ALTERNATIVE APPROACHES TO ANTIMICROBIAL USE AND INJECTION SITE IN CATTLE

by

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Abstract

Bovine herpesvirus-1 (BHV-1) is a viral pathogen that contributes to bovine respiratory disease (BRD) complex. Characterized by inflammation of the upper respiratory tract and trachea and accompanied by nasal lesion and discharge; BHV-1 has the ability to undergo latency in neurological tissue and recrudesce upon stress-induced immunosuppression. Immunostimulatory products are recently available for control of cattle diseases and may reduce the impact of stress-induced immunosuppression, but their efficacy to control the various pathogens involved in BRD is poorly understood. Furthermore, most injectable cattle products have a label indication for intramuscular or subcutaneous administration in the neck but some producers, primarily dairy, choose to administer injections in the ischiorectal fossa (RF); therefore, research on the efficacy and tissue reactivity of alternative injection sites is needed. Experiment 1 investigated the effect of a DNA immunostimulant (Zelnate, Bayer Animal Health) on recrudescence of BHV-1 after dexamethasone challenge administered for 3 consecutive days in beef cattle and Experiment 2 determined the efficacy of the RF as an alternative injection site. In Experiment 1, steers (n=10) and heifers (n=10) were administered 40 mg of dexamethasone i.v. 166-d subsequent to a controlled BHV-1 challenge (1.0×10^8 PFU per nostril). On day 1, calves were administered 2 mL of DNA immunostimulant (Zelnate; ZEL) or sterile saline (CON) i.m. Hematological variables, BHV-1 isolation from nasal swabs, presence of nasal lesions, BHV-1-specific antibody titers and rectal temperature were evaluated daily (0600) for 12 days after dexamethasone challenge. Results indicate that the DNA immunostimulant altered

eosinophil concentration but did not mitigate BHV-1 recrudesce. In Experiment 2, 28 Jersey steers were administered a modified-live virus (MLV) respiratory vaccine (Pyramid 5, Boehringer Ingelheim Animal Health USA) s.c in the neck region (NECK) or in the RF. Blood samples were collected to analyze BVDV-specific antibody titer, performance data was analyzed, and injection site lesions at harvest were observed. Results indicate that MLV respiratory vaccine administration in the RF did not cause injection site lesions and the humoral vaccine response was similar to NECK. The use of a DNA immunostimulant did not mitigate recrudescence of BHV-1 in dexamethasone challenged beef calves previously administered BHV-1 and the RF may be an effective route of administration for MLV. Further research investigating the efficacy of DNA immunostimulants in different disease challenge models is needed to ensure safe use in beef calves and research determining the effects of other commonly administered animal health products in the RF should be further explored.

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Chapter I

Introduction and Literature Review

Introduction

Bovine respiratory disease (BRD) is multifaceted involving both viral and bacterial pathogens and includes predisposing factors associated with stress and inflammation. Transportation and marketing practices in the US beef industry can result in periods of stress, nutritional deficiency, and exposure to infectious agents when calves are commingled from various sources and transported (Step et al., 2008b). Infectious bovine rhinotracheitis virus, also referred to as bovine herpesvirus-1 (BHV-1), is a causative viral agent within the BRD complex and is characterized by inflammation of the upper respiratory tract and trachea, accompanied by nasal discharge and nasal lesions (Yates, 1982). Latency is a unique property of BHV-1; after initial infection the virus has the ability to become latent in neurological tissue and recrudesce upon subsequent immunosuppression. Reactivation of BHV-1 can be stimulated by the synthetic glucocorticoid analog dexamethasone, which has been previously used to stimulate stress-induced immunosuppression in cattle (Lippolis, 2008). Antimicrobials are commonly used as treatment for the bacterial agents involved in BRD; however, the use of antimicrobials in livestock is under intense scrutiny due to antimicrobial resistance concerns in bacteria affecting humans and animals. Therefore, the use of immunostimulants to control disease in cattle may have increasing justification as they provide an alternative to antimicrobials. Immunostimulants

or immunomodulators have the ability to modify the immune system and may benefit the health of the animal; however, the evaluation of the safety and efficacy of immunostimulants in cattle is

necessary to determine their utility under various circumstances. Furthermore, vaccines and antimicrobials have the potential to cause injection site lesions which are of concern because tissue damage can occur and affect the carcass (Roeber et al., 2002). Beef Quality Assurance guidelines exist to minimize occurrence of lesions and stipulate that injections should be given to cattle s.c. when possible. Injections are typically administered in the neck region of cattle but some injectable products are administered posterior due to the prevalent use of headlocks as a management tool in dairy operations (Holland et al., 2018); because of this the RF has been demonstrated as a possible alternative injection site for s.c. administration.

Review of Literature

Overview of Bovine Respiratory Disease

Bovine respiratory disease (BRD) is the most common and costly disease in feedlot cattle in the US, responsible for 70% of morbidity and 40% of cattle mortality (Snowder et al., 2006; Taylor et al., 2010). Bovine respiratory disease is a multi-factorial disease involving complex interactions between stress-induced immunosuppression, infectious viral agents and bacterial pathogens that result in bronchopneumonia (Galyean et al., 1999; Grissett et al., 2015). The Feedlot 2011 report indicated that 95.6% of feedlots with 1,000 to less than 8,000 head of cattle were affected by BRD and 100% of feedlots with greater than 8,000 head were affected (APHIS, 2011). Bovine respiratory disease continues to be an economically important disease in cattle with losses estimated at $23.60 per treated animal (APHIS, 2011), costing the industry over 1 billion dollars annually (Griffin, 1997).

Pathogenesis of Bovine Respiratory Disease

Transportation and marketing practices in the US beef industry can result in periods of stress, nutritional deficiency and exposure to infectious agents when calves are commingled from various sources and transported to distant sites (Step et al., 2008b). These physiological stressors combined with physical or environmental stressors interact to predispose cattle to BRD (Taylor et al., 2010).

Preconditioning

Calf management before marketing influences stress once calves enter the feedlot (Lalman and Smith, 2001). Preconditioning is a common practice performed at the origin ranch that is designed to reduce the impact of stress during the shipping and receiving period by weaning, castrating, dehorning, and training cattle to eat feed from a bunk before shipping (Lalman and Smith, 2001). This management practice is used to add value to calves as they have a greater potential to perform efficiently in a feedlot and be more profitable (Bailey and Stenquist, 1996). Preconditioning cattle is not practiced by all cow-calf operations and does not guarantee calf health but studies indicate that calves that have been preconditioned before entering the feedlot had lower production costs due to decreased morbidity and mortality and increased ADG (Roeber and Umberger, 2002). Due to the segmented infrastructure of the beef cattle industry, adoption of preconditioning is difficult (Babcock et al., 2010). For cow-calf producers to utilize preconditioning programs they must receive economic incentive, at the time of calf sale. It also appears to be important to feedlots to assess the benefit of preconditioned animals within their own production system and pay premiums for preconditioning programs where they see economic benefits (Dhuyvetter, 2004; Babcock et al., 2010).

Commingling

The background source of the animal (i.e., ranch direct versus auction market procurement), also contributes to the incidence of BRD in calves via commingling. Commingling results from buying calves from one or more auction barns and combining through an order buyer (Taylor et al., 2010). Calves purchased through the auction market system are at greater risk for BRD than those arriving directly from a single ranch source because exposure to pathogens and increased stress during relocation, marketing and commingling are evident for the auction market method (Step et al., 2008a; Taylor et al., 2010). Step et al. (2008) evaluated morbidity among ranch-direct calves, market calves, and commingled calves and found commingled steers have greater BRD rates than ranch calves but less than auction market calves. Richeson et al. (2012) reported that total BRD morbidity was greater for commingled auction market calves when compared to single-source preconditioned calves with 70.4 and 6.7% of calves, respectively treated for BRD. Other studies align with this research indicating calves of unknown health history had greater morbidity when compared to those with known health protocols administered before marketing (Macartney et al., 2003; Seeger et al., 2008).

Handling and Transportation

Animals may be stressed by either psychological factors such as restraint, handling or novelty; or physical stressors such as, hunger, thirst, fatigue or injury (Grandin, 1997). These stressors can manifest from the fight or flight response during the process of handing and transportation. Transportation is the most well-known non-infectious environmental risk factor for BRD as the nature of cattle production in North America requires that most beef calves are transported at least once in their lifetime (Taylor et al., 2010). Sanderson et al. (2008) found a positive correlation between the distance transported and cattle morbidity, indicating that sorting, loading and distance are the most stressful factors to transportation (Cole et al., 1988; Sanderson

et al., 2008). Their data indicated a 10% increase in initial BRD risk for each 160 km (100 miles) increase in transport distance (Sanderson et al., 2008).

BRD Control

Upon arrival or shortly after arrival is when cattle most frequently exhibit signs of BRD and are at the greatest risk of clinical presentation of BRD signs. More than half of all feedlots (59.3%) use metaphylaxis, or population-wide administration of an antimicrobial to control an anticipated outbreak of BRD (APHIS, 2011). Seventy-four percent of feedlots used metaphylaxis because of the unknown health history or lack of vaccination against respiratory pathogens and 74.1% of feedlots used metaphylactic treatment based of the appearance of the cattle once received (APHIS, 2011).

It was determined that 21.2% of cattle weighing under 700 lb developed BRD and 89.6% of those were treated based on clinical signs such as depression or fever (APHIS, 2011). Of cattle weighing 700 lb or more, 8.8% developed BRD and 84.1% of those cattle were treated (APHIS, 2011). The background of calves as discussed can play a crucial role in exposure to pathogens leading to disease and depending on the perceived cause of the disease feedlots may elect to not treat BRD, allowing the disease to resolve on its own or sending affected cattle to slaughter early (APHIS, 2011).

BRD Pathogens

Viral pathogens are capable of causing primary infection which plays an important role in immunosuppression, thereby increasing susceptibility to secondary bacterial infections (Ogilvie, 1998). Viral pathogens such as bovine viral diarrhea virus (BVDV genotype 1 and 2, bovine herpesvirus-1 (BHV-1), parainfluenza-3 virus (PI-3V) and bovine respiratory syncytial virus

(BRSV)are transmitted via aerosolization; however, PI-3V and BRSV are accepted as minor contributors to BRD when compared to the other viruses (Grissett et al., 2015).

Bovine viral diarrhea virus consists of two recognized genotypes, BVDV type 1 and BVDV type 2, with virus isolates within these different groups exhibiting considerable biological and antigenic diversity (Kalaycioglu, 2007). Cattle of all ages are susceptible to BVDV infection with clinical signs ranging from subclinical to fatal conditions such as mucosal disease (Kahrs, 1981). Acute infections occur primarily in younger calves and are typically associated with diarrhea and potentially predisposes calves to BRD due to an immunosuppressive effect of the virus (Baker, 1995). Persistent infection (PI) of BVDV affects approximately 1 to 2% of the cattle population (Bolin, 1995). In rare occasions, animals that are BVDV-PI can succumb to mucosal disease. The onset of mucosal disease may be so rapid that first signs are extremely morbid or dead animals with erosions found in the mucosa at various sites along the gastrointestinal tract (Bolin, 1995). Persistently infected calves tend to fail to thrive as well as provide a continual source of infective virus to other cattle; therefore, these cattle should be rapidly identified and removed from the herd (Kahrs, 1981).

Infectious bovine rhinotracheitis, caused by BHV-1 infection, can be described as an acute, contagious, febrile infection of cattle that is characterized by inflammation of the upper respiratory tract and trachea and accompanied by nasal discharge, nasal lesions and loss of body condition (McKercher, 1964; Yates, 1982). Bovine herpesvirus-1 infects a wide variety of tissues in the bovine and the replication cycle is short with progeny completed within 12 hours. The virus spreads rapidly in infected cell cultures, resulting in complete cell destruction and also establishing latent infections in neurons of sensory and autonomic nerve ganglia (McKercher, 1964; Luria et al., 1978). Latency is a common property of all herpesviruses and is defined by

the unique relationship with the host in which initial infection is followed by persistence of the agent for life (Stevens, 1978). The latent or inactive BHV-1 can be reactivated following treatment of dexamethasone, a synthetic glucocorticoid or adrenocorticotropic hormone (Narita et al., 1981; Thiry et al., 1985), and the virus can be shed and transmitted (Turin et al., 1999). During reactivation, the virus is translocated back to the initial site of infection where it can spread to other susceptible hosts (Winkler et al., 1999. Winkler et al. (2000) reported that a single dose of dexamethasone was sufficient to consistently induce reactivation of BHV-1 in infected calves causing apoptosis in the tonsils of latently infected calves but not uninfected calves. In their study, no BHV-1 DNA was detected before dexamethasone administration but DNA was detected 24 and 48 hours after dexamethason administration in calves with latent infection (Winkler et al., 2000). Naturally occurring stress may increase endogenous cortisol production which could lead to reactivation and shedding of BHV-1 (Narita et al., 1981) and it was reported that transportation-induced stress leading to reactivation of BHV-1 was detected in 42% of calves (Thiry et al., 1987).

Although vaccination can reduce the amount of BHV-1 excreted following reactivation (Mars et al., 2001), latency is probably solely responsible for the perpetuation and transmission of the virus (Rock, 1994). Cattle with uncomplicated BHV-1 infections have upper respiratory disease that can resolve in 7 to 10 days (Kiorpes et al., 1978). However, in most cases BHV-1-associated BRD cases are mixed with an infection of bacteria such as *M. haemolytica* or *P. multocida* that results in severe lower respiratory tract disease (Ellis, 2009).

Bovine parainfluenza-3 virus is a member of the genus *Respirovirus* in the paramyxoviridae family (Murphy, 1995) and was first discovered from the nasal discharge of cattle with shipping fever (Ellis, 2010a). Clinical signs after PI-3V infection vary ranging from

asymptomatic infections to severe respiratory disease (Horwood et al., 2008). Coughing, fever, and nasal discharge are observed in majority of cases and in instances where animals are subjected to chronic stress, tissue damage and immunosuppression result in severe bronchopneumonia and lesions from secondary bacterial infections can occur (Haanes et al., 1997). Active infection can be diagnosed by virus isolation or serum samples as the infection is often complicated with other viruses and bacteria (Ellis, 2010b).

Bovine respiratory syncytial virus is a part of the *paramyxoviridae* family responsible for causing respiratory disease in cattle, as a single disease agent or component of BRD (Larsen, 2000). Being responsible for morbidity rates of 60 to 80 % and mortalities of 20% in cattle, BRSV is genetically and antigenically related to human (H)RSV which is the single most important cause of lower respiratory tract disease in infants (Gershwin, 2012a). The bovine-specific disease is common in young calves and begins with fever, cough, nasal discharge, depression, and increased respiratory rate (Gershwin, 2012b) with virus shedding occurring from the nasal passages from day 4 through day 10 and reported as early as day 1.

