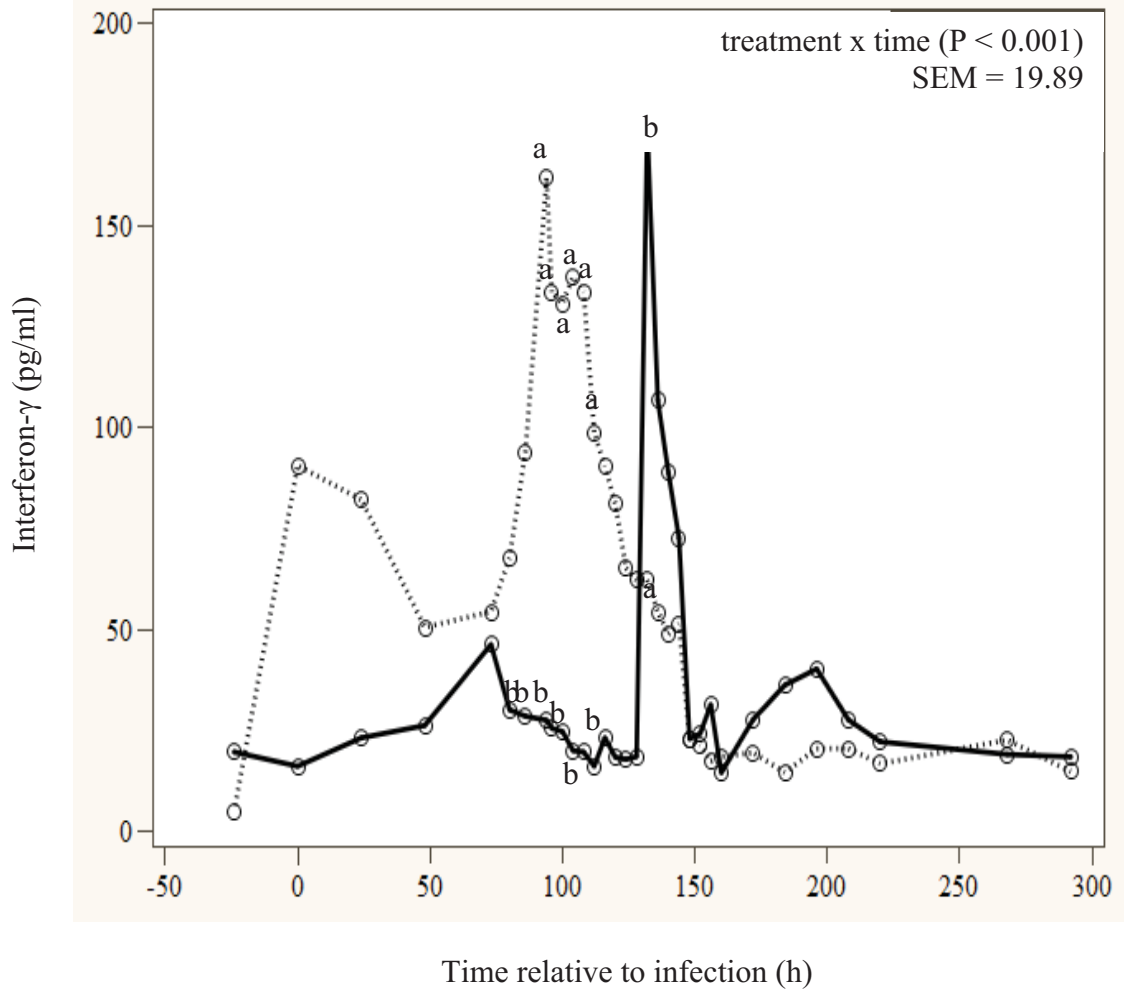


Figure 3.2 Mean serum concentrations of interferon- γ (IFN- γ) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ••○••) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl).

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

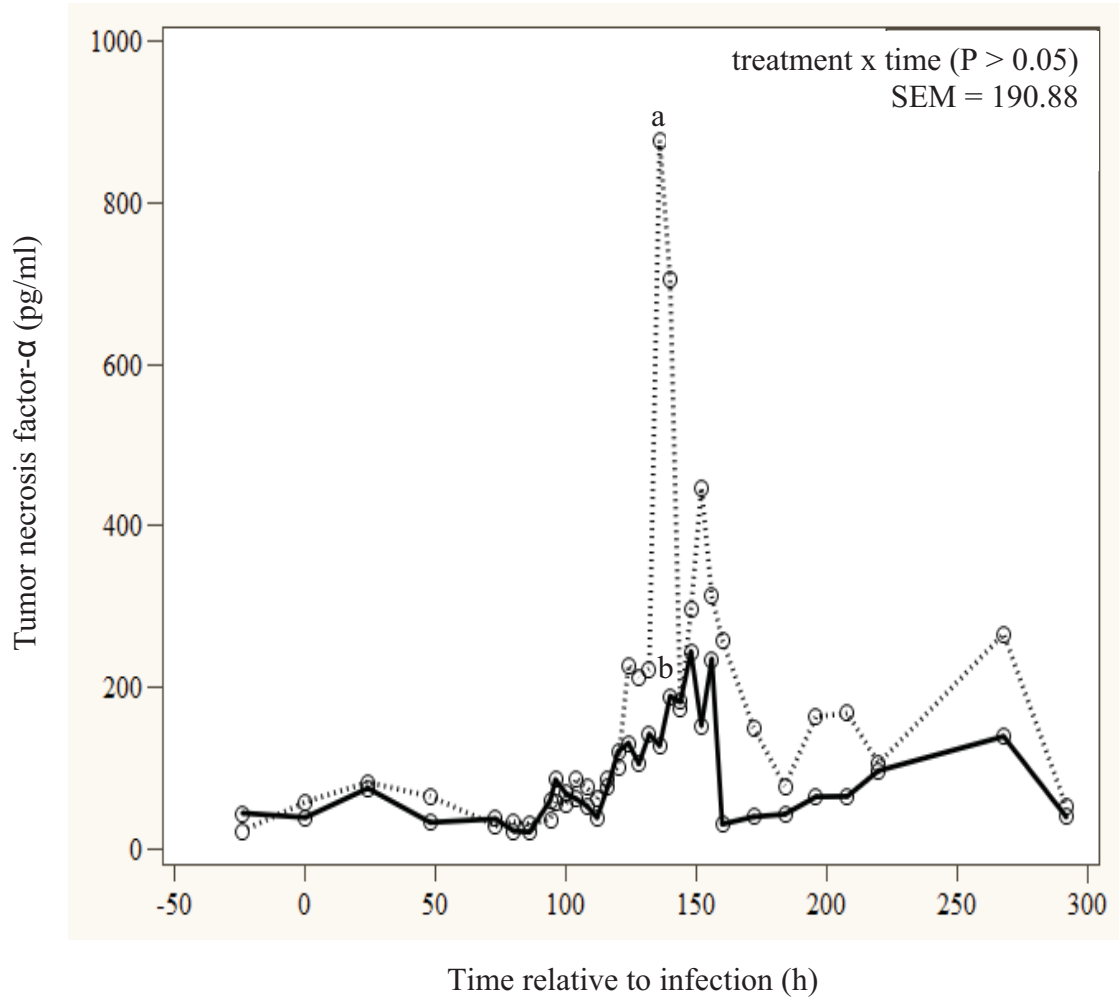


Figure 3.3 Mean serum concentrations of tumor necrosis factor- α (TNF- α) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ··○··) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl).

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

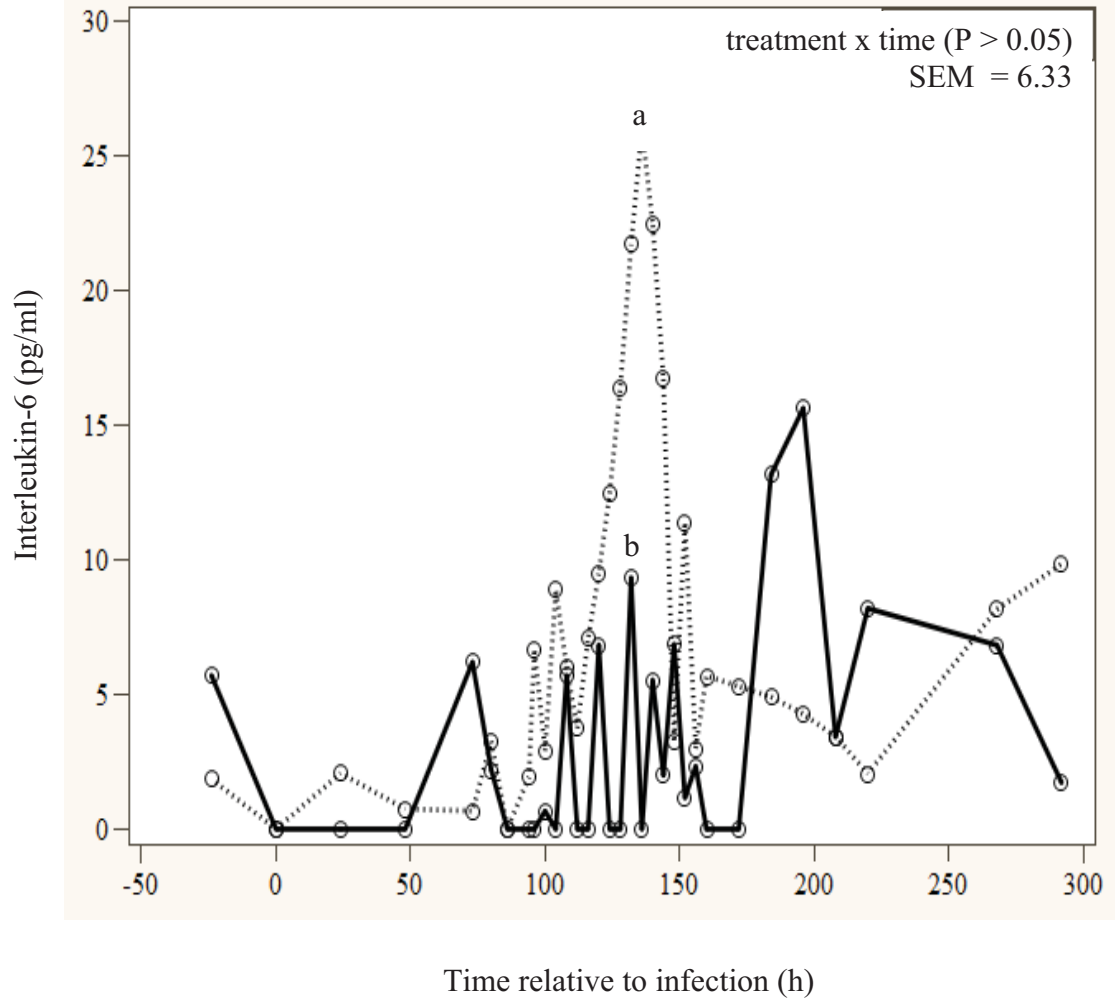


Figure 3.4 Mean serum concentrations of interleukin-6 (IL-6) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ···○···) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl).

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

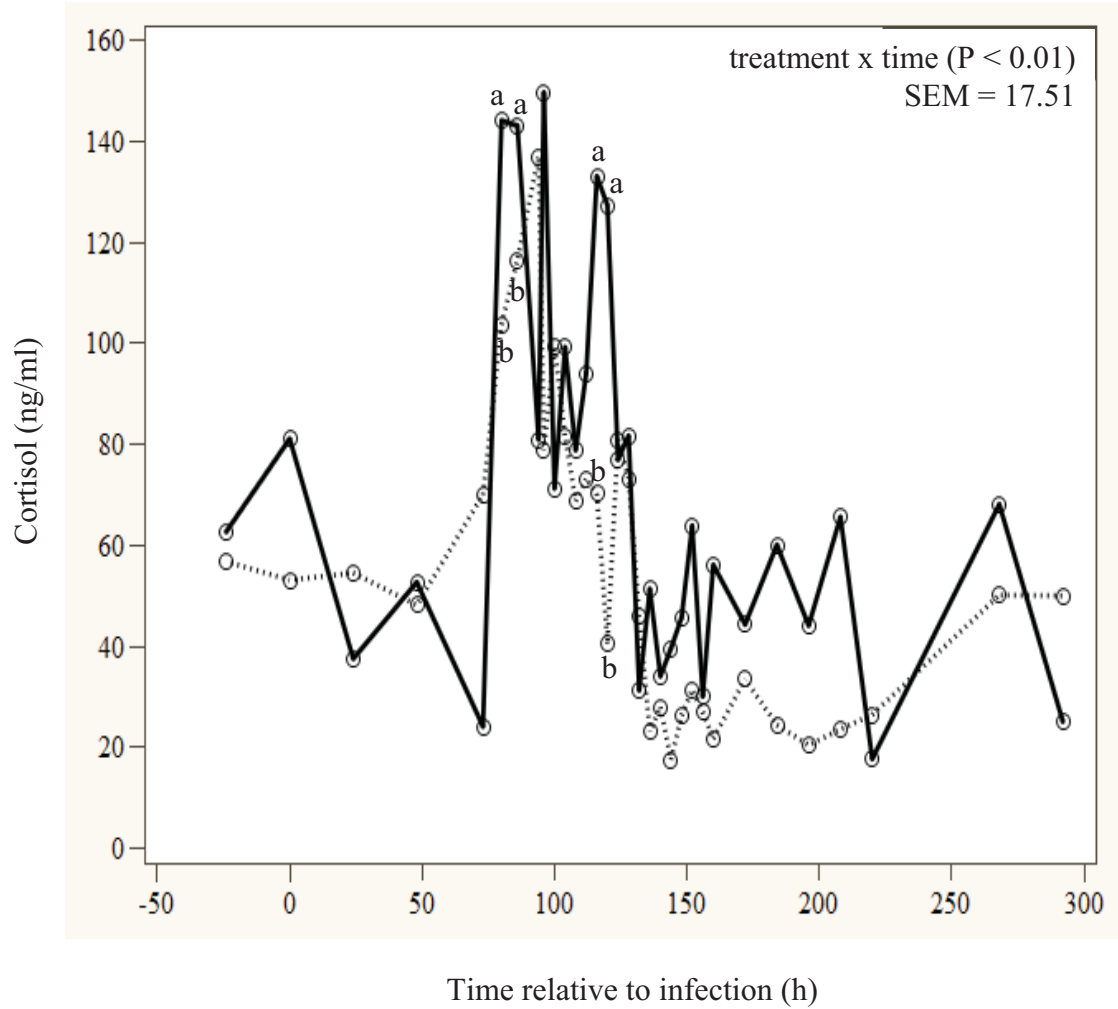


Figure 3.5 Mean serum concentrations of cortisol for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ··○··) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl)

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

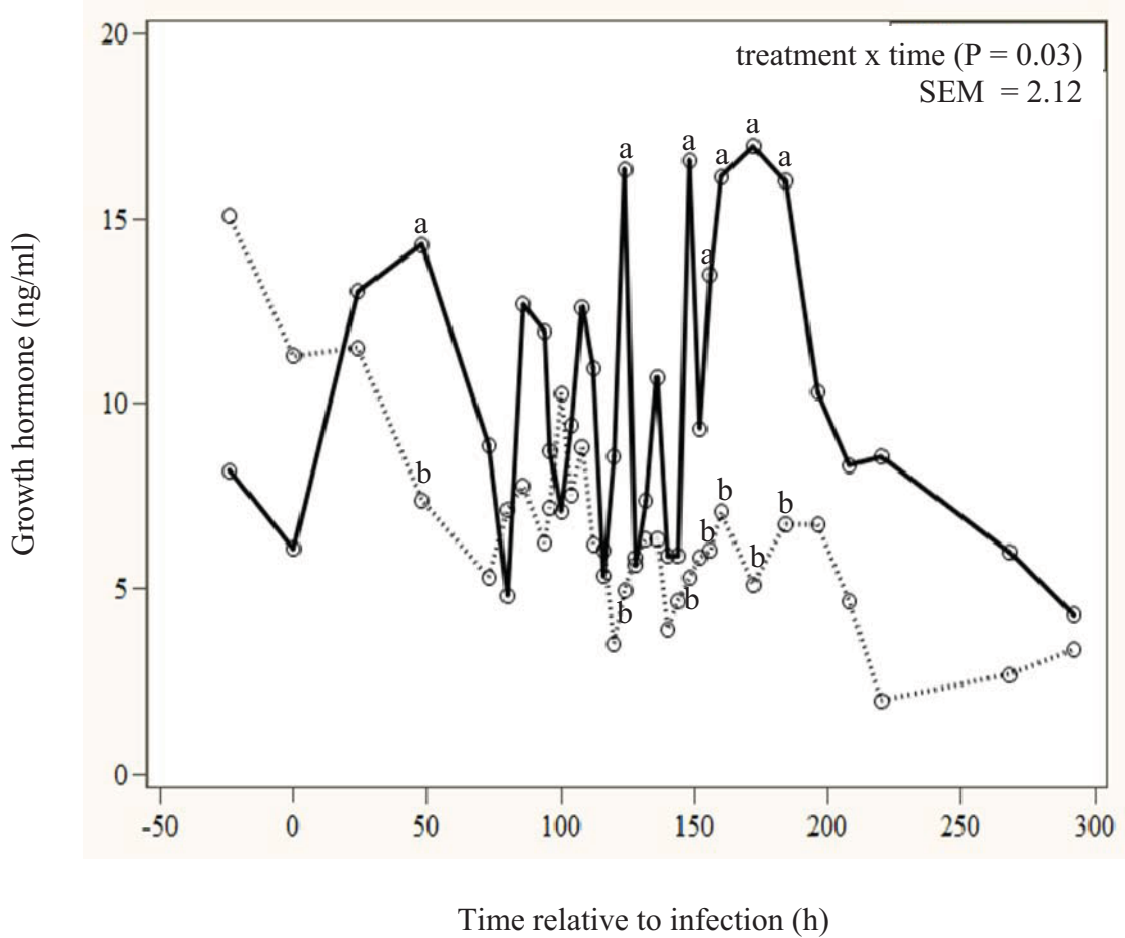


Figure 3.6 Mean serum concentrations of growth hormone (GH) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ··○··) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl)

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time points

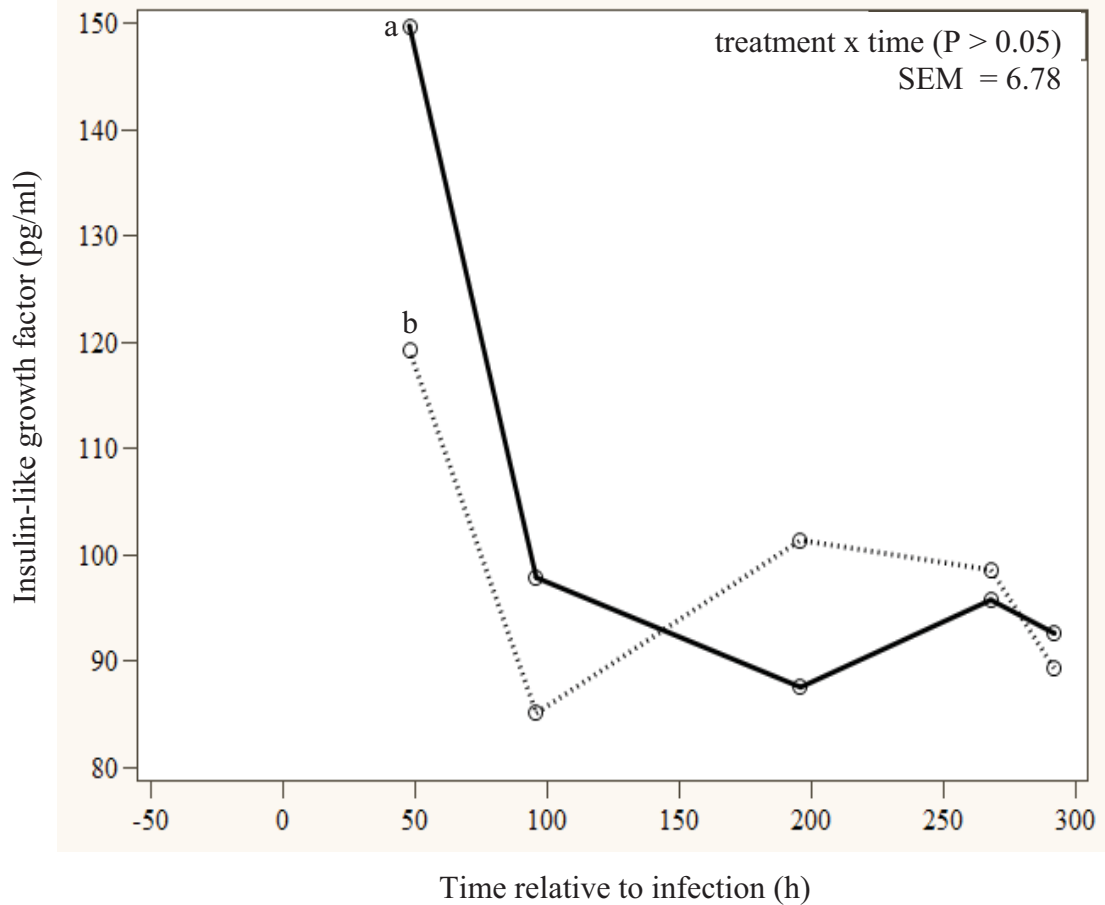


Figure 3.7 Mean serum concentrations of insulin-like growth factor-I (IGF-I) for Control (—○—) and infectious bovine rhinotracheitis virus (IBRV; ··○··) steers following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀/0.3 mL solution) or saline (0.9 w/v NaCl)

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

CHAPTER IV
EVALUATION OF VARYING VOLUMES OF INFECTIOUS BOVINE
RHINOTRACHEITIS VIRUS (IBRV) ON IMMUNE AND
PHYSIOLOGICAL PARAMETERS IN STEERS

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Abstract

Eighteen Holstein steers (450 ± 75.70 kg BW) were used to evaluate the immune response and the impact on endocrine growth parameters associated with varying volumes of infectious bovine rhinotracheitis virus (IBRV). Steers were randomly assigned to three groups; a Control (CON; 4 mL/nostril Saline), or one of two IBRV challenged treatments [4 mL/nostril (IBRV-2X) or 6 mL/nostril (IBRV-3X); Cooper strain 8.0 TCID₅₀ / 0.3 mL solution]. Prior to the challenge (-1 d) all steers were fitted with a rectal probe and a blood sample was obtained. On 0 d, steers received the respective assigned treatments and the IBRV treatment groups were moved to isolated paddocks. Blood was collected via jugular venipuncture every 24 h on 1 and 2 d; and every 12 h on 3, 4, 5, 6 and 7 d post challenge. Serum was analyzed for interleukin (IL) - 6, interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), GH, IGF-1. On the 2 d of the trial IBRV-2X and IBRV-3X treatment groups had increased rectal temperatures ($P <$

0.05) when compared to rectal temperature of steers in CON treatment group. Rectal temperature for steers in both the IBRV-2X and IBRV-3X treatment groups returned to or near baseline rectal temperatures on approximately 5 d after the challenge. An increase in IFN- γ was observed for steers in both the IBRV-2X and IBRV-3X treatment groups when compared to steers in the CON treatment group ($P < 0.05$), though no differences were observed for IFN- γ between steers in the IBRV-2X and IBRV-3X treatment groups. No differences ($P > 0.05$) in the mean concentrations of TNF- α and IL-6 were observed between steers in the IBRV-2X and IBRV-3X when compared to those concentrations in steers of the CON treatment group. Furthermore, no differences ($P > 0.05$) in mean concentrations of GH and IGF-I were observed between steers in the CON and those in the IBRV-2X and IBRV-3X treatment groups. Results indicate that inoculation of cattle with IBRV with 8 or 12 ml of IBRV at a concentration of 8.0 TCID₅₀ / 0.3 mL solution elicited an immune response; however there were no measured differences between the different volumes of IBRV utilized in this trial. Collectively, the data suggest that measurable immune responses to IBRV at the concentration evaluated may be selective in regard to cytokine and metabolic parameters.

Introduction

The bovine respiratory disease (BRD) complex continues to be a major cause of morbidity, mortality, and economic loss for all phases of cattle production. The BRD complex is comprised of bacterial and viral pathogens in combination with a variety of stressors cattle encounter as they progress through the production system. It has been estimated that the economic impact from morbidity and mortality associated with the

BRDC can range from \$480 million (Engelken, 1997) to \$624 million annually (Smith, 1996) and these costs are associated with treatment of infected cattle and losses in production of cattle due to morbidity and mortality of the BRDC. While the true cost of BRD may never be known due to all of the losses associated with the complex, the reality is that BRD is a devastating disease that has an enormous economical impact on the profitability of the American cattle industry.

The most common viruses involved in the BRD complex include bovine herpesvirus-1 or infectious bovine rhinotracheitis virus (IBRV), bovine parainfluenza virus type-3 (PI-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV). Typically these viruses infect the upper respiratory tract, resulting in rhinitis, tracheitis, and bronchitis. The viruses involved in the BRD complex typically do not cause major lung damage but rather predispose the lung to bacterial infections. Therefore, the primary role of these agents is to promote the bacterial challenge to the lungs by compromising the respiratory tract defense mechanisms.

