EVALUATION OF PHYSIOLOGICAL STRESS, RESPIRATORY VACCINATION, AND USE OF IMMUNOSTIMULANTS IN BEEF AND DAIRY CALVES

by

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ABSTRACT

Vaccination is a common practice to prevent bovine respiratory disease (BRD) and other diseases prevalent among the beef and dairy industries. A range in respiratory vaccination strategies are carried out with utilization of modified-live virus (MLV) and killed virus (KV) vaccines that may be advantageous under different conditions. More recently, immunostimulatory products have become available for control and prevention of BRD but their efficacy is poorly understood. Experiment 1 investigated the interaction of physiological stress and vaccine type (MLV vs. KV) in beef calves and Experiment 2 explored the safety and efficacy of different doses of an immunostimulant, pegbovigrastim, in recently weaned dairy calves. In Experiment 1, 48 crossbred beef steers were exposed to an acute (ACU) or chronic (CHR) stress model and were administered either a MLV or KV vaccine on d 0 of the study. This resulted in 4 treatments arranged in a 2 × 2 factorial consisting of ACU with killed virus vaccination (ACUKV), ACU with modified-live virus vaccination (ACUMLV), CHR with KV (CHRKV), and CHR with MLV (CHRMLV). Virus detection in nasal swabs, virus-specific antibody titers, haptoglobin, cortisol, and hematological variables were evaluated following stress model implementation and vaccination. Results indicated that CHR stress model and MLV vaccination may have more profoundly

induced immune dysfunction in beef calves due to altered hematological and endocrine responses. In Experiment 2, 33 weaned Jersey bull calves were administered a commercially available immunostimulant, pegbovigrastim, using 3 different doses in attempt to validate a safe and efficacious dose for use in calves. Treatments included s.c. administration of 1.11 mg (PEGA), 2.22 mg (PEGB), and 4.44 mg (PEGC) of pegbovigrastim injection. Blood samples were collected to analyze hematological variables and functional capacities of blood neutrophils. While functional capacities of blood neutrophils were not different between treatments, leukocyte variables were increased for all treatments with greatest increases in white blood cell and neutrophil responses among PEGC. Further research investigating the interactions between the duration and intensity of physiological stress and vaccine antigen type is needed to ensure safe use in stressed beef calves. In addition, clinical investigation is needed to validate the use of pegbovigrastim as a safe and effective aid in prevention of BRD.

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CHAPTER I

INTRODUCTION

Bovine respiratory disease (BRD) is multifaceted that involves risk factors associated with stress and inflammation, and causative agents. The disease is common among calves in both beef and dairy production systems throughout the United States. Management practices in both industries can have a major role in the incidence of BRD. In the beef marketing system, calves are often abruptly weaned from their dam and transported to an auction facility within hours to days of separation. Upon arrival to the auction facility, calves are commingled and may have limited access to feed and water. After sale, calves are typically relocated to a stocker, backgrounding or feedlot facility where they undergo initial processing. Once calves are weaned at the origin ranch, the animal begins to experience physiological stress and stressors are encountered throughout the relocation process. Acute stress, caused by stressors enduring ≤24 hours, is proposed to result in immune enhancement while chronic stress, caused by stressors enduring ≥24 hours, is known to cause immune dysfunction (Richeson et al., 2016). The highly segmented beef marketing system likely causes calves to reach a state of immunosuppression due to intense chronic stress and therefore they are at increased risk to develop signs of BRD. In addition, dairy calves that are housed and separated from

their dams within a few hours of birth and fed a milk substitute until weaning age are highly susceptible to respiratory and enteric diseases during this time.

Vaccination against respiratory pathogens is a common practice to prevent BRD; however, it is only beneficial when utilized appropriately and according to label directions. Different vaccine types are available for use in cattle including killed virus (KV) vaccines that contain non-replicating antigens and modified-live virus (MLV) vaccines that contain replicating antigens. It has been suggested that MLV vaccines have greater potential to cause subsequent disease when compared to KV vaccines due to possible over replication in the host when administered to chronically stressed, immunosuppressed animals (Richeson et al., 2016). Label directions for MLV vaccines indicate that MLV can be efficacious in healthy animals but a protective immune response may not be elicited if the animals are stressed. It has been proposed by Roth (1985) and Richeson (2015) that although glucocorticoids may cause decreased antibody titer concentration via suppressed antibody production or enhanced antibody protein metabolism, the effect depends upon the timing and duration of the elevated glucocorticoid concentration and nature of the antigen in question. Enhanced replication of MLV antigens as an artifact of stress-induced immunosuppression may result in antibody responses that are greater compared to non-stressed cohorts (Roth, 1985). Furthermore, antimicrobials are commonly utilized to treat bacterial infection associated with BRD, but due concerns with antibiotic resistance and consumer preferences, comprehensive disease management alternatives are desperately needed. Immunostimulants have recently been used in human medicine among cancer patients

receiving chemotherapy (Younes et al., 2004) and two new immunostimulatory products are available to the beef and dairy industries with aspirations to aid in disease prevention and potentially provide an alternative to antibiotic use in food producing animals. Critical evaluation of the safety and efficacy of respiratory vaccines and immunostimulants is necessary in cattle with considerations of adverse effects from stress events caused by current marketing and management practices.

CHAPTER II

REVIEW OF LITERATURE

Bovine Respiratory Disease

Demographics of the U.S. Beef Production System

Bovine Respiratory Disease (BRD) is responsible for the majority of morbidity and mortality in cattle within feedlots with less prevalence among forage-based cow-calf systems (Edwards, 2010; Murray et al., 2017) and continues to be the most costly disease among the North American beef system (Smith, 1998; NAHMS, 2000). Beef production in the United States is highly segmented, originating at the cow-calf operation, transitioning to a feedlot or stocker setting after purchase at an auction market, and finally ownership is exchanged to the packer once ideal feedlot finishing weight is reached (Hughes, 2015). Nearly all feedlots surveyed in the 2011 National Animal Health Monitoring Survey (NAHMS) Health and Management report with a capacity of greater than 1,000 head had at least some cattle affected by BRD. Respiratory disease affected more than 95% of feedlots with over 1,000 and less than 8,000 head while 100% of feedlots that contained greater than 8,000 head of cattle were affected by BRD (NAHMS, 2011). Feedlots in the Central region yielded twice the percentage of cattle affected with respiratory disease compared to feedlots in non-central regions (17.9 and 8.8% of cattle, respectively; NAHMS, 2011).

Small cow-calf operations that have less than 100 cows account for approximately 90.4% of all farms with beef cows and encompass 45.9% of beef cows in the United States (USDA – NASS, 2007). Unlike the effect of BRD on large-scale feedlots that contain more than 1,000 head, BRD has been reported to affect less cattle on cow-calf operations. USDA reported that only 25.5% of small-scale cow calf producers with 1 to 49 beef calves and 48.7% of cow calf producers with 50 to 99 beef calves strongly agreed calf pneumonia/shipping fever had a significant economic impact (USDA-APHIS, 2011c). Overall, 88.3% of these cow-calf operations market their animals through an auction facility (USDA-APHIS, 2011b). In 2007, only 26% of operations with 1 to 49 cattle and 63% of operations with 50 to 99 cattle vaccinated cattle or calves against respiratory disease from birth to sale (USDA-APHIS, 2011c). Nearly 64% of feedlots purchase cattle by auction with the distance of travel for cattle shipments averaging 339 miles due to most small cow-calf farms residing in the southeastern U.S. (USDA-APHIS, 2011a). Therefore, not only are a high percentage of cattle entering the feedlot unvaccinated, but cattle must also travel a great distance to reach the feedlot destination. Unfortunately, less than half of small cow-calf operations provide buyers with information about their calf health programs (Duff and Galyean, 2007; USDA-APHIS, 2011c); consequently, unless calves are marketed as “preconditioned”, feedlots will process incoming cattle with various products including vaccination, parasite control, application of growth promoting implants, or other procedures (USDA-APHIS, 2011a).