Bacterial pathogens such as *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis* are isolated from lung tissue in cattle with bronchopneumonia. These bacteria are ubiquitous in the cattle population within the nasopharynx and following stress or viral infection can proliferate and be inhaled into the lungs (Confer, 2009). Each bacteria has its own characteristics such as adhesions, toxins and enzymes that enhance ability to colonize and cause tissue damage and incite inflammatory response (Confer, 2009).

Mannheimia haemolytica, formally known as *Pasteurella haemolytica*, is a small gram-negative, facultative anaerobic bacterium that is arguably the most important of the group of bacteria (Confer, 2009; Griffin et al., 2010). The bacterium resides in the nasopharynx and

tonsillar crypts as calves acquire *M. haemolytica* through contact with their dams and other cattle early in life (Rice et al., 2007b; Confer, 2009). Rice et al. (2007) suggested that the bacteria maintains a commensal relationship with the host until the relationship is disrupted by stress or co-infection and the bacteria quickly becomes the predominate pathogenic organism leading to bronchopneumonia (Rice et al., 2007a; Griffin et al., 2010). *Mannheimia haemolyica* has many virulence factors including a capsule used for adherence and invasion, outer membrane proteins that result in protection from the host immune response, adhesions used for colonization, neuraminidase that reduces respiratory mucosal viscosity allowing bacteria to access the cell surface, and a lipopolysaccharide complex that causes hemorrhage along with acute inflammation (Gioia et al., 2006; Griffin et al., 2010). These factors allow the bacteria to evade clearance and avoid host defenses while rapidly reproducing in the lower respiratory tract. Leukotoxins produced during rapid growth of *Mannheimia haemolytica* are a significant virulence factor as they are responsible for lysis of leukocytes and platelets. The leukotoxin stimulates the host immune system to produce serum antibodies to the leukotoxin suggesting that animals with leukotoxin antibodies may have immunological protection against *Mannheimia haemolytica* (Fulton, 2009).

Pasteurella multocida, specifically serotype A:3, is commonly identified in respiratory disease affecting younger cattle during neonatal calf pneumonia and BRD in recently weaned, highly stressed calves (Apley, 2006b). Although it is often thought of as a secondary invader, there is evidence suggesting its role as a primary respiratory pathogen isolate from fatal cases of BRD (Griffin et al., 2010). Factors required for the development of *P. multicoida* infection include immune-modulating stressors such as adverse environmental conditions, adverse nutritional conditions, animal handling and transportation, and interaction of other infectious

agents (Hunt et al., 2000; Dabo et al., 2007a; Griffin et al., 2010). *Pasteurella multocida* is isolated from nasal secretions and deep pharyngeal collection (Dabo et al., 2007b), with reported isolation rates in clinically normal cattle between 20 and 60%, and twice the isolation rate of the bacteria in calves with BRD (Griffin et al., 2010).

Histophilus somni, formally *Haemophilus somnus*, is a commensal Gram-negative bacterium residing in the nasophayngeal region of calves and has the ability to colonize the lower respiratory tract (Apley, 2006a; Angen et al., 2009). The virulence factors are similar to *M. haemolytica* with the addition of histamine and an exopolysaccharide that are produced and allow for hematogenous transmission of the bacteria (Corbeil, 2007). The isolation rate in newly received cattle is reported to be greater than 50% and inversely related to the geometric mean of *H. somni* antibody titers for newly received calves, suggesting immunization that occurs before weaning and marketing may be a key management practice to minimizing the impact of *H. somni* (Griffin et al., 2010).

Mycoplasma bovis differentiates itself and is quite controversial from the other bacterial pathogens as its role in BRD is not as clear as other pathogens (Griffin et al., 2010). *Mycoplasma bovis* has a tri-layered membrane instead of a typical bacterial cell wall and is most often found in the lower respiratory tract and associated with arthritis in chronically ill cattle (Caswell and Archambault, 2007). Much of the controversy centers around whether *M. bovis* is a causative or opportunistic agent; for example 50% of cattle were positive for *Mycoplasma bovis* upon arrival at a feedlot and nearly 100% were positive within 12 hours after entry (Allen et al., 1992). Infected cattle exhibit characteristics in as little as one day and in most naïve cattle within a week (Apley, 2006a). Once in the respiratory tract, *M. bovis* can move from the respiratory cells and enter the blood and potentially persist in the cattle for life, likely the mechanism for the arthritis

most often associated with the respiratory form of mycoplasmosis (Apley, 2006a; Caswell and Archambault, 2007; Griffin et al., 2010). The prevalence of *M. bovis* increases as cattle are stressed and commingled, as the bacteria can survive in the environment for days to weeks if protected from ultraviolet light (Griffin et al., 2010).

Stress and Immune Interaction

Beef cattle are inevitably exposed to stress during their productive lives when relocated from cow-calf ranches to feedlots, including transport and feedlot entry (Carroll and Forsberg, 2007a; Cooke, 2017). The "stress response" is defined as the sum of all reactions of an individual to factors that potentially influence its homeostasis (Moberg, 2000). Stressors such as, weaning, commingling, exposure to novel environments, injury, thermal stress, fatigue, and feed and water deprivation during transportation either in combination or separately can directly decrease cattle performance and increase the risk of BRD (Carroll and Forsberg, 2007a; Duff and Galyean, 2007).

It has been demonstrated that stressors affect the immune and other systems such as the hypothalamic-pituitary adrenal (HPA) axis and the sympathetic nervous system (Elenkov et al., 2000). In response to stress, higher brain centers stimulate neurons in the hypothalamus, resulting in the secretion of corticotrophin-releasing hormone (CRH). Increased CRH concentration in the blood stimulates the anterior pituitary gland corticotropes to secrete adrenocorticotropic hormone (ACTH), stimulating the production of glucocorticoids that serve as an anti-inflammatory (Plotsky, 1991; Carrasco and Van de Kar, 2003). The stimulation of the adrenal gland causes the release of catecholamines into the circulatory system. Epinephrine, also known as adrenaline, and norepinephrine are primary catecholamines and cause an increased heart rate, blood vessel constriction, bronchiole dilation and increased metabolism (Carroll and

Forsberg, 2007b). These catecholamines have influence on the HPA axis and overall stress response due to the regulation and release of ACTH from the anterior pituitary (AP) and stimulation of cortisol (Axelrod and Reisine, 1984; Plotsky et al., 1989).

Inflammation

Inflammation is an adaptive response triggered by infection or tissue injury which involves the delivery of blood components (plasma and leukocytes) to the site of infection (Majno and Joris, 2004). The recognition of infection is mediated by resident macrophages that lead to the production of inflammatory mediators including chemokines and cytokines that mediate the arrival of neutrophils to the site of infection (Medzhitov, 2008).

Polymorphonuclear leukocytes (PMNs) also play an important role in both inflammatory response and control of bacterial infections. It has been observed that glucocorticoids affect PMNs to modulate the inflammatory response and predispose infections (Roth and Kaeberle, 1981). Dexamethasone (DEX) is a potent glucocorticoid that when administered to cattle caused recrudescence of IBR infection, suggesting that DEX suppresses the immune system (Davies and Carmichael, 1973; Roth and Kaeberle, 1981). Roth et al. (1981) reported that a single pharmacological dose of DEX administered to cattle resulted in the impairment of PMN function, showing the detrimental effects associated with the use of DEX. Results coincide with Richeson et al. who suggested the use of DEX blunts the acute phase response, and results in immunosuppression (Richeson et al., 2016).

Immune Response

The innate immune system is the first line of defense against an infectious challenge (Griffin et al., 2010) and provides the adaptive immune system time to develop an appropriate

and highly-specific antibody and cell-mediated response. Innate immunity effectiveness is influenced by the health or physiological status of the animal. For example, the innate immune system can be weakened by factors including wounds, dehydration, nutritional status, and other stressors that may allow increased replication and colonization of pathogens (Carroll and Forsberg, 2007b; Ackermann et al., 2010).

The upper respiratory tract may be colonized by a variety of pathogens that are inhaled and replicate in the tonsillar crypts and naris (Ackermann et al., 2010). The epithelial cells within the respiratory tract provide mechanical, chemical and microbiological barriers to prevent infection of BRD pathogens (Frank, 1984). Nasal passages of healthy and stressed calves may contain infecting pathogens, although stressed calves have a greater density of pathogens (Highlander, 2001). Once inhaled into the lungs, bacteria may then adhere to the epithelial cell surface and begin to colonize.

Cellular components of the innate immune system consist of phagocytic cells including, neutrophils, monocytes, macrophages and dendritic cells, as well as natural killer (NK) cells (Carroll and Forsberg, 2007b). At sites of infection, phagocytic cells are activated to attack and kill pathogens, ideally, before they cause widespread infection. Phagocytic cells recognize specific structures known as pathogen-associated molecular patterns (PAMPs) as they interact with receptors on the surface of the immune cells (Takeda et al., 2003). The binding of PAMPs to toll-like receptors, found on the surface of the cell, initiates killing mechanisms by the neutrophils and macrophages (Mann, 2001; Carroll and Forsberg, 2007b).

Indicators of Stress

Acute Phase Response

The acute phase response (APR) is a component of the innate immune system, stimulated by the presence of proinflammatory cytokines produced from macrophages at the site of inflammation or infection. Acute phase proteins (APP) are a component of the APR and are synthesized by hepatocytes in response to proinflammatory cytokines such as IL-1, IL-6 and TNF-α. Cytokine IL-1 is heavily involved in the febrile response that acts as a defensive response to destroy bacteria and microorganisms within the host. Inner leukin-6 contributes to antibody producing cells within the animal and TNF-α is involved in neutrophil activation (Carroll and Forsberg, 2007b).

Two physiologic responses associated with the APR are the febrile response and alteration in liver metabolism and gene regulation. The febrile response is a defensive one, associated with fever to impair growth of bacteria and other potentially harmful microorganisms within the host and accelerate the cellular proliferation of immune cell types. Alteration in liver metabolism and gene regulation involve the proinflammatory cytokines mediating the hepatic production and secretion of APP. When the production of these proteins is initiated by inflammation or infection, many of the APP play a crucial role in the immune response. In cattle, APP associated with respiratory infection include haptoglobin, serum amyloid A, α1-acid glycoprotein, and lipopolysaccharide binding protein (Godson et al., 1995; Nikunen et al., 2007).

Leukocyte Variables

Changes in different blood leukocyte concentrations have been historically used as a measure of stress before methods were available to directly assay the hormone cortisol (Hoagland et al., 1946). An increase in plasma cortisol concentration can result in neutrophilia, lymphopenia, and eosinopenia (Ramin et al., 1995). An automated hemocytometer is commonly used to evaluate physiological effects on hematological counts or concentration. Though

complete blood count analysis measures all leukocyte and erythrocyte variables, specific trends involving neutrophils, lymphocytes and eosinophils are most often reported within literature regarding the stress response.

Neutrophils are the first line of defense against most pathogens that affect cattle. These leukocytes originate from myeloid-lineage cells in bone marrow and upon maturation are released into circulation where they marginate through blood vessel endothelial cells and migrate to an area of infection through the process of translocation (Burton et al., 2005). Previous research indicates that increased blood levels of cortisol during stress is associated with increased susceptibility to infectious disease in cattle (Roth and Kaeberle, 1982; Burton and Erskine, 2003). Griebel et al., (2014) examined this comparing the effect of abrupt weaning and transportation versus a two-step weaning process. They concluded that calves that were abruptly weaned and transported had greater morbidity and enhanced inflammatory responses, such as cortisol production, when calves were challenged with BHV-1 (Griebel et al., 2014). Burton et al. (2005) evaluated the effects of administration of the synthetic glucocorticoid dexamethasone on bovine neutrophils and found pronounced neutrophilia occurred, indicating that glucocorticoids profoundly alter neutrophil homeostasis (Burton et al., 2005). These results are influenced by cattle possessing a high expression of glucocorticoid receptors in their leukocytes and other cells causing them to be highly sensitive to changes in circulating glucocorticoid concentrations (Chang et al., 2004). This aligns with a study comparing acute and chronic administration levels of DEX versus control; resulting in an increase in circulating neutrophils for both acute and chronic treatments, indicating physiological stress in the cattle (Hughes et al., 2017).

Research conducted on the effect of glucocorticoids on lymphocytes generally indicate a decrease in blood concentration resulting in lymphopenia (Roth and Kaeberle, 1982). In addition, decreased lymphocytes were reported by Hughes et al. (2017) in cattle that received DEX to mimic chronic stress; however it was important to note that the decrease in lymphocytes did not qualify as lymphopenia and that lymphocyte concentrations were within the normal reference range for cattle (Hughes et al., 2017). These findings are confirmed in other studies in cattle that demonstrated lymphopenia following DEX administration (Lan et al., 1995; Anderson et al., 1999; Thanasak et al., 2004; Hughes et al., 2017).

A decrease in circulating eosinophils can result in eosinopenia during cortisol release, and has been reported as an effective indicator in identification of calves with increased risk for development of BRD (Richeson et al., 2013). Results from Richeson et al. (2013) indicates calves with a low or intermediate eosinophil concentration on arrival had a greater risk for treatment of BRD when compared to calves that had a greater eosinophil count (Richeson et al., 2013). A plausible hypothesis could rely on stressed calves having altered hematopoiesis from eosinophils being redirected from circulation to a source of inflammation (Griffin, 1989; Richeson et al., 2013).

Antimicrobials

Antimicrobials are commonly used in food animal production to control or treat bacterial disease and to promote growth (McEwen and Fedorka-Cray, 2002). In the beef industry, various antimicrobials are administered to cattle for reasons including treatment of BRD, control of liver abscesses, and to enhance feed efficiency. The use of antimicrobials may result in selective pressure that acts on the microbial community, selecting for resistance gene determinants and antimicrobial-resistant bacteria (AMR) that may be residents in the bovine microflora (Cameron

and McAllister, 2016b). Although the microbiota includes many harmless bacteria, it also contains opportunistic pathogens that may acquire resistance genes via horizontal gene transfer (Soucy et al., 2015). These resistant pathogens can inhibit the prevention and treatment of infectious diseases, and are involved in the transmission of AMR genes to bovine-associated human pathogens, potentially causing a public health concern (Cameron and McAllister, 2016b). Pathogens such as *Campylobacter, Salmonella, E. coli* and *Enterococcus spp.* are typically studied in relation to AMR because they are of importance to human disease via transfer through the food chain. Focus on these enteric pathogens is due to them being easy to culture and isolate and having an established AMR minimum inhibitory concentration breakpoints for human infections (Cameron and McAllister, 2016a). However, the development of AMR is a highly complicated process and some species acquire and are able to maintain resistance more easily than others (McEwen and Fedorka-Cray, 2002). Two conditions are needed for AMR to develop, first, the organism must come into contact with the antimicrobial, then resistance against the agent must develop along with the mechanism to transfer resistance to other organisms (Khachatourians, 1998). Once this resistance is developed it can easily be transmitted between animals via the movement and commingling of infected animals (McEwen and Fedorka-Cray, 2002).