Viral pathogens play a critical role by suppressing the immune responses and allow opportunistic bacteria the ability to overcome the immune defenses and promote the BRD complex need to be explored further. More information regarding the participation viral pathogens have on physiological and immunological responses need to be defined to better understand their contribution in the BRD complex. Babiuk et al. (1996) suggested that in most cases of IBRV the peak activity of immune cellular responses occurs at 7-10 days post-infection and correlates with recovery from infection and before significant antibody is detected. Orr et al. (1990) inoculated calves with IBRV and reported a change in clinical symptoms and morbidity in the infected calves on

four days after infection and this was concurrent with the maximal nasal secretion of IBRV. Cattle infected with IBRV have also been implicated to have increased rectal temperature (RT) and clinical illness scores on days three through seven of the challenge period as well as having increased concentrations of acute phase proteins (APP) on days five through seven (Schmidt et al., 2006). When cattle were infected with bovine syncytial virus (BRSV), another viral agent found in the lungs of cattle suffering from the BRDC, tumor necrosis factor – alpha (TNF- α) was observed to be greater on day six and eight post infection (p.i.; Rontved et al., 2000). Results reported by Grell et al. (2005) when BRSV was used, reported rectal temperature started peaking on day four and cytokine mRNA expression was detected as early as day one.

One of the most common strains of the IBRV used by researchers is the IBR Cooper strain from USDA-APHIS Center for Veterinary Biologics, Ames, IA. This challenge virus has been utilized in a large number of research trials, with variable results. In previous research (Falkenberg, et al., 2011) use of the IBR Cooper strain challenge virus at a volume of 8.0 TCID₅₀/0.3 mL solution; 4 ml total volume; 2ml/nostril resulted in a febrile response, however, no symptoms of clinical illness were observed. Due to the limited immune and physical response associated with viral challenge at the 4 ml volume observed in previous research, the objective of this study was to evaluate the effect of two times (2x) or three times (3x) the volume of IBRV in cattle on immune and endocrine parameters.

Material and methods

The experiment was conducted at the Leveck Animal Research Facility at Mississippi State University (MSU) during October 2009 and all procedures involving animals were reviewed and approved (08-041) by the Animal Care and Use Committee at Mississippi State University.

Eighteen Holstein steers (242.44 ± 43.65 kg BW) were used to characterize physiological, endocrine, and innate immune response following an IBRV intra-nasal challenge administered at two volumes. All steers were obtained from the Bearden Dairy Research Center at Mississippi State University at approximately 6 m of age and transported approximately 16.1 km to the Leveck Animal Research facility at Mississippi State University. Steers were placed on pasture over the summer in isolated paddocks. Steers were supplemented while on pasture with a 50/50 (soyhull/corn gluten) mix for a programmed gain of approximately 0.45 kg/d, with *ad libitum* access to water and bermudagrass hay (*Cynodon dactylon*).

Twenty four h prior to administering the IBRV (USDA-APHIS Center for Veterinary Biologics, Ames, IA; IBR challenge virus, Cooper strain, Lot # 05-08) challenge, all steers were fit with automatic rectal temperature monitoring devices (Reuter et al., 2010) and BW was obtained. Rectal temperature was recorded in 1-min intervals as described by Reuter et al. (2010). Steers were randomly assigned to treatment groups and placed into isolated paddocks. On 0 d, IBRV-2X and IBRV-3X treatment groups received a 8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution) or a 12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution), respectively. The control (CON) steers received an 8 mL placebo of saline solution (0.9

w/v NaCl; 4 mL/nostril). Challenge dosage for each steer was prepared in pre-labeled 10 mL syringes and maintained at 23°C until administered. Control steers were processed first to prevent cross contamination. Viral and placebo doses were administered by use of a MADgic Laryngo-Tracheal Atomizing device (MAD700; Wolfe Tory Medical, Inc., Salt Lake City, UT) attached to a 10 mL syringe. The head of each steers was restrained so that the atomizing device could be inserted into the nasal cavity, with the tip of the syringe visible at the opening of the nostril. After the atomizing device was placed into the nostril, the opposing nostril was physically closed to facilitate adequate dispersal into the nasal cavity. Upon inhalation the syringe was discharged simultaneously releasing the pre-determined volume of IBRV into the nasal cavity of each steer (required approximately 10 sec). The same procedure was repeated in the opposing nostril for each steer. A new sterile atomizing device and syringe was utilized for each steer, regardless of treatment.

Prior to inoculation (-24 h), on 0 d (0 h) and subsequently on 1 through 14 d via a single venipuncture blood samples were collected. Blood samples were collected every 24 h on 1 and 2 d; and every 12 h on 3, 4, 5, 6, and 7 d post challenge. Each day the CON group was processed first to prevent contamination from the two IBRV treatment groups. Each steer was individually restrained in the working chute and the head was positioned so that the jugular vein was exposed and the blood sample could be obtained. Blood samples were allowed to clot for 30 minutes at 21°C and then were centrifuged at 3,000 x g for 30 minutes (4°C) and serum was separated. Serum was collected and transferred into 1.5 mL microcentrifuge tubes for storage and frozen (-80°C) for later analysis.

During the challenge period, after blood samples were collected and steers were returned to their paddocks, the same 50/50 (soy hull/corn gluten) supplement was offered to each group at 0800 hr. Control steers were fed first to ensure no cross-contamination between paddocks. *Ad libitum* access to clean water and bermudagrass hay (*Cynodon dactylon*) was provided throughout the trial. Clinical illness scores as defined by Schmidt et al. (2006) were also assigned to each steer at approximately 1800 hours by trained personnel from Mississippi State University blinded to treatment.

Serum concentrations of the pro-inflammatory cytokines, TNF- α , IL-6, and IFN- γ were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines (SearchLight; Pierce Biotechnology Inc., Rockford, IL). For all cytokines, the intra-assay variation was less than 5 %, and the inter-assay variation was less than 7 %.

Serum samples were analyzed for GH and IGF-1 as previously described (Elsasser et al., 1989). For the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined at 150 pg bGH/assay tube with 50% binding of tracer achieved at 1,800 pg/tube. Increasing volumes of plasma displaced tracer counts in a fashion parallel to that of the standard curve. Recovery of nonlabeled bGH averaged 97% for 300, 600 and 1,200 pg added to 200 microliters serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$.

Concentrations of IGF-I were determined following acidification for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual sample was diluted and neutralized with a 1:80 dilution with assay buffer. Anti

human/bovine IGF-1 primary serum was procured from GroPep (Adelaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of nonlabeled IGF-1 added to plasma before acidification averaged 95% with intra- and interassay coefficients of variation $\leq 10\%$.

Statistical analysis

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC) as a completely random design; and the model included sampling time, treatment, and sample time x treatment. Sample time x treatment was used as the error term to test main plot effects. Rectal temperature was initially recorded at 1-minute intervals, but subsequently averaged over 1-hour intervals to facilitate comparisons to other immune and physiological parameters. Analysis for GH and IGF-I are expressed as percent change when compared to the time 0 (0 h) sample. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pairwise differences among least squares means at various sample times were evaluated with the PDIFF option of SAS.

Results

While no clinical symptoms of BRD were observed any steer within any of the three treatment groups, there was a treatment x time interaction ($P = 0.01$) between the steers in the IBRV-2X, IBRV-3X and CON treatment groups for RT (Figure 1). No differences ($P > 0.05$) in initial RT were observed between all the treatments groups. The two groups of steers challenged with IBRV had an increased RT ($P < 0.05$) indicating a febrile response to the viral insult. An increase ($P < 0.05$) in RT was evident in the two IBRV infected groups of steers starting at 48 h post infection. The IBRV infected steers had a sustained increased temperature at 48 h post challenge continuing through 96 h post challenge compared to the CON steers. At 100 h post challenge, RT did not differ between the three treatment groups and returned to values observed prior to the IBRV challenge. No differences were observed between IBRV-2X and IBRV-3X groups, suggesting that regardless of the volume, IBRV was able to initiate a febrile response.

There was no main plot effect of treatment x time interaction ($P = 0.09$) observed for IL-6 between the steers in IBRV-2X, IBRV-3X, and CON treatment groups. When evaluating pairwise comparisons between the treatment groups at specific time points there were notable differences (Figure 2). At time zero, mean IL-6 concentrations were greater in the IBRV-2X steers at time 0 h ($P < 0.003$) and 24 h ($P < 0.001$) post-infection when compared to the CON and IBRV-3X steers. By 48 h post challenge, there were no differences ($P > 0.05$) in IL-6 concentration between the three treatment groups, and no differences between the treatment groups continued throughout the remainder of the study (7 d).

The main plot treatment x time interaction was not observed for TNF- α ($P = 0.32$), but pairwise comparisons between the treatment groups at specific time points revealed differences between treatment groups (Figure 3). No differences ($P > 0.05$) in initial TNF- α concentration were observed between the three treatment groups, but the steers in IBRV-2X treatment group had an greater ($P < 0.001$) TNF- α concentration 24 h post challenge when compared to the CON and IBRV-3X steers. At 48 h post challenge, there were no detectable differences in TNF- α ($P > 0.05$) between steers in the three treatment groups and by 84 h post challenge all values were similar to concentrations observed prior to the challenge.

A treatment x time interaction ($P < 0.001$) was observed for IFN- γ (Figure 4). No differences ($P > 0.05$) were observed at 24 h post challenge between the steers in the three treatment groups as compared to the two other pro-inflammatory cytokines IL-6 and TNF- α . An increase concentration ($P < 0.05$) of IFN- γ was observed for steers in both the IBRV-2X and IBRV-3X treatment groups at 48 – 96 h post challenge, when compared to the steers in the CON treatment groups. Steers in the both the IBRV-2X and IBRV-3X treatment groups had a sustained response for INF- γ starting at 48 h continuing through 96 h post challenge, peak concentration of INF- γ was observed at 84 h p.i.. The peak concentration observed at 84 h post challenge in the IBRV-3X steers was greater ($P = 0.02$) than the IBRV-2X group. While the steers in the IBRV-2X treatment group had greater ($P < 0.05$) concentrations of IL-6 and TNF- α at 24 h post challenge, the IBRV-3X steers had an increased IFN- γ response 84 h post challenge At 108 h post challenge there were no detectable differences ($P > 0.05$) between the steers in the three treatment

groups, and by 120 h post challenge values were comparable to those observed prior to the challenge.

There were no main effect treatment x time interactions ($P = 0.79$) for GH, but pairwise comparisons between the treatment groups at specific time points revealed differences between treatment groups (Figure 5). No differences ($P > 0.05$) in initial concentrations between the steers in the three treatment groups was observed for GH. At 48 h post challenge the steers in the IBRV-3X treatment group had the greatest ($P = 0.02$) increase for GH and was greater than the CON steers, but difference ($P > 0.05$) was observed between the IBRV-2X and 3x steers. Steers in the IBRV-3X treatment group also had greater ($P < 0.05$) increase for GH at 96 and 168 h post challenge when compared to the CON and IBRV-2X steers. At 120 h post challenge it was observed that all treatment groups had a decrease in GH. In addition, it was observed that the steers in the CON and IBRV-2X treatment groups at 72 h post challenge ($P < 0.05$) had a decrease in GH, and this decrease was evident throughout the remainder of the experiment.

Insulin-like growth factor-I was similar among the three treatment groups throughout the duration of the experiment and there was no treatment x time interaction ($P = 0.49$), but pairwise comparisons between the treatment groups at specific time points revealed differences between treatment groups (Figure 6). There were no differences in initial concentrations between the steers in the three treatment groups, and the IBRV-2X and IBRV-3X steers remained relatively constant. Throughout the experiment, the largest decrease in IGF-I was observed at 72 h post challenge for steers in the two IBRV treatment groups, concentrations of IGF-I for all steers in all three treatment groups remained relatively constant until approximately 120 h post challenge A greater ($P <$

0.05) increase in IGF-1 was observed for steers in the CON treatment group, greatest increase in IGF-I concentrations for steers in the CON treatment group were observed at 156 h post challenge; at which time point coincides with the greater ($P = 0.03$) increase in GH concentration for steers in the CON treatment group when compared to steers in the IBRV-3X treatment group.

Discussion

There are at least 9 viral agents that have been associated or isolated from the lungs of cattle diagnosed with BRD. Of these 9 viral pathogens; bovine herpesvirus I (BHV-1), also known as infectious bovine rhinotracheitis (IBR); parainfluenza 3 virus; bovine viral diarrhea virus; and bovine respiratory syncytial virus have been reported to be capable of causing symptoms associated with acute respiratory disease without interactions with other pathogens or factors such as stress. Bovine herpesvirus-I has been recognized as causing severe upper respiratory lesions, ranging from hemorrhage to diphthermic membranes (Caswell and Williams, 2007). Typically the role of viral pathogens is to assist in establishing a respiratory environment that is favorable to colonization and replication by several pathogenic bacteria resulting in pneumonia (Panciera and Confer, 2010). Generally this is accomplished by two major mechanisms; 1) is by alteration on mucosal surfaces such that adhesion of bacteria to virus-infected cells is enhanced, and 2) is modification of the innate and adaptive immune systems through altered alveolar macrophage function, suppression of lymphocyte proliferation and induced apoptosis, and modified cytokine and other inflammatory mediator release (Panciera and Confer, 2010).

The incubation period for IBRV is generally 2 to 6 days, and the recovery occurs 4 to 5 days after clinical signs are observed (Aiello and Mays, 1998). While the incubation period for IBRV is only a few days typically outbreaks of BRD occur 10 to 14 days after cattle are commingled, stressed, vaccinated, castrated, or other production practices that utilized and effects of BRD can last 2 to 3 weeks (Wiske, 1990). Steers in the present study were not confronted with novel experiences and did not have the stressful experiences that cattle in the production setting would have been exposed to. Therefore, the febrile response observed 48 h after the IBRV inoculation in the present study were typical of what would be expected with the short incubation associated with the virus. The transient increase in rectal temperature for groups infected with IBRV was an expected result of the viral challenge and provided evidence of an effective challenge that elicited a febrile response. Interpretation of these results suggests that a sufficient challenge was elicited and an adequate immune challenge was achieved to compare between the treatment groups. Previous research using IBRV as an immune challenge model also reported similar changes in rectal temperature during the challenge period (Schmidt et al., 2006). While there were no differences in rectal temperature between the two IBRV groups, to our knowledge this was the first trial to investigate the effect of different doses of IBRV and there are no reports of optimal or dose-responses to varying viral amounts.

Of the three pro-inflammatory cytokines measured, IFN- γ had the greatest response to the IBRV challenge. Carroll et al. (2008) characterized the bovine cytokine and acute phase response to an endotoxin challenge (LPS), and reported increased concentrations for TNF- α and IL-6 but only a minimal response for IFN- γ . The

differences in cytokine profiles between the IBRV and endotoxin challenge could be attributed to the signaling cascades used for the different pathogens. Typically major pathogens (viral, bacterial, and parasitic) produce some type of pathogen-associated molecular pattern (PAMP) that are recognized by epithelia, alveolar macrophages, and intravascular macrophages. The lungs, like other organs, express a variety of extracellular, cell surface, endosomal, and cytoplasmic receptors termed pattern recognition receptors (PRRs) that recognize microbial PAMPs. Toll-like receptors (TLRs) are a class of PRRs that activate nuclear factor (NF)-kappa B or the interferon regulatory factors (IRF)-3 and 7 inducing inflammatory gene transcription. Cattle have 10 TLRs that have specific and sometimes overlapping PAMP affinities with TLR-3, 7, 8 and 9 have affinity for viral pathogens and TLR 2 and 4 being more specific for gram negative endotoxin challenges (Ackerman et al., 2010). While results from this study did not detect a robust response for TNF- α or IL-6, which have been reported to be associated with viral infections (Rontved et al., 2000). There was a greater IFN- γ for the IBRV infected calves during the time frame we would have expected to see differences in immune parameters. Schmidt et al. (2006) while reporting similar increases in rectal temperature there was no increase in acute phase proteins until approximately 5 days after the IBRV challenge. While Schmidt et al. (2006) reported an acute phase response on day 5, this would be expected since our data indicates that the peak concentration of IFN- γ was observed approximately 3-4 days post-challenge and the acute phase response is generally initiated by pro-inflammatory cytokines. The differences in cytokine production observed between the different challenge model warrants further investigation to understand what TLR or cascades are activated with the different pathogens.

Hammond (1944) was one of the first to describe the concept of prioritization of nutrients to different tissues to ultimately ensure the survival of the animal. Elsasser et al. (2008) expanded on the concept of redirecting metabolism during the pro-inflammatory state, and implied that surviving an immune insult could take priority over other biological needs. In the case of young production animals that are growing, growth and tissue accretion would have a decreased priority fueling the immunological insult. Generally at the onset of the pro-inflammatory response, several of the first responder cytokines are purely catabolic in nature, promoting a physiological state of readiness to retrieve needed energy substrates from storage depots like adipose and in drastic states muscle (Elsasser et al., 2008). These first responders are subsequently downregulated and reversed back towards anabolic state and function as the later arriving anti-inflammatory cytokines and acute phase proteins reestablish normal function in the body. The initial responses are generally catabolic for two main reasons (Elsasser et al., 2008): 1) when fever is present the caloric demand can increase as much as 30% for each 1°C increase in core body temperature (Baracos et al., 1987) and 2) if the increase in temperature is accompanied by a decrease in feed intake that occurs during pro-inflammatory stress (as is usually the case), the needed calories are channeled away from other resources and processes in a prioritized manner that is proportional to the severity of the response initialized due to the immune insult. Sartin et al. (1998) suggested that the treatment of GH during an immune response improves protein anabolism, enhance nitrogen retention, improve protein synthesis, reduces urea generation, and have improved wound healing capabilities. Elsasser et al. (1994) gave a low dose (0.2 microgram/kg, iv) LPS endotoxin bolus and demonstrated that the use of GH decreased

the TNF- α response to the endotoxin challenge and decreased membrane content of TNF- α receptors. While major differences were not observed for GH and IGF-1 between the IBRV steers and the CON steers, there were trends similar to that of other disease models. The overall increase in GH for the IBRV-3X steers suggests that there could have been an increase demand from the greater immune response observed in the IBRV-3X steers. Similarly, the IGF-I values for the IBRV steers were generally less than the CON steers throughout the challenge period. Collectively, these results would suggest a redirection of metabolic demands especially in the IBRV-3X steers which had a greater disconnect in GH and IGF-1 axis.

The only observable difference between the IBRV-2X and IBRV-3X steers was the peak IFN- γ concentration approximately 84 h post challenge and the magnitude of change in GH and IGF-I. We anticipated a greater difference between the IBRV-2X and IBRV-3X but the lack of differences observed could be in part due to the amount of solution dispensed into the nasal cavity of each steer. While the infectious dose was held constant per mL, the total amount of fluid given to the IBRV-3X was greater than the IBRV-2X steers. Since cattle have a relatively long tracheobronchial tree, which increases the amount of dead space volume in comparison to other species (Ackermann et al., 2010). Increase dead space may not affect respiratory tract immunity, but it could be a contributory factor allowing for increased surface area for particulate deposition and increased transit time of inhaled vapors, gases, and particulate matter (Ackermann et al., 2010). The IBRV-3X steers should have received a greater infectious dose (150% greater), but due to the more volume of solution administered for the challenge, they may not have received the entire dose and expired more than the IBRV-2X steers.

Based on the results from this trial we concluded that IBRV challenge method used in this study was effective in eliciting an adequate febrile and cytokine response to the infection with IBRV. However, the majority of response to the viral challenge was observed over a two to three day range and more intensive sampling during this time frame should be evaluated to help characterize the immune response associated with IBRV infections.

Conclusion

Based on the results from this trial we concluded that IBRV challenge method used in this study was effective in eliciting an adequate febrile and cytokine response to the infection with IBRV. While two different dose concentrations were utilized to challenge the steers, both evoked similar responses and the dose concentration did not alter the response associated with the challenge. However, the majority of response to the viral challenge was observed over a two to three day range and more intensive sampling during this time frame should be evaluated to characterize the immune response associated with the IBRV infection. A more intensive sampling period during the immune response period would also be useful to characterize the GH response and quantify any alterations in GH production. While it would be useful to have a more intensive sampling period, the multiple dose concentrations did not provide differences in pro-inflammatory cytokine production. Therefore, it would appear that the IBRV1 dose would be sufficient in stimulating an adequate febrile immune response.

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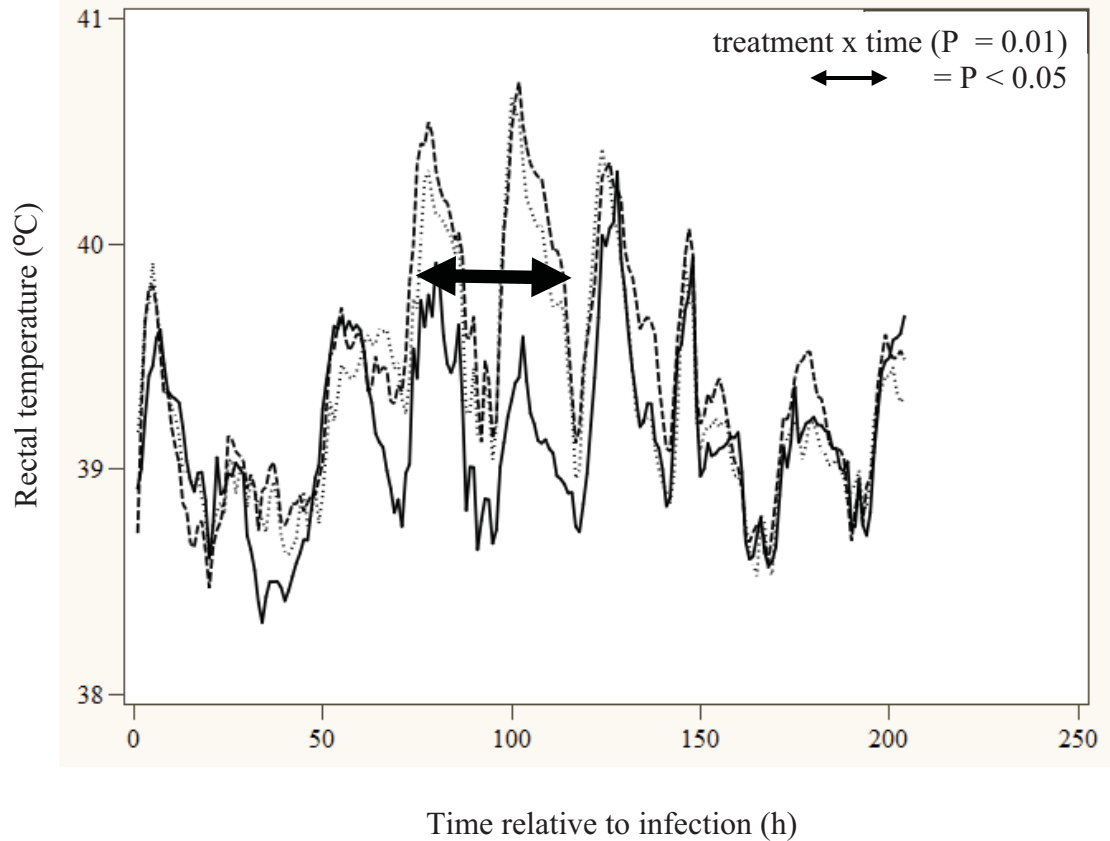


Figure 4.1 Mean rectal temperature (RT) for Control^a (—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (---) and IBRV-3X^c (·····) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl). The IBRV challenge caused changes over time in RT (P = 0.01), and the largest differences (P < 0.05) observed 72 to 120 h post infection (p.i.).