On average, 60% of feedlot operations process cattle within 24 hours upon arrival while only 27% of feedlot operations delay processing some cattle more than 72 hours

after arrival (USDA-APHIS, 2011a). This stressful transition of cattle from their original environment to that of the feedlot causes most clinical illness to occur early in the feeding period (USDA-APHIS, 2011a). Of the many practices carried out during initial processing, the most common practice is vaccination against respiratory disease with 96% of feedlots utilizing this practice (USDA-APHIS, 2011a). In addition to administering a respiratory vaccine, about 50% of feedlots administer an antibiotic metaphylaxis regimen as part of the initial processing procedure (USDA-APHIS, 2011a). The decision of when to apply metaphylactic treatment may be dependent on several factors related either to the current state of the animals or to the animals' management history (USDA-APHIS, 2011a), but the decision is typically made using limited information and not on an individual animal basis. Multiple antimicrobials are currently available and used for metaphylaxis treatment intended to decrease negative effects of BRD in groups of feedlot cattle and the decision to implement a specific antimicrobial is based upon efficacy and cost effectiveness (Nickell and White, 2010; Ives and Richeson, 2015).

Typically, cattle are observed for clinical signs of illness via pen-riding or walking to determine treatment for BRD (USDA-APHIS, 2011a). Unfortunately, this diagnostic approach is not an objective method (White and Renter, 2009) as cattle will consequently mask signs of sickness, especially in presence of humans (Weary et al., 2009). Therefore, a proportion of cattle with BRD are never detected by pen riders, resulting in false negatives (Timsit et al., 2016). Clinical signs typically used to diagnose BRD include depression, anorexia, and fever; however, these signs are not specific to this

disease condition (Portillo, 2014). While 97% of all feedlots observe animals at least once daily during the cattle's first 14 days in the feedlot (USDA-APHIS, 2011a), the diagnostic accuracy of clinical illness detected by pen-riders remains largely unknown and person-to-person variability is problematic (Timsit et al., 2016).

Demographics of the U.S. Dairy Production System

Mastitis continues to be one of the most common and detrimental diseases cows can experience and threatens the image of the dairy sector because of animal welfare and milk quality issues (De Vliegher et al., 2012). Cow-to-cow transmission of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. generally colonize the teat skin and mammary gland that eventually causes the chronic intramammary infection known as mastitis (USDA-APHIS, 2008). Clinical mastitis was detected in about one-fourth of all cows at some point during 2013 with less than 5% of cows resulting in death from the disease (USDA-APHIS, 2014a). The majority of cows identified with clinical mastitis are treated with antimicrobials with nearly three-fourths of operations utilizing cephalosporins (USDA-APHIS, 2014a). It is important to prevent heifer mastitis due to the economic losses instigated by lost milk production, premature culling, additional labor, management, veterinary needs, use of drugs and risk of residues, and production of nonsalable milk (Huijps et al., 2009).

While mastitis poses a major concern in the dairy industry, post-weaning bovine respiratory disease (BRD) has been reported by the 2007 US NAHMS to be the predominant cause of reported deaths of weaned heifers (USDA-APHIS, 2010). The primary source of data on BRD prevalence in US adult dairy cattle is producer surveys

provided by NAHMS (Guterbock, 2014); NAHMS 2002 and 2007 (USDA-APHIS, 2002, 2009) surveys used producer interviews to find that 2.4 and 2.9% of cows were diagnosed with respiratory disease in 2002 and 2007, respectively. While these estimates appear low, they indicate that approximately 3% of dairy cows die on the farm as a result of BRD or go to slaughter with severe BRD (Guterbock, 2014). It is important to note that these data excluded cows that are treated for BRD and retained in the herd. In comparison to BRD incidence in dairy cows, the primary manifestation of BRD on dairy farms is in young calves (Guterbock, 2014). The NAHMS 2007 (USDA-APHIS, 2010) surveyed dairies in 17 states that represented approximately 80% of US dairy farms and cows and estimated that 12.4% of preweaned heifer calves had BRD and that 11.4% were treated for BRD. Surveys based on heifer raisers estimated that 2.3% of preweaned heifers, 1.3% of weaned open heifers, and 0.2% of pregnant heifers died from pneumonia and that 16.4% of preweaned heifers, 11% of weaned heifers, and 1.2% of pregnant heifers were reported to be treated for BRD (USDA-APHIS, 2012). Guterbock (2014) evaluated the "Gold Standard" for BRD treatment on dairy farms that was previously established by the Dairy Calf and Heifer Association (Young and Rood, 2010) which stated less than 10% of heifer calves from 1 to 60 days of age, less than 15% from 61 to 120 days of age, and less than 2% from 121 to 180 days were treated for BRD. If these rates are additive, Guterbock (2014) concluded the cumulative rate to meet the Gold Standard would be 27%; however, this is not always true as even on large calf raising operations with excellent colostrum programs BRD treatment rates have been observed to reach 50% for certain periods.

Incidence of BRD among dairy calves has been linked to colostrum quality and volume fed (Van Donkersgoed et al., 1993; Virtala et al., 1999; Gorden and Plummer, 2010). In addition, the effect of milk source, volume, and frequency of feeding before weaning on BRD prevalence remains unclear, as some studies showed significant effects (Morrison et al., 2012; Araujo et al., 2014) while others showed no significant effects (Kehoe et al., 2007; Bach et al., 2013). Effect of weaning age on BRD incidence has not been directly studied (Guterbock, 2014); however, later weaning ages have been shown to result in greater average daily gain (ADG) and body weight (BW) at weaning in organically raised calves (Bjorklund et al., 2013). Therefore, calf management practices may influence BRD incidence among dairy calves yet further research is needed to quantify the relationships between herd practices and the occurrence of BRD (Love et al., 2016).

Holsteins are the predominant dairy breed comprising 86% of all U.S. dairy cows while Jerseys are the second most common breed but only represent less than 8% of dairy cows (USDA-APHIS, 2014b). Unlike the U.S. beef production system, the dairy production system implements biosecurity recommendations that include quarantining new arrivals for 30 to 60 days to allow for testing observation of animals for infectious diseases (USDA-APHIS, 2014b). While the biosecurity recommendations would reduce disease susceptibility, only 9.6 % of operations follow these guidelines and quarantine new additions upon arrival because lactating cattle are difficult to quarantine due to the need to be milked and most operations do not have separate housing or milking facilities for new additions (USDA-APHIS, 2014b). About 30% of operations introduced new

cattle to the operation during 2013 with pregnant dairy heifers and lactating dairy cows accounting for 11.9% of the total new cattle introduced, respectively (USDA-APHIS, 2014b). Heifer raising operations obtain dairy heifers from different sources including other dairy operations, auction markets, and dealers (USDA-APHIS, 2011d). Small operations tend to utilize two sources for heifers when compared to large operations (38.6 vs. 16.4%) while large operations tend to utilize five to nine sources for heifers when compared to small operations (35.85 vs. 5.3 %; USDA-APHIS, 2011d). As the number of sources increases, the potential for introducing and transmitting disease among the herd also increases, if contact of cattle from different sources is allowed without an established quarantine period (USDA-APHIS, 2011d).