Beef Industry Use of Antimicrobials

Antimicrobials are used for therapeutic practices for treatment of infection or non-therapeutic treatment including growth promotion. Antimicrobials used to control an anticipated disease outbreak are classified as prophylaxis or metaphylaxis treatments. Prophylaxis and metaphylaxis are classified by the US Food and Drug Administration (FDA) as therapeutic drug uses where prophylaxis is used to prevent disease by administration of antimicrobial to an

individual animal; and metaphylaxis, typically applied to high-risk, newly received beef calves, refers to the treatment of a larger group or entire herd to provide therapy to infected animals or those susceptible to disease (Morton, 1989; McEwen and Fedorka-Cray, 2002; Cameron and McAllister, 2016a). Antimicrobial growth promoters are administered for an extended duration to improve feed efficiency and have the ability to enhance the immune system of animal receiving treatment by affecting hormones, cytokines and other immune factors (Cunningham-Rundles et al., 2000). Research suggests that although metaphylatic approaches may expose more bacteria to antimicrobial selection pressure, they may also reduce pathology and eliminate pathogens more effectively than therapeutic approaches (Zaheer et al., 2013).

Antimicrobial Alternatives

Alternatives to antimicrobials are becoming more important as AMR concerns arise, but the use of alternatives such as direct-fed microbials (DFM) can be challenging because of the complexity of the gastrointestinal tract (GIT) ecosystem (Allen et al., 2013). The challenge exists as the microbiota of the GIT compete with intestinal pathogens for nutrients and binding sites, produces chemical modulators of intestinal health and influences immune maturation. A healthy microbiota is important to animal health as the microbiota modulates innate immune responses to prevent barrier dysfunction and regulates the function of adaptive immune mediators (Artis, 2008).

The use of feed additives, such as DFM have been used as an antimicrobial alternative because of their ability to modulate the gut microbiota to benefit the health of the animal. Examples of living cells used in DFM are *Lactobacillus, Streptococcus, Bifidobacterium, Bacillus* and yeasts (Dunne et al., 2001). The DFM strains utilized have important traits including being nonpathogenic, resistant to stomach acids and bile, having potential to colonize

the host, production of nutrients, and being free of antibiotic resistance genes or having reduced gene transfer functions making them useful alternatives to antimicrobials (Allen et al., 2013).

Immunostimulants

The innate immune system plays a crucial role in the health of the animal by providing defense against infectious agents (Blecha, 2001). The ability to positively modify the innate immune response via stimulation of neutrophils, macrophages and natural killer cells to provide immediate defense against infection is the primary objective of immunomodulation; and the substances that exert this control are called immunostimulants (Blecha, 2001; Nickell, 2016). Over the past 20 years, numerous studies have been conducted on the use of immunostimulants in livestock but only 2 immunostimulants have been approved by either the FDA or USDA for use in beef cattle (FDA, 2018). Pegbovigrastim (Imrestor, Elanco Animal Health) is FDA approved, labeled as an immunomodulatory agent, and is a prescribed product. Whereas, another product (Zelnate, Bayer Animal Health) is USDA regulated, approved and labeled as a DNA immunostimulant (APHIS, 2011).

Within the animal, innate immune cells are activated to fight infection and produce cytokines and co-stimulatory molecules that are necessary for proper T and B cell activation, which mediate the adaptive immune response (Lippolis, 2008). The adaptive immune system provides memory that allows the host to more effectively defend against the same antigen encountered in the future. It has been recognized that immunostimulants are often natural or synthetic PAMPs and activate the innate immune system similar to a pathogen but without the possibility of infection (Nickell, 2016). These PAMPs bind to pathogen recognition receptors (PRR) on innate leukocytes. The binding of PAMP to PRR activates leukocytes to fight pathogens and stimulate the adaptive immune response (Lippolis, 2008). The use of an

immunostimulant administered to an animal at risk of infection, post-infection, or clinically ill has been shown to increase activation of the innate immune system and decrease BRD-associated pathology or clinical signs following experimental infection or natural challenge (Nickell, 2016).

DNA Immunostimulant

Zelnate was introduced in 2015 (Ilg, 2017) and is categorized as a DNA immunostimulant that is unique in exploiting the cytosolic DNA recognition pathways in cattle (Ilg, 2017). Zelnate contains an innovative cationic lipid delivery system combined with non-coding bacterial DNA rich in non-methylated CpG motifs, that is intended to stimulate the innate immune response in cattle (Nickell, 2016). Bacterial and viral DNA possess immunostimulatory potential, therefore qualifying them as PAMPs (Janeway, 1989) and cationic lipids that interact with PRR and activate innate immune cells similar to PAMPs. The first PRR to recognize DNA was type 1 transmembrane protein Toll-like receptor (TLR9; . Toll-like receptor-9 is present in dendritic cells, B cells, monocytes and macrophages (Mestas and Hughes, 2004) and recognition of DNA by TLR9 requires the presence of non-methylated CpG dinucleotides in a specific sequence (Hemmi et al., 2000). Zelnate specifically consists of cationic DOTIM-cholesterol liposomes that contain pMB75.6 plasmid DNA to activate the immune system. Cellular uptake and intracellular routing of plasmid DNA complexed with or enclosed by cationic lipid mixtures has been reported to involve endocytosis residence in endosome, and subsequent degradation or escape into the cytosol (Zabner et al., 1995; Lechardeur et al., 2005; Wasungu and Hoekstra, 2006; Duan et al., 2009). Within TLR9 expressing cells, Zelnate interacts with the CpG-DNA receptor in the endosome but also gains access to cytosolic DNA recognition pathways activating the innate immune response (Ilg, 2017).

The use of the DNA immunostimulant Zelnate is indicated for control of BRD due to *M. haemolytica* in cattle 4 months of age or older at the time of, or within 24 hours after a perceived stress event (Nickell, 2016). Nickell et al. (2016) evaluated the efficacy and safety of DNA immunostimulant in a *M. haemolytica* challenge model and reported that the DNA immunostimulant significantly improved lung pathology when administered concurrently with, or 24 hours after *M. haemolytica* challenge when compared to control treatment. The use of DNA immunostimulant also decreased mortality among cattle administered ZEL relative to the CON group (Nickell, 2016). These results coincide with Rogers et al. (2016) who determined the administration of a DNA immunostimulant on arrival at the feedlot reduced total mortality at days 60, 116, and close out, resulting in a 22% reduction in overall death loss (Rogers et al., 2016). These findings are relevant given the high incidence of BRD in the beef industry and the negative impact it has on economic returns as well as ongoing efforts to identify non-antimicrobial alternative treatments for BRD.

Pegbovigrastim

Pegbovigrastim (Imrestor, Elanco Animal Health, Greenfield, IN) is an immunostimulant that contains recombinant granulocyte colony-stimulating factor (rbG-CSF) in a buffered sodium acetate solution, and is approved for use in dairy cows 7 days before and 24 hours after calving to help restore the bovine innate immune system by increasing the number of circulating neutrophils lost during the transition period (Health, 2016). Mastitis is recognized as a major cause of morbidity in dairy herds that can lead to reduced conception rates, lost milk production through lactation and increased risk of transmission (Santos et al., 2004; Wilson et al., 2004; Pantoja et al., 2009). Clinical trials report pegbovigrastim reduces the incidence of clinical mastitis by 35% (Hassfurther et al., 2015; Canning et al., 2017).

Mastitis is known to be associated with decreased polymorphonuclear neutrophil (PMN) and lymphocyte functions due to the increase in nutrient demand during calving (Kimura et al., 2014). Multiple studies have investigated the effects of pegbovigrastim injection and each resulted in a reduction of mastitis and increase in performance among dairy herds (Kimura et al., 2014; Canning et al., 2017; McDougall et al., 2017; Ruiz et al., 2017). Kimura et al. (2014) reported that cows injected with pegbovigrastim, 6 days before calving and 24 hours after calving had increased PMN counts, indicating an increase in the cows ability to defend against clinical disease during the periparturient period (Kimura et al., 2014). Canning et al. (2017) reported injection of pegbovigrastim reduced incidence of clinical mastitis by 35% among treated animals aligning with McDougal et al. (2017) who reported increased total WBC, PMN, lymphocyte, and monocyte counts after injection; with increases as early as one day after initial injection and 7 days following the second injection (Canning et al., 2017; McDougall et al., 2017). The use of pegbovigrastim has shown to reduce clinical mastitis while also reducing use of antimicrobial treatment for disease by increasing total PMN counts (Kimura et al., 2014).

Beef Quality Assurance (BQA): Injection Site Lesions

Beef Quality Assurance is a national program facilitated by the National Cattlemen's Beef Association and compelled by cattle producers who assume responsibility for producing beef that is a healthy, wholesome, quality product free from defects such as injection-site lesions and bruises. In the 1990's it was reported that one in five beef cattle rounds and one in three dairy cattle rounds had injection site lesions, costing over $9 million annually; however, this has been improved drastically (Roeber et al., 2002). In 1991, the incidence of injection site lesions was 21% and improved to 2.1% in 2000 and 0.5% in 2016 (NCBA, 2016). Although minimal research has been conducted; vaccines, antimicrobials, anthelmintics and vitamins injected into

muscle have been shown to cause injection site lesions and reduce tenderness of beef cuts depending on the calf's age, volume of product injected, anatomical site of injection, route of injection, and the product (Van Donkersgoed et al., 2000). Injection sites are important to producers and consumers because if given incorrectly it could lead to injection site lesions, causing tissue scaring due to irritation from the injection. Needle size, type of injection (s.c. or i.m.), type of vaccine, and injection site are all important to consider when processing or vaccinating cattle. Approval of injection sites is monitored by the FDA and the BQA depending on the injection. Injection sites are typically in the neck region "triangle" zone in beef cattle due to the head restraint availability and the RF in dairy cows and heifers (Roeber et al., 2002).

Beef Quality Assurance Guidelines

Current beef quality assurance guidelines recommend that the administration of animal health products should be "tissue friendly" and low volume; should be injected in the neck only and never top hip or thigh (Van Donkersgoed et al., 2000). To minimize lesions in the more valuable cuts of meat, BQA guidelines stipulate that injections should be given to cattle s.c. Previously, injections were primarily administered i.m. until it became an issue with meat quality. Many products are still administered in the semimembranosis, semitendinosis, and gluteal muscle of dairy cows due to the prevalent use of headlock systems as a management tool on many dairies, but are administered s.c. instead of i.m. (Holland et al., 2018). It has been stated that the provision of an acceptable injection site that can be accessed from the rear of the animal may improve BQA compliance (Holland et al., 2018).

Ischiorectal fossa

The RF has been demonstrated as one possible site for s.c. administration. Located next to the tailhead, the RF is a fat-filled, roughly triangular area between the ischium and rectum (Holland et al., 2018). An anatomical study of the RF concluded that injections given in the RF with a 1-inch needle were considered to be given s.c. (Holland et al., 2018). Considering the anatomy the injection should be performed in the center with the needle directed cranially (Holland et al., 2018). Holland et al. (2018) also evaluated the administration of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the RF to determine whether administration would result in a similar physiological response to an intramuscular injection. They concluded that injection of $PGF_{2\alpha}$ in the RF for synchronization of estrus and luteolysis did not differ from i.m. injection in the neck (Holland et al., 2018). However, it was suggested that possible chronic damage could occur to nerves and ligaments if many injection were given in the RF. These results coincide with Colazo et al. (2002) who conducted 3 experiments to investigate the RF as route of administration of $PGF_{2\alpha}$ and determined the RF was a simple, practical and useful site for the injection (Colazo et al., 2002).

Conclusions from the Literature

Bovine respiratory disease is the most common disease in the beef industry with a complex interaction between stressed-induced immunosuppression and viral and bacterial pathogens resulting in bronchopneumonia. The beef production and marketing system plays a vital role in initiating immunosuppression with the stressors of weaning, transportation, marketing, handling and processing of cattle. Immunosuppression provides opportunity for viral and bacterial pathogens to colonize and challenge the immune system of naïve calves. The activation of the innate immune system is critical in defense against pathogens. Antimicrobials are commonly used for BRD control and treatment; however, with the growing concern of

antimicrobial resistance, focus has shifted to using antimicrobial alternatives. Immunostimulants have shown to decrease BRD-associated mortality and may be a useful antimicrobial alternative as they stimulate the innate immune system to provide defense against infectious pathogens. It is important that injections follow guidelines provided by the BQA program to insure the health and safety of the animal and the consumer, but evidence suggest RF as a safe and effective alternative injection site. Further research is necessary to improve the understanding of the complex relationship of stress, disease, immune health, and antimicrobial use and its involvement with respiratory disease in the beef industry.

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Chapter II

Administration of a DNA immunostimulant does not mitigate bovine herpesvirus-1 recrudescence in dexamethasone challenged beef cattle

Abstract

The study objective was to determine the effect of a DNA immunostimulant on recrudescence of bovine herpesvirus-1 (BHV-1) after dexamethasone challenge in beef cattle. It was hypothesized that the DNA immunostimulant would mitigate stress-induced immunosuppression; thereby, reducing the incidence of BHV-1 recrudescence. Steers (n=10) and heifers (n=10; initial BW = 489 kg ± 57 kg) were stratified by pre-existing BHV-1 antibody titer, sex and initial BW and randomly assigned to treatment (n=4 pens/treatment; 2 or 3 animals/pen). Calves were administered 40 mg of dexamethasone i.v. at 0600 hour from day 0 to 2, 166-days subsequent to BHV-1 challenge with 1.0×10^8 plaque-forming units per nostril. On day 1 calves were administered 2 mL of DNA immunostimulant (Zelnate; ZEL) or sterile saline (CON). Whole blood was obtained via jugular venipuncture for complete blood count (CBC) analysis, BHV-1 specific antibody titers, and nasal swabs were collected to determine BHV-1 prevalence via virus isolation testing. Additionally, each animal received a SCR ear tag to measure activity and rumination minutes. A repeated measures mixed model was used to test effects of treatment, day and their interaction for CBC variables, BHV-1 antibody titers, and SCR activity and rumination; day was the repeated statement. Binomial virus detection data generated from nasal swab collection and nasal lesion presence was analyzed using Fisher's exact test via PROC FREQ. There was a treatment × day interaction for eosinophils ($P = 0.02$) and percent

eosinophils ($P = 0.03$). Eosinophils were greater ($P < 0.01$) for ZEL on d 3 and 6 post-dexamethasone challenge. On days 11 and 12, eosinophils for CON rebounded such that their concentration was greater than ZEL ($P < 0.01$). Lymphocytes, neutrophil and monocyte concentration did not differ ($P \geq 0.44$); however, a day effect ($P \leq 0.01$) existed such that each variable increased transiently after dexamethasone challenge. All cattle had BHV-1 present in a nasal swab sample on at least one sample day, with prevalence of BHV-1 in nasal swab samples being greatest on day 5 (80% positive; $P = 0.01$). However, no treatment differences were detected for BHV-1 prevalence in this study. Additionally, there was no treatment × day interaction ($P = 0.50$) or treatment effect ($P = 0.11$) observed for BHV-1 specific antibody titers from serum samples collected on day 0 and 12. However, a day effect ($P \leq 0.01$) was observed, as BHV-1 antibody titer increased with time after dexamethasone administration. There was a treatment × day interaction ($P \leq 0.01$) for hourly activity and a treatment × day interaction ($P = 0.05$) for daily activity. There was no treatment × day interaction ($P = 0.43$) for daily rumination minutes; however, there was a treatment ($P = 0.07$) and day ($P \leq 0.01$) effect with greater rumination minutes in CON animals on day 12. There was a treatment × day interaction ($P < 0.01$) for hourly rumination minutes that was greater for CON on hour 0800 and 2400. Results indicate the DNA immunostimulant altered eosinophil concentrations and impacted animal rumination and activity but did not mitigate BHV-1 recrudesce after dexamethasone challenge.