^a Control [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)]

^b IBRV1 [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^c IBRV2 [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

↔ Denotes differences $P \leq 0.05$

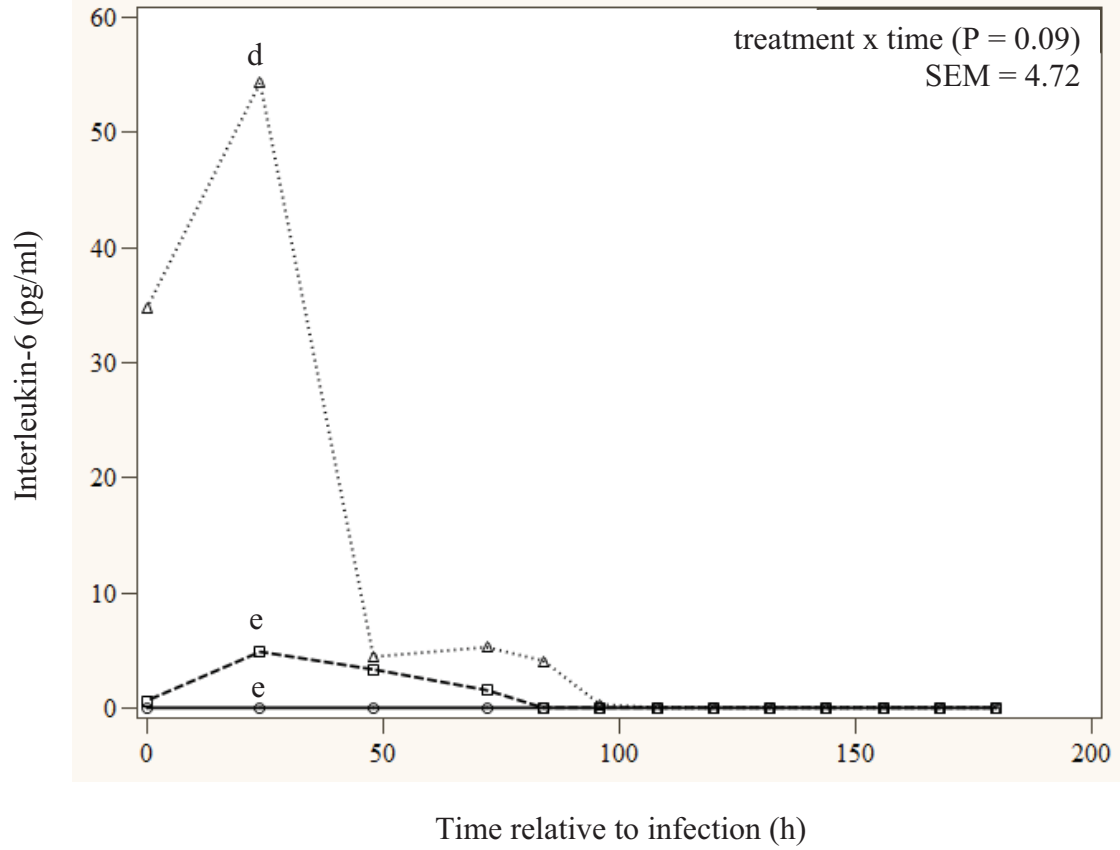


Figure 4.2 Mean serum concentrations of interleukin-6 (IL-6) for Control^a (—○—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (—□—) and IBRV-3X^c (···△···) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl).

^aControl [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)]

^bIBRV-2X [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^cIBRV-3X [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^{d,e}Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

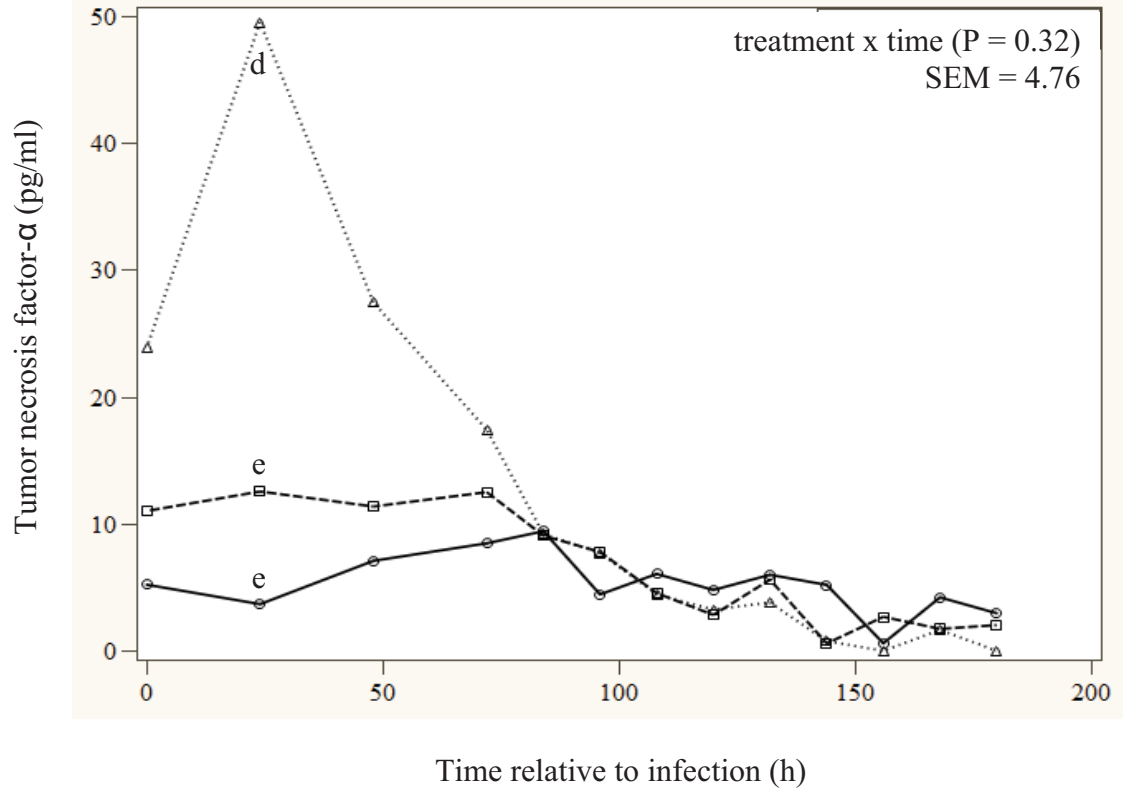


Figure 4.3 Mean serum concentrations of tumor necrosis factor- α (TNF- α) for Control^a (—○—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (—□—) and IBRV-3X^c (···△···) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl)

^a Control [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)].

^b IBRV-2X [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^c IBRV-3X [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^{d,e} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

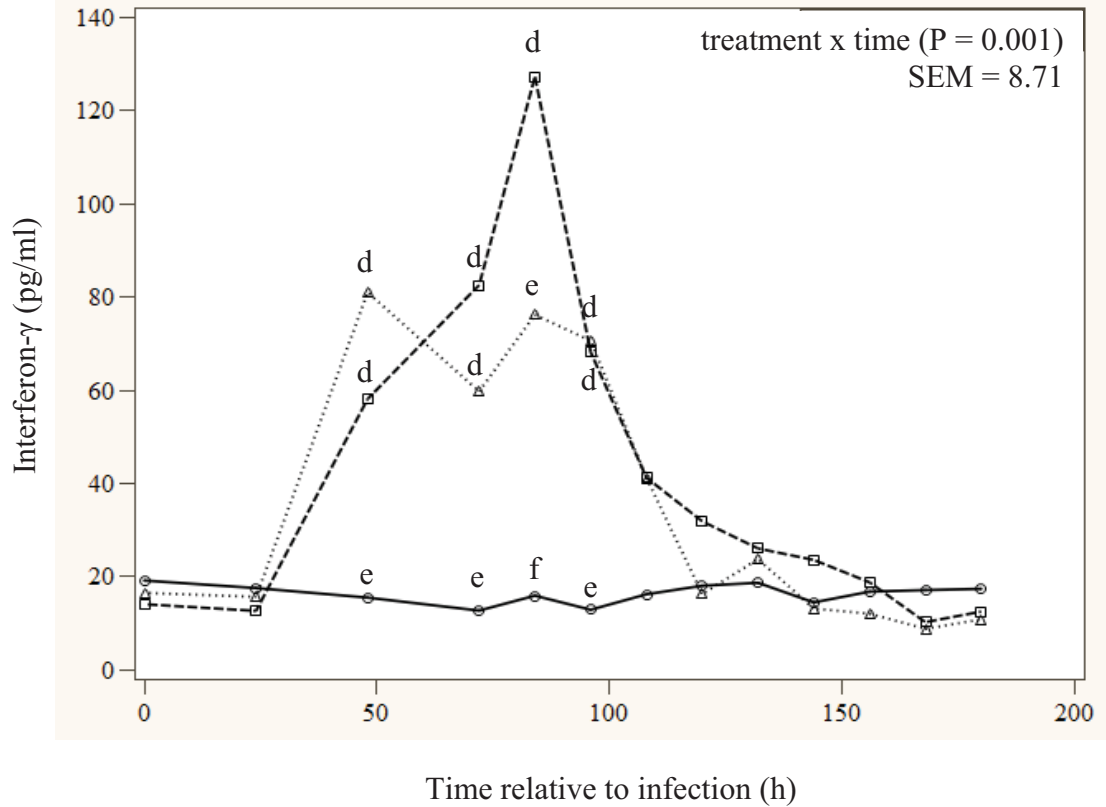


Figure 4.4 Mean serum concentrations of interferon- γ (IFN- γ) for Control^a (—○—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (—□—) and IBRV-3X^c (···△···) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl)

^aControl [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)]

^bIBRV-2X [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^cIBRV-3X [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^{d,e,f} Denotes differences $P \leq 0.05$ between treatment groups at the specific time point

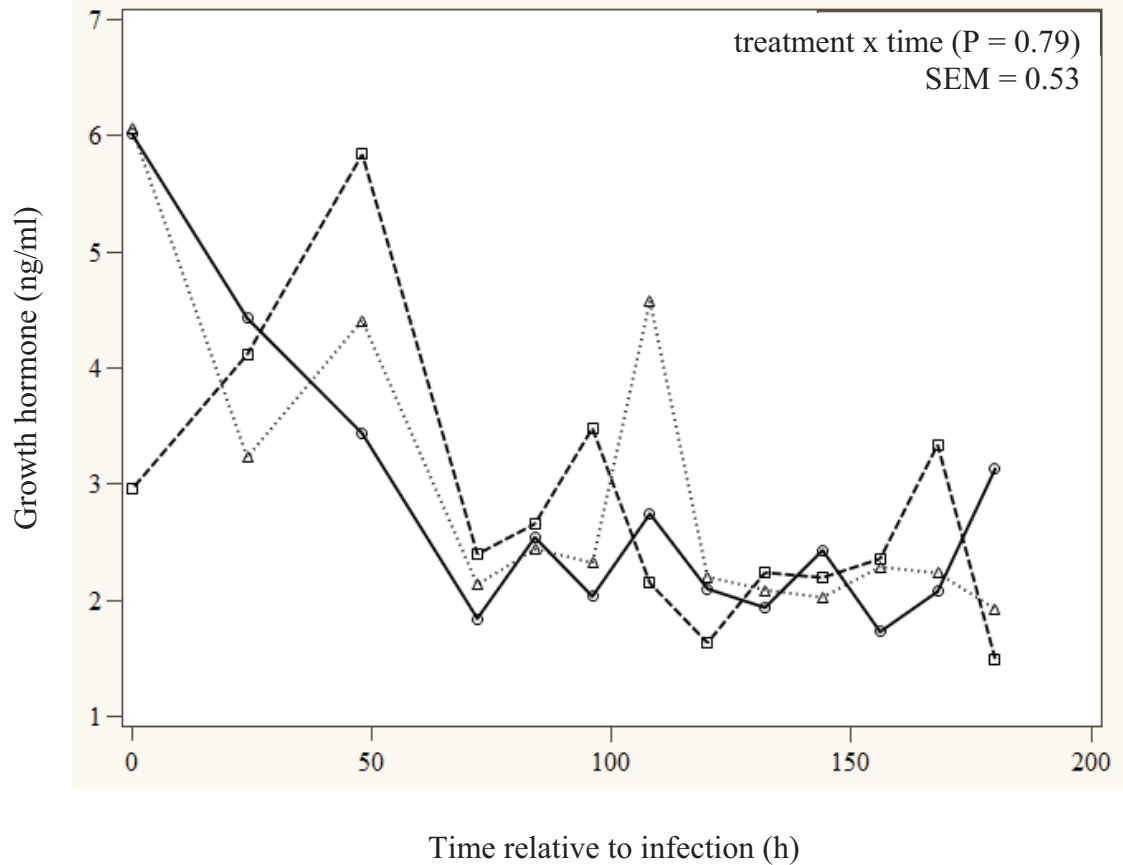


Figure 4.5 Mean serum concentrations of growth hormone (GH) for Control^a (—○—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (—□—) and IBRV-3X^c (···△···) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl)

^aControl [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)].

^bIBRV-2X [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^cIBRV-3X [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

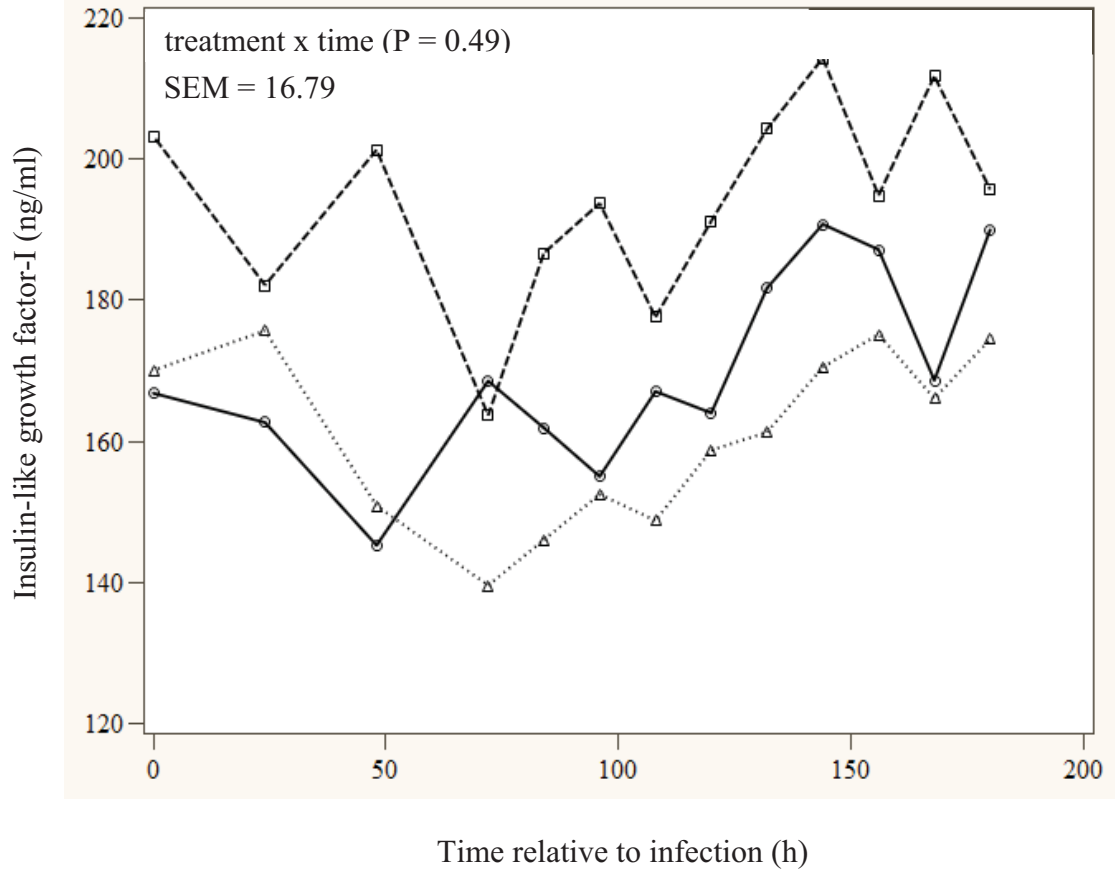


Figure 4.6 Mean serum concentrations of insulin-like growth factor-I (IGF-I) for Control^a (—○—), infectious bovine rhinotracheitis virus (IBRV)-2X^b (---□---), and IBRV-3X^c (···△···) steers (n = 6/treatment) following an intra-nasal challenge with IBRV (Cooper strain 8.0 TCID₅₀ / 0.3 mL of solution) or saline (0.9 w/v NaCl)

^aControl [8 mL placebo dose of saline (0.9 w/v NaCl) solution (4 mL/nostril)]

^bIBRV-2X [8 mL dose of IBRV (4 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

^cIBRV-3X [12 mL dose of IBRV (6 mL/nostril, 8.0 TCID₅₀ at 0.3/mL of solution)]

CHAPTER V
EVALUATION OF THE ENDOCRINE RESPONSE OF CATTLE DURING THE
RELOCATION PROCESS

A manuscript to be submitted to *The Journal of Animal Science*

Abstract

To evaluate the endocrine response associated with the relocation process; 22 Holstein heifers (326.4 ± 46.8 kg BW) were randomly assigned to control (CON) or transport (RELOC) treatment groups. On 0 d, 12 h prior to transportation, heifers from both treatment groups were weighed, fitted with indwelling rectal temperature (RT) probe, and jugular catheters. On 1 d, heifers were haltered and placed into individual tie stalls 2 h prior to transportation. This 2 h period was utilized to obtain time baseline blood samples prior to the transportation event. At conclusion of the baseline period, heifers from both treatment groups were weighed, CON heifers were returned to the tie stalls and RELOC heifers were loaded on a modified stock trailer with 12 individual stanchions for a 4 h transportation event. Blood samples were simultaneously obtained from both treatment groups throughout the first 4 h transport event (TE-I; 0-4.25 h) at 30-min intervals. After transport, RELOC heifers were unloaded at an unfamiliar location and weighed. After unloading and placing RELOC heifers in tie stales, a 2 h post

transportation period was started to collect blood samples post-transport. At the conclusion of the 2 h post transportation period, heifers from both treatment groups were placed into two separate holding paddocks with access to water and hay for a period of 4 h. After 4 h, access to the hay and water was terminated; heifers remained in the holding paddock for an additional 8 h. At 2200 h on 1 d, heifers were prepared for a second 4 h transport event (TE-II; 23.5-27.75 h). Time line and procedures of transport event II was identical to transport event I. Serum was analyzed for cortisol, GH and IGF-I. Transport event-I resulted in a 6% reduction in BW for the RELOC heifers as compared to a 2.5% reduction for the CON heifers ($P < 0.001$). Overall BW loss was 2% greater ($P > 0.02$; TE-I and TE-II combined) for RELOC compared to CON heifers. During TE-I RELOC heifers had increased RT compared to CON heifers. There was a main effect interaction of treatment x time observed for cortisol with the RELOC having greater cortisol concentration at multiple time points throughout TE-I and II. No differences ($P > 0.05$) in area under the curve for cortisol were observed during TE-I, but area under the curve was different for total cortisol production during TE-II. Heifers in the RELOC treatment group had greater cortisol concentrations compared to CON. There were no differences in area under the curve for GH between the RELOC and CON heifers for TE-I or II, but a transient decline ($P < 0.05$) within each group (RELOC and CON) was observed from 1 to 2 d. Similarly there were no differences ($P > 0.05$) between the RELOC and CON heifers for IGF-I concentrations at specific time points during the trial period. Results of this study indicate that the relocation process, and more specifically the actual event of transportation can elicit a stress response; as indicative of increased cortisol concentrations, increased RT and increased BW losses.

Introduction

Most stressful stimuli induce two types of responses, a general stress response or an individual stress response (Pacak and Palkovits, 2001). A general stress response, common to all stressors, involves the release of adrenal corticotrophic hormone (ACTH) followed by adrenal secretion of cortisol. An individual stress response is mediated by conditioning factors, such as genetic and predisposition factors (Curley et al., 2006). Secretion of cortisol during perceived stressful events has been shown to suppress appetite, reduce growth rate, alter digestive function, and compromise immune function (Loerch and Fluharty, 1999).

The relocation process (loading, transporting and unloading) has been implicated to be one of the major stressors associated with newly received cattle (Grandin, 1997) and contribute to the bovine respiratory disease or shipping stress complex (Loerch and Fluharty, 1999). The stress associated with transportation and arrival at the new facility can contribute to physiological changes such as; transient endocrine responses, altered metabolic enzymes and metabolites (NEFA, glucose, blood urea nitrogen, creatine phosphokinase, and lactic dehydrogenase) associated with energy and protein metabolism, and changes in growth parameters of newly received cattle (Loerch and Fluharty, 1999). Potent stressors such as immobilization in rats were found to increase stress corticosterone concentrations and decrease growth hormone concentrations (Kant et al., 1983). The relationship associated with transportation stress and endocrine growth parameters to our knowledge has not been elucidated.

During the relocation process from the farm to the next phase of production either stockering or feedlot segment, cattle can be subjected to two consecutive transports

coupled with feed and water deprivation. Cattle that leave the farm are loaded and transported to the salebarn, potentially held off feed and water for approximately 12 h prior to being loaded and transported to the stockering or feedlot facility. Based on the assumption that cattle will leave the farm and will enter a salebarn prior to moving to the next segment of production, a transportation model was established to evaluate the following objectives.

One stressor most/if not all cattle encounter in a typical management system is stress associated with the relocation process (handling, loading, transporting, and unloading of cattle). The relocation process, specifically the act of transportation has been shown to elicit physiological responses such as elevated rectal temperature during the transportation process. Burdick et al. (2010) reported no alteration in cortisol concentrations between pre- and post-transport samples after a 9 h transport, but did observe an increase in rectal temperature within 30 min after the onset of the transport event and declining during the transport period with the least mean temperature observed at 400 min after initiation of transport. Blecha et al. (1984) also utilized a 8 - 9 h transport event also reported no alteration in cortisol concentrations pre- and post-transport. While the initial increase in rectal temperature reported by Burdick et al. (2010) suggests alterations in physiological parameters, no changes were observed in cortisol concentrations when utilizing only pre and post transport sample. The differences in rectal temperature observed by Burdick et al. (2010) occur early (< 2 h) in the transport event and the post-transport blood samples were obtained 8 - 9 h after the onset of transport. These results suggesting that while there may be an endocrine response associated with transportation, the alteration occurs relatively early in the

transport process and due to possible acclimation to the event, allow cortisol concentrations to returned to similar concentrations observed prior to transportation. In a second transportation trial, Burdick et al. (2011) reported similar results for rectal temperature during a transport event with the exception that blood samples were collected in transit via the Ice Sampler[®]. Regardless of treatment (Calm, Intermediate, and Temperamental temperament scores) rectal temperature during the transportation process increased over time. Cortisol concentrations observed during the transport process revealed an increase in the calm bulls, there was no alteration in cortisol concentrations for temperamental bulls (Burdick et al., 2011). Other researchers have reported an increase in circulating glucocorticoids after transport (Crookshank et al., 1979; Locatelli et al., 1987). Differences in these results suggest timing of the sample collection could impact the differences observed in physiological and endocrine parameters. While changes in physiological function have been reported pre-and post-transport; however, there is limited available data detailing the physiological and endocrine response during the actual transportation process. Therefore, transportation remains a concern within the cattle industry and is generally recognized as a stressor.

The objectives of this study were to profile the stress response associated with transportation and elucidate the endocrine growth disruption of cattle while being subjected to two separate transportation events. The second transportation event is after feed and water withdrawal to simulate normal industry practices associated with cattle being held at sale barns or sorting facilities prior to transport.

Material and methods

The use of animals and all procedures conducted were approved by the IACUC committee at Mississippi State University (IACUC #08-041).

Twenty-two Holstein heifers (326.4 ± 46.8 kg BW) were housed on pasture at the Bearden heifer development unit for a period of 10 weeks prior to the start of the study. During this 10 week period, heifers were acclimated to human contact and restraint in tie stalls. Seven days prior to initiation of relocation process, heifers were weighed and randomly assigned to one of two treatment groups; control (CON) and relocation (RELOC). The RELOC treatment consisted of two transport events (TE) 24 h apart with a 12 h feed and water deprivation period prior to TE-II (Table 1). On 0 d, heifers were weighed, fitted with indwelling rectal temperature monitors (Reuter et al., 2010) and indwelling jugular catheter for serial blood collection. Rectal temperature was recorded at 1 min intervals for each heifer using an automatic rectal temperature monitoring device (Reuter et al., 2010) for the duration of the project. Jugular catheters consisted of approximately 150 mm of PTFE tubing (6417-41 18TW; Cole-Palmer; o.d. = 1.66 mm) that was inserted into the jugular vein using a 14-gauge x 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). The catheter was maintained in place using tag cement and a 5.1 cm wide porous surgical tape. The catheters was fitted with extensions made of sterile plastic tubing (Tygon S-50 HL; VWR Scientific; i.d. = 1.59 mm; o.d. = 3.18 mm) for collection of blood samples without disturbing the heifers. Prior to collection of each blood sample, approximately 5 ml of waste fluid was removed from the catheter to ensure a clean blood sample was collected (removing any heparinized saline in the tubing). After which, a 14 ml sample tube was immediately connected to the

catheter and a sample was obtained from each heifer. After the sample was obtained, catheters were flushed with 5 mL of saline followed by 3 mL of heparinized saline to ensure the catheter remained functioning for the duration of the trial (3 d).