Preweaned heifers are individually housed to reduce the transmission of disease-causing pathogens and to monitor individual feed intake (USDA-APHIS, 2014b). Over 50% of operations administer vitamin A-D-E or selenium by injection or in feed to preweaned heifers and 41.1% of operations administer probiotics (USDA-APHIS, 2011d). The percentage of operations that vaccinate preweaned heifers against any disease increases as herd size increases, ranging from 37% of small operations to 81.3% of large operations (USDA-APHIS, 2014b). Most operations vaccinate weaned and pregnant heifers against infectious bovine rhinotracheitis (IBR; 64.1%), bovine viral diarrhea (BVD; 63.8%), parainfluenza type 3 virus (PI3V; 58.4%) and bovine respiratory syncytial virus (BRSV; 56.8%; USDA-APHIS, 2011d).

Pathogenesis of Bovine Respiratory Disease

The segmented beef production system and management practices of the dairy production system contribute to BRD prevalence in both industries. Cattle with BRD may not exhibit clinical signs or lesions definitive for specific etiology and diagnosis involves multiple agents (Fulton et al., 2016). The disease is multi-factorial but comprises three primary factors including stress-induced immunosuppression, infectious viral pathogens and bacterial pathogens that ultimately result in bronchopneumonia (Grissett et al., 2015).

Calf Origin

Management practices at the origin ranch contributes to the stress and inflammation induced in calves entering the feedlot (Lalman and Mourer, 2014). An industry known practice to combat increased stress and inflammation upon feedlot arrival involves the concept of preconditioning (Hilton, 2015). The goal of preconditioning is to decrease morbidity and mortality in the backgrounding lot or feedlot by marketing a calf that is immunized, castrated, dehorned, dewormed, weaned, and trained to eat from a bunk and drink from a water tank (Hilton, 2015). While this concept is not practiced by every cow calf producer and still does not guarantee benefits of decreased morbidity and mortality, calves with improved health have greater ADG, decreased cost of gain, and increase profitability when compared to calves that undergo disease (Cravey, 1996; Gardner et al., 1998; Ensley, 2001; Schneider et al., 2009). Calves that arrive at the feedlot without prior vaccination against respiratory viruses have potential for low antibody concentrations to these viral antigens which has been associated with increased

morbidity, increased number of total treatments leading to greater treatment costs, and an overall decreased net value to feedlot producers (Fulton et al., 2011). The Texas Ranch to Rail program has conveyed data to suggest that the timing of vaccination affects feedlot morbidity and mortality rates with lowest rates within calves who were administered a respiratory vaccine 9 weeks prior to feedlot entry rather than administering additional immunizations throughout the feeding period (McNeill and McCollum, 2000).

Weaning

Weaning has been evolutionarily designed to be stressful, as it is inherent for the youngster to maintain proximity to its mother for achievement of protection, comfort, and nourishment (Henderson, 2016). When referring to calves, weaning can involve several methods including abrupt separation of calf from its dam, fence-line separation, and two-step weaning (Kaufman, 2017). It has been reported by Griebel et al. (2014) that calves weaned abruptly had greater serum haptoglobin concentration when compared to calves that were weaned from a two-step program that allowed calves to graze during weaning. This suggests that abrupt weaning has potential to cause greater inflammatory responses when compared to other weaning methods. Maternal separation forces the calf to transition to self-care with availability of a non-milk diet and causes psychological stress due to dam separation and milk deprivation. It has been observed that weaning alone does not increase cortisol levels in calves (Lefcourt et al., 1995; Hickey et al., 2003); however Hickey et al. (2003) reported an increase in the neutrophil:lymphocyte (N:L) ratio by increasing the proportion of neutrophils which is thought to act as a biological indicator of stress and disease susceptibility (Herzog, 2007). While an increase in cortisol

concentration was not detected after weaning by Lefcourt et al. (1995) or Hickey et al. (2003), the increase in the N:L indicated stressful conditions signifying that an initial increase in cortisol may have been lost in laboratory measurement due to infrequent sampling. Stress induced from weaning causes alterations in calf immunity with potential to increase incidence and severity of respiratory disease (Duff and Galyean, 2007).

Commingling

Commingling involves social reorganization of cattle and is a psychological stressor (Kelley, 1980) that begins at the auction market and is maintained or exacerbated when calves are relocated to a stocker facility or feedlot. McVeigh et al. (1982) observed social mixing among cattle to increase rectal temperature and heart rate while decreasing muscle glycogen most likely the result of amplified physical activity from continuous hierarchy establishment. These results directly correspond with performance observations made by Step et al. (2008) who reported overall greater ADG for single source cattle when compared to auction market calves. While Veissier et al. (2001) found no change in plasma cortisol concentrations between mixed animals and non-mixed animals, observed plasma cortisol concentrations had a greater integrated response for mixed animals after an injection of adrenocorticotropic hormone (ACTH) was administered. It is apparent that commingling acts as an additive stressor among the beef marketing process and may contribute to immune dysfunction in cattle.

Transportation and Handling

Studies investigating the amount of stress on farm animals during transport and routine handling often yield highly variable results, making it difficult to determine from

an animal welfare standpoint (Grandin, 1997). Stress involved in transportation and handling activities include psychological stress from restraint or novelty with possibility of physical stresses from hunger, thirst, fatigue, or injury (Grandin, 1997). Cattle are fear-conditioned animals which motivate them to avoid predators (LeDoux, 1994; Grandin, 1997). When an animal is suddenly confronted with novelty, it acts as a very strong stressor (Stephens and Toner, 1975; Moberg and Wood, 1982) as it is often associated with strange sights or sounds which are habitually a sign of danger (Grandin, 1993). Truck transportation and handling in new facilities activate the hypothalamic-pituitary-adrenal (HPA) axis that results in a dramatic increase in plasma glucocorticoid concentrations (Murata et al., 1987; Gupta et al., 2007; Sporer et al., 2008). Cole et al. (1998) reported that when calves were transported less than 24 hours, the greatest stressor was the loading and unloading of calves from a truck; however, time spent in the trailer may be stressful in itself with varying effects dependent on the experience of the driver, age and origin of the animal, heat or cold stress, and the time when cattle are loaded onto the trailer (Gonzalez et al., 2012). The elevation of cortisol caused by stressors associated with transportation and handling has been linked to respiratory disease (Murata et al., 1987).