Introduction

The multi-factorial bovine respiratory disease (BRD) is the most common and costly disease in feedlot cattle, responsible for 70% of feedlot morbidity and 40% of feedlot mortality (Snowder et al., 2006; Taylor et al., 2010). Bovine respiratory disease involves a complex interaction between stress-induced immunosuppression and infectious viral and bacterial pathogens that result in bronchopneumonia (Galyean et al., 1999; Grissett et al., 2015). Infectious bovine rhinotracheitis virus (IBRV), also referred to as bovine herpesvirus-1 (BHV-1), is described as an acute, contagious, febrile infection of cattle characterized by the inflammation of the upper respiratory tract and trachea and accompained by nasal discharge and nasal lesions (McKercher, 1964; Yates, 1982). Latency is a unique property of BHV-1 in which the initial infection is followed by persistent infection of the viral agent for life (Stevens, 1978). Latent BHV-1 can be reactivated following the treatment of the synthetic glucocorticoid, dexamethasone, which has been used in previous research to stimulate stress-induced immunosuppression in cattle (Lippolis, 2008), where BHV-1 can recrudese and be transmitted (Turin et al., 1999).

Immunostimulants have the ability to modify the immune response to benefit the health and production of an animal. Zelnate (Zelnate, ZEL; Bayer HealthCare, Shawnee Mission, KS), the first commerical DNA immunostimulant was approved for use in beef cattle in 2015 to aid in the treatment of BRD due to *Mannheimia haemolytica* in cattle at least 4 months old when administerd at the time of, or within 24 hours after a percieved stressed event. Zelnate is unique as it contains an innovative catonic lipid delivery system combined with non-coding bacterial deoxyribonucleic acids (DNA) that is intended to stimulate the innate immune response in cattle (Nickell, 2016). Zelnate's liposome outer layer protects the DNA as it is engulfed by phagocytes.

The DNA has a pathogen associated molecular pattern (PAMP) that the phagocyte recognizes as forgein and allows the PAMP to attach to TLR9, the only receptor known in cells of the innate immune system that recognize sequence elements in DNA. This attachment activates the immune cell to release cytokines that stimulate leukocytes to resolve infection (Nickell, 2016), but the efficacy of Zelnate to control stress-induced BHV-1 recrudescense is unclear. Therefore, the study objective was to determine the effect of a DNA immunostimulant on recrudescence of BHV-1 after dexamethasone challenge of previously infected beef calves.

Materials and Methods

The study was conducted from March 2018 to April 2018, over a 12 d period, at the West Texas A&M University (WTAMU) Research Feedlot in Canyon, TX. Cattle were received from USDA-ARS Livestock Issues Research Unit near Lubbock, TX succeeding research involving a bovine herpesvirus-1 (BHV-1) challenge with 1.0×10^8 plaque-forming units per nostril 166 days subsequent to the study being conducted at WTAMU. Animal procedures and experimental protocols were approved by the animal care and use committee at WTAMU (protocol number 01-02-18).

Animals and Treatments

On day -1, steers (n = 10) and heifers (n = 10; initial BW = 489 kg ± 57 kg) were stratified by pre-existing BHV-1 titer, sex and initial body weight and randomly assigned to treatment (n = 4 pens/treatment; 2 or 3 animals/pen). The treatments consisted of: 1) Control (CON) with administration of 2 mL sterile saline injection; or 2) DNA immunostimulant (ZEL) with 2 mL injection of Zelnate, both administered intramuscularly. On days 0, 1, and 2, cattle were administered 40 mg of dexamethasone (Dexasone; Aspen Veterinary Resources, Location;

DEX) i.v. at 0600 hour with designated treatments administered on day 1. One heifer from ZEL treatment died on day 8 and necropsy findings indicated the cause of death to be bronchopneumonia. Heifers were fed 0.48 mg/head/day of melengestrol acetate (Add product info and manufacturer) for the duration of the study until slaughter.

Blood Collection and Serology

Blood was collected daily from day 0 to 12 at 0600 h via jugular venipuncture into a 4 mL evacuated tube containing EDTA (BD Vacutainer K2EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ). These samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) at the WTAMU Animal Health Laboratory to determine red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), white blood cell count (WBC), and concentrations of neutrophil (NEU), lymphocyte (LYMPH), monocyte (MONO), eosinophils (EOS), basophil (BASO), and neutrophil percent (NEU%), lymphocyte percent (LYMPH%), monocyte percent (MONO%), eosinophil percent (EOS%), and basophil percent (BASO%).

Additionally, jugular blood was collected into a 10 mL evacuated tube without additive (BD Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) to harvest serum used to determine BHV-1-specific antibody titer. Samples were placed in an insulated cooler after sample collection without ice to achieve storage temperature of approximately 20ºC and transported to the WTAMU Animal Health Laboratory. Samples were allowed to clot ≥ 30 min before centrifugation at 1,500 × *g* for 20 minutes at 20ºC. After centrifugation, serum was harvested and stored in duplicate aliquots at -20ºC until subsequent analyses. One aliquot of frozen sera from d 0 and 12 was packaged on ice and transported to the Texas A&M Veterinary

Medical Diagnostic Laboratory (TVMDL) located in Amarillo, TX, to determine IBRV specific antibody titers using the virus neutralization assay as described by Rosenbaum et al. (1970).

Nasal Swab Collection and Virus Detection

Nasal swab specimens were collected daily from days 0 to 12 by inserting 1 nylon-flocked swab (HydraFlock; Puritan Medical Products, Guilford, ME) in the mid-naris region and rotating until the swab was completely saturated. Nasal swabs were clipped, placed into a sterile polystyrene tube (Falcon; Corning, Inc., Corning, NY) with preservative (500 ML premade minimum essential media, 10 ML penicillin/streptomycin solution, 2 ml ciprofloxacin, and 4 ml amphotericin B), sealed, and stored at -80ºC until subsequent analysis. Swabs were transported on ice to TVMDL in Amarillo, TX to detect the presence of IBRV via virus isolation assay (Hierholzer et al., 1996). In addition to nasal swab collection, a photograph was taken of each naris from day 0 to 12 and evaluated to determine the presence of nasal lesions.

Behavior and Rectal Temperature

Each animal received an ear tag containing a 3-axis accelerometer (Allflex Livestock Intellegence, Madison, WI) to monitor animal behavior and rumination activity. Data was recorded in real-time and transmitted via Wi-Fi to computer software housed on site. Rectal temperature was recorded from days 0 to 12, using a digital thermometer (GLA Agriculture Electronics, San Luis Obispo, CA).

Statistical Analyses

This completely randomized design experiment used animal as the experimental unit for all analyses of dependent variables. Data analyzed from CBC variables, BHV-1 antibody titers, rectal temperature, and activity and rumination values were analyzed using the MIXED

procedure of SAS (SAS inst. Inc., Cary, NC) with repeated measures. The model for these variables included effects of treatment, day and treatment × day interaction. The repeated statement was day, and the covariance structure with the lowest Akaike information criterion for each dependent variable was used. Complete blood count variables were tested for normal distribution using the UNIVARIATE procedure and nonparametric data were log_2-transformed and again tested for normal distribution; if log_2 transformation improved normality, the log-transformed data were statistically analyzed, and back-transformed means were subsequently generated and shown. Differences of least square means were determined using the PDIFF option in SAS, and Tukey adjustment was made for behavior variables. Statistical significance was established for treatment, day, and treatment × day effects if a resulting *P*-value was ≤ 0.05. If a significant treatment × day interaction existed for a repeated variable, treatment mean comparisons within day were evaluated and considered statically significant for a given *P*-value ≤ 0.05. Binomial virus detection data generated from nasal swab collection and naris photographs of lesions, were analyzed using the Fisher's Exact test via PROC FREQ in SAS. The frequency of BHV-1 positive nasal swabs and nasal lesions were determined within day with significance established if a *P*-value was ≤ 0.05.

Results and Discussion

Complete Blood Count

There was a treatment × day interaction ($P = 0.02$) for EOS and PEREOS ($P = 0.03$). Eosinophils were greater ($P < 0.01$) for ZEL on day 3 and 6 post-DEX challenge. On day 11 and 12, EOS for CON rebounded such that their concentration was greater than ZEL ($P < 0.01$, Figure 3.1), suggesting that the administration of a DNA immunostimulant could have prevented

eosinopenia. Administration of DEX has been reported to decrease EOS (Roth, 1985; Anderson et al., 1999; Hughes et al., 2017; Hudson, 2018) concentration in cattle blood resulting in eosinopenia. Furthermore, research suggests that calves with lower eosinophil concentrations upon arrival at a stocker facility are at a higher risk for BRD and greater eosinophil concentrations may help resolve inflammation (Isobe et al., 2012; Richeson et al., 2013). Remaining CBC variables did not differ due to treatment × day or treatment ($P \geq 0.44$); however, a day effect ($P \leq 0.01$) existed such that WBC, NEU and MONO increased and LYMP decreased transiently after DEX challenge (Figures 3.2 to 3.7; Table 3.1). Table 3.1 demonstrates individual leukocyte variables by treatments indicating eosinophil interaction and day effects. These results are in agreement with other studies in cattle that demonstrated leukocytosis following DEX administration (Lan et al., 1995; Anderson et al., 1999; Thanasak et al., 2004; Hughes et al., 2017). Table 3.2 demonstrates contrast effects of DNA immunostimulant administration were tested for hematolytic variable from day 0 to 12. There was a linear effect for MCV ($P < 0.01$), MCH ($P <0.01$), PLT ($P <0.01$), WBC ($P <0.01$), NEU ($P <0.01$), LYM ($P = 0.03$), MONO ($P <0.01$), EOS ($P = 0.03$), NEU% ($P <0.01$), LYM% ($P <0.01$), MONO% ($P <0.01$) and EOS% ($P <0.01$) variables. There was a quadratic effect for HGB ($P = 0.03$) and MCH ($P = 0.05$) variables; and no effect of day ($P \geq 0.21$) for BASO and BASO% variables.

Virus Detection in Nasal Swabs

The prevalence of BHV-1 in nasal swab specimens collected from cattle from day 0 to 12 is shown Table 3.3. No BHV-1 was detected in nasal specimens on day 0 to 4. On day 4 virus was isolated from both CON and ZEL calves with 65% of calves testing positive; the greatest overall rate of BHV-1 isolation was day 5 with 80% positive for BHV-1 present in nasal swabs (Table 3.4). However, there was no treatment difference in BHV-1 isolation detected for any of

the days ($P \geq 0.35$). By day 10, no BHV-1 was isolated from nasal swab samples for the remaining sample days. These results align with the BHV-1 incubation period of 2 to 6 days and typically shedding of the virus in nasal secretions for 10-16 days after initial infection (Caswell, 2016). It should be noted that all cattle were positive for BHV-1 on at least on sample day. This could be contributed by the experimental design and housing as BHV-1 could have been transmitted from infected animal to non-infected animal due to natural transmission. To reduce risk of transmission of the virus, each time a new animal entered the chute chlorhexidine was sprayed. Prevalence of BHV-1 in nasal secretions suggests that immunosuppression induced by DEX administration caused recrudescence, replication and shedding of BHV-1. This could potentially cause challenges in the feedlot as cattle arriving could have exposure to the virus before entering the feedlot; and if exposed to stress induced immunosuppression the virus may recrudesce causing a decrease in health and performance of the animal and possible transmission to other animals.

Nasal Lesions

The prevalence of observed BHV-1 nasal lesions are shown in Table 3.5 and Table 3.6**.** Presence of nasal lesions was first detected on day 2 and remained present through the duration of the study with the greatest prevalence on day 9 with 79% of cattle having visible nasal lesions. Similar to BHV-1 isolation results, all cattle showed signs of nasal lesions on at least one sample day; however, there was no treatment difference ($P \geq 0.17$).

BHV-1 Specific Antibody Response

There was not a treatment × day interaction ($P = 0.50$) or treatment effect ($P = 0.11$) observed from serum samples from day 0 and 12 (Figure 3.8). A day effect ($P \leq 0.01$) was

observed; as expected BHV-1 antibody titer increased with time after DEX-induced recrudescence of BHV-1. The DEX challenge model used in the current study may cause a greater severity of immunosuppression than immunosuppression induced by natural stress conditions (Richeson et al., 2016). However, in previous research evaluating replicating viruses, natural stress enhanced viral-bacterial synergy and recrudescence of BHV-1 (Hodgson et al., 2005).

SCR Tag Rumination and Activity

Daily and hourly rumination and activity was recorded for the duration of the study in 2 hour increments. There was a treatment × day interaction ($P \leq 0.01$) for hourly activity (Figure 3.9) with no treatment difference within any given hour. There was also a treatment × day interaction ($P = 0.05$) for daily activity (Figure 3.10); however there was no treatment differences within day. There was no treatment × day interaction ($P = 0.43$) for daily rumination minutes; however, there was a treatment ($P = 0.07$) and day ($P \leq 0.01$) effect with greater rumination minutes in CON animals on day 12 (Figure 3.11). There was a treatment × day interaction ($P < 0.01$) for hourly rumination minutes greater for CON on hour 0800 and 2400 (Figure 3.12). These results could be due to a low replication in the experimental design or to the impact the DNA immunostimulant had on pro inflammatory cytokine production and inflammation.

Rectal Temperature

There was no treatment × day interaction ($P = 0.69$) or treatment effect ($P = 0.18$) for rectal temperature (Figure 3.13). A day effect ($P \leq 0.01$) was observed, which was expected due to administration of dexamethasone. These results align with previous research that indicates the

anti-inflammatory effects of DEX administration inhibits the febrile response in cattle (Raekallio et al., 2005; Danek, 2006; Hughes et al., 2017). However, DNA immunostimulant did not alter rectal temperature ($P = 0.69$).

Conclusions

Results of the present study suggest longstanding BHV-1 recrudescence is induced by administration of 40 mg dexamethasone for 3 consecutive days as recrudescence of BHV-1 was found in all animals on at least one sample day, 6 months after initial BHV-1 challenge. The DEX challenge model appeared to cause stress-induced immunosuppression because LYM decreased and WBC, NEU, and MONO concentrations increased following DEX administration. Previous research indicates the use of Zelnate decreases overall mortality in calves administered Zelnate at feedlot arrival (Nickell, 2016; Rogers et al., 2016). However in the current study, the administration of DNA immunostimulant resulted in maintaining eosinophil concentration compared to control but did not mitigate BHV-1 recrudescence. Further research is warranted to elucidate the mode of action of the immunostimulant and determine its efficacy in different disease challenge models and production environments.