Transport events

Transport event-I (TE-I)

On 1 d (0800), heifers from both treatment groups were weighed, haltered and placed into individual tie stalls within the heifer development unit. Once all 22 heifers were in tie stalls, collection of blood samples commenced at 30-min intervals for 2-h (2400-0200 h) prior to loading the heifers on the trailer (baseline). At the conclusion of the 2-h pre-transport period, RELOC heifers were loaded onto a modified stock trailer with 12 individual 5'-X-24" stanchions. Once all RELOC heifers were secured in the stanchions and catheters lines were secured, a blood sample was obtained simultaneously on RELOC and CON heifers at 0 h (0330 h of 1 d); this was the initiation of the TE-I. For the next 4 h, RELOC heifers were exposed to transportation with serial collection of blood throughout the TE. Collection of blood samples was achieved by stationing three trained individuals within the trailer with the heifers; the individuals had access to jugular catheters during transit. During the serial sampling period, blood samples were collected at 15 min intervals for the first 60 min; followed by 30 min intervals for the remaining 180 min (3 h). Upon completion of TE-I, RELOC heifers were unloaded at the Bearden Dairy Unit (an unfamiliar working facility) and placed into individual tie stalls. After all heifers were secured in the tie stalls, blood samples were collected for 2-h post-transport

at 30-min intervals on both treatment groups. After the 2-h post transport blood collection, heifers (CON and RELOC) were untied, weighed and placed in a dry lot for 16 h. For the first 4 h of this period heifers had *ad libitum* access to water and bermudagrass hay (*Cynodon dactylon*; 1100 to 1500 h), after which access to water and hay was prohibited. Heifers experienced a 12-h withdrawal (1500 to 0300 h) from feed and water prior to the second transport event.

Transport event-II (TE-II)

After experiencing a 12-h deprivation of hay and water on 2 d, heifers in the CON and RELOC treatment groups were weighed, haltered and moved to their tie stalls (2400 h on 2 d; RELOC heifers were placed into tie stalls at the unfamiliar location). Similar to the 2 h baseline period prior to TE-I, in 30 min intervals for a period of 2 h, blood samples were taken from all heifers (baseline for TE-II was ~20.5 h post TE-I). After the 2 h baseline sample for TE-II were collection period, RELOC heifers were loaded on the same modified stock trailer. Transport event-II was conducted following the exact same procedures (route traveled, time line, and sample collection) reported in TE-I. The first blood sample for TE-II was collected approximately 23.5 h after the initiation of TE-I. Upon conclusion of the TE-II, RELOC heifers were return to the unfamiliar working facility, unloaded, and moved to individual tie stalls. Once RELOC heifers were unloaded the 2 h post-TE-II blood samples were collected (CON and RELOC heifers, simultaneously). At the conclusion of the 2 h post transport blood collection, all heifers (CON and RELOC) were untied, weighed, catheters removed, and returned paddocks.

Serum cortisol concentration was determined by radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA) per the manufacture's protocol in a single assay with a detection limit of 2-ng/mL and less than 5% intra-assay coefficient of variation.

Serum samples were analyzed for GH and IGF-I as previously described (Elsasser et al., 1989). For the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined at 150 pg bGH/assay tube with 50% binding of tracer achieved at 1,800 pg/tube. Increasing volumes of plasma displaced tracer counts in a fashion parallel to that of the standard curve. Recovery of nonlabeled bGH averaged 97% for 300, 600 and 1,200 pg added to 200 microliters serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$.

Concentrations of IGF-I were determined following acidification for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual sample was diluted and neutralized with a 1:80 dilution with assay buffer. Anti human/bovine IGF-I primary serum was procured from GroPep (Adalaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of nonlabeled IGF-1 added to plasma before acidification averaged 95% with intra- and interassay coefficients of variation $\leq 10\%$.

Statistical analysis

The data consisted of repeated measurements of heifers over time to evaluate changes in cortisol, GH, IGF-I, as well as rectal temperature over time. The response to the transportation over time was analyzed by repeated measures with ANOVA in the MIXED procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The model included sampling time, treatment and treatment x sample time as a fixed effect, and a BY statement was used to partition each day. In a separate analysis day was added to the model to compare variables of interest between TE-I and II. Therefore, the response to the transportation over time was analyzed by repeated measures with ANOVA in the MIXED procedure of SAS and the model included sampling time, treatment, day and treatment x sample time x day as a fixed effect. Rectal temperature was recorded at 1-min intervals, but subsequently averaged over 30-min intervals to facilitate comparisons to other physiological measures. When F-test were significant ($p < 0.05$) for treatment x sample time on each day means were separated using LSD.

Area under the curve for cortisol and GH were calculated by $[(\text{Time 1} + \text{Time 2}) * (\text{time between Time 1 and Time 2})] / 2$ to determine the ng*h/ml concentration of each hormone over a given time period. Results from these calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

Results

Body weight comparisons

During TE-I, RELOC heifers had a 6% loss in BW as compared the 2.5% loss for CON heifers ($P \leq 0.001$; Table 2). There was no difference in percent of BW loss between RELOC and CON heifers during TE-II ($P > 0.05$). When total weight loss was compared, BW loss was 2% greater ($P = 0.02$) for RELOC heifers as compared to CON heifers.

Rectal temperature

There was a treatment x time interaction ($P \leq 0.001$) observed for rectal temperature (RT); with differences in RT observed at specific time points during TE-I between RELOC and CON heifers. Prior to the first transport there were no differences in RT between RELOC and CON heifers ($P > 0.05$; Figure 1). Rectal temperature peaked (39.1°C) for the RELOC heifers at the beginning of the TE-I (< 1 h) and began to decline again approximately 90 min after TE-I began. While RELOC heifers had a peak in RT early in the transport period, the difference in RT ($P < 0.05$) remained between the RELOC and CON heifers beginning 30 min after TE-I began and sustaining the entire 240-min TE-I period. The increase in RT for the RELOC heifers sustained for 250 min, or 10 min after the initial TE-I was completed.

During TE-II, there were no differences ($P > 0.05$) in rectal temperature prior to transport, during transport and post-transport (Figure 2) for CON and RELOC heifers. Rectal temperature gradually declined for both CON and RELOC heifers from initial

temperature observed at -120-min pre-transport (39.05°C and 38.9°C, respectively) to 390-min post-transport (38.7°C and 38.65°C, respectively).

Cortisol

There was a main plot effect of treatment x time interaction ($P = 0.003$) observed for cortisol during TE-I (Figure 3), with RELOC heifers having a biphasic response. During the collection of baseline samples and up to approximately 90 min into TE-I, there was no differences ($P > 0.05$) in cortisol concentrations between the two treatment groups. After approximately 120 min of exposure to transportation, RELOC heifers had a biphasic response in cortisol concentrations. Cortisol concentrations for RELOC heifers were greater ~120 h after initiation of transport, and remained greater ($P < 0.05$) until ~180 min of transportation, at which time there was no difference ($P > 0.05$) when compared to CON heifers. Heifers in the RELOC treatment group had greater cortisol concentrations at the conclusion of the 240 min TE-I ($P = 0.02$), no differences ($P > 0.05$) were observed in post-transport cortisol concentrations between RELOC and CON heifers.

A treatment x time interaction was observed for cortisol during TE-II. There were no differences ($P > 0.05$) in pre-transport cortisol concentrations between RELOC and CON heifers (Figure 4). At the onset of TE-II (25 h after TE-I), there were no differences ($P > 0.05$) between the heifers; 60 min into TE-II (26 h), RELOC heifers had greater ($P < 0.05$) cortisol concentrations when compared to CON heifers. This alteration in cortisol continued until ~ 120 h into TE-II, with the RELOC heifers remaining greater ($P < 0.05$) than the CON heifers for 120 min (2 h) during TE-II (26 - 28 h). Thirty min

after the conclusion of TE-II (h 29.5) RELOC heifers had greater cortisol concentrations ($P = 0.03$), but no differences ($P > 0.05$) were observed for any other post-transport cortisol concentrations between RELOC and CON heifers.

Area under the curve

While differences were observed at specific time points during TE-I, when the total concentration of cortisol produced during 0-60, 60-120, 120 -180, and 180-240 min of transport was calculated (area under the curve), there were no differences between the groups ($P > 0.05$; Table 3). Cortisol concentration as a function of ng*h/ml did not differ at any of the time intervals during the 240 min of transportation, as well as total cortisol produced during the TE-I.

The differences that were observed at specific time points during TE-II translated into differences in total concentration of cortisol produced during the calculated time intervals during the transportation process (area under the curve) between the CON and RELOC heifers (Table 3). Cortisol concentration as a function of ng*h/ml was different ($P < 0.05$) at time intervals 60-120 and 120-180 min as well as total circulating cortisol during TE-II ($P = 0.01$).

While the total circulating concentration of cortisol was different ($P = 0.01$) between the RELOC and CON heifers during TE-II, with the RELOC heifers having greater cortisol concentrations. When the total circulating cortisol within the RELOC heifers was compared for TE-I and TE-II was, there was no difference ($P = 0.09$; Table 3) between the two days. There were also no difference ($P = 0.58$) for total circulating cortisol in the CON heifers between TE-I compared to TE-II.

Growth hormone comparisons

There was the main effect of treatment x time for GH between the RELOC and CON heifers however there was no clear pattern to the alteration in GH. Evaluation of total concentration of GH produced at time intervals of 0-60, 60-120, 120-180, and 180-240 min (area under the curve, ng*h/ml) during TE-I, also indicated that there were no differences between CON and RELOC ($P > 0.05$; Table 4).

Area under the curve

There was no main effect interaction of treatment x time for GH during TE-II and both the RELOC and CON heifers had very similar patterns of circulating GH concentrations. Therefore, there were no differences ($P > 0.05$) in GH concentrations as a function of ng*h/ml at any of the hour intervals (0-60, 60-120, 120-180, and 180-240 min) as well as total GH produced during TE-II for RELOC and CON heifers.

The total concentration of GH produced during TE-I was decreased ($P < 0.05$) when compared to the total GH produced during TE-II within each treatment group (Table 4). It would appear that withdrawing feed and water for 12 h prior to TE-II impacted GH production in both the CON and RELOC heifers. Growth hormone production was greater on d 2 for both the CON and RELOC heifers.

Insulin-like growth factor-I comparisons

There was no main plot effect of treatment x time interaction observed for IGF-I on d 1 or 2 ($P > 0.05$). There were no differences in initial IGF-I concentrations between the treatment groups, as well as no difference for change in pre- and post- transport

concentrations between the groups (Table 5). Transportation did not alter IGF-I concentrations, neither did transportation process after fasting the animals prior to transportation on 2 d.

Discussion

In this study heifers were subjected to a relocation event that included two 4 h transport events 23.5 h apart to examine the role the relocation process has on physiological and endocrine parameters. The two TE were used to simulate an experience that cattle might experience if transported to the sale barn and then held for a period of time at the sale barn prior to being transported to another location (background or feedlot facility). The relocation process is generally regarded as stressful to cattle and includes both physical and psychological stimuli that can cause detrimental physiological and endocrine changes. These physiological and endocrine changes can often potentiate or alter other physiological, immunological, or endocrine responses. Consequently, the release of cortisol associated with the relocation process can cause cattle to be more susceptible and vulnerable to disease, and have an increased incidence of morbidity or mortality. Preventive measures have been studied to help condition and mitigate the detrimental effects associated with transportation stress. While research has focused on newly received incoming cattle that have been transported to the feedlot or stocker facility pre- and post-transport, little data has established the changes that occur during the actual transportation process. Data from this study helps establish biological changes associated with transportation and the contribution transportation has on the overall health and well-being of cattle that undergo this type of stress.

Rectal temperature observed during TE-I is similar to data reported by Burdick et al., (2010) in which Brahman bulls transported for a period of 8 h displayed a peak in RT 30 min after the onset of transportation. Similarly, the decrease in RT observed over time by Burdick et al. (2010) is consistent with data in the current study for both TE-I and II. While there was not an initial increase in RT during TE-II, this could be associated with acclimation to the handling procedures the cattle experienced on d 1. Conversely, Burdick et al., (2011) observed an increase in RT overtime beginning during the transport event continuing through the post-transport event but some of the increase in RT during the trial could be attributed to the correlation with the increase in ambient temperature. Other research using a digital thermometer did not detect differences before or after a 9 h transport of bulls (Buckham Sporer et al., 2008). However, RT was not measured during transportation, therefore, it is unknown if any changes during transport were observed by Buckham Sporer et al. (2008).

Limited data is available that has evaluated the stress response associated with transportation during the actual transportation process. Burdick et al. (2011) evaluated calm and temperamental bulls during a transportation event and only reported a difference in cortisol for the calm bulls. Other studies have evaluated pre-and post-transportation cortisol concentrations in cattle and have determined that there were no differences in cortisol concentration (Blecha et al., 1984; Burdick et al., 2010). Conversely, other research has suggested that pre-transport samples differ from samples taken at different time points during transportation (Buckham Sporer et al., 2008; Crookshank et al., 1979; Odore et al., 2004), but a complete profile of the physiological and endocrine response during the entire transportation process was not determined. The

conflicting results observed between these studies suggest that transportation time or length as well as sampling time and intervals can influence the overall conclusions that can be made. Temperament can also impact cortisol concentrations (Burdick et al., 2010), therefore, Holstein cattle were used in the current study to help minimize the amount of variability in the stress response. While it is important to understand the fluctuations in the cortisol during the sample period, the total amount of circulating concentration of cortisol at different time intervals during the transportation process should be considered. Since the majority of the previous research lacked samples throughout the transportation period it is unknown the total amount of cortisol produced (ng*h/ml) while subjected to transportation stress. There were differences in cortisol at specific time points during TE-I, but these spikes in cortisol differing between the RELOC and CON heifers did not translate into differences in the total amount of cortisol produced during specific time intervals during the transportation process. Whereas cortisol concentrations during TE-II were greater than concentration observed during TE-I and the fluctuations in cortisol were greater. The differences in fluctuations of cortisol coincided with differences in the total amount of cortisol produced during specific time intervals during the transportation process. The greater concentration of circulating cortisol produced over a period of time for the RELOC heifers during the transportation process could be more concerning, rather than small fluctuations in cortisol that are not sustained. The sustained response of cortisol over a period of time needs to be evaluated in comparison to short lasting perturbation and fluctuations to determine when greater cortisol responses over time impact performance of cattle. Data from the current study would suggest that transportation does cause activation of the HPA axis and transported

animals have greater concentrations of cortisol during the transportation process.

Cortisol does return to baseline concentrations by the end of a 4 h transport. Therefore if samples were only obtained pre-and post-transport, it would appear that there is no change in cortisol concentration. Furthermore, the initial response during the onset of transportation could be coupled with the handling and loading of the cattle onto the trailer which is an unfamiliar space. The CON heifers were handled to obtain a weight at the same time the RELOC heifers were weighed and loaded on the trailer, but handling the CON heifers did not appear to impact cortisol concentrations, possibly due to returning the heifers to their original tie stalls. Whereas, the transported heifers exhibited greater RT and cortisol concentrations early in the transportation process and it would appear the handling a loading process could have impacted the response to transportation since they were put in an unfamiliar stall.

Transportation stress has been implicated to be one of the major stressors associated with newly received cattle (Grandin, 1997), the actual transportation event does not appear to be a stressful event. While transportation is part of the relocation process, there are multiple other factors such as; pathogen exposure, commingling, diet changes, handling, processing, and acclimation that contribute to the shipping stress complex generally associated with newly received cattle. Therefore, after evaluating the initial data generated it would appear the term transportation or shipping stress should be regarded as relocation stress to encompass the whole process associated with arrival and acclimation to a new environment.

The increase in cortisol due to transportation has been implicated to alter metabolic functions in beef cattle. Buckham Sporer et al. (2008) reported metabolic

alterations in beef calves demonstrated by the differences observed in plasma concentrations of albumin, globulin, urea, total protein, and creatine kinase due to the effects of transportation. Immobilization stress in rats has been reported to cause suppression of GH, but increased concentrations of corticosterone were observed for prolonged periods (Kant et al., 1983). Similarly, GH suppression is observed in humans that received prolonged exposure to glucocorticoid treatment which lead to IGF-I insensitivity (Jux et al., 1998; Allen et al., 1998). While these results indicate that transportation stress can alter protein metabolism, data from TE-I and II did not elicit differences in GH or IGF-I concentration between the RELOC and CON heifers. Cortisol concentrations did not remain increased for prolonged periods of time during the transport events, thus it is not surprising no changes in GH or IGF were observed for TE-I or II. Therefore, it would appear that transportation stress does not impact growth parameters when cattle a subjected to transportation and transportation after a 12 h feed and water withdrawal. While cortisol did not appear to impact GH concentrations, withdrawal from feed and water for 12 h did alter GH responses in both groups. The alteration in GH associated with feed and water withdrawal may have masked any changes that could have been associated with the actual transportation process.

Conclusion

In conclusion, RT and cortisol do increase during the initial transportation process. While there are differences during the 4-h, there still does not appear to be differences in samples taken pre-and post-transport. Therefore, this data does not completely explain the stressor associated with transportation and more research needs to

be conducted to understand the relationship handling has on the initial increase in physiological parameters during transportation. Nonetheless, handling is an aspect of the transportation process and therefore the current study would suggest that transportation does cause a stress response, but physiological parameters return to baseline within 4 hours after the onset of transportation. It would also appear the short period the heifers were subjected to greater cortisol concentrations did not impact endocrine factors associated with the somatotrophic axis. While transportation is associated with the relocation process, the multiple stressors associated with the relocation process that result in; transient endocrine responses, altered products of energy and protein metabolism, and changes in growth rate are not directly related the transport event. Transportation does not appear to be the driving force behind the relocation process. A better understanding of the components associated with the relocation process is needed to identify the major stressors or at what point multiple stressors begin to impact endocrine, immunological and metabolic functions resulting in the BRD complex.

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Table 5.1 Overall timeline for the three day study to evaluate the potential endocrine disruption in cattle during the relocation process

	Time	Hour ^a	Duration	Event
Day 0	0800	-19.5 h	6 hr	Weigh, insertion of catheters and rectal probes
	1500	-12.5 h	14 hr	No human contact
	2300	-4.5 h	1 hr	Halter and placed in tie stalls
Day 1	2400	-3.5 h	2 hr	2 h pre transport blood collection
	0200	-1.5 h	1.5 hr	Load heifers onto trailer
	0330	0 h	4.25 hr	Transport Event I
	0800	4.5 h	1 hr	Unloaded heifers
	0900	5.5 h	2 hr	2 hr post transport blood collection
	1100	7.5 h	4 hr	Returned to paddock; access to hay and water
	1500	11.5 h	8 hr	Denied access to hay and water
2300	19.5 h	1 hr	Halter and placed in tie stalls	
Day 2	2400	20.5 h	2 hr	2 h pre transport blood collection
	0200	22.5 h	1 hr	Load heifers onto trailer
	0300	23.5 h	4.25 hr	Transport Event II
	0715	27.75 h	0.75 hr	Unload and weigh heifers
	0800	28.5 h	2 hr	2 h post transport blood collection
	1000	30.5 h	X	End of trial: Heifers weighed

^a Time from when the project initiated (insertion of catheters and rectal probes)

Table 5.2 Least squares means for weight loss (kg) for Control and Transport treatment groups during transport event (TE)-I^b and II^c and total weight loss after both TE-I and II

Variable of Interest	Treatment groups ^a		SEM	P-value
	Control	Transport		
Transport Event-I ^b	8.20	18.83	5.27	0.001
Transport Event-II ^c	3.30	4.25	3.79	0.45
Total Loss for Transport Event-I and II	26.70	33.00	7.31	0.02

^aMeans within a row different between treatment groups if $P \leq 0.05$

^bTE-I = transport period from 0 to 4.25 h on d 1

^cTE-II=transport period from 23.5 to 27.75 h on d 2

Table 5.3 Least squares means for circulating cortisol concentrations (ng*h/ml) at hour intervals during transport event-I^b and II^c for Control and Transport treatment groups

Hour intervals	Treatment groups ^a		SEM	P-value
	Control	Transport		
Cortisol (ng*h/ml) Transport event-I ^b				
0 – 1 h	9.71	8.99	1.42	0.71
1 – 2 h	10.27	15.80	2.85	0.16
2 – 3 h	11.35	17.20	2.17	0.06
3 – 4 h	12.43	16.77	1.90	0.10
Total (0 – 4 h)	43.74	58.76	6.85	0.12

Cortisol (ng*h/ml) Transport event- II^c

25 – 26 h	10.18	14.80	1.93	0.09
26 – 27 h	13.40	21.09	2.39	0.03
27 – 28 h	13.88	23.35	2.28	0.006
28 – 29 h	11.64	14.98	2.20	0.28
Total (25 – 29 h)	49.10	74.21	6.85	0.01

^aMeans within a row different between treatment groups if $P \leq 0.05$

^bTE-I = transport period from 0 to 4.25 h on d 1

^cTE-II=transport period from 23.5 to 27.75 h on d 2

Table 5.4 Least squares means for circulating growth hormone concentrations (ng*h/ml) at hour intervals during transport event-I^c and II^d for Control and Transport treatment groups

Hour intervals	Treatment groups		SEM	P-value
	Control	Transport		
Growth hormone (ng*h/ml) Transport event-I ^c				
0 – 1 h	5.33	6.75	0.95	0.28
1 – 2 h	4.46	5.63	0.82	0.29
2 – 3 h	3.85	4.80	0.80	0.40
3 – 4 h	6.23	5.32	0.96	0.48
Total (0 – 4 h)	19.87 ^a	23.71 ^a	2.71	0.33
Growth hormone (ng*h/ml) Transport event- II ^d				
25 – 26 h	9.94	8.72	1.28	0.50
26 – 27 h	9.29	7.26	1.11	0.22
27 – 28 h	7.46	7.34	0.92	0.92
28 – 29 h	6.43	6.99	0.87	0.65
Total (25 – 29 h)	33.13 ^b	32.07 ^b	2.92	0.78

^{a, b}Means within a column are different within treatment groups if $P \leq 0.05$

^cTE-I = transport period from 0 to 4.25 h on d 1

^dTE-II=transport period from 23.5 to 27.75 h on d 2

Table 5.5 Least squares means for circulating insulin-like growth factor-I concentrations at specific time points and time intervals during transport event-I^b and II^c for Control and Transport treatment groups

	Treatment groups ^a		SEM	P-value
	Control	Transport		
Insulin-like growth factor-I (ng*h/ml)				
Transport event-I ^b	1229.59	1285.05	104.23	0.68
Transport event-II ^c	883.95	804.75	74.30	0.16
Insulin-like growth factor-I (ng/ml)				
-120 min	159.23	139.91	12.55	0.26
390 min	140.61	162.46	11.16	0.18
1440 min	145.96	145.85	14.17	0.99
1770 min	173.87	140.54	13.57	0.10

^aMeans within a row different between treatment groups if $P \leq 0.05$

^bTE-I = transport period from 0 to 4.25 h on d 1

^cTE-II=transport period from 23.5 to 27.75 h on d 2

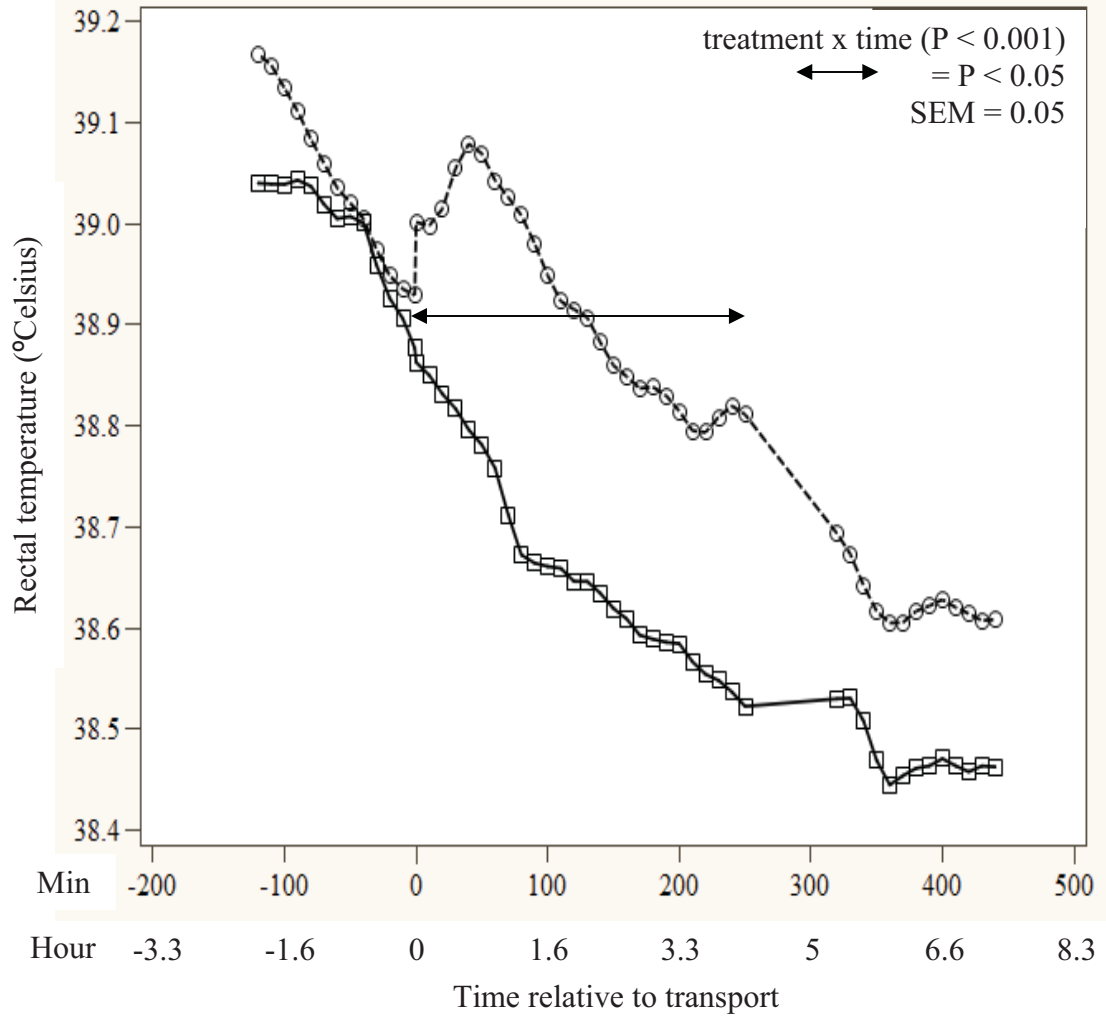


Figure 5.1 Mean rectal temperature (RT) for Control (—□—) and Transport (••○••) heifers prior to, during, and after transport event-I. The transportation event elicited a change in RT ($P < 0.001$) over time. Transport event-I began at min 0 and ended at min 240 on d 1