Castration and Dehorning

Often included in initial processing upon feedlot arrival, castration and dehorning practices reduce aggressive behavior and injury to other animals, respectively. For multiple reasons, some cow calf producers do not market steers due to perceived reductions in weaning weight (Smith et al., 2000), despite the increased value for steers

(Ratcliff et al., 2014). Richeson et al. (2013) reported increased risk for BRD in cattle that were castrated following feedlot arrival which may be attributable to increased cortisol levels following castration reported by Roberts et al. (2015). Increased cortisol levels in surgically castrated cattle were reported as soon as 30 minutes following castration with a second increase after cattle were returned to their home pen after handling (Roberts et al., 2015). This indicates that prolonged pain caused by castration continually activated the HPA axis and caused extended cortisol release and inflammatory responses. Whereas cortisol has been conveyed to increase following castration, most studies do not analyze morbidity as an outcome of interest and therefore association between castration and BRD is by extrapolation (Taylor et al., 2010). Nonetheless, while a direct correlation between these practices and BRD have been inconsistent, castration and dehorning are additive stressors among the beef marketing system (Ratcliff et al., 2014) known to decrease growth performance (Taylor et al., 2010; Pinchak et al., 2004; Ratcliff et al., 2014) and increase morbidity (Berry et al., 2001; Taylor et al., 2010;); however, these negative effects observed in the feedlot setting are typically transient (Berry et al., 2001). Despite the negative performance effects, it is still necessary to castrate and dehorn cattle to implement best management practices in the feedlot setting.

It is clear that BRD is a multifaceted disease with several disposing factors. The complexity of the disease cross-links common beef marketing practices with immunosuppression caused by intense stress experienced by cattle before and upon arrival at the feedlot. While causes and susceptibility to BRD has been widely

investigated, addition exploration is warranted for a complete understanding of its pathogenesis and link to stress.

Viral Pathogens

The most common viruses associated with the BRD complex include infectious bovine rhinotracheitis virus (IBRV; Baker et al., 1960; Curtis et al., 1966), PI3V (Betts et al., 1964, Omar et al., 1966), bovine vial diarrhea virus genotypes I and II (BVDV I and II; Potgieter, 1997; Shahriar et al., 2002; Fulton et al., 2003; Tuncer and Yesilbag, 2015), and BRSV (Paccaud and Jacquier, 1970, Babiuk et al., 1988; Larsen et al., 2001). One or more of these viral agents have been reported in the etiology of BRD (Härtel et al., 2004) and are considered primary pathogens of the disease (Tuncer and Yesilbag, 2015). While these are the most common viruses associated with BRD, bovine rhinovirus, bovine coronavirus and bovine reovirus serotype 3 have infrequently been isolated from BRD-affected animals (Kurogi et al.,1976; Richer et al.,1988; Decaro et al., 2008).

Infectious bovine rhinotracheitis virus is a member of the *Herpesvirus* group and is analogous with bovine herpesvirus-1 (BHV-1); these viruses share common properties with other members of deoxyribonucleic acids (Curtis et al., 1966; Biswas et al., 2013). The clinical manifestation of BHV-1 infection is dependent on the nature of various subtypes with potential to cause respiratory infection, genital infection, and neurological disease (Biswas et al., 2013). The virus initially infects the epithelial cells of the upper respiratory tract and then spreads to the lower respiratory tract (Griffin et al., 2010). It is estimated that 1 percent of cattle infected with IBRV die of secondary bacterial pneumonia (Blood et al., 1979). While losses due to IBRV are usually not reported

separately from total BRD mortality, Church and Radostits (1981) reported that respiratory diseases, including BRD and IBR were responsible for approximately two-thirds of morbidity and mortality in an Alberta feedlot study. These differences in mortality reports may be due to the multi-factorial properties of the virus in relation to pneumonia infection and differences in population or individual host effects. The importance of IBRV contribution within the BRD complex is still under investigation; however, Straub (1978) commented that out of all the viruses involved in the BRD complex, IBRV repeatedly caused disease following experimental inoculation.

The bovine-specific PI3V is a RNA virus in the genus *Respirovirus* in the family Paramyxoviridae (Chanock et al., 2001). The virus was first isolated from cattle with shipping fever in 1959 (Reisinger et al., 1959) and is now recognized as one of the most important of the known viral respiratory pathogens in both young and adult cattle (Ide, 1970). Strains of PI3V have produced clinical and histological (Betts et al., 1964; Dawson et al., 1965; Omar et al., 1966) evidence of respiratory disease and while clinical signs may be mild and subacute, the macroscopic and microscopic lung lesions were usually severe (Ide, 1970). Lesions resulting from PI3V infection from several experiments have been observed to range from rhinitis and tracheitis to severe pneumonia (Dawson et al., 1965; Omar et al., 1966; Frank and Marshall, 1971; Bryson et al., 1979); however, it is important to point out that lesions specifically attributable to PI3V can be challenging to differentiate due to involvement of multiple respiratory pathogens (Ellis, 2010). Primary PI3V infections occur in the epithelial cells in the trachea, bronchi, and alveoli, causing necrosis of the ciliated epithelium (Griffin et al., 2010). The incidence of

PI3V is thought to be extensive with more than 80 percent of the cattle population possessing antibodies to PI3V (Harbourne, 1966). Its prevalence among the cattle feeding industry brings rise to its potential to cause cross-species infection among sheep and humans (Abinanti et al., 1960; Hore et al., 1968; Ellis, 2010).

Regarding BVDV, the virus belongs to a heterogenous group of viruses of 2 different genotypes including BVDV I and BVDV II that are within the *Pestivirus* genus of the Flaviviridae virus family (Potgieter, 1997; Larson, 2015). Two biotypes of the virus are distinguishable in the laboratory including the noncytopathic type that is predominant biotype in nature with no causes of cell culture degeneration and the cytopathic biotype that induces cellular degeneration (Malmquist, 1968; Ames, 1986; Bolin, 1992; Ridpath, 2008). The BVDV is associated with several feedlot diseases including contribution to BRD through suppression of the immune system and synergism with other pathogens (Peterhans et al., 2003; Chase et al., 2004; Ridpath, 2010) and to a less extent digestive tract disease (Larson, 2015). Once the host is exposed to BVDV, the virus replicates in and impairs function or destroys alveolar macrophages inducing immunosuppression (Babiuk et al., 2004). Infections caused by BVDV in cattle may also result in bovine viral diarrhea (or primary postnatal infections), mucosal disease, and fetal disease (Ames, 1986; Bolin, 1992; Baker, 1995; Bezek, 1995). Fetal infections may result in fetal malformations, death, or persistently-infected (PI), immunotolerant calves (Potgieter, 1997). Regarding PI calves, noncytopathic BVDV invades the fetus early in its intrauterine development before the development of a competent immune response and establishes immunotolerance that is specific for the persisting viral strain (Peterhans

et al., 2003). Cattle PI with BVDV are a primary reservoir for the virus (Larson, 2015) and can develop mucosal disease if they acquire a cytopathic biotype of the same genotype (Potgieter, 1997).

Another major cause of respiratory disease in young cattle is BRSV, and it is responsible for significant economic losses around the world (Valarcher and Taylor, 2007). The virus is closely related to human (H)RSV, which is the single most important cause of lower respiratory tract disease in young infants (Guzman and Taylor, 2015). Both BRSV and HRSV are enveloped, non-segmented, negative stranded RNA viruses that belong to the genus *Pneumovirus* within the Paramyxoviridae family (Guzman and Taylor, 2015). Infections associated with BRSV result in morbidity rates of 60 to 80 percent and mortality rates as high as 20 percent (Larsen, 2000; Gershwin, 2008). Direct transmission between herds is frequent when there is an introduction of infected animals and natural clinical disease signs have been observed in calves younger than 6 months of age with uncomplicated BRSV infection (Urban-Chmiel et al., 2015). Upon transmission of BRSV, the virus replicates primarily in ciliated airway epithelia cells and type II pneumocytes (Johnson et al., 2007; Viuff et al., 1996; Welliver et al., 2008). This causes direct expression of cellular adhesion molecules and recruitment of leukocytes to the lungs (Guzman and Taylor, 2015) while depressing phagocytosis and opsonization by alveolar macrophages (Griffin et al., 2010), resulting in bronchiolitis and interstitial pneumonia (Guzman and Taylor, 2015).