Table 3.1. Overall effect of DNA immunostimulant administration on hematology of beef cattle sampled daily from day 0 to d 12.

Item[1]	Treatment[2]			*P*-value[3]		
	CON	ZEL	SEM	TRT	Day	TRT × Day
Red Blood Cells, M/µL	9.60	10.13	0.33	0.27	< 0.01	0.33
Hemoglobin, g/dL	14.19	14.87	0.36	0.20	< 0.01	0.39
Hematocrit, %	43.82	45.16	1.16	0.43	< 0.01	0.34
Mean corpuscular volume, fL	45.97	44.69	1.22	0.43	< 0.01	0.81
Mean corpuscular hemoglobin, pg	14.87	14.73	0.33	0.75	< 0.01	< 0.01
Mean corpuscular hemoglobin concentration, g/dL	32.41	32.96	0.29	0.19	< 0.01	0.89
Platelet , K/µL	356.12	321.46	26.65	0.37	< 0.01	0.73
Whole Blood Count, K/µL	10.01	9.41	0.37	0.27	< 0.01	0.88
Neutrophil, K/µL	4.39	4.21	0.27	0.64	< 0.01	0.57
Lymphocyte, K/µL	4.24	3.88	0.23	0.30	< 0.01	0.67
Monocyte, K/µL	1.29	1.20	0.10	0.57	< 0.01	0.44
Eosinophil, K/µL	0.94	0.09	0.02	0.76	< 0.01	0.01
Basophil, K/µL	< 0.01	< 0.01	< 0.01	0.35	0.41	0.50
Neutrophil, %	40.08	40.68	2.02	0.83	< 0.01	0.52
Lymphocyte, %	45.20	44.92	1.88	0.92	< 0.01	0.51
Monocyte, %	13.65	13.30	0.78	0.76	< 0.01	0.70
Eosinophil, %	1.06	1.06	0.16	0.99	< 0.01	0.03
Basophil, %	0.01	0.03	0.01	0.24	0.32	0.47

[1] Complete blood count variables analyzed from Idexx, ProCyte Dx Hematology Analyzer.
[2] CON= Control, 2 mL saline administered i.m. on day 1.
ZEL= Zelnate, 2 mL, DNA immunostimulant administered i.m. on day 1.
[3] PROC MIXED with repeated measures was used to determine effects of DNA immunostimulant on hematological variables.

Table 3.2. Contrast effects of DNA immunostimulant administration on hematology of beef cattle sampled daily from day 0 to d 12.

	Day[4]														Contrast *P*-value		
Item[1]	0	1	2	3	4	5	6	7	8	9	10	11	12	SEM	Linear	Quadratic	Cubic
Red Blood Cells, M/µL	10.25^{e}	9.90^{bcde}	9.64^{ab}	9.28^{a}	9.70^{bc}	9.75^{bcd}	10.08^{cde}	10.13^{de}	9.88^{bcde}	9.81^{bcd}	9.97^{bcde}	10.04^{bcde}	9.81^{bcd}	0.27	0.38	0.15	< 0.01
Hemoglobin, g/dL	15.12^{e}	14.59^{bcde}	14.17^{ab}	13.61^{a}	14.25^{bc}	14.33^{bcd}	14.78^{cde}	14.87^{de}	14.56^{bcde}	14.45^{bcd}	14.77^{cde}	14.83^{cde}	14.61^{bcde}	0.33	0.15	0.03	< 0.01
Hematocrit, %	45.80^{bc}	44.39^{bc}	43.68^{ab}	42.10^{a}	43.40^{ab}	43.76^{ab}	45.19^{bc}	45.95^{c}	44.55^{bc}	44.05^{b}	45.12^{bc}	45.71^{bc}	44.67^{bc}	1.06	0.09	0.10	< 0.01
Mean corpuscular volume, fL	44.87^{a}	45.04^{ab}	45.50^{bc}	45.52^{bc}	44.95^{ab}	45.14^{ab}	45.03^{ab}	45.57^{bc}	45.31^{b}	45.15^{ab}	45.54^{bc}	45.87^{c}	45.77^{c}	0.80	< 0.01	0.08	0.01
Mean corpuscular hemoglobin, pg	14.79^{ab}	14.80^{b}	14.76^{ab}	14.74^{ab}	14.76^{ab}	14.78^{ab}	14.73^{a}	14.75^{ab}	14.79^{ab}	14.80^{b}	14.89^{c}	14.88^{c}	14.97^{d}	0.23	< 0.01	< 0.01	0.38
Mean corpuscular hemoglobin concentration, g/dL	32.99^{c}	32.88^{bc}	32.44^{ab}	32.39^{a}	32.86^{bc}	32.76^{bc}	32.75^{bc}	32.39^{a}	32.69^{b}	32.81^{bc}	32.76^{bc}	32.46^{ab}	32.75^{bc}	0.23	0.18	0.05	0.02
Platelet , K/µL	254.45^{a}	271.35^{ab}	260.75^{a}	261.00^{a}	347.15^{b}	331.15^{b}	317.25^{ab}	332.05^{b}	364.67^{b}	434.43^{c}	458.25^{c}	384.29^{bc}	387.51^{bc}	29.97	< 0.01	0.42	0.04
Whole Blood Count, K/µL	9.76^{c}	13.89^{d}	15.35^{e}	14.53^{de}	9.45^{bc}	7.41^{a}	7.51^{a}	7.37^{a}	7.34^{a}	7.84^{ab}	8.23^{ab}	8.55^{b}	8.99^{bc}	0.45	< 0.01	< 0.01	≤0.01
Neutrophil, K/µL	1.90^{a}	8.70^{d}	9.86^{e}	8.52^{d}	5.17^{c}	3.27^{b}	2.86^{ab}	1.88^{a}	2.03^{ab}	2.50^{ab}	3.03^{b}	3.04^{b}	3.17^{b}	0.43	< 0.01	0.07	≤0.01
Lymphocyte, K/µL	6.32^{c}	3.56^{ab}	3.93^{ab}	4.63^{b}	3.43^{ab}	3.20^{a}	3.49^{ab}	4.00^{ab}	3.84^{ab}	3.95^{ab}	3.90^{ab}	4.13^{b}	4.40^{b}	0.34	0.03	< 0.01	≤0.01
Monocyte, K/µL	1.34^{c}	1.62^{d}	1.55^{d}	1.31^{c}	0.78^{a}	0.85^{a}	1.07^{b}	1.41^{cd}	1.33^{c}	1.29^{c}	1.20^{bc}	1.24^{c}	1.23^{bc}	0.09	< 0.01	< 0.01	< 0.01
Eosinophil, K/µL	0.20^{c}	0.01^{a}	0.02^{ab}	0.07^{ab}	0.07^{ab}	0.09^{b}	0.09^{b}	0.07^{ab}	0.12^{bc}	0.08^{b}	0.08^{b}	0.11^{bc}	0.17^{c}	0.03	0.03	< 0.01	0.09
Basophil, K/µL	<0.01	<0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.96	0.27	0.87
Neutrophil, %	19.48^{a}	62.53^{d}	64.13^{d}	59.11^{cd}	53.18^{c}	43.88^{b}	36.28^{b}	25.84^{ab}	26.73^{ab}	29.87^{b}	34.48^{b}	34.43^{b}	35.00^{b}	3.08	< 0.01	0.12	≤0.01
Lymphocyte, %	64.81^{d}	25.75^{a}	25.56^{a}	31.43^{ab}	37.74^{b}	43.69^{bc}	47.92^{c}	53.94^{c}	53.29^{c}	52.4^{c}	49.91^{c}	49.82^{c}	49.55^{c}	2.80	< 0.01	0.04	≤0.01
Monocyte, %	13.66^{c}	11.60^{b}	10.12^{ab}	9.07^{a}	8.38^{a}	11.26^{b}	14.43^{c}	19.26^{e}	18.40^{d}	16.64^{d}	14.49^{c}	14.36^{c}	13.52^{c}	0.85	< 0.01	0.04	< 0.01
Eosinophil, %	2.06^{c}	0.10^{a}	0.14^{a}	0.39^{ab}	0.69^{ab}	1.18^{bc}	1.35^{bc}	0.89^{b}	1.55^{c}	1.05^{bc}	1.09^{bc}	1.35^{bc}	1.91^{c}	0.25	< 0.01	≤ 0.01	< 0.01
Basophil, %	<0.01^{a}	0.02^{ab}	0.06^{b}	0.01^{ab}	0.02^{ab}	0.01^{ab}	0.02^{ab}	0.08^{b}	0.02^{ab}	0.02^{ab}	0.02^{ab}	0.03^{ab}	0.01^{ab}	0.02	0.83	0.21	0.78

[1] Complete blood count variables analyzed from Idexx, ProCyte Dx Hematology Analyzer.

[2] CON= Control, 2 mL saline administered i.m. on day 1.

ZEL= Zelnate, 2 mL, DNA immunostimulant administered i.m. on day 1.

[3] PROC MIXED with repeated measure and orthogonal contrast was used to determine effects of immunostimulant on hemolytic variables.

[4abcde] Means within a row without a common superscript differ $P \leq 0.05$.

Table 3.3. Prevalence of BHV-1 isolated from nasal swabs by day and treatment in beef cattle administered 40 mg of dexamethasone/animal i.v. on day 0, 1 and 2.

Item	Treatment		
Day	CON	ZEL	*P*-value[2]
0	0 of 10	0 of 10	-
1	0 of 10	0 of 10	-
2	0 of 10	0 of 10	-
3	0 of 10	0 of 10	-
4	7 of 10	6 of 10	1.00
5	7 of 10	9 of 10	0.58
6	2 of 10	5 of 10	0.35
7	2 of 10	3 of 10	1.00
8	3 of 10	1 of 9	0.58
9	1 of 10	1 of 9	1.00
10	0 of 10	0 of 9	-
11	0 of 10	0 of 9	-
12	0 of 10	0 of 9	-

[1]CON= Control, 2 mL saline administered i.m. on day 1. ZEL= Zelnate, 2 mL, DNA immunostimulant administered i.m. on day 1.
[2]Firsher's exact test was used to determine the probability of treatment effect within day.

Table 3.4. Prevalence of BHV-1 isolated from nasal swabs by day in beef cattle administered 40 mg dexamethasone/ animal i.v. on day 0, 1 and 2.

Day	Positive BHV1 Isolation, %[1]
0	0.0
1	0.0
2	0.0
3	0.0
4	65.0
5	80.0
6	35.0
7	25.0
8[2]	21.1
9	10.5
10	0.0
11	0.0
12	0.0

[1]Percent of positive BHV1 virus isolation, n=20.

[2]Percent of positive BHV1 virus isolation, n=19.

Table 3.5. Presence of BHV-1 nasal lesions by day in beef cattle administered 40 mg/animal of dexamethasone/animal i.v. on day 0, 1 and 2.

Day	Positive BHV1 Isolation[1,3]
0	0.00
1	0.00
2	5.00
3	5.00
4	30.00
5	75.00
6	75.00
7	75.00
8[2]	73.68
9	78.95
10	57.89
11	47.37
12	52.63

[1]Percent of positive BHV1 virus isolation, n=20.

[2]Percent of positive BHV1 virus isolation, n=19.

Table 3.6. Presence of BHV-1 nasal lesions by day and treatment in beef cattle administered 40 mg of dexamethasone/animal i.v. on day 0, 1 and 2.

Item	Treatment		
Day	CON	ZEL	*P*-value[2]
0	0 of 10	0 of 10	-
1	0 of 10	0 of 10	-
2	0 of 10	1 of 10	-
3	0 of 10	1 of 10	-
4	3 of 10	3 of 10	-
5	6 of 10	9 of 10	0.30
6	7 of 10	8 of 10	-
7	7 of 10	8 of 10	-
8	3 of 10	7 of 9	-
9	8 of 10	7 of 9	-
10	4 of 10	7 of 9	0.17
11	4 of 10	5 of 9	0.66
12	4 of 10	6 of 9	0.37

[1]CON = Control, 2 mL saline administered i.m. on day 1.
ZEL= Zelnate, 2 mL, DNA immunostimulant administered i.m. on day 1.
[2]Fisher's exact test in SAS was used to determine effect of treatment within day.

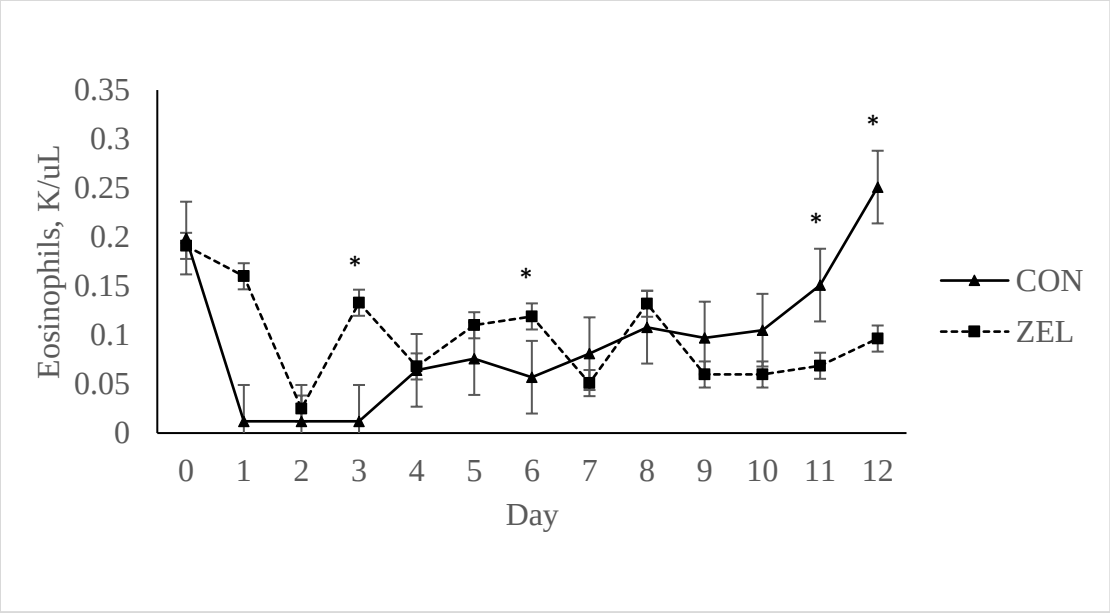

Figure 3.1. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on Eosinophil concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment × day ($P < 0.01$), treatment ($P < 0.01$), and day ($P < 0.01$). *Means within day differ ($P < 0.01$).