↔ Denotes time period difference observed between treatment groups

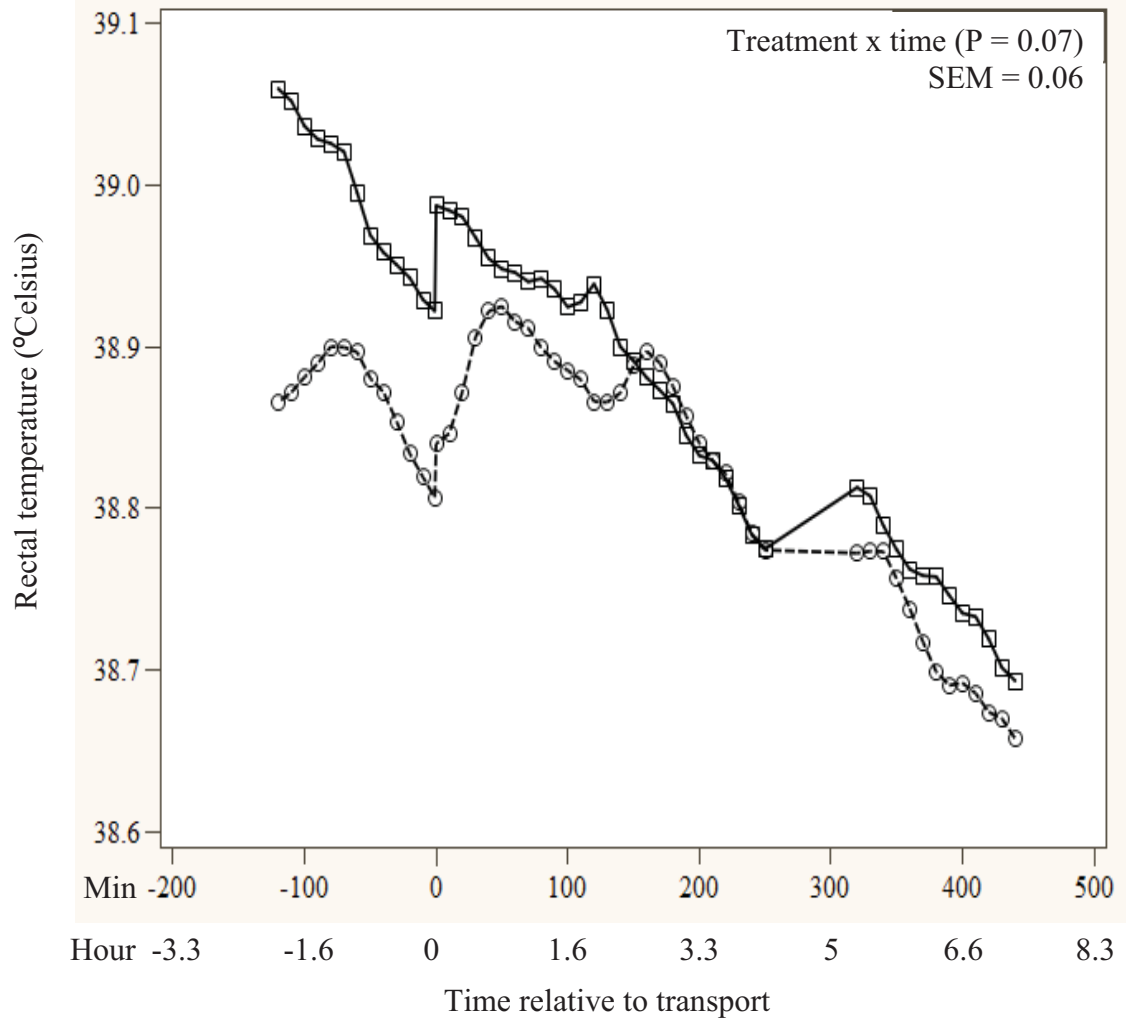


Figure 5.2 Mean rectal temperature (RT) for Control (—□—) and Transport (···○··) heifers prior to, during, and after transport event-II. The transportation event did not elicit a change in RT ($P = 0.07$) over time. Transport event-II began at min 0 and ended at min 240 on d 2

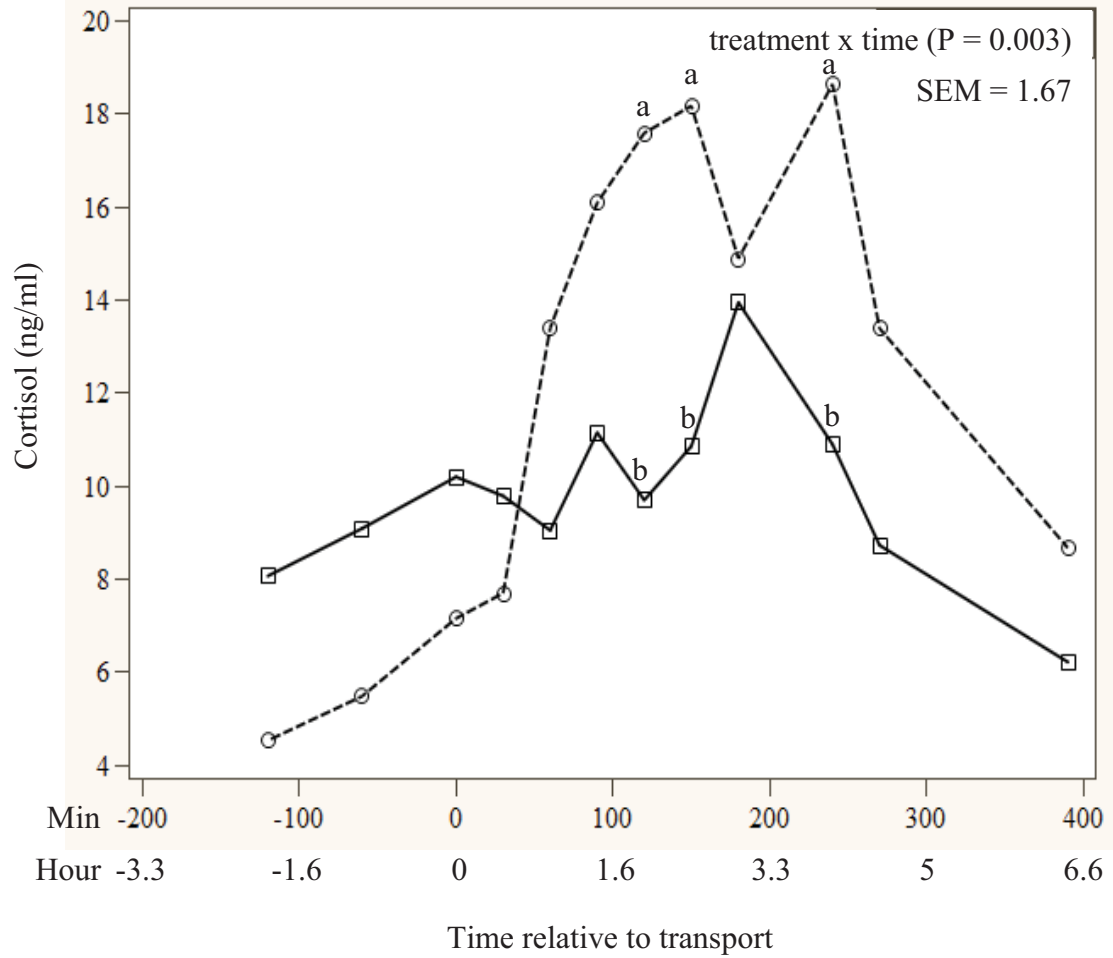


Figure 5.3 Mean serum concentration of cortisol for Control (—□—) and Transport (---○---) heifers prior to, during, and after transport event-I. The transportation event elicited a change in cortisol ($P < 0.001$) over time. Transport event-I began at min 0 and ended at min 240 on d 1

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time points

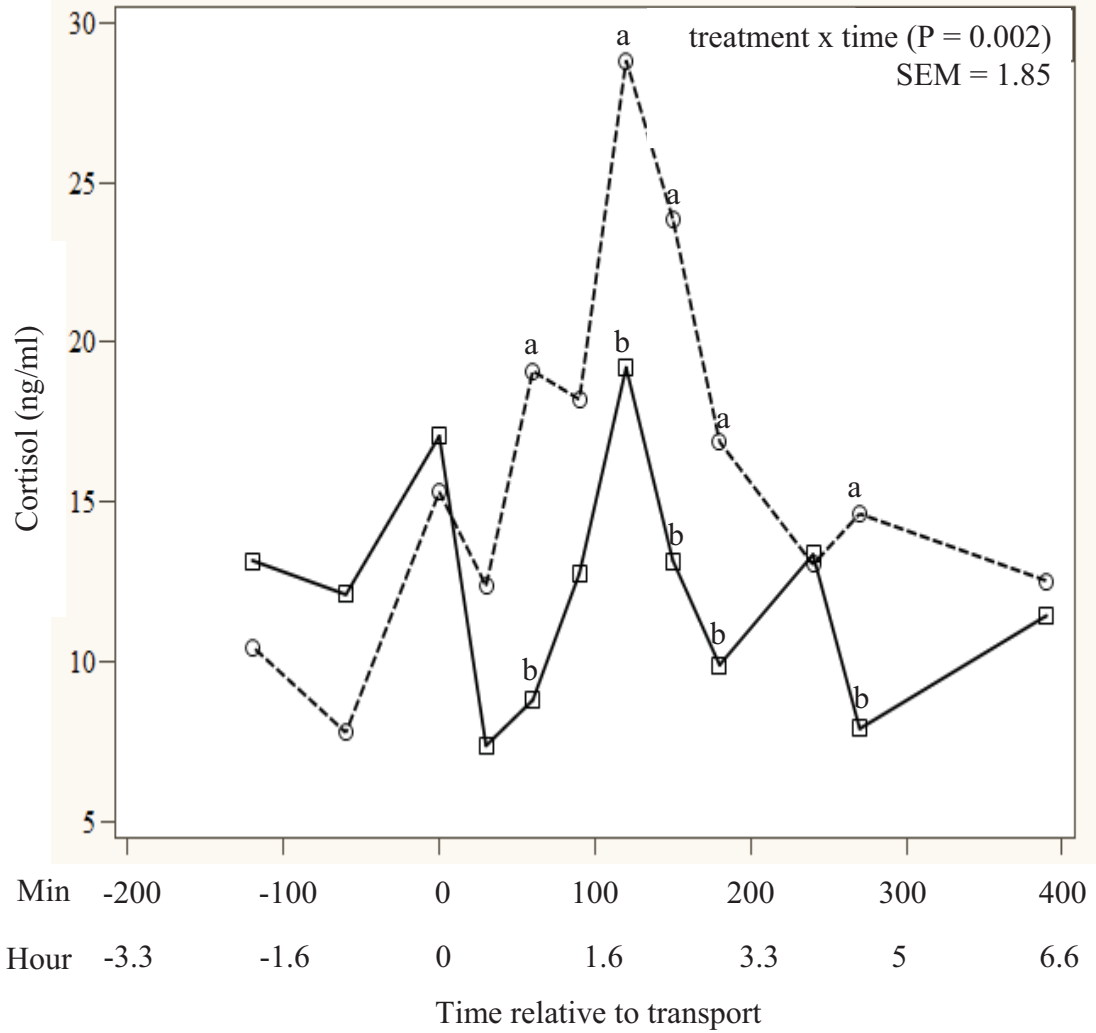


Figure 5.4 Mean serum concentration of cortisol for Control (—□—) and Transport (---○---) heifers prior to, during, and after transport event-II. The transportation event elicited a change in cortisol (P = 0.002) over time. Transport event-II began at min 0 and ended at min 240 on d 2

^{a,b} Denotes differences $P \leq 0.05$ between treatment groups at the specific time points

CHAPTER VI
EVALUATION OF THE TIMING OF CORTISOL RELEASE (SINGLE STIMULI) ON
THE SOMATOTROPHIC AXIS DURING AN *M. HAEMOLYTICA*
CHALLENGE IN BEEF STEERS

A manuscript to be submitted to *The Journal of Animal Science*

Abstract

The objective of this trial was to characterize the potential impact of stimulating release of cortisol (SRC) prior to, simultaneously and post *Mannheimia haemolytica* (Mh) challenge on the somatotrophic axis. Twenty-four beef steers (208.30 ± 22.1 kg BW) were randomly assigned to 1 of 4 treatments: 1) Mh only, no SRC (NC) 2) SRC at -7.5 h + Mh (Prior); 3) SRC at 0 h + Mh (same time, ST); and 4) Mh followed by SRC at 7.5 h (Post). To SRC, corticotropin releasing hormone (bCRH at $0.1 \mu\text{g}/\text{kg}$ of BW) + arginine vasopressin (AVP at $0.5 \mu\text{g}/\text{kg}$ of BW) were administered in tandem via indwelling jugular catheter. *Mannheimia haemolytica* (D-152) challenge was administered at time point 0 h via intra-tracheal injection (5.4×10^8 CFU/mL in 50 mL of the saline-diluted inoculum). Three days prior to the Mh challenge, steers were fitted with jugular catheters and rectal temperature (RT) probes, moved to tie stalls, and allowed 24 h to acclimate (-2 d). Baseline RT and blood samples were obtained on -1 d,

and the challenge was given on 0 d. Prior to the Mh challenge there were no differences ($P > 0.05$) in RT (-44 to 0 h post Mh challenge). The NC, Prior and Post treatment groups had greater ($P < 0.05$) percent change in RT compared to the steers in the ST treatment group. The increase in RT was observed for all four treatment groups at 8 h post Mh challenge. When the respective treatment groups received the bCRH + AVP to SRC (Prior, ST, and Post), there was an increase ($P < 0.05$) in circulating cortisol concentration during the specific time intervals. Total cortisol production (TCP) was not different ($P > 0.05$) between the three treatment groups that received a bCRH + AVP challenge; steers in the NC treatment group did not have an increase in cortisol production compared to all other treatment groups. Total GH production (TGHP) was decreased ($P < 0.05$) in Post compared to NC, Prior and ST steers. There was a difference (within treatment, $P \leq 0.006$) in IGF-I concentrations at the conclusion of the trial compared to IGF-I concentrations prior to the start of the trial for steers in all four treatment groups. The results suggest that the release of cortisol can impact the overall regulation of GH when release occurs after infection with Mh. It would also appear that the Mh challenge caused a reduction in IGF-I for all treatment groups regardless of timing of bCRH + AVP challenge.

Introduction

Thus there is a great deal of interest in understanding the impact the release of cortisol, a glucocorticoid, has on production characteristics of cattle. The release of cortisol is the primary hormonal response of animals that experience a stressful stimulus that causes activation the HPA axis (Salak-Johnson and McGlone, 2007). The release of

glucocorticoids is very important in the innate immune response. Glucocorticoid receptors are expressed on a variety of immune cells and will bind cortisol and interfere with the function of nuclear factor-kB (NF-kB), which regulates the activity of cytokine-producing immune cells (Paul, 2008). Carroll et al. (2006) utilized corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) to stimulate the release of cortisol as a model to mimic a stress response, and were able to elicit an acute stress response as observed by cortisol secretion. Utilizing a stress model similar to this allows some consistency in cortisol production, and repeatable responses over time.

Researchers have reported that cortisol can have both pro- and anti-inflammatory properties depending on the timing of the stressor and the immune insult experienced by the animal. Frank et al. (2010) evaluated the issue of time course of glucocorticoids exposure in relation to an immune challenge in rats. Cortisol concentrations were increased either 24 or 2 h pre- or 1-h post lipopolysaccharide (LPS) immune challenge. Increased cortisol concentrations pre-LPS potentiated the inflammatory response (tumor necrosis factor- α , interleukin (IL)-1 and IL-6) in the hippocampus and liver. In contrast, increased cortisol concentrations post-LPS were anti-inflammatory and inhibited the inflammatory responses as expected. This research would indicate that timing of the stressor could play a major role in the animal's immune response to cortisol production. The intricate cross-talk between the immune system and HPA axis suggests there is the potential for alteration in the innate immune response. Stimulation of the HPA axis and more specifically the release of the glucocorticoid cortisol is of extreme importance within the cattle industry due to the implication of stress within the multi-factorial etiology of the bovine respiratory disease complex.

The impact of glucocorticoid release on the immune response is intriguing for the cattle industry due to the occurrence and repetition of stressors that cattle experience throughout the various phases of the cattle industry. During the typical relocation process of cattle from the farm/ranch to the feedlot, cattle may experience a wide range of stressors, (relocation, nutritional deprivation, environmental changes, handling and processing). Understanding the impact that stimulation of the HPA axis may have on cattle prior to, simultaneously, or post exposure to pathogens associated with the BRD complex could provide insight in the ability of cattle to mount an effective immune response. Therefore, the objectives of this study are as follows: 1) Determine the impact acute release of cortisol has on the pro-inflammatory response when administered prior-to, simultaneously, or post-immune *M. haemolytica* challenge in beef steers, and 2) evaluate endocrine growth parameters prior-to, during and after stimulated cortisol release and an immune challenge to elucidate the implications of the interaction of the HPA and immune system on endocrine growth parameters.

Material and methods

The use of animals and all procedures conducted were approved by the IACUC committee at Mississippi State University (IACCUC #10-029). Twenty-four Holstein steers (208.30 ± 20.80 kg) were utilized to characterize the potential impact of stimulating release of cortisol (SCR) prior to, simultaneously, and post *M. haemolytica* (Mh) challenge on the somatotrophic axis. Steers were randomly stratified by BW into four treatment groups -4 d prior to the initiation of the trial. The immune challenge for the trial was *Mannheimia haemolytica* (D-152; Mh) and to SRC, corticotropin releasing

hormone (bCRH at 0.1 $\mu\text{g}/\text{kg}$ of BW) + arginine vasopressin (AVP at 0.5 $\mu\text{g}/\text{kg}$ of BW) in tandem were administered. The timeline for this study was anchored at 0 h. Zero h is time point when all treatment groups received the Mh challenge. The timing of bCRH+AVP was administered at -7.5 h pre, simultaneously with Mh challenge (at 0 h), and 7.5 h post Mh challenge (depending upon the treatment group). Treatments groups consisted of 1) Mh only, no SRC (NC) 2) SRC at -7.5 h prior to Mh challenge (Prior); 3) SRC simultaneously with Mh challenge (0 h; ST); and 4) SRC at 7.5 h post Mh challenge (Post). All treatment groups received the Mh challenge at 0 h.

Seventy-two h prior (0600 on - 3 d) to the Mh challenge, steers were fitted with indwelling jugular vein catheters for serial blood collection and rectal temperature monitoring device described by Reuter et al. (2010) that recorded temperature at one minute intervals for the duration of the trial. Catheters consisted of approximately 150 mm of PTFE tubing (6417-41 18TW; Cole-Palmer; o.d. = 1.66 mm) that was inserted into the jugular vein using a 14-gauge x 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). Catheters were maintained in place using tag cement and a 5.1 cm wide porous surgical tape. Catheters were fitted with extensions made of sterile plastic tubing (Tygon S-50 HL; VWR Scientific; i.d. = 1.59 mm; o.d. = 3.18 mm) for collection of blood samples without disturbing the steers. After being fitted catheters, steers were moved to respective individual tie stall. During the collection of blood samples, approximately 5 ml of waste fluid was pulled from the catheter to ensure a clean blood sample was flowing. After which the sample tube was connected to the catheter and a 14 mL sample was be obtained from each steer. After the sample was obtained the

catheter was flushed with 5 mL of saline followed by 3 mL of heparinized saline to serve as a lock on the catheter.

Steers were allowed to acclimate to the tie stalls and environmental changes for a period of 24 h (duration of -2 d). At 0600 and 1800 h each day during the trial period, 4.5 kg of 60/40 (soy hull/corn gluten) mixture was offered to each steer (base diet steers were on for 60 d prior to trial). Steers also had ad libitum access to hay and water. Steers were offered the same feed mixture during the duration of the experiment (-3 to 1 d).

At -25.5 h prior to Mh-challenge (-1 d) baseline blood samples were obtained beginning 0600 h (-31.5 h prior to Mh challenge) and continuing every 6 h at -25.5, -19.5, -13.5, and -8 h prior to the Mh challenge. The first tandem bCRH + AVP bolus was administered at -7.5 h prior to Mh-challenge for steers in the Prior group. Blood samples were collected every 30 min starting at -7 h and concluding -0.5 h. At the conclusion of the pre-Mh sample collection period (0 h) the Mh challenge was administered to all treatment groups and well as the bCRH + AVP challenge to the steers in the ST treatment group. Samples were collected every 30 min starting at 0.5 h and concluding 7.0 h post-Mh challenge. Seven and half h post-infection, the last bCRH + AVP bolus was administered to steers in the Post treatment group. Samples were collected every 30 mins starting at 8 h post-Mh challenge and concluding 14.5 h post-Mh challenge. Additional samples were also collected at 16.5 h and 22.5 h post-Mh challenge. Immediately after collection of the blood sample at 22.5 h, catheters and RPs were removed and all steers were moved via an enclosed stock trailer to a quarantine pasture to be monitored for signs of illness for a period of 45 d.

The *Mannheimia haemolytica* strain NADC D152, a serotype 1 isolate from bovine pneumonic lung originally recovered in Iowa was grown in Brain-Heart Infusion (BHI) broth overnight with shaking. The culture was diluted in physiological saline (0.9%) to yield approximately 10^7 CFU/mL based on OD (600) without washing the cells. The challenge consisted of intra-tracheal deposition via tracheal puncture with an 18 gauge needle of 60 mL of the saline-diluted inoculum. Colony counts before administration were 1.06×10^6 CFU/ml; after challenge, aliquots were taken of each challenge dose, returned to the lab and determined to be at 2.67×10^4 CFU/ml. Based on the counts from the samples collected before and after the challenge it could be determined that a total dose of 5.4×10^8 CFU were administered to each steer.

Serum cortisol concentration was determined by radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA) per the manufacture's protocol in a single assay with a detection limit of 2-ng/mL and less than 5% intra-assay coefficient of variation. Serum concentrations of the pro-inflammatory cytokines, TNF- α , IL-6, and IFN- γ were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines (SearchLight; Pierce Biotechnology Inc., Rockford, IL). For all cytokines, the intra-assay variation was less than 5 %, and the inter-assay variation was less than 7 %.

Serum samples were analyzed for GH and IGF-I as previously described (Elsasser et al., 1989). For the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined at 150 pg bGH/assay tube with 50% binding of tracer achieved at 1,800 pg/tube. Increasing volumes of plasma displaced tracer counts in

a fashion parallel to that of the standard curve. Recovery of non-labeled bGH averaged 97% for 300, 600 and 1,200 pg added to 200 microliters serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$. Concentrations of IGF-I were determined following acidification for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual sample was diluted and neutralized with a 1:80 dilution with assay buffer. Anti human/bovine IGF-I primary serum was procured from GroPep (Adelaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of nonlabeled IGF-1 added to plasma before acidification averaged 95% with intra- and inter-assay coefficients of variation $\leq 10\%$.

When percent change was calculated for rectal temperature, calculations were determined by the following formula. Percent change = $[(\text{Time point of interest} - 0 \text{ h value}) / 0 \text{ h value}] * 100$. This calculation was used to express the percent change from 0 h as a result of the Mh challenge or determine the variability between the days and time of day.

Area under the curve was calculated for variables of interest by using specific time point samples $[(\text{Time 1} + \text{Time 2}) * (\text{time between Time 1 and Time 2})] / 2$ to determine the ng or pg*h/ml concentration of each variable over a specific time interval. Area under the curve was calculated for three different time intervals during the trial for cortisol, GH, and IGF-I.