Bacterial Pathogens

Coinfection with virus and bacteria may exploit the animals' immunosuppressed state and synergistically contribute to the persistence or severity of BRD. Upon viral replication in the host, otherwise commensal bacteria may cause development of clinical disease. *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and *Mycoplasma bovis* are all frequently implicated in BRD with *M. haemolytica* considered the most important of the group (Confer, 2009; Griffin et al., 2010; Gershwin et al., 2015). These bacterial pathogens facilitate immune stimuli via pathogen-associated molecular patterns and are initially recognized by innate immune cells via pattern recognition receptors; activated phagocytes then engulf the intruding pathogens (Pauwels et al., 2017). While macrophages and neutrophils have potential to counteract bacterial infection, each bacterium has unique defense mechanisms and virulence factors (Murray et al., 2016).

Mannheimia haemolytica, formerly known as *Pasteurella haemolytica*, is a gram-negative, facultatively anaerobic bacterium member of the family *Pasteurellaceae* (Griffin et al., 2010; Boukahil and Czuprynski, 2016). It otherwise exists as a commensal bacteria in the upper respiratory tract of healthy cattle with potential to descend into the lungs to cause pneumonia after exposure to stress and/or respiratory viruses (Caswell, 2014; Moore et al., 2015). Infection with *M. haemolytica* includes a capsule that is used for adherence and invasion, outer membrane proteins that produce protective immune defense, adhesions used for colonization, and neuraminidase that reduces respiratory mucosal viscosity that allows the bacteria to access the cell surface (Hodgins and

Shewen, 2004). Because *M. haemolytica* is gram-negative, it contains lipopolysaccharide (LPS) that causes hemorrhage, edema, hypoxemia, and acute inflammation and produces a leukotoxin that is responsible for lysis of ruminant leukocytes and platelets (Gioia et al., 2006). *M. haemolytica* leukotoxin is a potent calcium dependent cytolytic toxin that causes oncosis of bovine leukocytes at high concentrations with activation and apoptotic effects on bovine leukocytes at low concentrations (Tumbikat et al., 2005).

Pasteurella multocida has been isolated from up to 40% of the cases of enzootic and shipping fever pneumonia (Welsh et al, 2004) and is more commonly identified in respiratory disease affecting younger cattle (Griffin et al., 2010). The most common serotype involved in bovine pneumonia includes A:3; however, infections caused by *P. multocida* are often difficult to discern from pneumonia associated with other bovine bacterial pathogens (Confer, 2009). *P. multocida* A:3 virulence factors are less numerous than those associated with *M. haemolytica* but include several adhesins with a thick LPS capsule (Confer, 2009). These adhesins are responsible for adherence of the bacteria to cell surfaces and aid in *P. multocida* LPS invasion (Harper et al., 2006). These invading factors cause overall effects of endotoxin shock with antiphagocytic properties.

Histophilus somni, formerly known as *Haemophilus somnus,* also exists as a gram-negative bacterium and is associated with numerous pathological processes including pneumonia, septicemia, myocarditis, and abortion (Confer, 2009). Whereas *H. somni* is associated with BRD, there is marked variation in its prevalence when compared to *M. haemolytica* or *P. multocida* (Corbeil, 2007). Massive fibrin deposition is the most common observation upon gross examination of affected lungs, but it is important to note

that this has also been frequently observed with *M. haemolytica* and *P. multocida* infections (Griffin et al., 2010). The bacterium is non-encapsulated, and the virulence factors include lipooligosaccharide and various heat-modifiable outer membrane proteins, especially transferrin-binding proteins and immunoglobulin-binding proteins (Confer, 2009). In addition to inhibiting phagocytosis, *H. somni* has been observed to produce histamine which may account for early respiratory lesions (Corbeil, 2007).

Mycoplasma bovis has been under much investigation; however, its ultimate role in bacterial pneumonia is controversial. *M. bovis* has been isolated from up to 45% of histologically normal bovine lungs (Gagea et al., 2006) and is predominantly an extracellular pathogen that is present on respiratory epithelial surfaces (Confer, 2009). The bacterium causes infection by gaining entrance into the respiratory tract with further migration between the respiratory cells to gain access to the blood stream (Caswell and Archambault, 2008). Unlike the previous causative bacterial pathogens, *M. bovis* has a trilayered membrane instead of a cell wall (Griffin et al., 2010). Some strains may produce hydrogen peroxide that form oxygen free radicals and causes host lipid peroxidation and production of heat shock proteins have been observed, but their role in virulence is unknown (Confer, 2009).

Stress and Immune Interaction

Beef cattle are unavoidably exposed to stress during their productive lives (Carroll and Forsberg, 2007). Some of these stressful situations include physiologic, psychologic, and physical stressors associated with management and marketing procedures currently practiced among the North American beef production system

(Cooke, 2017). A classic and repeatable example occurs during transfer of beef calves from origin cow-calf ranches to commercial feedlots, when cattle are exposed to several stressors over several days (Araujo et al., 2010). Some of these stressors include weaning, commingling with new animals, exposure to novel environments, possible injury from excessive handling and transport, thermal stress, fatigue, feed and water deprivation during road transport, as well as the resultant disruption in endocrine or neuroendocrine function characterized by activation of the HPA axis (Carroll and Forsberg, 2007). Stress is an important factor in the animal feeding industry due to direct links between intensity of stress and effects on growth, reproduction, meat quality, animal welfare, and disease susceptibility (Schaefer et al., 1997; Yamane et al., 2009).

Inflammation

Inflammation is a multicomponent antigen-nonspecific stereotyped reaction to infection that recruits leukocytes and immune activity to the site of infection or tissue damage (Medzhitov and Janeway, 1997). Its function is to combat dangers of all types, not simply to recognize non-self and self (Piccinini and Midwood, 2010). Principle effects include an increase in blood supply to the site of infection by vasodilation of arterioles, increased vascular permeability to large plasma molecules for antibody production, and pro-inflammatory cytokine production to enhance migration of leukocytes across the local vascular endothelium into the infected tissue (Holliman, 1992; Roitt, 1994). Inflammatory responses engage the innate immune system by recognizing a highly conserved set of pattern recognition receptors; these receptors are the key to initiate inflammation and can be induced exogenously by microbial or non-microbial

inducers, or endogenously by signals from stressed, damaged, or otherwise malfunctioning tissues (Medzhitov, 2008). Inducers act on macrophages resident in tissue, on mast-cells and on specific tissue cells to trigger the production of inflammatory mediators (Luster et al., 2005). Some of these mediators include vasoactive amines and peptides, complement fragments, lipid mediators such as prostaglandins, thromboxanes, leukotrienes and lipoxins, pro-inflammatory cytokines such as TNFα, IL-1 and IL-6, chemokines, and proteolytic enzymes (Medzhitov and Janeway, 1997).