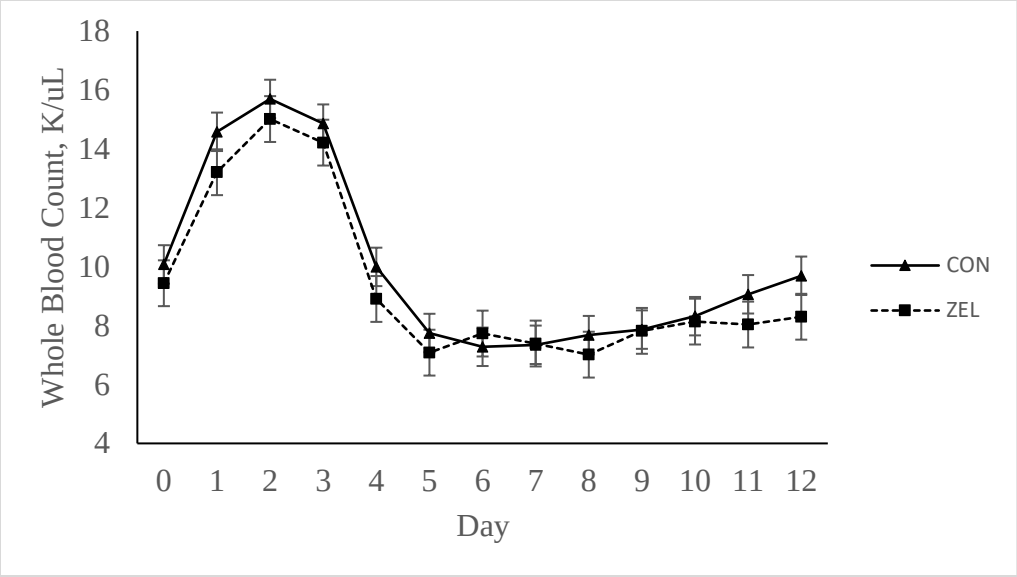

Figure 3.2. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on whole blood count concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment ($P = 0.27$), day ($P < 0.01$) and treatment × day ($P = 0.88$).

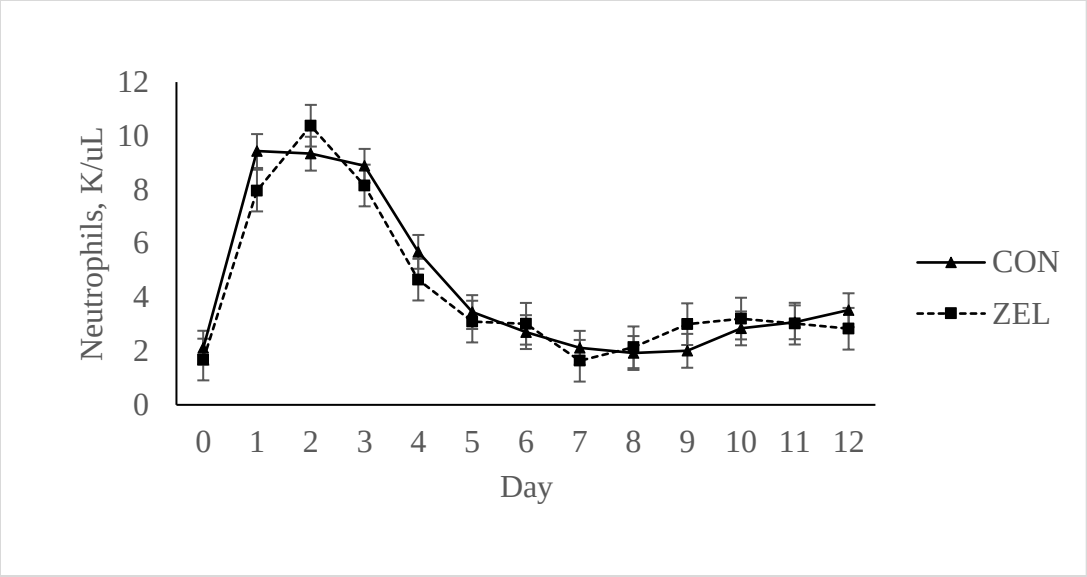

Figure 3.3. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on neutrophil concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment ($P = 0.64$), day ($P < 0.01$) and treatment × day ($P = 0.57$).

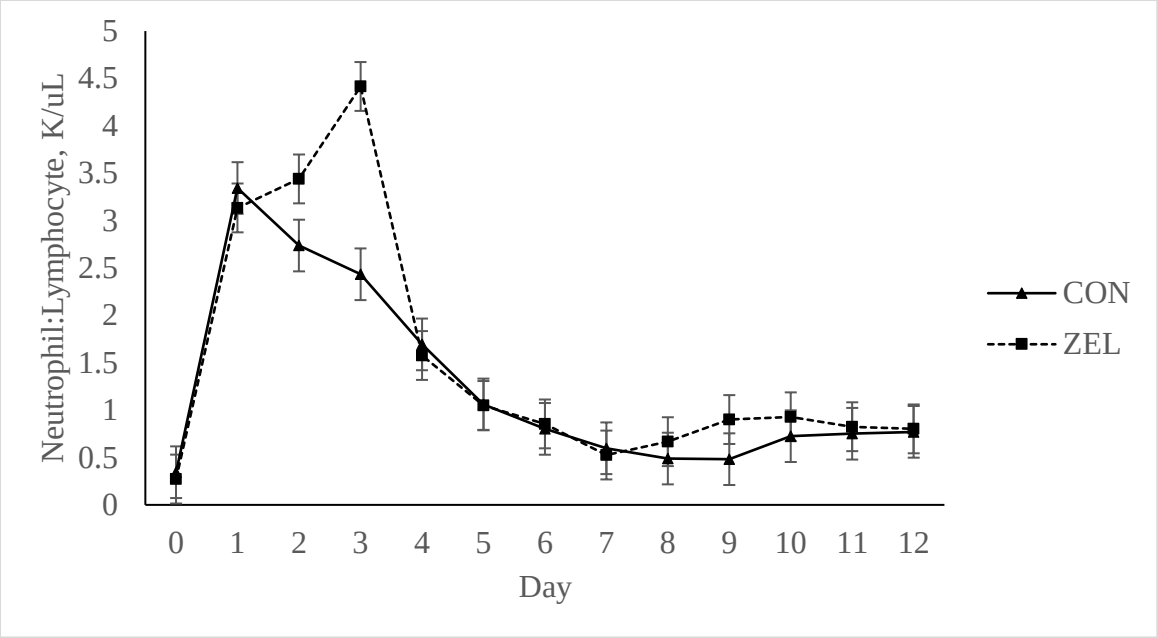

Figure 3.4. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on neutrophil:lymphocyte after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Blood was collected daily at 0600.Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment (P = 0.53), day ($P < 0.01$) and treatment × day (P = 0.89).

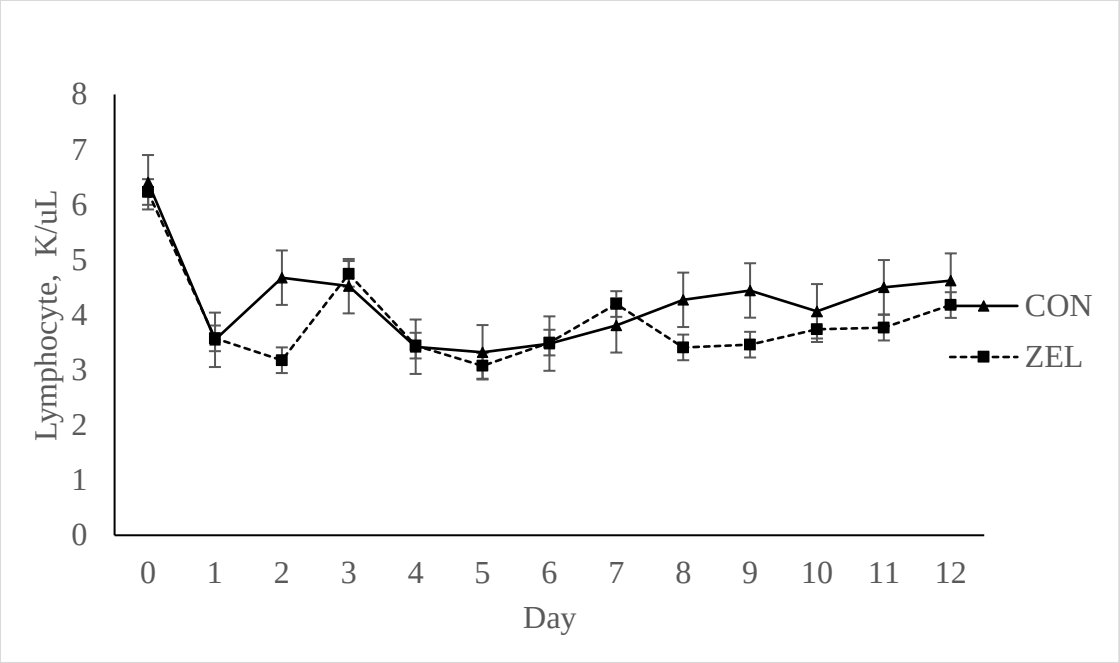

Figure 3.5. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on lymphocyte concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment ($P = 0.29$), day ($P < 0.01$) and treatment × day ($P = 0.67$).

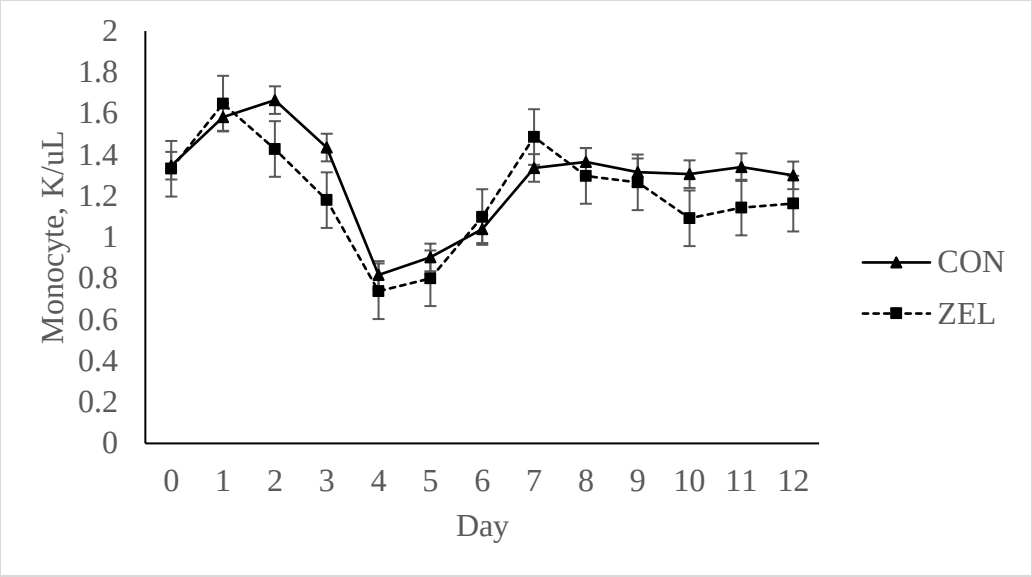

Figure 3.6. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on monocyte concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment ($P = 0.56$), day ($P < 0.01$) and treatment × day ($P = 0.44$).

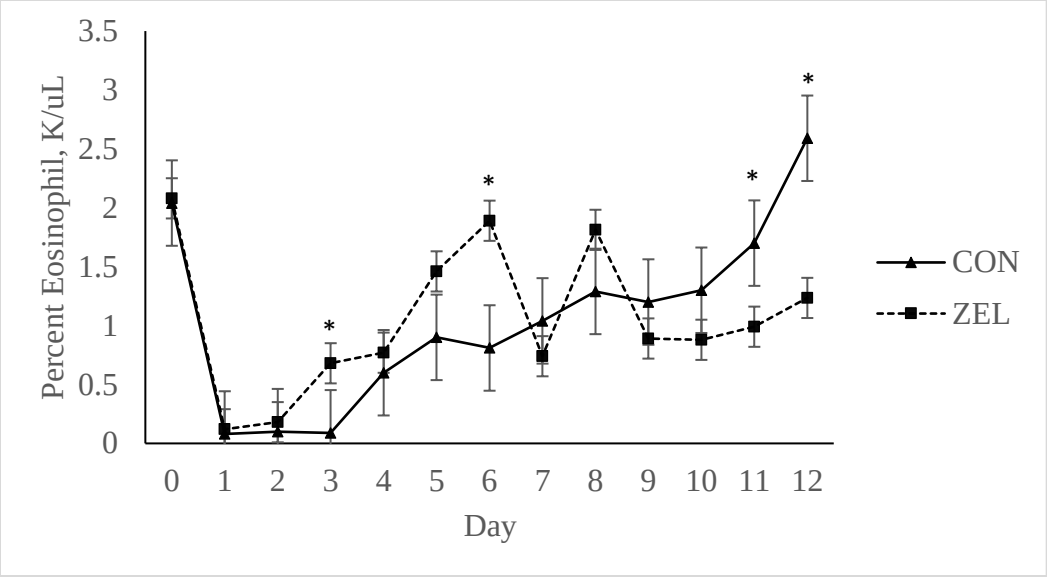

Figure 3.7. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on percent eosinophil concentration after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Samples were analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) for complete blood count analysis. Effect of treatment ($P = 0.99$), day ($P < 0.01$) and treatment × day ($P = 0.03$). *Means within day differ ($P < 0.01$).

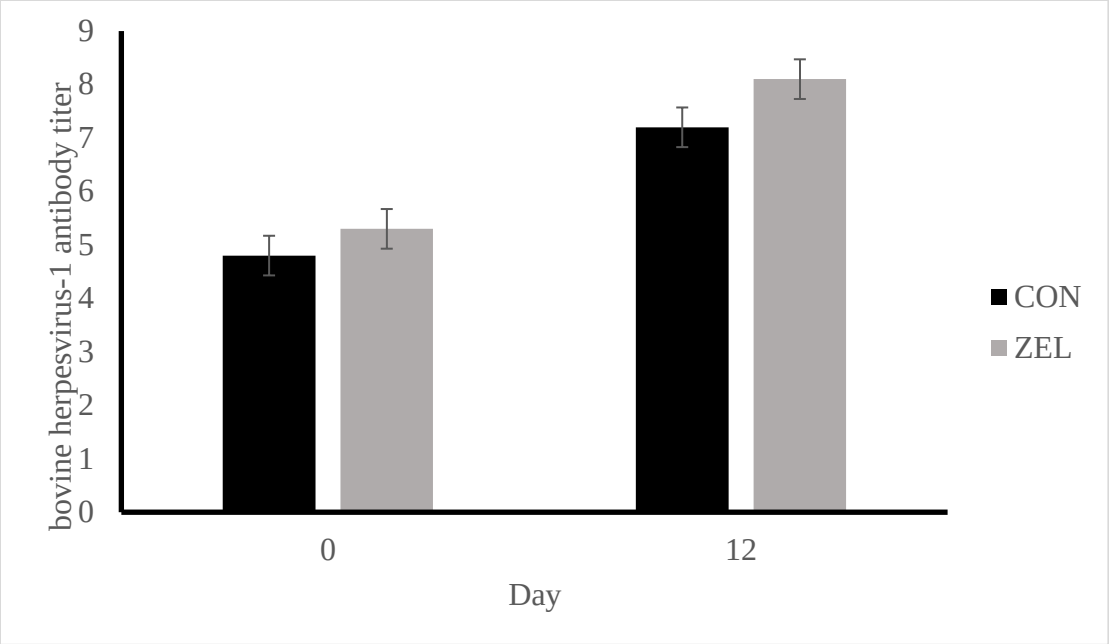

Figure 3.8. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on bovine herpesvirus-1 antibody titer after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Effect of treatment ($P = 0.11$), day ($P < 0.01$) and treatment × day ($P = 0.50$).