Time intervals (Area under the curve) for cortisol are as follows: 1) Time Interval Prior (TI-Cort-Prior) = - 8 h to -5.5 h prior to the challenge, 2) Time Interval ST (TI-

Cort-ST) = -0.5 to 2.5 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-Cort-Post) = 7 to 9.5 h post *M. haemolytica* challenge. Total cortisol production (TCP) during the 3 time intervals (TI-Cort-Prior, TI-Cort-ST and TI-Cort-Post) was calculated by adding the three time intervals together.

Time intervals (Area under the curve) for GH are as follows:; 1) Time Interval Prior (TI-GH-Prior) = - 13.5 h to -4.0 h prior to the challenge, 2) Time Interval ST (TI-GH-ST) = -0.5 to 3.5 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-GH-Post) = 7 to 10.5 h post *M. haemolytica* challenge. Total growth hormone production (TGHP) during the 3 time intervals (TI-GH-Prior, TI-GH-ST and TI-GH-Post) were calculated by adding the three time intervals together.

Time intervals (Area under the curve) for IGF-I are as follows:; 1) Time Interval Prior (TI-IGF-Prior) = - 25.5 h to -0.5 h prior to the challenge, 2) Time Interval ST (TI-IGF-ST) = -0.5 to 7.0 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-IGF-Post) = 7 to 22.5 h post *M. haemolytica* challenge. Total insulin-like growth factor-I production (TIGFP) during the 3 time intervals (TI-IGF-Prior, TI-IGF-ST and TI-IGF-Post) was calculated by adding the three time intervals together

Statistical analysis

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS as a completely random design; and the model included sampling time, treatment, and sample time x treatment. Sample treatment x time was used as the error term to test whole plot effects.

Rectal temperature was initially recorded at 1-minute intervals, but subsequently averaged over 30-min intervals to facilitate comparisons to other immune and physiological parameters. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pairwise differences among least squares means at various sample times were evaluated with the PDIFF option of SAS. Results from the area under the curve calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

Results

Rectal temperature

There was a treatment x time interaction ($P = 0.02$) observed for RT when the steers were administered the Mh challenge (Table 1) indicating a possible febrile response associated with the Mh challenge. No differences ($P > 0.05$) in percent change from 0 h were observed for RT prior to (-44 to 0 h) the Mh challenge between the NC, Prior, ST, and Post groups. An increase ($P < 0.05$) in percent change for RT from 0 h was evident at 4 h post Mh challenge for the NC, Prior and Post steers, while the increase in percent change in RT for ST steers increased at 8 h post Mh challenge. At 16 h post Mh challenge, RT returned to similar values observed prior to administering the Mh challenge for the NC steers, and at 12 h post Mh challenge for the steers in the Post treatment group. Percent change from 0 h for RT decreased by 20 h post Mh challenge for the ST steers, but had not decreased for the Prior steers by 24 h post Mh challenge.

Cortisol

There was a treatment x time interaction observed for cortisol ($P < 0.001$). As expected, circulating cortisol concentrations increased approximately 30 min after administering the bCRH + AVP challenge (Table 1) for steers in the three treatment groups that received the bCRH + AVP challenges. Steers in the NC treatment group had circulating cortisol concentrations ($P < 0.05$) that were decreased compared to the steers that received the bCRH + AVP challenge (Prior, ST, and Post). For steers in the Prior treatment, cortisol concentrations were elevated ($P = 0.001$) starting at -7 h (30 min after stress challenge), and remained greater ($P = 0.05$) until -5.5 h, when compared to the NC, ST, and Post steers. For steers in the ST treatment group, cortisol concentrations were increased ($P = 0.001$) at 0.5 h (30 minutes post bCRH + AVP challenge), and remained greater ($P = 0.001$) until 2.0 h, when compared to NC, Prior, and Post steers. Finally, steers receiving the Post bCRH + AVP challenge had increased concentrations of circulating cortisol at 8 h (30 min post bCRH + AVP challenge), however, cortisol concentrations were only increased until 8.5 h, when compared to NC, Prior, and ST. Again, these alterations in circulating cortisol concentrations were expected due to the role of both CRH and AVP have in the secretion of ACTH (Carroll et al. 2006).

When area under the curve ($\text{ng}\cdot\text{h}/\text{ml}$) was calculated for three different time intervals during the trial, steers receiving the bCRH + AVP bolus had elevated ($P < 0.05$) cortisol concentration during the specific time intervals, as anticipated. While there were differences between time intervals, total cortisol production (Total Cortisol Production; sum of the three time intervals) was not different ($P > 0.05$) between the treatment groups receiving bCRH + AVP challenges (158.13, 167.20, and 158.55 $\text{ng}\cdot\text{h}/\text{ml}$ for Prior, ST,

and Post, respectively). Cortisol production for steers in the NC treatment group was decreased ($P = 0.007$; 81.49 ng*h/ml) compared to steers in all other treatment groups.

Tumor necrosis factor- α

There was no treatment x time interaction observed for TNF- α ($P = 0.28$) post Mh challenge (Table 3). While there was no main effect interaction, there was a difference observed at time point 0.5 h post Mh challenge with the ST steers having the greatest concentration of TNF- α compared to the steers in the other treatment groups (NC, Prior and Post). There were no other alterations in TNF- α concentrations during the course of the trial for all treatment groups.

Interleukin-6

There was a main effect of treatment x time interaction observed for IL-6 ($P = 0.001$) post Mh challenge (Table 4), however, this interaction was at a single specific time point (22.5 h post Mh challenge. At 22.5 h post Mh challenge there were differences in concentrations of IL-6, steers in the Prior treatment group had the greatest production of IL-6 concentrations, while steers in the NC treatment group had the least production of IL-6 compared to all treatment groups that received the bCRH + AVP challenge.

Interferon- γ

There was a main effect interaction of treatment x time observed for IFN- γ ($P = 0.001$) post Mh challenge (Table 5). As with IL-6, the interaction for interferon- γ was

only observed at one time point, 22.5 h post Mh challenge. In terms of treatment response, INF- γ production was greatest in steers in the NC treatment group compared to steers in the Post treatment group; steers in the Prior and ST treatment groups were intermediate).

Growth hormone

There was a main effect interaction of treatment x time observed for GH ($P = 0.01$). Differences in GH concentrations were observed between the four treatment groups (-6 and -4 prior to Mh challenge and 5, 13 and 14.5 h post Mh challenge; Table 6). There was however, no clear pattern to the variation observed for GH concentrations. Growth hormone concentrations were also divided into the three distinct time intervals similar to cortisol to investigate total GH production during the trial (Table 2). Prior to the Mh challenge, there was no difference in the concentration of GH between the four treatment groups (TI-GH-Prior; $P = 0.33$). During TI-GH-ST, steers in the NC treatment groups had greater production of GH compared to steers in the Post treatment group (Prior and ST were intermediate). After the Mh challenge (TI-GH-Post), steers in the ST treatment group had GH concentrations that were less ($P = 0.04$) than steers in the NC treatment group (Prior and Post were intermediate). Total GH production (TGHP) was decreased ($P < 0.05$) in steers within the Post treatment group (59.01 ng*h/ml) when compared to steers in the NC and Prior treatment group, steers in the ST treatment group were intermediate.

Insulin-like growth factor-I

There was not a main effect interaction of treatment x time for IGF-I ($P = 0.17$). There was no difference ($P > 0.17$) in hourly concentrations and time interval concentrations of IGF-I for any four treatment groups for the duration of the trial (Table 7). There were differences ($P < 0.05$) observed in IGF-I concentrations within treatments. For all four treatment groups (NC, ST, Prior and Post) there was a decrease ($P \leq 0.006$) in IGF-I concentrations at time 22.5 post Mh challenge compared to baseline IGF-I concentrations observed at -25.5 h prior to Mh challenge. When IGF-I was divided into three distinct time intervals similar to cortisol and GH, there were no differences observed between the treatment groups and time intervals.

Discussion

Activation of the HPA axis results in the release of hormones, neuropeptides, and neurotransmitters with immuno-modulating activity that can impact host resistance to immune insults or challenges. One of the most indicative responses associated with an immune insult is characterized by the febrile response. The febrile response is a component of the innate immune response and the innate immune response can be considered one of the first lines of defense against a pathogen. In the course of the innate immune response, a cascade of pro-inflammatory cytokines including IL-1, IL-6, TNF- α , and IFN- γ provide a communication network between immune cells and other cells of the body (Paul, 2008). A fever is triggered by the release of endogenous pyrogens from different types of macrophage-like cells (Kluger et al., 1998). These pyrogens include pro-inflammatory cytokines that act at the anterior hypothalamus to raise the

thermoregulatory set point (Kluger et al., 1998). Numerous bacterial species have been isolated from the lungs of cattle diagnosed with BRD, but *Mannheimia haemolytica* is considered one of the most important pathogens (Callan and Garry, 2002). All of the bacterial pathogens that are considered important for the BRD complex can be isolated from the upper respiratory tract of healthy cattle, and in the absence of predisposing factors these agents are not of major significance (Callan and Garry, 2002). Based on the multifactorial etiology of the disease, when a combination of these factors occur cattle exposed develop interstitial pneumonias. All steers in the present study were given a Mh challenge and all treatment groups; NC, Prior, ST, and Post had an increase in RT. The change in RT for each group was compared to 0 h values and when the first difference at a specific time point was observed that was the onset of temperature. The treatment group (ST) that received the stress challenge during the Mh challenge had a delayed onset of temperature at 8 h. The other steers in treatment groups NC, Prior and Post had an observed increase in RT at 4 h post Mh challenge. All treatment groups had a sustained temperature greater than values observed at 0 h suggesting an adequate febrile response to the Mh challenge. In an effort to help quantify the febrile response associated with the increase in RT, circulating cytokine concentrations were also measured to evaluate systemic response to the Mh challenge. The main effect interaction of treatment x time was observed for IL-6 and IFN- γ , but the difference observed for these parameters was only observed at the conclusion of the sampling period at 22.5 h post Mh challenge. The NC steers had the greatest circulation concentration of IFN- γ , while the Prior steers had the greatest for IL-6. The only difference observed between the treatment groups initially after the Mh challenge was for TNF- α , but the main effect of treatment x time was not

significant. There was only a specific time point at 0.5 h p.i that was different between the treatment groups with the steers in the ST treatment group having the greatest concentration. While the circulating concentrations observed for the cytokines were not as large as concentrations in an endotoxin (Elsasser et al. 1995) and other Mh challenge trials (Burciaga-Robles et al., 2010), there are a few possible explanations. The infectious dose used in our present trial was small and diluted in a larger volume of fluid when administered to the steers. This could have helped disperse the bacterial challenge and not caused a small localized infection in the respiratory tract. Therefore a large immune response was not needed initially, but the increase in cytokines initiated at conclusion of the sampling period could have been attributed to the colonization of Mh and the immune cells in the respiratory tract trying to eliminate the bacteria.

In addition to the release of endogenous pyrogens, endogenous antipyretics or cyrogens are also released (Kluger et al., 1998). Some of the cyrogens that can modulate the febrile rise in body temperature and prevent temperatures from becoming too elevated; include AVP, α -melanocyte stimulating hormone, and glucocorticoids (Kluger et al., 1998). The ST steers that had the delayed response in RT till 8 h post Mh challenge, and had increased cortisol concentrations till 1.5 post Mh challenge h, therefore cortisol concentrations were just returning to baseline at 2 p.i h. The other treatment groups (NC, Prior, and Post) did not have increased cortisol concentrations initially after the Mh challenge. It would appear that the stress challenge simultaneously with the Mh challenge could have caused temporary suppression of RT in the ST steers. The Prior steers were given a CRH+AVP challenge at 7.5 h post Mh challenge, which was while the steers still had increased RT, and RT was decreased at 12 h post Mh

challenge compared to the other treatment groups NC, Prior and ST which still had increase RT values. Therefore, it appears that the CRH+AVP challenge administered once the febrile response had been initiated could cause alteration in RT response. The stress challenge that was administered -7.5 h pre-Mh challenge did not appear to have any impact on the RT response, as indicated by the NC and Prior had very similar RT responses to the Mh challenge.

There can be a severe cost to the cattle when developing a fever. It was been demonstrated that the innate immune response is more nutritionally demanding than the acquired immune response (Klasing and Calvert, 2000). An immune response can impact the nutritional status of an animal by several mechanisms. For example, modulation of hormone profiles by cytokines and glucocorticoids can significantly impact growth and production. Hormonal modulation by the glucocorticoids and pro-inflammatory cytokines provide an effective mechanism for changes in endocrine growth hormones and substrate modification to facilitate nutrient partitioning. Studies utilizing immune challenge models have reported uncoupling of the somatotrophic axis (Thissen et al. 1999), as well as reductions in GH and IGF-I concentration (Elsasser et al., 2008). Daniels et al. (2002) also suggested that the increase in cortisol observed during immune challenges may have a role as well in reduced GH release. While endotoxin challenges have reported a reduction in GH and IGF-I concentrations, endotoxin challenges elicit a rapid and substantial increase in pro-inflammatory cytokines. There is limited data utilizing a challenge model that elicits a sustained febrile response without overwhelming the immune system and the pro-inflammatory cytokine release overshooting the needed response. Circulating cytokine concentrations in the current trial would suggest the Mh

challenge did not overwhelm the immune defense. Steers that were administered the stress challenge 7.5 h post Mh challenge (Post) had decreased TGHP, suggesting increase in cortisol during the febrile response causes the overall alteration in GH response. While the Post steers had the greatest reduction in GH production, there were no differences in overall production of IGF-I between the treatment groups. Over approximately a 48 h window all groups did have a reduction in IGF-I from -25.5 h to 22.5 h.

Conclusion

In order to understand how bidirectional communication among these physiological systems affects the pathogenesis of immune insults and challenges it is imperative to find methods to mimic HPA and immune disruptions similar to that found in production. Generally cattle will experience more than one stressor when they are susceptible to developing the BRD complex, understanding the individual contribution of timing prior-to, during or post-exposure can help in understanding the contribution stress plays. Stress in beef cattle production will always be a pre-disposing factor because it is part of the everyday routine processes involved in production, and cattle will always be exposed to some type of stressor. Therefore the contribution it has on immune and growth parameters in growing beef cattle should be explored further to understand these interactions.

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Table 6.1 Least squares means for percent change in rectal temperature values of 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-44.0	0.06	0.25	-0.29	-0.49	0.15	0.30
-40.0	0.39	0.42	0.11	-0.30	0.15	0.29
-36.0	-0.08	0.30	-0.34	-0.40	0.15	0.36
-32.0	0.28	0.16	-0.06	-0.44	0.15	0.33
-28.0	-0.15	-0.28	0.04	-0.44	0.15	0.69
-24.0	-0.43	0.07	0.06	-0.32	0.15	0.52
-20.0	0.60	0.71	0.02	0.12	0.15	0.28
-16.0	0.59	0.79	0.07	0.12	0.15	0.26
-12.0	-0.24	0.21	0	-0.59	0.15	0.28
-8.0	0.13	0.31	0.21	0.12	0.15	0.97
-4.0	-0.28	-0.37	-0.27	-0.20	0.15	0.98
0	0	0	0	0	0.15	0.99
4.0	1.56 ^f	0.97 ^{fg}	0.43 ^g	1.08 ^{fg}	0.15	0.05
8.0	1.11 ^f	2.19 ^g	1.25 ^f	1.38 ^{fg}	0.15	0.06
12.0	1.35 ^{fg}	2.02 ^g	1.31 ^{fg}	0.60 ^f	0.15	0.01
16.0	0.51	1.24	1.27	0.77	0.15	0.20
20.0	0.11 ^f	1.29 ^g	0.14 ^f	0.27 ^f	0.15	0.02
24.0	0.47 ^f	1.37 ^g	0.12 ^f	-0.06 ^f	0.15	0.01

^a Time interval is hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^{f,g,h} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 6.2 Least squares means at sample time points and sample time intervals for cortisol concentrations of 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-8.0	2.27	9.86	5.15	8.16	4.58	0.56
-7.0	3.83 ^j	82.02 ^k	9.55 ^j	9.33 ^j	4.58	0.001
-6.5	6.84 ^j	59.91 ^k	6.46 ^j	4.33 ^j	4.58	0.001
-6.0	4.42 ^j	19.85 ^k	1.55 ^j	6.12 ^j	4.58	0.005
-5.5	9.17	11.00	2.22	7.89	4.58	0.42
-0.5	10.55	6.58	3.59	10.53	4.58	0.54
0.5	5.98 ^j	7.64 ^j	77.06 ^k	13.15 ^j	4.58	0.001
1.0	5.25 ^j	8.46 ^j	78.94 ^k	8.27 ^j	4.58	0.001
1.5	19.61 ^j	14.20 ^j	47.39 ^k	21.64 ^j	4.58	0.001
2.0	18.83	11.67	21.73	12.32	4.58	0.20
2.5	9.17	6.08	11.04	15.59	4.58	0.38
7.0	11.09	7.72	4.77	15.27	4.58	0.26
8.0	7.50 ^j	8.19 ^j	4.45 ^j	79.84 ^k	4.58	0.001
8.5	23.87 ^j	9.28 ^j	4.98 ^j	50.81 ^k	4.58	0.001
9.0	18.30	6.65	14.61	17.77	4.58	0.14
9.5	13.55	13.61	4.09	7.68	4.58	0.25
12.5	15.36	6.77	5.37	8.76	4.58	0.44
TI-Cort-Prior ^f	11.90 ^j	109.07 ^k	14.29 ^j	17.62 ^j	8.61	0.001
TI-Cort-ST ^g	33.91 ^j	27.70 ^j	136.37 ^k	40.15 ^j	7.20	0.001
TI-Cort-Post ^h	35.64 ^j	21.36 ^j	16.54 ^j	103.72 ^k	6.33	0.001
TCP ⁱ	81.49 ^j	158.13 ^k	167.20 ^k	158.55 ^k	17.41	0.007

^a Time interval is hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^f Time Interval Prior (TI-Cort-Prior) = -8 to -5.5 h pre-*M. haemolytica* challenge

^g Time Interval ST (TI-Cort-ST) = -0.5 to 2.5 h relative to *M. haemolytica* challenge

^h Time Interval Post (TI-Cort-Post) = 7 to 9.5 h post-*M. haemolytica* challenge

ⁱ Total Production (TCP) = TI-Cort-Prior + TI-Cort-ST + TI-Cort-Post

^{j,k} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 6.3 Least squares means for TNF- α at sample time points for 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-13.5	0	0	12.46	2.85	2.38	0.19
-6.0	0	0.28	7.07	4.12	2.29	0.65
-0.5	0 ^f	8.07 ^f	27.33 ^g	7.43 ^f	2.38	0.001
0.5	0	0.88	10.40	8.90	2.31	0.26
1.0	0	1.07	8.12	6.37	2.29	0.53
2.0	0	0.27	14.75	4.40	2.29	0.08
4.0	0	0	9.65	3.42	2.29	0.39
8.0	0	0	10.17	5.35	2.29	0.33
12.0	0	0	11.22	6.62	2.31	0.23
22.5	0	0	8.67	6.85	2.29	0.40

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^{f,g} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 6.4 Least squares means for IL-6 at sample time points for 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Prior ^e	SEM	P-value
-13.5	0	0	0	0	2.16	0.99
-6.0	0.86	0.65	0	0	2.16	0.99
-0.5	0	0	0	0	2.16	0.99
0.5	0	0	0	0	2.16	0.99
1.0	1.12	0	0	0.88	2.16	0.99
2.0	0	6.10	0	0	2.16	0.67
4.0	0	0	0	0	2.16	0.99
8.0	0	1.63	0	0	2.16	0.99
12.0	0	2.77	5.10	1.95	2.16	0.87
22.5	0.02 ^f	53.42 ^g	23.52 ^h	10.33 ⁱ	2.23	0.001

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^{f,g,h,i} Means within row lacking commons superscripts are different at $P \leq 0.05$

Table 6.5 Least squares means for IFN- γ at sample time points for 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-13.5	10.64	14.23	20.63	12.2	1.70	0.17
-6.0	12.96	21.67	14.97	19.47	1.69	0.25
-0.5	16.84	14.45	19.45	17.75	1.69	0.75
0.5	16.64	13.80	18.52	17.07	1.69	0.78
1.0	15.14	16.45	20.15	18.18	1.69	0.75
2.0	17.52	16.30	21.33	16.89	1.69	0.71
4.0	12.66	9.28	12.42	12.83	1.69	0.86
8.0	16.94	8.33	12.68	11.20	1.69	0.36
12.0	18.80	8.20	15.65	7.88	1.69	0.06
22.5	25.50 ^f	10.37 ^{gh}	18.13 ^{fg}	8.62 ^h	1.69	0.002

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^{f,g,h} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 6.6 Least squares means at sample time points and sample time intervals for growth hormone concentrations for 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-13.5	7.57	5.83	4.81	6.04	1.30	0.52
-8.0	8.34	5.21	6.03	5.04	1.30	0.27
-7.0	5.97	3.82	7.89	7.91	1.30	0.06
-6.0	5.46 ^k	7.26 ^{kl}	3.32 ^{jk}	2.71 ^j	1.30	0.04
-5.0	6.47	5.95	6.85	4.02	1.30	0.39
-4.0	7.55 ^{kl}	10.25 ^l	6.68 ^{jk}	3.48 ^j	1.30	0.002
-0.5	6.94	5.32	3.87	5.08	1.30	0.42
0.5	6.92	6.12	2.64	5.31	1.30	0.09
1.5	8.34	9.66	7.26	5.73	1.30	0.15
2.5	9.39	6.62	7.64	6.29	1.30	0.33
3.5	7.25	8.51	5.51	5.70	1.30	0.28
5.0	9.28 ^k	4.84 ^j	4.60 ^j	2.64 ^j	1.30	0.004
5.5	6.95	6.61	5.16	6.74	1.30	0.73
7.0	8.11	9.34	5.58	7.22	1.30	0.18
8.5	6.44	6.52	3.32	4.56	1.30	0.21
9.5	5.93	7.63	6.48	5.30	1.30	0.59
10.5	7.67	6.31	4.78	9.27	1.30	0.07
13.0	9.82 ^k	5.86 ^j	5.03 ^j	5.85 ^j	1.30	0.05
14.5	9.28 ^l	8.59 ^{kl}	5.87 ^{jk}	4.67 ^j	1.30	0.03
22.5	5.66	6.76	5.97	5.62	1.30	0.91
TI-GH-Prior ^f	25.84	24.75	24.40	18.30	3.11	0.33
TI-GH-ST ^g	31.75 ^j	29.30 ^{jk}	22.23 ^k	22.72 ^k	2.79	0.05
TI-GH-Post ^h	23.89 ^j	25.95 ^j	17.20 ^k	21.04 ^{jk}	2.28	0.04
TGHP ⁱ	81.48 ^j	80.01 ^j	63.84 ^{jk}	59.01 ^k	6.76	0.05

^a Time interval is hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^f Time Interval Prior (TI-GH-Prior) = -13.5 to -4.0 h pre-*M. haemolytica* challenge

^g Time Interval ST (TI-GH-ST) = -0.5 to 3.5 h relative to *M. haemolytica* challenge

^h Time Interval Post (TI-GH-Post) = 7 to 10.5 h post-*M. haemolytica* challenge

ⁱ Total Production (TGHP) = TI-GH-Prior + TI-GH-ST + TI-GH-Post

^{j, k, l} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 6.7 Least squares means at sample time points and sample time intervals for insulin-like growth factor-I concentrations for 24 Holstein steers receiving a single corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenge to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior ^c	ST ^d	Post ^e	SEM	P-value
-25.5	180.14 ^j	174.33 ^j	201.44 ^j	206.44 ^j	13.35	0.17
-0.5	158.38 ^{jk}	151.61 ^{jk}	174.15 ^{jk}	165.80 ^k	14.03	0.63
7.0	149.52 ^k	152.86 ^{jk}	166.66 ^k	153.80 ^k	13.35	0.75
22.5	150.90 ^k	127.64 ^k	148.96 ^k	138.13 ^k	13.35	0.49
P-value	0.006	0.001	0.001	0.001	11.86	
TI-IGF-Prior ^f	4311.16	4136.80	4660.33	4652.98	371.41	0.64
TI-IGF-ST ^g	1005.53	1036.99	1103.87	1038.69	98.32	0.88
TI-IGF-Post ^h	2328.21	2173.87	2446.00	2262.42	159.48	0.68
Total Production ⁱ	7650.82	7443.37	8234.69	7954.09	648.19	0.80

^a Time interval is hours prior to and post *M. haemolytica* intra-tracheal challenge

^b No stimulating release of cortisol and *M. haemolytica* challenge

^c Stimulating release of cortisol at -7.5 h pre-*M. haemolytica*

^d Stimulating release of cortisol at 0 h during *M. haemolytica*

^e Stimulating release of cortisol at 7.5 post-*M. haemolytica*

^f Time Interval Prior (TI-IGF-Prior) = -25.5 to -0.5 h pre-*M. haemolytica* challenge

^g Time Interval ST (TI-IGF-ST) = -0.5 to 7.0 h relative to *M. haemolytica* challenge

^h Time Interval Post (TI-IGF-Post) = 7 to 22.5 h post-*M. haemolytica* challenge

ⁱ Total Production (TIGFP) = TI-IGF-Prior + TI-IGF-ST + TI-IGF-Post

^{j,k} Means within column lacking common superscripts are different at $P \leq 0.05$

CHAPTER VII
EVALUATION OF THE TIMING OF CORTISOL RELEASE (MULTIPLE STIMULI)
ON THE SOMATOTROPHIC AXIS DURING AN *M. HAEMOLYTICA*
CHALLENGE IN BEEF STEERS

A manuscript to be submitted to *The Journal of Animal Science*

Abstract

The objective of this trial was to characterize the potential impact of stimulating release of cortisol (SRC) multiple times prior to, simultaneously, and post *M. haemolytica* (Mh) challenge on the somatotrophic axis. Thirty beef steers (207.30 ± 22.10 kg BW) vaccinated against Mh were randomly assigned to 1 of 5 treatments: 1) Mh only, no SCR (NC); 2) SRC at -7.5 h and 0 h + Mh (Prior-ST); 3) SRC at -7.5 h and 7.5 h + Mh (Prior-Post); 4) SRC at 0 h and 7.5 h + Mh (ST-Post); and 5) SRC at -7.5 h, 0 h and 7.5 h + Mh (Prior-ST-Post). To stimulate the release of cortisol, corticotropin releasing hormone (bCRH at $0.1 \mu\text{g}/\text{kg}$ of BW) + arginine vasopressin (AVP at $0.5 \mu\text{g}/\text{kg}$ of BW) were administered in tandem via indwelling jugular catheter. *Mannheimia haemolytica* (D-152) challenge was administered at time point 0 h via intra-tracheal injection (5.4×10^8 CFU/mL in 50 mL of the saline-diluted inoculum). Steers were fitted with indwelling jugular catheters and rectal temperature (RT) probes on -3 d, moved to

tie stalls and allowed 24 h to acclimate (-2 d) to the new environment. Baseline samples (RT and blood) were obtained on -1 d, and the challenge was given on 0 d. There were treatment x time interactions or treatment effects ($P < 0.05$) observed for cortisol, interferon- γ (IFN- γ), GH, IGF-I and RT. Treatment groups NC, Prior-ST, Prior-Post and ST-Post had greater percent change in RT compared to RT values observed at 0 h. There was no change in RT for steers in the Prior-ST-Post when compared to RT at 0 h. The Prior-ST-Post steers had the greatest ($P = 0.001$) total production of cortisol, and the NC steers had the least ($P = 0.001$) total production of cortisol. There were no differences ($P > 0.05$) in cortisol production between the Prior-ST, Prior-Post and ST-Post steers. The NC steers did not have increased circulating concentrations of cortisol, but did have a greater IFN- γ concentrations at 22.5 h compared to the other treatment groups. While there was no clear pattern to the differences observed in GH concentration, there was a tendency ($P = 0.09$) for the NC steers to have greater circulating GH concentrations when compared to the Prior-ST-Post steers. Differences in IGF-I concentrations were observed at -25.5 h and 22.5 h p.i. between the treatment groups. The results suggest that multiple stressors when given during an immune challenge can impact GH and IGF-I secretion compared to steers that do not have an increase in cortisol.