Effects of inflammatory mediators can be local or systemic. Local responses are characterized by redness, heat, swelling and pain while systemic responses result in symptoms of fever, endocrine and brain effects (Medzhitov and Janeway, 1997). Pro-inflammatory cytokines have potential to induce catabolism that increases lipolysis of adipose tissue and muscle proteolysis with varying degrees of involvement; nonetheless, the negative effects can be very important for quality of life because of their detrimental effect at a physical and mental level (Dantzer et al., 2008). For example, the development of decreased motor activity and reduction in appetite in sick animals is reflective of depression-like behavior induced by infection (Dantzer et al., 2008) and reduce productivity in food producing animals. Increases in blood glucose from catabolism of tissue may be due to increased cortisol production caused by stress as well as insulin resistance (Medzhitov and Janeway, 1997). Whereas, inflammatory responses are essential to reduce sensitivity to infections and to increase survival, inflammation may be viewed as beneficial and detrimental in that it protects the host from infection but also can potentially be very harmful (Sorci and Faivre, 2009). A “neutrophil paradox” exists

when normally beneficial leukocytes gain potential to contribute to the pathogenesis of infectious disease if their proinflammatory properties are not appropriately regulated; neutrophils' ability to degranulate and release harmful proteolytic enzymes and reactive oxygen species can cause excessive damage to otherwise healthy tissue at the infection site (Sporer et al., 2007). This alleviates the interaction between inflammatory responses and glucocorticoid release with question as to whereas the interaction is beneficial or detrimental to immune responses.

Stress Impact

Acute stress occurs when an animal experiences a stressor for a short period of time and has been suggested to prime the immune system and increase resistance to infectious disease (Dragos and Tanasescu, 2010; Hughes et al., 2013). During a response to an acute stressor, an animal initiates restraining forces to prohibit an over-reaction from the central and peripheral components of the stress system (Hughes et al., 2013). While leukocytes have been observed to decrease in circulation in the presence of an acute stressor, the response is most likely due to redistribution rather than immune suppression. When animals experience a transient stressor, leukocytes exit circulation and infiltrate sites of wounding and lymphoid tissues (Dhabhar and McEwen, 1999). Prolonged subjection to stress and therefore prolonged secretion of glucocorticoids can lead to the development of pathological conditions (Hughes et al., 2013). Chronic stress manifests when an animal experiences a prolonged insult to its homeostatic state with potential to shift the stress response from one that is preparatory to one that is suppressive across the entire immune system (Roth, 1985; Carroll and Burdick Sanchez, 2013;

Hughes et al., 2013). The stress calves undergo as a result of the beef marketing process from abrupt weaning, transportation, commingling with calves from other cow calf operations, feedlot processing, and adaption to a novel environment manifests into chronic stress (Richeson et al., 2016); however, the shift from acute to chronic stress is dependent upon the animal and its perception and duration of the stressor as well as its ability to overcome a stressful event based on previous exposure, genetics, gender, temperament, and other contributing factors (Habib et al., 2001; Chrousos, 2007). The transition from acute to chronic stress condition in cattle is largely unknown and requires further research to better understand this phenomenon.

Inhibition of L-selectin and consequently reduced translocation of neutrophils into the epithelial tissue mediated by the release of glucocorticoids, such as cortisol from the adrenal glands, causes immune dysfunction in calves that undergo chronic stress (Burton et al., 1995, 2005). Sapolsky et al. (2000) described a diminished immune response during stress as a coping mechanism for the animal to reserve and redirect resources towards activity more immediately valuable to survival. Therefore, stress has the potential to suppress immune activity due to catabolism of immune cells and tissues to provide protein and glucose (Martin, 2009). It has been reported for calves that have not been previously administered a respiratory vaccine, weaned and allowed an adequate adaption period to feed, and furthermore transported, and commingled with new calves from other cow calf operations are at increased risk for developing signs of BRD (Ribble et al., 1995; Stanton, 2009).

Indicators of Stress

The Acute Phase Response

During immune system stimulation, an extruding insult will stimulate macrophages to release pro-inflammatory cytokines, including interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF-α; Klasing, 1988; Werling et al. 1996) while double stranded viral RNA induces type 1 interferons (IFNα/β; Fossum, 1998). These cytokine messengers initiate the acute phase response, which is characterized by dramatic changes in synthetic activity of the liver to produce acute phase proteins (APPs; Colditz, 2002). While APPs are synthesized in the liver, some APPs are also synthesized in the gut during the acute phase response (Wang et al., 1998). The major positive APPs in cattle, meaning increased concentrations during the acute phase response, include haptoglobin (Hp), serum amyloid-A (SAA), and fibrinogen (Fb; Humblet et al., 2004; Nikunen et al., 2007). In contrast, concentrations of albumin (negative APP), high density lipoproteins, and low-density lipoproteins all decrease during the acute phase response (Colditz, 2002). These changes in concentrations result from increased protein turnover due to immune system stimulation; positive APPs increase protein synthesis with a lower rate of degradation while negative APPs increase protein synthesis with a high rate of utilization. Acute phase proteins have also been used as prognostic markers or assessing severity of diseases (Horadagoda et al., 1999; Humblet et al., 2004; Schneider et al., 2013). Plasma proteins involved in the acute phase response include C-reactive protein, mannose binding protein and serum amyloid P (Colditz, 2002). These are pentameric lectin-like molecules that bind to microbial, rather than self, polysaccharides and promote

engulfment of microbes by the phagocytic leukocytes, macrophages, and neutrophils (Roitt, 1994).

In addition to modifying the metabolic activity in the liver and gut, proinflammatory cytokines including IL-6, IL-1β, and TNFα induce further systematic effects. IL-1β and IL-6 activate the hypothalamus to induce fever, and IL-1β and TNFα reduce protein accretion in the muscle by redirecting amino acids via deamination to energy production (Klasing, 1988). This leads to an increase in oxygen consumption as well as an increase in metabolic rate (Klasing, 1988). These cytokines also induce anorexia and sickness behavior (Johnson, 1998) with lipolysis in some adipocyte depots, which is an antagonistic effect by glucocorticoids and may be elevated later in the immune response (Wynn et al., 1994).

Cortisol

Animals respond to stress with activation of behavioral and physiological responses centrally controlled by the hypothalamus with synthesis of corticotropin-releasing hormone (CRH; Smith and Vale, 2006). In response to stress, CRH is released from the hypothalamus into portal vessels that access the anterior pituitary gland (Smith and Vale, 2006). Upon binding to its receptor on pituitary corticotropes, ACTH is released from the anterior pituitary gland and stimulates the adrenal cortex to increase the synthesis and secretion of cortisol (hydrocortisone) (Roth, 1985; Smith and Vale, 2006). Under basal conditions, cortisol interacts mostly with high-affinity mineralocorticoid receptors, which are important for normal homeostatic control of metabolic processes (King and Hegadoren, 2002); however, when the HPA axis is activated during a stressful

experience, it has been reported that cortisol levels can increase at least 10-fold in mice (Schimmer and Parker, 1996). Since cortisol is the end product of induction of the HPA axis, it is plausible that measuring cortisol concentration in the laboratory can serve as an adequate indicator of stress. Cortisol concentration can be measured from saliva, serum, hair, feces and urine (King and Hegadoren, 2002) and has been long used to indicate stress (Grandin, 1997; Queyras and Carosi, 2003) due to its easily accessible peripheral measure that provides reliable quantification (Baum and Grunber, 1997).