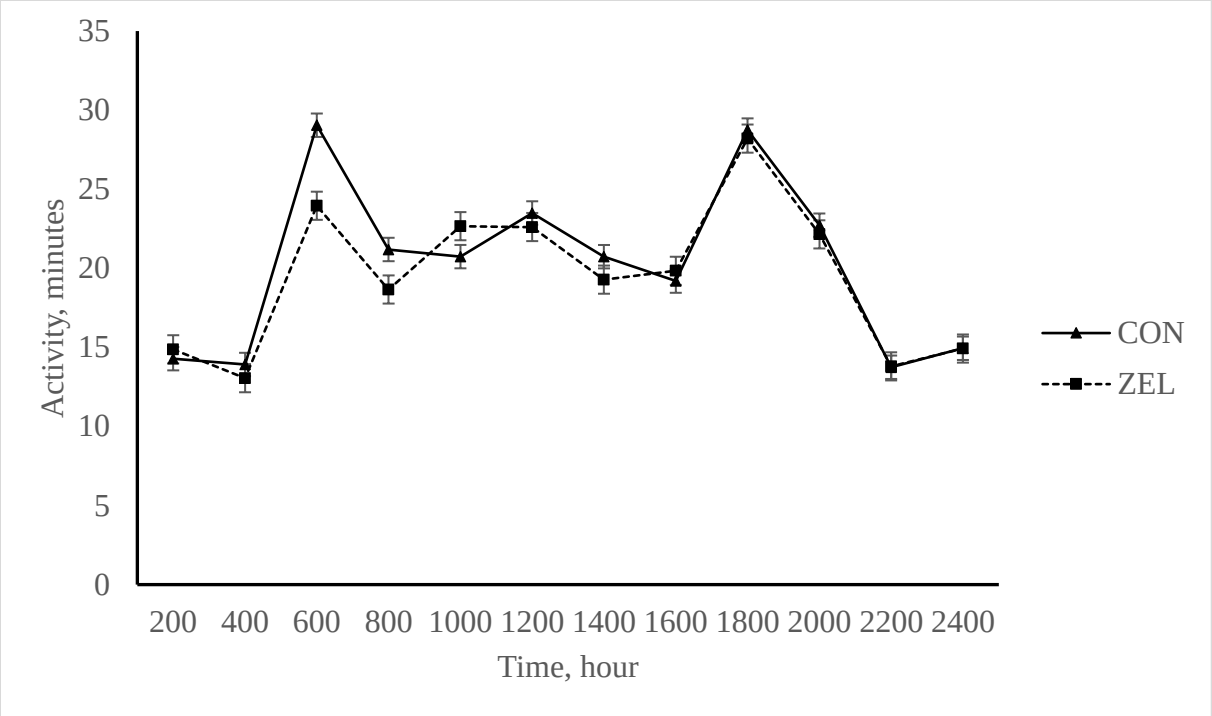

Figure 3.9. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on hourly active minutes after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Effect of treatment ($P = 0.02$), day ($P < 0.01$) and treatment × day ($P = 0.0001$).

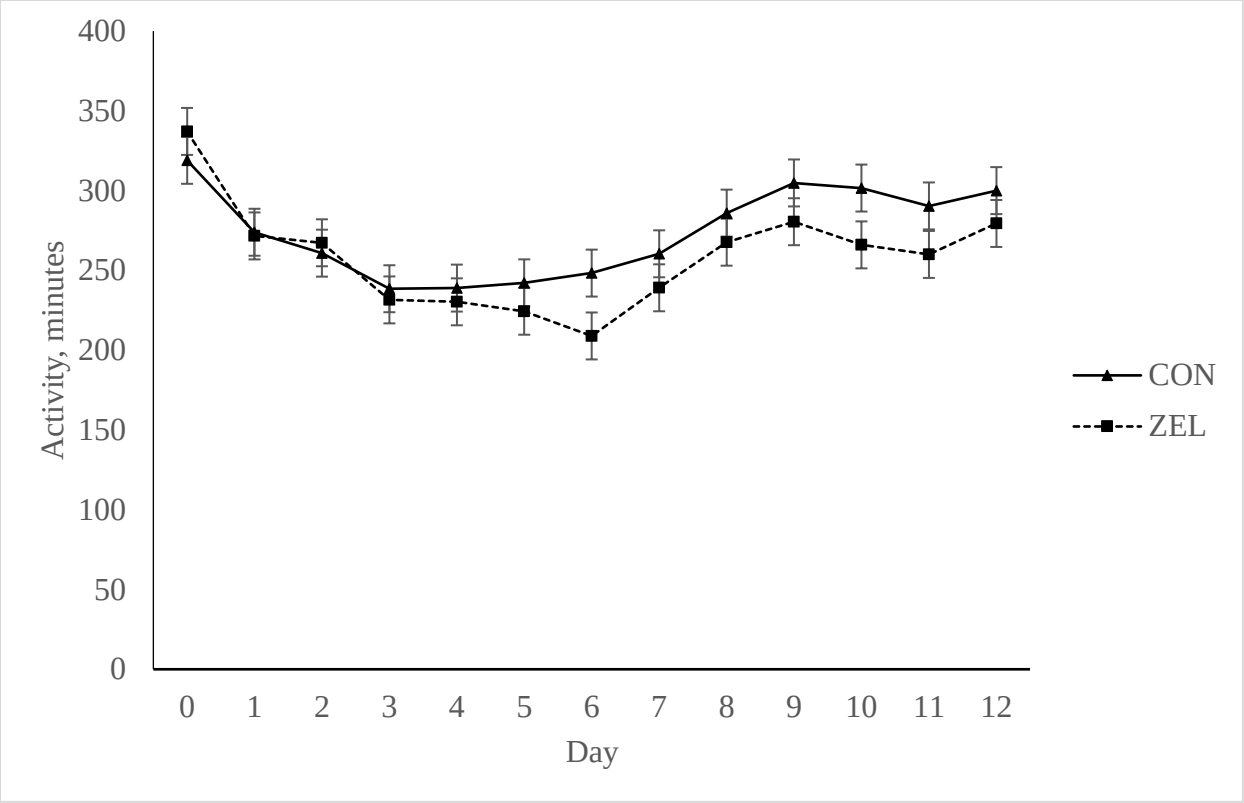

Figure 3.10. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on average daily active minutes dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Effect of treatment ($P = 0.38$), day ($P < 0.01$) and treatment × day ($P = 0.05$).

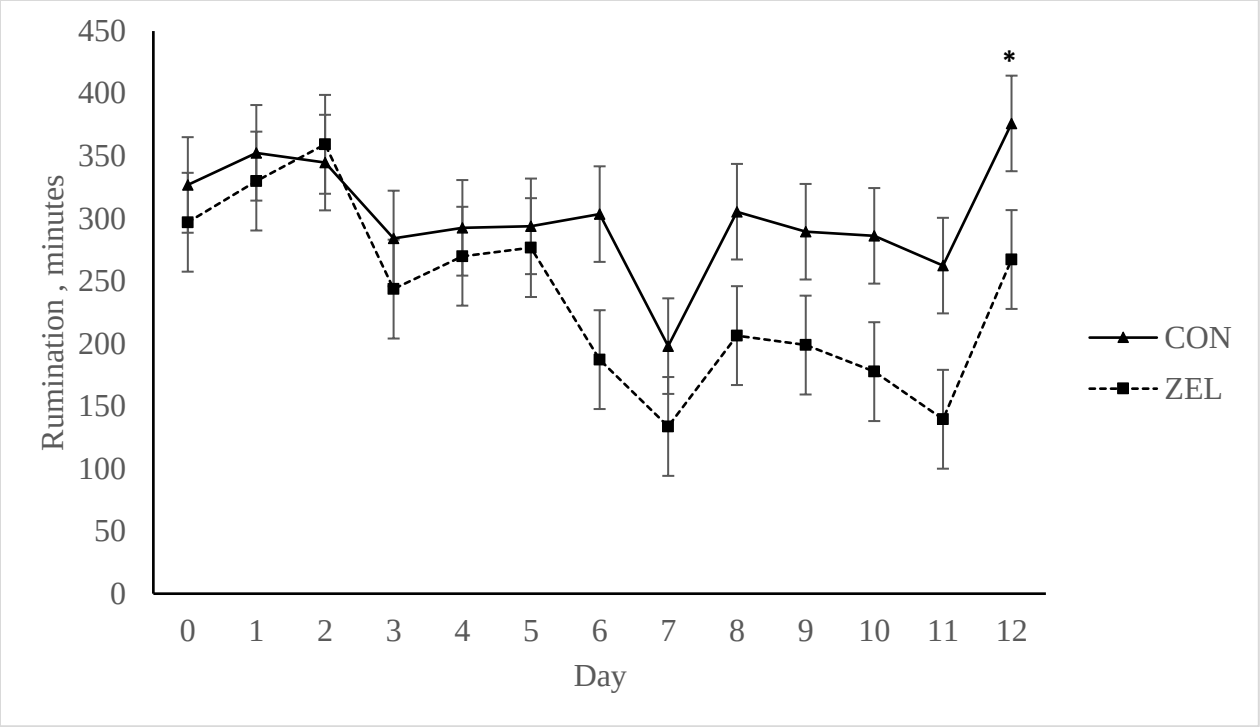

Figure 3.11. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on daily rumination minutes after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Effect of treatment ($P = 0.07$), day ($P < 0.01$) and treatment × day ($P = 0.43$). *Means within day differ ($P < 0.01$).

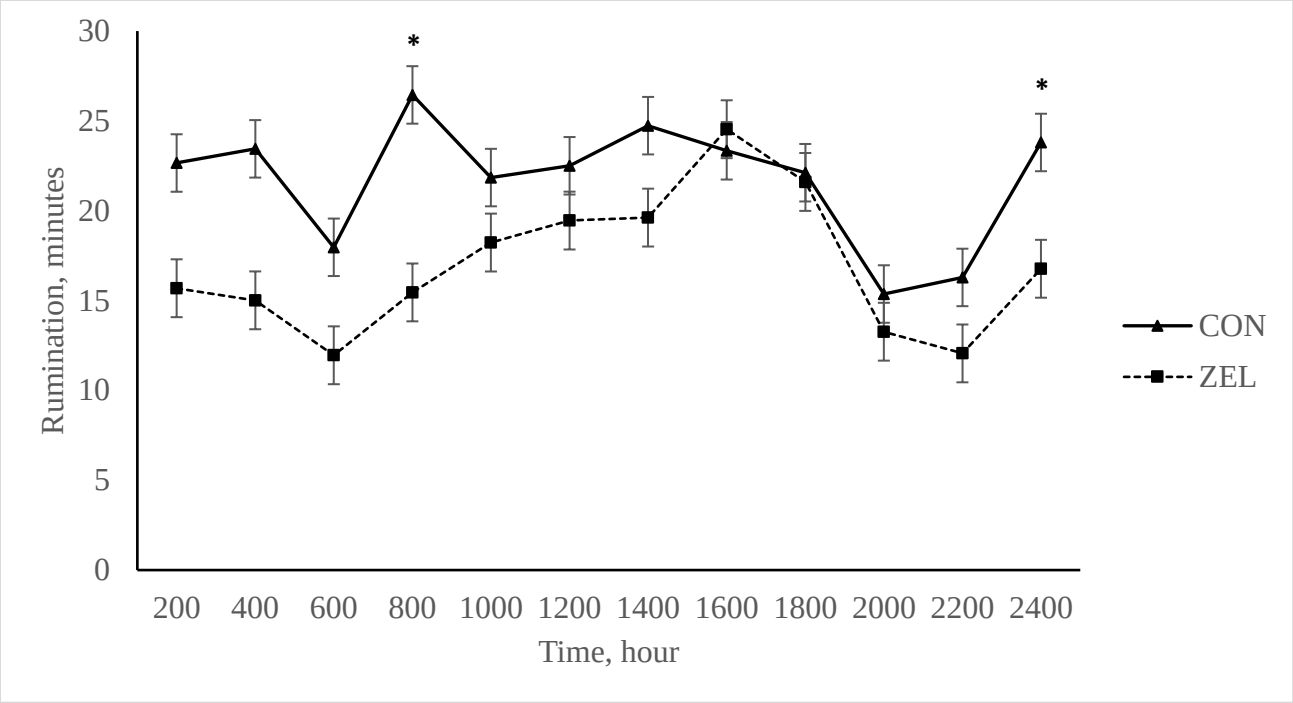

Figure 3.12. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on hourly rumination minutes after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Effect of treatment ($P < 0.01$), day ($P < 0.01$) and treatment × day ($P < 0.01$). *Means within hour differ ($P < 0.01$).

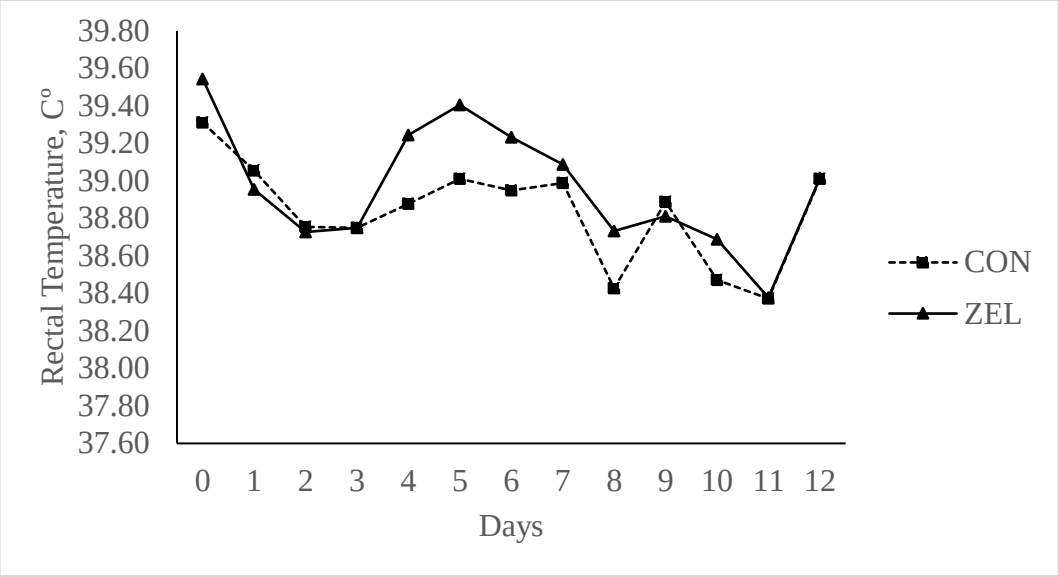

Figure 3.13. Effect of DNA immunostimulant (Zelante; ZEL; Bayer Animal Health) on rectal temperature after dexamethasone (DEX) administration in beef cattle. On day 0 to 2 cattle were administered 40 mg of DEX (Dexasone; Aspen Veterinary Resources, LTD) intravenous at 0600 hour with designated treatments administered on day 1. On day 1 ZEL treatment received 2 mL of DNA immunostimulant i.m. and CON treatment received 2 mL of sterile saline i.m. Rectal temperature was recorded daily, using the GLA M700 Digital Thermometer on continuous temperature mode (GLA Agriculture Electronics, San Luis Obispo, CA). Effect of treatment ($P = 0.18$), day ($P < 0.01$) and treatment × day ($P = 0.69$).