Introduction

The Bovine Respiratory disease (BRD) complex is one, if not the most devastating disease to impact the cattle industry, costing the beef industry million's if not billions of dollars in lost revenue annually. Bovine respiratory disease is a devastating disease due to its multi-factorial etiology which includes stress and exposure to novel

viral and bacterial pathogens. This disease is the perfect storm for the cattle industry as it takes advantage of the complexity and centralization of the industry. There are a variety of stressors cattle can experience when centralization occurs. These stressors can range from handling, commingling with non-herdmates, psychological stress of past experiences (processing procedures and handling), nutritional changes, weaning, routine processing procedures, and relocation to new facilities, just to name a few. The reality when considering the stressors cattle experience is that it is generally not just one stressful situation, rather multiple stressful situations. One of the most common events that cattle will experience is the relocation process. As cattle arrive at a new facility new events arise such as, new environment/surroundings, handling, and processing before being placed into new a environment (pen/ paddock depending upon place in the various phases of the cattle industry). Upon arrival or prior to arrival at a new facility, cattle can be exposed to bacterial and viral pathogens that can compromise the immune defenses. Being exposed to pathogens, along with being subjected to multiple stressors, allows pathogens the opportunity to overwhelm the immune defenses and cause infection. This complex multifaceted etiology of stress, pathogen exposure, and health status of the cattle are generally characterized by the Bovine Respiratory Disease (BRD) complex. The predisposing factors, such as stress that cause the development of the BRD complex act synergistically, and are most commonly identified in combination rather than as a single causative problem.

Due to the devastating effects of BRD, there has been a great deal of research devoted to understanding the viral and bacterial pathogens associated with BRD, however, in comparison, there has been very limited research related to the one true

common variable of the BRD complex, stress. In terms of livestock, the term stress is difficult to analyze for a multitude of reasons (in ability to communicate, variation in temperament, and previous exposure, to name a few). Thus, in livestock, the term stress is actually a measurement of cortisol concentrations (as of today it is the best endocrine marker for what we estimate to be a stressor to livestock). Currently, there is a great deal of interest in understanding the impact the release of cortisol (stress), a glucocorticoid, has on production characteristics of cattle. The release of cortisol is the primary hormonal response of animals that experience a stressful stimulus that causes activation of the HPA axis (Salak-Johnson and McGlone, 2007). The release of glucocorticoids is not only the primary endocrine signal for the assumption of a stressful event, cortisol is also an important signal for the innate immune response as observed with increases in cortisol during an endotoxin challenge (Carroll et al., 2009). Glucocorticoid receptors are expressed on a variety of immune cells and will bind cortisol and interfere with the function of nuclear factor- κ B (NF- κ B; which regulates the activity of cytokine-producing immune cells; Paul, 2008). Researchers have also reported that cortisol can have both pro- and anti-inflammatory properties depending on the timing of the stressor and the immune insult experienced by the animal. Frank et al. (2010) evaluated the issue of time course of glucocorticoid exposure in relation to an immune challenge in rats. Cortisol concentrations were increased either 24 or 2 h pre- or 1-h post lipopolysaccharide (LPS) immune challenge. Increased cortisol concentrations pre-LPS potentiated the inflammatory response (tumor necrosis factor- α , interleukin (IL)-1 and IL-6) in the hippocampus and liver. In contrast, increased cortisol concentrations post-LPS were anti-inflammatory and inhibited the inflammatory responses as expected. This research would

indicate that timing of the stressor could play a major role in the animal's immune response to cortisol production.

Therefore, the objective of this study was to evaluate the relationship between stimulation of cortisol at a combination of multiple time points during a *M. haemolytica* challenge on endocrine growth parameters, total circulating cortisol concentrations and the febrile response of the cattle. As well as, understanding the impact the febrile response has on the release of cortisol and endocrine growth parameters. Understanding the contribution both the immune and endocrine systems have will provide a better understanding of the intricate cross-talk between the systems.

Material and methods

The use of animals and all procedures conducted were approved by the IACUC committee at Mississippi State University (IACCUC #10-029). Thirty Holstein steers (207.30 ± 22.10 kg BW) were utilized to characterize the potential impact of stimulating release of cortisol (SRC) multiple times prior to, simultaneously, and post *M. haemolytica* (Mh) challenge on the somatotrophic axis. Steers were randomly stratified by BW into the 5 treatment groups -4 d prior to the initiation of the trial. The immune challenge for the trial was *Mannheimia haemolytica* (D-152; Mh) and to stimulate the release of cortisol, corticotropin releasing hormone (bCRH at $0.1 \mu\text{g/kg}$ of BW) + arginine vasopressin (AVP at $0.5 \mu\text{g/kg}$ of BW) in tandem was administered. The timeline for this study was anchored at 0 h. Zero h is the time point when all treatment groups received the Mh challenge. The timing of bCRH+AVP was administered at -7.5 h pre-Mh, simultaneously with Mh challenge (at 0 h), and 7.5 h post-Mh challenge

(depending upon the treatment group). Treatments groups consisted of 1) Mh only, no SRC (NC); 2) SRC at -7.5 h and 0 h + Mh (Prior-ST); 3) SRC at -7.5 h and 7.5 h + Mh (Prior-Post); 4) SRC at 0 h and 7.5 h + Mh (ST-Post); and 5) SRC at -7.5 h, 0 h and 7.5 h + Mh (Prior-ST-Post).

Seventy-two h prior (0600 on - 3 d) to the Mh challenge, steers were fitted with indwelling jugular vein catheters for serial blood collection and rectal temperature monitoring device described by Reuter et al. (2010) that recorded temperature at one minute intervals for the duration of the trial. Catheters consisted of approximately 150 mm of PTFE tubing (6417-41 18TW; Cole-Palmer; o.d. = 1.66 mm) that was inserted into the jugular vein using a 14-gauge x 5.1 cm thin-walled stainless steel biomedical needle (o.d. = 2.11 mm). Catheters were maintained in place using tag cement and a 5.1 cm wide porous surgical tape. Catheters were fitted with extensions made of sterile plastic tubing (Tygon S-50 HL; VWR Scientific; i.d. = 1.59 mm; o.d. = 3.18 mm) for collection of blood samples without disturbing the steers. After being fitted catheters, steers were moved to respective individual tie stall. During the collection of blood samples, approximately 5 ml of waste fluid was pulled from the catheter to ensure a clean blood sample was flowing. After which the sample tube was connected to the catheter and a 14 mL sample was be obtained from each steer. After the sample was obtained the catheter was flushed with 5 mL of saline followed by 3 mL of heparinized saline to serve as a lock on the catheter.

Steers were allowed to acclimate to the tie stalls and environmental changes for a period of 24 h (duration of -2 d). At 0600 and 1800 h each day during the trial period, 4.5 kg of 60/40 (soyhull/corn gluten) mixture was offered to each steer (base diet steers

were on for 60 d prior to trial). Steers also had ad libitum access to hay and water. Steers were offered the same feed mixture during the duration of the experiment (-3 to 1 d).

At -25.5 h prior to Mh-challenge (-1 d) baseline blood samples were obtained beginning 0600 h and continuing every 6 h at -19.5, -13.5, and -8 h prior to the Mh challenge. The first tandem bCRH+AVP bolus was administered at -7.5 h prior to Mh-challenge for steers in the Prior-ST, Prior-Post and Prior-ST-Post groups (via jugular catheters). Blood samples were collected every 30 min starting at -7 h and concluding -0.5 h. At the conclusion of the pre-Mh sample collection period (0 h) the Mh challenge was administered to all 5 treatment groups and the bCRH + AVP bolus for the Prior-ST, ST-Post and Prior-ST-Post steers. Samples were collected every 30 min starting at 0.5 h and concluding 7.0 h post-Mh challenge. Seven and half h post-challenge, the last bCRH + AVP bolus was administered to steers in the Prior-Post, ST-Post and Prior-ST-Post treatment groups. Samples were collected every 30 mins starting at 8 h post-Mh challenge and concluding 14.5 h post-Mh challenge. Additional samples were also collected at 16.5 h and 22.5 h post-Mh challenge. Immediately after collection of the blood sample at 22.5 h, catheters and RPs were removed and all steers were moved via an enclosed stock trailer to a quarantine pasture to be monitored for signs of illness for a period of 45 d.

The *Mannheimia haemolytica* strain NADC D152, a serotype 1 isolate from bovine pneumonic lung originally recovered in Iowa was grown in Brain-Heart Infusion (BHI) broth overnight with shaking. The culture was diluted in physiological saline (0.9%) to yield approximately 10^7 CFU/mL based on OD (600) without washing the cells. The challenge consisted of intra-tracheal deposition via tracheal puncture with an

18 gauge needle of 50 mL of the saline-diluted inoculum. Colony counts before administration were 1.06×10^6 CFU/ml; after challenge, aliquots were taken of each challenge dose, returned to the lab and determined to be at 2.67×10^4 CFU/ml. Based on the counts from the samples collected before and after the challenge it could be determined that a total dose of 5.4×10^8 CFU were administered to each steer.

Serum cortisol concentration was determined by radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA) per the manufacture's protocol in a single assay with a detection limit of 2-ng/mL and less than 5% intra-assay coefficient of variation. Serum concentrations of the pro-inflammatory cytokines, TNF- α , IL-6, and IFN- γ were assayed per the manufacture's protocol using a custom developed multiplex ELISA validated for bovine cytokines (SearchLight; Pierce Biotechnology Inc., Rockford, IL). For all cytokines, the intra-assay variation was less than 5 %, and the inter-assay variation was less than 7 %.

Serum samples were analyzed for GH and IGF-I as previously described (Elsasser et al., 1989). For the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined at 150 pg bGH/assay tube with 50% binding of tracer achieved at 1,800 pg/tube. Increasing volumes of plasma displaced tracer counts in a fashion parallel to that of the standard curve. Recovery of non-labeled bGH averaged 97% for 300, 600 and 1,200 pg added to 200 microliters serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$.

Concentrations of IGF-I were determined following acidification for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual

sample was diluted and neutralized with a 1:80 dilution with assay buffer. Anti human/bovine IGF-I primary serum was procured from GroPep (Adelaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of nonlabeled IGF-1 added to plasma before acidification averaged 95% with intra- and inter-assay coefficients of variation \leq 10%.

When percent change was calculated for rectal temperature, calculations were determined by the following formula. Percent change = $[(\text{Time point of interest} - 0 \text{ h value}) / 0 \text{ h value}] * 100$. This calculation was used to express the percent change from 0 h as a result of the Mh challenge or determine the variability between the days and time of day.

Area under the curve was calculated for variables of interest by using specific time point samples $[(\text{Time 1} + \text{Time 2}) * (\text{time between Time 1 and Time 2})] / 2$ to determine the ng or pg*h/ml concentration of each variable over a specific time interval. Area under the curve was calculated for three different time intervals during the trial for cortisol, GH and IGF-I.

Time intervals (Area under the curve) for cortisol are as follows: 1) Time Interval Prior (TI-Cort-Prior) = - 8 h to -5.5 h prior to the challenge, 2) Time Interval ST (TI-Cort-ST) = -0.5 to 2.5 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-Cort-Post) = 7 to 9.5 h post *M. haemolytica* challenge. Total cortisol production (TCP) during the 3 time intervals (TI-Cort-Prior, TI-Cort-ST and TI-Cort-Post) was calculated by adding the three time intervals together.

Time intervals (Area under the curve) for GH are as follows;; 1) Time Interval Prior (TI-GH-Prior) = - 13.5 h to -4.0 h prior to the challenge, 2) Time Interval ST (TI-GH-ST) = -0.5 to 3.5 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-GH-Post) = 7 to 10.5 h post *M. haemolytica* challenge. Total growth hormone production (TGHP) during the 3 time intervals (TI-GH-Prior, TI-GH-ST and TI-GH-Post) were calculated by adding the three time intervals together.

Time intervals (Area under the curve) for IGF-I are as follows;; 1) Time Interval Prior (TI-IGF-Prior) = - 25.5 h to -0.5 h prior to the challenge, 2) Time Interval ST (TI-IGF-ST) = -0.5 to 7.0 h relative to *M. haemolytica* challenge, and 3) Time Interval Post (TI-IGF-Post) = 7 to 22.5 h post *M. haemolytica* challenge. Total insulin-like growth factor-I production (TIGFP) during the 3 time intervals (TI-IGF-Prior, TI-IGF-ST and TI-IGF-Post) was calculated by adding the three time intervals together.

Statistical analysis

Summary statistics were calculated for each variable, and these summary statistics were averaged across each treatment. Response to the challenge over time was analyzed by ANOVA for repeated measures with the MIXED procedure of SAS as a completely random design; and the model included sampling time, treatment, and sample time x treatment. Sample treatment x time was used as the error term to test whole plot effects. Rectal temperature was initially recorded at 1-minute intervals, but subsequently averaged over 30-min intervals to facilitate comparisons to other immune and physiological parameters. When results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pairwise differences among

least squares means at various sample times were evaluated with the PDIFF option of SAS. Results from the area under the curve calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

Results

Rectal temperature

There was a treatment x time ($P = 0.002$) interaction observed when the steers were administered the Mh challenge (Table 1). Four treatment groups (NC, Prior-ST, Prior-Post and ST-Post) had an increased RT ($P < 0.05$) suggesting a febrile response associated with the Mh challenge. Steers in the Prior-ST-Post treatment group did not differ in percent change ($P > 0.05$) from 0 h over time for RT. No differences for percent change ($P > 0.05$) in initial RT (-44 to 0 h) were observed prior to the Mh challenge between the NS, Prior-ST, Prior-Post, ST-Post and Prior-ST-Post steers. An increase ($P < 0.05$) in RT was evident starting at 4 h post-Mh challenge for NC and Prior-Post steers and a difference ($P < 0.05$) was observed at 8 h post-Mh challenge for Prior-ST and ST-Post, no difference in percent change was observed for the Prior-ST-Post when compared to 0 h temperatures. Steers in the NC, Prior-ST, Prior-Post and ST-Post all had a sustained increased temperature at 8 h post-Mh challenge. Twelve h post-Mh challenge RT returned to similar temperatures observed at 0 h for treatment groups Prior-Post and ST-Post. There was no difference ($P > 0.05$) from 0 h at 16 h for the NC, 20 h for the Prior-ST, and 12 h for the Prior-Post and ST-Post group.

Cortisol

There was a treatment x time interaction observed for cortisol ($P < 0.001$) post bCRH + AVP bolus administration. Circulating concentrations of cortisol were observed approximately 30 min after administering the bCRH + AVP bolus (Table 1). Steers in the Prior-ST, Prior-Post and Prior-ST-Post treatment group had an augmentation of ($P < 0.05$) cortisol concentrations 30 min post bCRH + AVP bolus (-7 h prior to Mh challenge). Cortisol concentrations remained greater ($P > 0.05$) for a period of 2.0 h (-5.5 h prior to Mh challenge) when compared to NC and ST-Post treatment groups. For steers in the Prior-ST, ST-Post and Prior-ST-Post treatment groups, cortisol concentrations were also augmented ($P = 0.001$) 30 min post bCRH + AVP bolus and remained greater ($P = 0.001$) for a period of 2.0 hr (2.0 h post-Mh challenge) when compared to the NC and Prior-Post steers. The Prior-Post, ST-Post and Prior-ST-Post treatment groups had greater ($P < 0.05$) cortisol concentrations at 8 and 8.5 h post-Mh challenge compared to the NC and Prior-ST steers. The change in cortisol observed after administering the bCRH + AVP bolus prior to and in conjunction with the Mh challenge had a sustained response for approximately 2 h after administering the bCRH + AVP bolus. When bCRH + AVP was administered 7.5 h post-Mh challenge, the cortisol response was only sustained for 1.5 h. These alterations in circulating cortisol concentrations were anticipated due to the role both bCRH and AVP have in the secretion of ACTH, and previous research reported by Carroll et al. (2006).

When area under the curve ($\text{ng}\cdot\text{h}/\text{ml}$) was calculated for three different time intervals during the trial, steers receiving the bCRH + AVP bolus had greater ($P = 0.001$) cortisol concentration during the specific time intervals, as anticipated. Total cortisol

production (TCP; sum of the 3 time intervals) was different ($P < 0.05$) between the treatment groups. The Prior-ST-Post steers had the greatest ($P = 0.001$) TCP production while NC steers had the least TCP when compared to the remaining treatment groups (Prior-ST, Prior-Post and ST-Post). There were no differences ($P > 0.05$) in TCP between the Prior-ST, Prior-Post and ST-Post steers.

Tumor necrosis factor- α

There was no treatment x time interaction observed for TNF- α ($P = 0.93$) post-Mh challenge (Table 3). While there was no main effect difference, there was a difference observed at time points 0.5 and 1.0 h post-Mh challenge for the ST-Post steers. Steers in the ST-Post had increased concentrations of TNF- α from 0.5 to 1.0 h post-Mh challenge when compared to 0 h; there was no increase in TNF- α concentrations at the specific time points for treatment groups NS, Prior-ST, Prior-Post and Prior-ST-Post during the duration of the trial period.

Interleukin-6

There was no main effect of treatment x time interaction observed for IL-6 ($P = 0.13$) post-Mh challenge (Table 4). While there was no main effect difference, there was a difference observed at time point 22.5 h post-Mh challenge for the Prior-Post and ST-Post treatment groups. Steers in these two groups had increased concentrations of IL-6 when compared to the other treatment groups (NC, Prior-Post, and Prior-ST-Post) at this specific time point (22.5 h post-Mh challenge). The steers receiving the bCRH+AVP

during or after the Mh challenge had an increase in IL-6 at conclusion of the sampling period.

Interferon- γ

There was a main effect interaction of treatment x time observed for IFN- γ ($P = 0.001$) at 22.5 h post-Mh challenge (Table 5). Steers in NC treatment group had greater ($P = 0.001$) concentrations of IFN- γ compared to all other treatment groups at 22.5 h post-Mh challenge. Timing of the SRC did not elicit differences ($P > 0.05$) were between the treatment at other specific time points throughout the duration of the trial period.

Growth hormone

There was a main effect interaction of treatment x time observed for GH ($P = 0.05$; Table 2). Concentrations of GH were similar between all treatment groups at the start of the sampling period (13.5 h prior to Mh challenge). Starting at approximately 5 h prior to Mh challenge alterations in GH concentrations were observed for the treatment groups. There was however, no clear pattern to the variation observed for GH concentrations at these specific sample points. Growth hormone concentrations were also divided in three time intervals similar to cortisol to investigate total GH production during the trial (Table 2). Growth hormone concentrations during TI-GH-ST and TI-GH-Post were not different ($P > 0.05$) between the treatment groups. Growth hormone concentrations for Prior-ST-Post and Prior-ST during TI-GH-Prior were decreased ($P < 0.05$) compared to steers in the NC, Prior-Post and ST-Post treatment groups. For TGHP,

there was a tendency for steers in the NC treatment group to have greater ($P=0.008$) circulating GH concentrations when compared to the Prior-ST-Post steers.

Insulin-like growth factor-I

There was a main effect interaction of treatment x time for IGF-I ($P = 0.02$). There was variability ($P= 0.05$) in the initial concentrations of IGF-I between the treatment groups -25.5 h prior to the Mh challenge (Table 3); steers in the ST-Post treatment group had greater ($P = 0.005$) IGF-I concentration when compared to steers in the Prior-ST and NC treatment groups (Prior-Post and Prior-ST-Post being intermediate). Concentrations of IGF-I tended ($P = 0.08$) to follow the same pattern -0.5 h prior to the Mh challenge (ST-Post greater than Prior-ST and NC); Seven h post-Mh challenge there was no difference ($P= 0.29$) in IGF-I concentrations between all treatment groups. At the conclusion of the trial (22.5 h post-Mh challenge) ST-Prior steers had greater ($P= 0.03$) IGF-I concentrations compared to steers in the Prior-ST and NC treatment groups. When comparing IGF-I concentrations within treatment groups, 25.5 h prior to Mh challenge, steers in treatment groups NS, Prior-ST, Prior-Post and ST-Post had a greater ($P<0.05$) IGF-I concentration compared to IGF-I concentrations 22.5 h post-Mh challenge (Figure 2).

When area under the curve ($\text{ng}\cdot\text{h}/\text{ml}$) was calculated for three different time intervals during the trial, there was a tendency ($P= 0.06$) for steers in the ST-Post treatment group to have greater IGF-I concentrations compared to steers in the Prior-ST and NC treatment groups during TI-Prior; there were no differences ($P = 0.19$) during TI-ST. Steers within the ST-Post treatment group had greater ($P= 0.04$) IGF-I

concentrations during TI-Post when compared to Prior-ST and NC treatment groups (Prior-Post and Prior-St-Post were intermediate). Total IGF-I production was tended to be greater ($P= 0.09$) for steers within the ST-Post treatment group when compared to steers within Prior-ST and NC treatment groups (Prior-Post and Prior-St-Post were intermediate).

Discussion

Rectal temperature is considered to be a reliable diagnostic tool to measure a febrile response associated with an infection or activation of immune parameters (Duff and Galyean, 2007; Reuter et al., 2010). When immune cells become activated in response to a pathogen, a cascade of pro-inflammatory cytokines (IL-6, TNF- α , and IFN- γ) are released from these cells (Paul, 2008). These pro-inflammatory cytokines are considered pyrogens and function within the anterior hypothalamus to elevate the thermoregulatory set point (Kluger et al., 1998). Therefore, a fever is initiated by the release of endogenous pyrogens from the immune cells resulting in an increase in body temperature, which can be monitored through rectal temperature of cattle.