It is important to note the measure of cortisol as an indicator for stress also has several disadvantages. Queyras and Carosi (2003) elaborated on the diurnal rhythm in which cortisol is naturally secreted with increases in the morning with a low point reached around midnight. In addition, as previously described in this thesis, each stressor involved in the beef production system elicits variable degrees of HPA stimulation with supplementary effects mediated by host differences. When utilizing serum cortisol concentration as a stress indicator, handling is necessary to retrieve a blood sample prior to laboratory processing and measurement. Handling an animal causes an increase in blood cortisol in as little as 2 minutes (Queyras and Carosi, 2003). In addition, during times of intense stress, cortisol can return to baseline in as little as 90 minutes due to the negative feedback cortisol has on the HPA axis (Queyras and Carosi, 2003). This indicates that cortisol may not be an effective measurement during chronic or long-term stress.

Leukocyte Variables

A common practice to evaluate physiological effects on hematological status includes conducting a complete blood count using an automated hemocytometer. Changes in blood leukocyte concentration and/or percentage have been historically used as a measure of stress before methods were available to directly assay the cortisol hormone (Hoagland et al., 1946). Increased plasma cortisol concentrations can result in neutrophilia, lymphopenia, and eosinopenia (Ramin, 1995). While complete blood count analysis measures all leukocyte and erythrocyte variables, these specific trends are the most investigated within the literature regarding the stress response.

Neutrophils are widely investigated because they are the first line of immunity against most pathogens that infect cattle (Roth, 1985; Burton et al., 2005) and are the most thoroughly understood phagocytic cell type (Roth, 1985). Pronounced neutrophilia has been observed following administration of an artificial glucocorticoid (Burton et al., 1995; Weber et al., 2001; Weber et al., 2004; Chang et al., 2004; Hughes et al., 2017) because of down-regulated expression of surface adhesion molecules during the early phase of glucocorticoid exposure (Burton et al., 1995; Weber et al., 2004). In addition to a down-regulation in surface adhesion molecules, bovine circulating neutrophils possess high expression of glucocorticoid receptors when compared to other species and therefore are highly sensitive to changes in circulating glucocorticoid concentrations (Burton et al., 2005). Observation in an increase in the N:L has been reported (Hickey et al., 2003) by increasing the proportion of neutrophils which is thought to act as a biological indicator of stress and disease susceptibility (Herzog, 2007) as previously discussed in this thesis.

Lymphopenia and eosinopenia have been reported with less investigation than neutrophilia throughout times of glucocorticoid increase. During times of lymphopenia, depressed in vitro lymphocyte blastogenic response to mitogens because of increased plasma cortisol concentration induced by ACTH administration (Roth et al., 1982). In addition, decreased lymphocytes were reported by Hughes et al. (2017) in cattle that received exogenous dexamethasone to mimic chronic stress; however, the decrease reported was not considered lymphopenia as lymphocytes were still in the normal reference range for cattle (Hughes et al., 2017). It has been hypothesized that this depressed response may be attributable to a direct effect of cortisol on lymphocytes to inhibit mitosis (Roth, 1985); however, it is important to note that this does not necessarily indicate immune function is impaired as lymphocytes are probably sequestered to lymphoid tissues to aid in infection and more efficiently interact with antigens (Roth, 1985). Confirmed eosinopenia during cortisol release (Roth, 1985) has been reported as an effective indicator in identification of calves with a high risk for development of BRD (Richeson et al., 2013). Richeson et al. (2013) hypothesized the relationship between eosinopenia and BRD risk may have been a contribution of stressed calves having altered hematopoiesis from eosinophils being redirected from circulation to a source of inflammation. Roth et al. (1981) further demonstrated this phenomenon when eosinopenia was observed three days following a BVDV challenge in cattle.

Vaccination in Beef Cattle

Respiratory Vaccinations

Vaccination is one of the most cost-effective measures to prevent disease and vaccines are primarily developed to prevent disease as a result of infection (Van Oirshcnot, 1999). Vaccine products are available commercially in an array of MLV, inactivated killed organisms, or inactivated toxins of organisms known to cause a particular disease (Faries, 2005). When compared to total production costs, expenditures for both preventatives and treatment only account for 2 to 6 percent of the total production cost per animal (Griffin, 1997). Unfortunately, the complexity of factors associated with BRD frequently creates inconsistency in morbidity reports and despite the development of new vaccines and therapeutics, the disease continues to be the primary health problem of feedlot cattle (Griffin, 1997).

When discussing vaccines, it is imperative to evaluate the importance of vaccine efficacy and vaccine efficiency. Vaccine efficacy includes the reduction in disease incidence in a vaccinated group when compared to an unvaccinated group and should be assessed in a vaccination-challenge experiment in the target host in an animal for which the vaccine is meant (Van Oirschot, 1999). The vaccine must show to be biologically active and safely stimulate an active immune response against the agents contained in the vaccine (Richeson et al., 2015). Ideally, exploration of vaccine efficacy should be followed by a transmission experiment that trials the ability of the vaccine to prevent or reduce transmission of the challenge virus (DeJong and Kimman, 1994). Vaccine efficiency or effectiveness describes the ability of a vaccine to prevent negative outcomes

of interest in a desired species (Fedson, 1998) and should result in a reduction in clinical illness, improvement in weight gain, and show a clear economic advantage in the commercial production setting (Richeson et al., 2015). Effectiveness is historically assessed retrospectively, often in a cohort study with rigorous risk adjustment to ensure the comparability of study populations (Fedson 1998).

Viral Vaccines

Viral vaccines deliver antigens that stimulate the body's immune response through the production of antibodies (Faries, 2005) and cell-mediated factors. Bovine respiratory vaccines available in today's market include trivalent protection against IBRV, BRSV, and PI3V, pentavalent protection against IBRV, BRSV, PI3V, BVDV I and II, single protection against one respiratory antigen, or protection against viral and bacterial antigens in combination vaccines. Upon feedlot arrival, nearly all calves are vaccinated against at least one of these respiratory viruses (USDA-APHIS, 2011a). Various experiments have been performed to evaluate the efficacy of live and killed vaccines, particularly against BVDV, (McClurkin et al., 1975; Harkness et al., 1987; Brownlie et al., 1995; Van Oirschot, 1999) and it is apparent that the type of vaccine, number of vaccinations, challenge viral strain, and interval time between vaccinations confound results. Van Oirschot (1999) concluded that vaccines do not always achieve complete protection and therefore opportunity exists to improve the efficacy of vaccines. Nonetheless, several MLV and KV vaccines containing the 4 major viruses that contribute to BRD are licensed by the USDA and are commercially available (Inglis et

al., 2003). These vaccines may contain numerous strains and concentrations of viruses with potential to induce various levels and types of immune responses (Platt et al., 2006).

Modified-Live Virus Vaccine

Live vaccines contain an attenuated strain of virus, with the most common avenue for attenuation achieved by multiple passaging viral strains in cell cultures from bovine and/or non-bovine origin, that replicate in the host (Van Oirschot, 1999). Advantages of live-attenuated vaccines include a strong immune response from a single administration of a replicating antigen and induction of both cell-mediated and humoral immunity; however, live vaccines have potential to over replicate in the host and increase the risk of reverting to full virulence following administration (Potter and Gerdts, 2008). To promote immunization rather than disease in vaccinated hosts, the process of attenuating mutation is important (Webster et al., 2003). Lobmann et al. (1984) formed a basis for a vaccine against BVDV by treating live BVDV with nitrous acid and selecting a temperature-sensitive mutant by limiting dilutions at permissive and restrictive temperatures. This provides one example of the possible manufacturing involved in producing live vaccines that contain attenuated strains of a virus. Platt et al. (2006) carried out a study to measure antigen-specific T-cell subset activation to BRD viruses by administration of a MLV virus vaccine. They concluded that the vaccine induced antigen-specific serum virus neutralization, T-helper cells, and cytotoxic T cells persisted from 1 to 6 months after vaccination (Platt et al., 2006). In addition, the vaccine reduced viral shedding of BHV-1 and febrile responses in vaccinated calves when compared to control calves after a controlled BHV-1 challenge (Platt et al. 2006). This shows the efficacy of a

MLV vaccine in which the immunity induced by MLV vaccination decreased viral replication and febrile responses 6 months after a single dose was administered.