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Chapter III

Antibody response, lesions, and performance do not differ between injection site (ischiorectal fossa or neck) after multivalent modified-live virus vaccination in Jersey steers

Abstract

The study objective was to explore the feasibility of an alternative injection site, ischiorectal fossa (RF), for modified-live virus (MLV) vaccination in Jersey steers. We hypothesized administration of MLV in the RF would not cause injection site lesions and result in similar antibody response against bovine viral diarrhea virus (BVDV) compared to the neck. Jersey steers (n = 28; BW = 517 ± 116 kg) were stratified by a previously assigned growth implant treatment and pre-existing (day-35) BVDV antibody titer and randomly assigned to 1 of 2 treatments equivalently represented in each of 2 pens. Treatments consisted of: 1) 2 mL MLV vaccine administered s.c. in the neck (NECK); or 2) 2 mL MLV vaccine (Pyramid 5, Boehringer Ingelheim Animal Health USA) administered s.c. in the RF. Blood was collected on days -35, 0, 35, 70, and 105 to determine BVDV-specific antibody titers using the virus neutralization assay and BW was recorded on the same days to determine gain performance. Steers were harvested on day 106 and examined for lesions respective to injection site. Antibody titers and performance variables were statistically analyzed using mixed models with animal as the experimental unit. Concentration of BVDV antibody increased ($P = 0.05$) with time, but there was no treatment difference ($P = 0.94$) or treatment × day interaction ($P = 0.70$). There was no treatment difference for BW on any day ($P \geq 0.78$). There was also no treatment difference in ADG ($P \geq$

0.45). However, there was a period effect, where ADG was reduced markedly ($P <$ 0.0001) for the day 0 to 35 interim period immediately following MLV vaccination compared to day -35 to 0 (0.79 vs. 0.18 kg/d). Only one injection site lesion was observed during harvest and results from BVDV antibody titers and performance were similar; therefore, RF may be a potential alternative vaccination location for MLV vaccines.

Introduction

The Beef Quality Assurance (BQA) program is driven by cattle producers who acknowledge the importance of injection site lesions. The BQA recommends the administration of animal health products should be tissue friendly and low volume (Van Donkersgoed et al., 2000). Guidelines stipulate that injections should be administered to cattle s.c. in the neck, which is a less valuable meat product if an injection site lesion was to develop. However, oftentimes drugs and vaccines are administered in the semimembranosis, semitendinosis, and gluteal muscle of cows due to the use of headlocks in dairies (Holland et al., 2018). It is reported that i.m. injection of clostridials and antibiotics may cause severe damage evident in beef muscle 7.5 to 12 months later (George et al., 1995). It has been stated that an acceptable injection site that can be accessed from the rear of the animal may improve BQA compliance (Holland et al., 2018). The RF is located next to the tailhead and has been demonstrated as one possible alternative site for s.c. administration. Holland et al. (2018) and Colazo et al. (2002) validated administration of $PGF_{2\alpha}$ in the RF and concluded that injection of $PGF_{2\alpha}$ in the RF for synchronization of estrus and luteolysis did not differ from i.m. injection in the neck; however, multiple injections administered in the RF could result in chronic nerve damage (Colazo et al., 2002; Holland et al., 2018). Therefore the objective of the study was to explore the feasibility of an alternative injection site, RF, for MLV vaccination in Jersey steers. Our hypothesis was that the

administration of MLV in the RF would not cause injection site lesions and result in similar antibody response against bovine viral diarrhea virus (BVDV) compared to the neck.

Materials and Methods

This study was conducted from August 2018 to November 2018 at the West Texas A&M University (WTAMU) Research Feedlot in Canyon, TX. Jersey bulls were received in June 2017 from a single commercial dairy facility located near Plainview, TX and castrated following completion of a previous study. Cattle were simultaneously used to examine effects of growth implant strategy on Jersey steers. All animal procedures and experimental protocols were approved by the animal care and use committee at WTAMU (protocol number 02-09-17).

Animals and Treatments

On day 0, steers (n = 28; BW 517 ± 116 kg) were stratified by previous implant treatment and pre-existing BVDV antibody titer and randomly assigned to receive treatments consisting of: 1) 2 mL pentavalent modified-live virus (MLV) vaccine (Pyramid 5; Boehringer Ingelheim Animal Health USA, Inc. [BIVI]) s.c. in the neck (NECK) region; or 2) 2 mL pentavalent MLV vaccine (BIVI) s.c. in the ischiorectal fossa (RF). This completely randomized design resulted in 14 animals per treatment for NECK and RF, respectively, with animal serving as the experimental unit. One steer assigned to NECK treatment died before day 35 of the study due to peritonitis as determined from necropsy findings.

Blood Collection and Serology

Blood was collected on day -35, 0, 35, 70, and 105 at 0600 hour via jugular venipuncture into a 10 mL evacuated tube without additive (Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) to harvest serum used to determine BVDV titer. Samples were

placed in an insulated cooler after sample collection without ice to achieve storage temperature of approximately 20ºC and transported to the WTAMU Animal Health Laboratory. Samples were allowed to clot ≥ 30 min before centrifugation at 1,500 × *g* for 20 minutes at 20ºC. After centrifugation, serum was harvested and stored in duplicate aliquots at -20ºC until subsequent laboratory analyses were performed. One aliquot of frozen sera from each sample day was packaged on ice and transported to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) located in Amarillo, TX, to determine BVDV-specific antibody titers using the virus neutralization assay as described by Rosenbaum et al. (1970). Additionally, BW was recorded on each collection day and ADG was calculated.

Animals were harvested at the WTAMU Meat Lab on day 106. Each animal was examined for lesion respective to injection site; if a lesion was present it was removed, packaged and sent to TVMDL for histopathological analysis.

Statistical Analyses

This completely randomized design experiment used animal as the experimental unit for analyses of all dependent variables. The BVDV antibody titers were analyzed using the MIXED procedure of SAS (SAS inst. Inc., Cary, NC) with repeated measures. The model for these variables included fixed effects of treatment, day and treatment × day interaction. The repeated statement was day and the covariance structure with the lowest Akaike information criterion for the dependent variable was used. Statistical significance was established for treatment, day, and treatment × day F-tests if the resulting *P*-value was ≤ 0.05. Performance data was analyzed using the MIXED procedure of SAS. The model included fixed effect of treatment and the random effect of pen. Statistical significant was established if a resulting *P*-value was ≤ 0.05, tendencies were noted for a *P*-value of 0.05 ≥ 0.10.

Results and Discussion

BVDV Specific Antibody Response

Figure 4.1 indicates a day effect ($P = 0.05$) for BVDV-specific antibody titers; however, there was no difference in treatment ($P = 0.94$) or a treatment × day interaction ($P = 0.70$). The increase in BVDV serum antibody was expected as calves receiving MLV with BVDV antigens have increased antibody titers after vaccination (Grooms and Coe, 2002; Hudson, 2018). Cattle receiving the NECK treatment had numerically greater BVDV antibody titer versus RF steers, but there was not a statistical difference ($P = 0.94$). The similar vaccine response suggests that the RF may be a potential alternative site for MLV vaccine administration.

Performance

Results indicate there was no treatment difference for BW on any day ($P \geq 0.78$). There was also no difference in ADG ($P \geq 0.45$). Table 4.1 displays performance variables and ADG. Table 4.2 shows BW and ADG of all animals indicating that ADG decreased from day -35 to 0 to ADG from day 0 to 35 ($P < 0.001$) and then increased from day 35 to 70 ($P \leq 0.02$). The reduced ADG for all animals from day 0 to 35, compared to the other time periods is probably due to an increased inflammatory response to the MLV vaccine administered on day 0. Administration of a vaccine causes an inflammatory response that provokes the production of pro inflammatory cytokines such as IL-6, TNF-α, and IFN- γ, that in turn activates acute phase protein production (APP) that may have an anorexic effect on the animal (Dinarello, 1984; Hughes et al., 2017) resulting in a decreased growth performance (Exton, 1997). These findings are supported by Arthington et al. (2005) who reported that calves with a lesser APP response experienced two-fold greater feed efficiency during a 28 day receiving period. This was further

supported by Arthington et al. (2013) that demonstrated a correlation between the vaccination-induced APP reaction and reduced cattle performance.

Injection-site Lesions

Upon harvest on day 106, each animal was examined for injection site lesion respective to location of injection. There was one observable lesion noted for the RF treatment but histopathological analysis determined a granulomas lesion; a common vaccination reaction. No other injection site lesions were found on any other steers indicating RF as potentially safe location of MLV vaccine administration with respect to injection lesion. These results agree with previous research that evaluated the RF as an injection site for $PGF_{2\alpha}$ and determined the RF was a simple, practical and useful site for injection (Colazo et al., 2002; Holland et al., 2018). However, the sample size in the current study was very small and further research using a larger population is needed to make confident conclusions about potential differences in injection site lesions between the injection sites.

Conclusions

Results suggested that using the RF as an injection site for a MLV respiratory vaccine had similar immunological responses as animals injected with MLV in the neck region. There was no treatment differences noted for vaccine response; both treatments had an increase in BVDV-specific antibody titers with time. After vaccination, there was an overall decrease in ADG, but no treatment effect. Only one injection-site lesion was found indicating the RF as a promising alternative site of administration for MLV vaccines. Further research in a larger population is warranted to determine the effect of other vaccines or products administered in the RF in dairy cattle.

Table 4.1. Effect of MLV respiratory vaccine injection site on growth performance in Jersey steers.

	Treatment[1]			
Item	Neck	RF	SEM[2]	*P*-value
BW, kg				
Day -35	490.53	492.62	33.48	0.89
Day 0	520.30	517.77	31.80	0.87
Day 35	524.41	522.88	21.58	0.78
Day 70	555.11	551.23	20.60	0.84
Day 105	569.00	587.31	25.14	0.93
ADG, kg				
Day -35 to 0	0.85	0.73	0.11	0.45
Day 0 to 35	0.16	0.15	0.34	0.92
Day 35 to 70	0.80	0.83	0.08	0.82
Day 70 to 105	0.39	0.45	0.15	0.47
Day 0 to 105	0.45	0.48	0.09	0.64

[1]Neck= MLV respiratory vaccine (Pyramid 5; Boehringer Ingelheim Animal Health USA) injection administered in neck region.
RF= MLV respiratory vaccine administered in ischiorectal fossa.
[2]Standard error of the mean.

Table 4.2. Day and period effects of pentavalent MLV respiratory vaccine injection site on growth performance in Jersey steers.

Day[1]	-35	0	35	70	105	SEM[3]	P
BW, kg	490.21[a]	518.74[b]	525.94[bc]	554.35[d]	568.71[d]	26.18	< 0.01
Period[2]	-35 to 0	0 to 35	35 to 70	70 to 105	-		
ADG, kg	0.79[a]	0.18[c]	0.80[a]	0.40[b]	-	0.10	< 0.01

[1]BW of all animals on the indicated study day.
[2]ADG of all animals by time period.
[3]Standard error of the mean.
[abcde]Means within a row without a common superscript differ, $P \leq 0.05$.

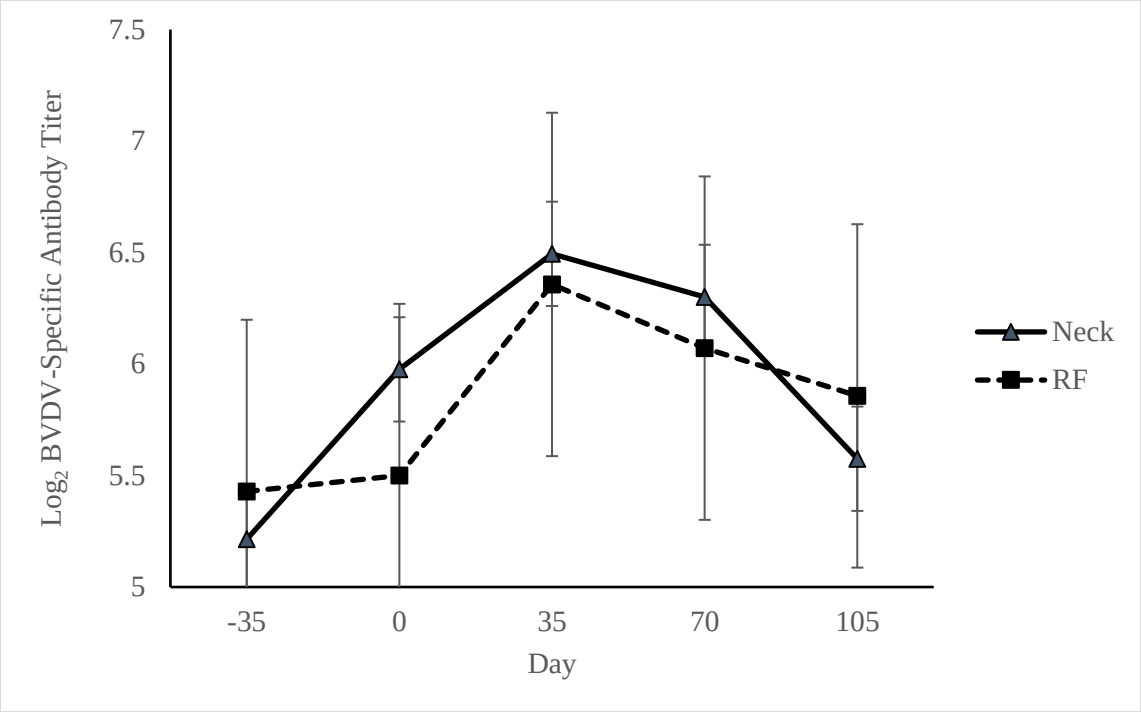

Figure 4.1. Effect of location of pentavalent modified-live viurus vaccination (Pyramid 5; Boehringer Ingelheim Animal Health USA, Inc.) on bovine viral diarrhea virus specific antibody titers. On day 0 steers were administered 2 mL MLV vaccine s.c. in either the neck region (NECK) or ischiorectal fossa (RF). Effect of treatment × day (P = 0.70), treatment (P = 0.94), and day (P = 0.05).

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