Numerous pathogenic bacteria have been isolated from the lungs of cattle diagnosed with BRD, but *Mannheimia haemolytica* is considered one of the most common pathogens (Callan and Garry, 2002). All of the bacterial pathogens that are considered important for the BRD complex can be isolated from the upper respiratory tract of healthy cattle, and in the absence of predisposing factors these agents are of minor significance (Callan and Garry, 2002). Based on the multi-factorial etiology of

BRD, a combination of a bacterial pathogen with viral/or diminished health status due to stressor within cattle may result in interstitial pneumonias.

In contrast, endogenous antipyretics or cyrogens are also released to combat the over stimulation and increase in rectal temperature (Kluger et al., 1998). The cyrogens which can cause suppression of the febrile response are AVP, α -melanocyte stimulating hormone, and glucocorticoids (Kluger et al., 1998). Gander et al. (1980) reported stressed animals might not develop a fever due to the suppressive effects of glucocorticoids. Glucocorticoids are typically greater during times of stress, and stressful events could modulate or suppress the actions and release of endogenous pyrogens that typically signal inflammation and increase temperature. In the present study, timing of the administration of the bCRH + AVP challenge in comparison to the Mh challenge or administration of multiple bCRH + AVP challenges did not alter the observed cortisol responses at specific time intervals. When each bCRH + AVP challenge was administered, the duration and concentration of the cortisol release was similar, regardless of the time prior or post Mh challenge. The total production of cortisol was greatest for the Prior-ST-Post steers when compared to the other treatment groups receiving the bCRH + AVP challenge (Prior-ST, Prior-Post, and ST-Post steers). Steers (regardless of treatment group) receiving the bCRH + AVP challenge had greater total cortisol production when compared to steers not receiving the bCRH + AVO challenge.

The Mh challenge elicited a febrile response as indicated by the RT response observed in the NC group. Steers in the NC treatment group had a sustained RT greater than temperatures observed at 0, 4, 8 and 12 h post-Mh challenge. In contrast, steers in the Prior-ST, Prior-Post, ST-Post and Prior-ST-Post had a delayed, reduced and/or no

response in RT to the Mh challenge. The Prior-ST steers had a similar RT response to the Mh challenge as the NC steers, while the steers in the Prior-Post and ST-Post both had similar RT responses to the Mh challenge. The Prior-Post and ST-Post had an increased RT response at 8 h p.i. when compared to values at 0 h p.i. This suggests when given a stressor prior-to or during the Mh challenge and another stressor during the febrile response, RT is suppressed and was increased for a shorter duration. When steers were given all 3 stress challenges in the Prior-ST-Post treatment group there was no difference in RT response from values observed at 0 h through the duration of the febrile response observed in the other treatment groups. Based on the data from the present study it would appear that when the stress challenges were administered and an increase in cortisol was observed, the febrile response was altered as indicated by RT. When considering the cytokine response in comparison to the RT observed with the Mh challenge, there were little differences in circulating cytokines between the treatment groups. The main differences were observed for IFN- γ , with the NS steers having an increase in IFN- γ at conclusion of the sampling period. There was also a trend for the ST-Post steers to have greater concentrations of TNF- α initially after the challenge and increased IL-6 at conclusion of the sampling period (22.5 h p.i.). The increase in pro-inflammatory cytokines to the Mh challenge was minimal compared to an endotoxin challenge in cattle (Elsasser et al., 1995) as well as other Mh challenge models (Burciaga-Robles et al., 2010). The differences observed in our study compared to other challenge models could be attributed to the infectious dose. The infectious dose used in our study was less than the dose utilized by Burcia-Robles et al. (2010) and was diluted in a larger volume to help disperse the solution in the respiratory track. The larger volume and

smaller dose in our study could have potentially been credited with the lack of systemic response in circulating cytokines. The smaller infectious dose used in our study was chosen to allow the increase or lack of cortisol present at the different time points the opportunity to elicit changes rather than overwhelming the immune system.

Generally at the onset of the febrile response, several of the first responder cytokines are purely catabolic in nature, promoting a physiological state of readiness to retrieve needed energy substrates from storage depots like fat and in some instances muscle (Elsasser et al., 2008). These first responders are subsequently downregulated and reversed back towards anabolic state and function as the later arriving anti-inflammatory cytokines and acute phase proteins reestablish normal function in the body. The initial responses are generally catabolic because when fever is present the caloric demand can increase as much as 30% for each 1°C increase in core body temperature, the needed calories are channeled away from other resources and processes in a prioritized manner that is proportional to the severity of the response initialized due to the immune insult (Elsasser et al., 2008). Therefore the main priority is not to grow, but rather redistribute those resources to fuel the immune system to help fight the infection. Growth hormone and IGF-I are two of the endocrine growth parameters associated with muscle accretion and overall growth of the animal. Studies utilizing immune challenge models have reported uncoupling of the somatotrophic axis (Thissen et al. 1999), as well as reductions in GH and IGF-I concentration (Elsasser et al., 2008). Daniels et al. (2002) also suggested that the increase in cortisol observed during immune challenges may have a role as well in reduced GH release. While endotoxin challenges have reported a reduction in GH and IGF-I concentrations, endotoxin challenges elicit a rapid and

substantial increase in pro-inflammatory cytokines. In the present study a model was utilized that would evaluate the endocrine growth response associated with multiple stressors in combination with a sustained febrile response that wouldn't overwhelm the immune defenses. In the present study it is reasonable to assume the immune system was not overwhelmed as indicated by the circulating concentrations of cytokines evaluated during the trial period. There was not a large increase in the cytokines evaluated, but it still appears the Mh challenge still elicited a febrile response as evident by the increase in RT. Growth hormone concentrations in the present study were different at specific sample time points, but no distinctive patterns could be established for treatment groups that had differences in GH concentrations at sample time points during the trial period. The multiple stressors in combination with the *M. haemolytica* challenge caused perturbations in GH secretion for all groups with a consistent pattern since all groups were getting subjected to multiple challenges. Since there were no patterns or alterations in GH secretion, the lack of differences observed in IGF-I concentrations between the treatment groups is reasonable. There were differences in IGF-I concentrations at h -25.5 prior to the challenge and at h 22.5 h p.i., but no differences between the treatment groups during the challenge. The lack of differences during the trial could also be explained due to the steers being subjected to the Mh challenge and the challenge causing suppression of IGF-I for all the treatment groups. This is evident when comparing the IGF-I concentrations at -25.5 h pre-Mh challenge and 22.5 h p.i. within each treatment group and evidence of a decline in IGF-I over the 48 h period. Although no difference was observed in the Prior-ST-Post treatment group that received all of the stress challenges, but this group also had a lower circulating GH

concentration when examined at the multiple time intervals. Therefore, the lack of difference observed in the Prior-ST-Post treatment group could be attributed to this group having greater total circulating cortisol concentrations, suppressed GH concentrations at multiple time points and intervals during the challenge period resulting in a non-significant decline in IGF-I.

Conclusion

Activation of the HPA axis eliciting secretion of cortisol appears to have an impact on the febrile response as measured by rectal temperature as well as endocrine growth parameters. While there were no clear differences in GH concentrations during the time intervals or at specific time periods, this could be attributed to the combination of the stressors. Results from Chapter VI suggest stress post-immune challenge causes suppression of GH, but when stressors occur prior to the challenge as well as after these effects are not as noticeable between the groups. It is unclear the role the Mh challenge had on the IGF-I response for all the treatment groups, as indicated by the lack of differences during the challenge period between all treatment groups. It would appear based on the results from this study as well as results from Chapter VI, acute increases in cortisol do appear to modulate the febrile response as well as endocrine growth parameters, but when multiple increases in cortisol occur prior-to, during, and post-Mh challenge the differences are less detectable.

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Table 7.1 Least squares means for percent change in rectal temperature values of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-44.0	0.06	-0.07	-0.37	-0.72	-0.45	0.15	0.48
-40.0	0.39 ^{gh}	0.88 ^g	-0.40 ^h	-0.05 ^h	-0.06 ^h	0.15	0.06
-36.0	-0.08	0.19	-0.14	-0.23	-0.10	0.15	0.91
-32.0	0.28	0.19	-0.28	-0.15	-0.14	0.15	0.69
-28.0	-0.15	0.13	-0.53	-0.60	-0.66	0.15	0.32
-24.0	-0.43 ^{gh}	0.19 ^h	-0.97 ^g	-0.25 ^{gh}	-0.91 ^g	0.15	0.06
-20.0	0.60 ^h	0.39 ^h	-0.82 ^g	0.05 ^h	-0.03 ^h	0.15	0.06
-16.0	0.59	0.60	0.01	0.21	0.12	0.15	0.59
-12.0	-0.24	-0.01	-0.28	-0.10	-0.29	0.15	0.96
-8.0	0.13	0.64	-0.11	0.48	-0.09	0.15	0.37
-4.0	-0.28	-0.38	-0.32	0.25	-0.23	0.15	0.74
0	0	0	0	0	0	0.15	0.99
4.0	1.56 ^g	0.66 ^h	0.85 ^{gh}	0.60 ^h	0.19 ^h	0.15	0.03
8.0	1.11 ^g	1.81 ^g	1.03 ^g	1.19 ^g	0.26 ^h	0.15	0.01
12.0	1.35 ^g	1.44 ^g	0.64 ^h	0.50 ^h	-0.21 ⁱ	0.15	0.001
16.0	0.51 ^{gh}	1.24 ^h	0.003 ^{gi}	-0.10 ^{gi}	-0.38 ⁱ	0.15	0.002
20.0	0.11	0.24	0.08	-0.25	-0.07	0.15	0.87
24.0	0.47 ^g	0.43 ^g	0.07 ^g	-0.45 ^h	-0.10 ^h	0.15	0.001

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^{g,h,i} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 7.2 Least squares means at sample time points and sample time intervals for cortisol concentrations of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-8.0	2.27	6.54	6.00	3.92	6.04	4.58	0.96
-7.0	3.83 ^k	71.29 ^l	76.71 ^l	2.01 ^k	74.84 ^l	5.03	0.001
-6.5	6.84 ^k	54.65 ^l	54.77 ^l	2.34 ^k	54.80 ^l	4.58	0.001
-6.0	4.42 ^k	21.72 ^l	18.01 ^l	4.29 ^k	19.62 ^l	4.18	0.005
-5.5	9.17	10.63	8.24	9.46	8.58	4.18	0.99
-0.5	10.55	5.98	4.02	4.08	11.24	5.03	0.67
0.5	5.98 ^k	74.19 ^l	8.52 ^k	62.89 ^l	76.79 ^l	4.58	0.001
1.0	5.25 ^k	72.45 ^l	9.13 ^k	59.88 ^l	71.61 ^l	4.58	0.001
1.5	19.61 ^l	29.01 ^l	4.04 ^k	24.04 ^l	27.19 ^l	4.58	0.001
2.0	18.83	14.94	8.70	10.25	13.71	4.58	0.51
2.5	9.17	7.78	14.25	5.94	6.85	4.58	0.67
7.0	11.09	10.45	11.96	6.12	1.57	4.58	0.38
8.0	7.50 ^k	4.70 ^k	77.43 ^l	66.28 ^l	75.24 ^l	4.58	0.001
8.5	23.87 ^k	7.12 ^l	63.00 ^m	59.46 ^m	74.10 ^m	4.58	0.001
9.0	18.30	16.09	26.65	21.77	29.59	4.58	0.13
9.5	13.55	8.48	11.13	9.43	12.69	4.58	0.92
12.5	15.36	11.27	4.45	2.52	1.36	5.03	0.19
TI-Cort-Prior ^g	11.93 ^k	97.58 ^l	98.75 ^l	9.22 ^k	98.50 ^l	7.32	0.001
TI-Cort-ST ^h	33.91 ^k	118.78 ^l	22.90 ^k	98.07 ^l	119.25 ^l	8.38	0.001
TI-Cort-Post ⁱ	35.64 ^k	22.47 ^k	111.96 ^l	95.75 ^l	112.24 ^l	8.92	0.001
TCP ^j	81.49 ^k	238.83 ^l	233.31 ^l	203.04 ^l	310.11 ^m	21.53	0.001

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^g Time Interval Prior (TI-Cort-Prior) = -8 to -5.5 h pre-*M. haemolytica* challenge

^h Time Interval ST (TI-Cort-ST) = -0.5 to 2.5 h relative to *M. haemolytica* challenge

ⁱ Time Interval Post (TI-Cort-Post) = 7 to 9.5 h post-*M. haemolytica* challenge

^j Total Production (TCP) = TI-Cort-Prior + TI-Cort-ST + TI-Cort-Post

^{k, l, m} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 7.3 Least squares means for TNF- α at sample time points of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-13.5	0	0	2.72	12.71	12.62	1.87	0.44
-6.0	0	0.64	1.80	13.02	4.14	1.37	0.62
-0.5	0	1.82	2.20	12.26	2.78	1.29	0.72
0.5	0 ^h	0 ^h	13.0 ^{gh}	29.56 ^h	8.34 ^h	3.50	0.01
1.0	0 ^g	1.2 ^g	8.05 ^g	25.64 ^h	3.76 ^g	2.69	0.04
2.0	0	0.66	5.18	9.76	4.68	1.40	0.83
4.0	0	0	8.37	16.02	3.64	1.97	0.35
8.0	0	0	1.73	10.56	5.08	1.22	0.75
12.0	0	0	3.33	15.80	7.04	1.80	0.41
22.5	0	0	4.23	7.74	3.72	1.10	0.90

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^{g,h} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 7.4 Least squares means for IL-6 at sample time points of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-13.5	0	0	0	0.25	0	0.95	0.99
-6.0	0.86	0	0	2.88	0	0.92	0.84
-0.5	0	0	0	0.86	0	0.92	0.99
0.5	0	0	0	3.96	0	0.92	0.59
1.0	1.12	0	0	3.10	0	0.92	0.80
2.0	0	0	0	0	0	0.94	0.99
4.0	0	0	0	1.86	0	0.92	0.96
8.0	0 ^h	0 ^h	0 ^h	8.96 ^g	0	0.94	0.008
12.0	0	0	1.57	0	0	0.95	0.97
22.5	0.02 ^g	1.57 ^{gh}	10.28 ^{hi}	16.10 ⁱ	0.28	0.95	0.001

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^{g,h,i} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 7.5 Least squares means for IFN- γ at sample time points for of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-13.5	10.64	18.62	12.27	14.14	18.04	1.34	0.26
-6.0	12.96	17.67	9.05	13.86	19.48	1.88	0.12
-0.5	16.84	17.67	7.95	11.62	14.90	1.88	0.12
0.5	16.64	19.83	13.15	14.14	21.38	1.37	0.24
1.0	15.14 ^{gh}	20.48 ^g	10.87	18.84 ^{gh}	21.80 ^g	2.23	0.07
2.0	17.52	14.12	9.70	10.50	18.44	1.66	0.16
4.0	12.66	9.33	12.23	16.52	15.46	0.88	0.47
8.0	16.94	10.68	10.85	13.58	15.34	0.82	0.51
12.0	18.80	9.95	11.43	8.10	11.02	1.69	0.15
22.5	25.50 ^h	14.30 ^g	8.75 ^g	7.68 ^g	16.24 ^g	5.24	0.001

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^{g,h} Means within row lacking common superscripts are different at $P \leq 0.05$

Table 7.6 Least squares means at sample time points and sample time intervals for growth hormone concentrations of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST-Post ^f	SEM	P-value
-13.5	7.57	6.75	6.27	5.69	4.10	1.44	0.40
-8.0	8.34	8.60	5.88	7.87	5.16	1.30	0.16
-7.0	5.97	2.96	5.21	6.67	3.17	1.44	0.15
-6.0	5.46	2.96	4.70	4.54	4.33	1.30	0.70
-5.0	6.47 ^{klm}	5.35 ^{kl}	8.32 ^{lm}	9.77 ^m	4.62 ^k	1.30	0.02
-4.0	7.55	5.48	8.68	5.93	6.56	1.30	0.34
-0.5	6.94	5.65	7.97	7.97	4.86	1.44	0.28
0.5	6.92 ^k	2.46 ^l	5.79 ^{km}	3.22 ^{lm}	2.36 ^l	1.30	0.03
1.5	8.34	7.14	9.05	8.29	5.98	1.30	0.40
2.5	9.39	7.62	5.78	7.15	6.68	1.30	0.34
3.5	7.25	5.59	7.00	4.94	7.28	1.30	0.57
5.0	9.28 ^k	5.08 ^{lm}	8.44 ^k	8.09 ^{km}	3.92 ^l	1.30	0.007
5.5	6.95	5.72	7.94	6.47	4.10	1.30	0.22
7.0	8.11	4.03	6.64	5.54	5.08	1.30	0.18
8.5	6.44	7.88	6.39	5.71	4.33	1.30	0.33
9.5	5.93 ^{kl}	5.28 ^{kl}	3.47 ^l	9.29 ^k	6.71 ^{kl}	1.30	0.02
10.5	7.67	5.72	8.23	6.98	6.46	1.30	0.61
13.0	9.82	6.08	7.34	6.41	6.60	1.30	0.24
14.5	9.28 ^k	7.03 ^{klm}	5.70 ^{lm}	6.74 ^{klm}	3.83 ^{lm}	1.30	0.04
22.5	5.66	8.67	5.41	7.91	5.18	1.44	0.15
TI-GH-Prior ^g	25.84 ^k	18.31 ^l	25.50 ^k	28.14 ^k	17.98 ^l	2.60	0.02
TI-GH-ST ^h	31.75	22.84	28.10	24.89	21.10	3.29	0.16
TI-GH-Post ⁱ	23.89	21.01	20.56	24.08	19.17	2.29	0.44
TGHP ^j	81.48 ⁿ	62.17 ^{op}	74.16 ^{no}	77.11 ^{no}	58.24 ^p	6.81	0.08

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^g Time Interval Prior (TI-GH-Prior) = -13.5 to -4.0 h pre-*M. haemolytica* challenge

^h Time Interval ST (TI-GH-ST) = -0.5 to 3.5 h relative to *M. haemolytica* challenge

ⁱ Time Interval Post (TI-GH-Post) = 7 to 10.5 h post-*M. haemolytica* challenge

^j Total Production (TGHP) = TI-GH-Prior + TI-GH-ST + TI-GH-Post

^{k,l,m} Means within row lacking common superscripts are different at $P \leq 0.05$

^{n,o,p} Means with row lacking common superscripts are considered tendencies at $P \leq 0.10$

Table 7.7 Least squares means at sample time points and sample time intervals for insulin-like growth factor-I concentrations of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

Sample Time ^a	NC ^b	Prior-ST ^c	Prior-Post ^d	ST-Post ^e	Prior-ST - Post ^f	SEM	P-value
-25.5 h	180.14 ^{kl}	160.67 ^k	208.70 ^{lm}	231.20 ^m	193.27 ^{klm}	14.04	0.005
-0.5 h	158.38 ^l	156.35 ^l	176.01 ^{kl}	203.51 ^k	159.78 ^l	14.04	0.08
7.0 h	149.52	156.40	151.14	174.84	182.76	13.35	0.29
22.5 h	150.90 ^{klm}	132.32 ^k	139.02 ^{kl}	186.64 ^m	172.71 ^{lm}	13.35	0.03
TI-IGF-Prior ^g	4311.16 ^{no}	3962.72 ⁿ	4833.63 ^{op}	5433.95 ^p	4412.44 ^{nop}	413.77	0.06
TI-IGF-ST ^h	1005.53	1016.43	1056.04	1229.66	1140.78	77.75	0.19
TI-IGF-Post ⁱ	2328.21 ^{kl}	2237.54 ^k	2248.75 ^k	2801.47 ^m	2754.92 ^{lm}	161.25	0.04
TIGFP ^j	7650.82 ^{no}	7216.68 ⁿ	8070.86 ^o	9465.07 ^p	8219.62 ^o	675.49	0.09

^a Time interval in hours prior to and post *M. haemolytica* intra-tracheal challenge

^b Stimulate release of cortisol and *M. haemolytica* challenge

^c Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*

^d Stimulate release of cortisol at -7.5 pre- and 7.5 h post-*M. haemolytica*

^e Stimulate release of cortisol at 0 h during and 7.5 post-*M. haemolytica*

^f Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*

^g Time Interval Prior (TI-IGF-Prior) = -25.5 to -0.5 h pre-*M. haemolytica* challenge

^h Time Interval ST (TI-IGF-ST) = -0.5 to 7.0 h relative to *M. haemolytica* challenge

ⁱ Time Interval Post (TI-IGF-Post) = 7 to 22.5 h post-*M. haemolytica* challenge

^j Total Production (TIGFP) = TI-IGF-Prior + TI-IGF-ST + TI-IGF-Post

^{k, l, m} Means within row lacking common subscripts are different at $P \leq 0.05$

^{n, o, p} Means with row lacking common superscripts are considered tendencies at $P \leq 0.10$

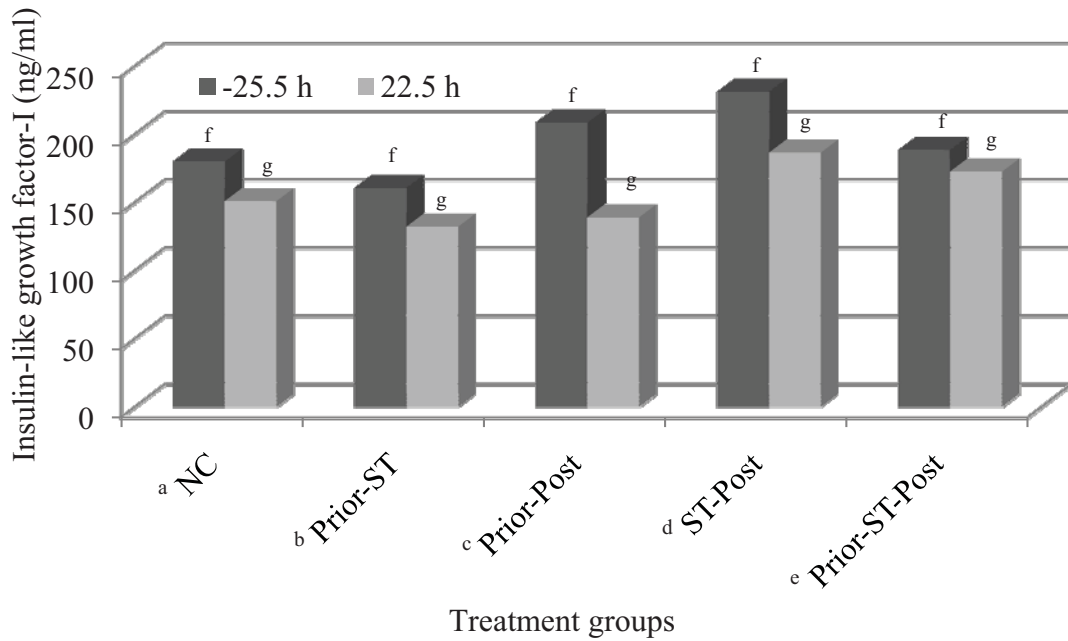


Figure 7.1 Insulin-like growth factor-I concentrations (ng/ml) at -25.5 h pre-*M. haemolytica* and 22.5 h post-*M. haemolytica* challenge of 30 Holstein steers receiving multiple corticotropin releasing hormone (CRH) + arginine vasopressin (AVP) challenges to stimulate release of cortisol at -7.5-pre, during, or 7.5-hr post *M. haemolytica* intra-tracheal challenge

- ^a No stimulated release of cortisol and *M. haemolytica* challenge
- ^b Stimulate release of cortisol at -7.5 h pre- and at 0 h during the *M. haemolytica*
- ^c Stimulate release of cortisol at -7.5 h pre- and 7.5 h post-*M. haemolytica*
- ^d Stimulate release of cortisol at 0 h during and 7.5 h post-*M. haemolytica*
- ^e Stimulate release of cortisol at -7.5 h pre-, 0 h during and 7.5 h post-*M. haemolytica*
- ^{f, g} Means within treatment group lacking common superscripts are different at $P \leq 0.05$

CHAPTER VIII

CONCLUSION

The Bovine Respiratory Disease complex appears to be the crossroads, where predisposing factors such as stressors and health status of the calf meet novel pathogens creating the perfect storm. The problem with the BRD complex is difficult to replicate with a challenge model to study the various interactions and synergistic contributions of each of the factors. While immune and stress models exist to stimulate the activation of these systems, it is difficult to replicate the actual pathogenesis of BRD. There are a multiple methods of infectivity and understanding the complexity of the multi-factorial etiology of BRD is a daunting task that requires understanding each and all the components associated with it. It does appear the activation of the HPA axis leading to increases in cortisol is a major component of the BRD complex and a deeper understanding of the interaction between the immune and HPA axis is still needed. Methods and doses for infectivity of the pathogens is critical rather than overwhelming the immune system with a large dose, and understand how the interaction of the factors build or suppress each other is another critical area that needs to be considered when building a model. There does not appear to be a “silver bullet” to solve the production losses, costs, and problems associated with the BRD complex. Therefore, a multi-

factorial approach needs to be utilized to study this multi-factorial disease to help mitigate the losses that keep occurring and have not changed.