While MLV vaccination has proven to be efficacious, the safety of MLV vaccination has received attention particularly in immunosuppressed animals and replacement heifers (Hantman et al., 1999; Perry et al., 2017). Stress is known to compromise immune function (Chirase et al., 2004) and label guidelines released by vaccine manufactures recommend to avoid vaccination to stressed cattle (Richeson et al. 2008). Richeson et al. (2008) conducted a study that evaluated the effects of on-arrival versus delayed (14-days) MLV vaccination of newly received beef calves and reported improved vaccine response and slightly improved performance for cattle that were vaccinated 14 days post-arrival. While morbidity and mortality were not different between arrival or delayed vaccination, improved gain performance of high-risk, newly received cattle in the delayed vaccination group suggests greater economic advantage to delay initial MLV vaccination (Richeson et al., 2008). Similar results were reported in a later study such that cattle receiving a pentavalent modified-live virus vaccine 14 days after arrival had greater average daily gain than cattle that were vaccinated upon arrival (Richeson et al., 2015). In addition, Richeson et al. (2016) conducted a study that utilized dexamethasone administration to mimic acute and chronic stress in beef calves and measured immunological responses to a multivalent respiratory vaccine that contained replicating and non-replicating agents. Their results indicated treatment differences in antigen-specific antibody concentration with IBRV-specific antibody titer concentrations and BVDV-specific antibody titer concentrations to be greatest for chronic stressed

animals because immunosuppression was greater (Richeson et al., 2016). Regarding replacement heifers, Perry et al. (2017) reported greater conception rates in heifers that received a chemically altered/inactivated BHV-1/BVD vaccine than heifers who were administered a MLV vaccine with the same antigens. These results cause concern as to if administration of MLV vaccines gain potential to over replicate in immunosuppressed calves that have undergone chronic stress or decrease conception rates in replacement heifers and emphasize importance of balance between safety and efficacy in the use of MLV vaccines.

Killed Virus Vaccine

Inactivated vaccines include viral components that have been rendered non-infectious most often via chemical treatment such as formaldehyde (Van Oirschot, 1999; Hughes, 2015). These viral antigens or components are incapable of replication and therefore lower the risk of adverse effects in host animals as well as the developing fetus in pregnant animals (Newcomer et al., 2017). When utilizing a killed vaccine, additional handling is required due to the need for initial vaccinates to be dosed multiple times, primary administration with administration of a secondary "booster", to achieve protective antibody levels in the host (Van Oirschot, 1999; Newcomer et al., 2017). The primary reason to utilize an inactivated vaccine is increased safety when compared to MLV products. Inactivated viral strains contained in KV vaccines are neither immunosuppressive nor pathogenic due to non-replicating feature and inability to over-replicate once injected into the host (Kelling, 2004); however, the onset of immunity may be delayed 4 to 6 weeks following initial dose administration and viral challenge before

administration of the booster or incomplete realization of the vaccine protocol may result in vaccine failure (Newcomer et al., 2017).

Inactivated vaccination reduces adverse effects in the host animal when compared to a MLV vaccine because the inactivation process increases potential to alter functionality of antigens, leading to production of predominantly non-neutralizing antibodies in immunized cattle (Ellis et al., 1995). Therefore, KV vaccines are considered a safer option in vaccination programs where stress may be present in the animals. Richeson et al. (2016) reported decreased antigen-specific antibody response to a non-replicating *Manheimia haemolytica* toxoid fraction of a combination respiratory vaccine for immunosuppressed animals induced by glucocorticoid administration to mimic chronic stress. This suggests possible challenges when utilizing inactivated vaccines in high-risk or immunosuppressed cattle as optimal immunization may not be achieved due to decreased antibody titer responses to KV versions. In addition, after exogenous cortisol administration in calves followed by simultaneous vaccination with a non-replicating antigen, *Salmonella dublin*, the antibody response was inhibited (Roth, 1985). Despite possible decreased antibody responses in high-risk or immunosuppressed cattle, KV vaccination has shown benefits when used in replacement heifers with greater reception rates than heifers that received MLV vaccination (Perry et al., 2017). It is important to understand effects of stress and condition of calves at vaccination due to apparent effects of vaccine antigen type on immune responses and reproductive performance.

Bacterins

A vaccine containing killed bacterial cells is called a bacterin (Faries, 2005). Bacterins contain adjuvants which are additives that increase effectiveness of the antigens by slowing the release of the antigen into the body and prolonging the immune response (Faries, 2005). Vaccines may contain single bacterin components or bacterin components paired with viral antigens. The vaccine utilized for determining effects of acute or chronic stress on the antibody response previously mentioned in Richeson et al. (2016) is an example of a bacterin paired with viral antigens. Vaccine manufacturing often includes toxoid or leukotoxin virulence factors for production of bacterins with *Manheimia haemolytica* leukotoxin (LKT) recognized as the most dominant virulence factor (Czuprynski et al., 2004; Welsh et al., 2004; Abdelsalam, 2008) and the most relevant and successfully applied antigen for bacterin vaccination (Oppermann et al., 2017). Effects of LKT are dose dependent and at lesser concentrations induce bovine cells to undergo respiratory burst and degranulation that in return cause production of inflammatory cytokines (Oppermann et al., 2017); however, at greater concentrations LKT induces apoptosis and the formation of transmembrane pores with outcomes involving breakdown of the pulmonary immune system (Rice et al., 2007). There may be increased risk when utilizing bacterins paired with LKT in high-risk or immunosuppressed calves but further research is warranted.

Immunostimulants

The beef and dairy industries continue to receive feedback from consumers regarding their concerns with antimicrobial use in these food production systems. One

way to potentially mitigate bacterial and/or viral infection is to administer substances exogenously that augment the immune response and increase disease resistance (Blecha, 1988). The ability to enhance an immune response to benefit the animal and production efficiency is the goal of immunostimulation in food producing animals and the substances that exert this control are known as immunostimulants (Blecha, 2001). Stimulation of the immune system may be either cellular or humoral and it is important to choose suitable relevance when experimenting and utilizing immunostimulatory products because each is applicable to a different type of immune challenge condition (Ganeshpurkar and Saluja, 2017).

Immunostimulants have raised much interest among beef cattle producers as a novel preventative measure that may reduce antimicrobial use in livestock; however, immunostimulants have had both positive and negative outcomes associated with dose and duration of usage as well as age and immune status of the host animal (Schultz, 1998). Broad categories of immunostimulants include cytokines and pharmaceuticals and as knowledge of these products become more well-known, scientists research intervention strategies to modulate the immune response to improve outcomes in the host (Blecha, 2001). While immunostimulants are intended to benefit the animal, only a limited number of immunomodulatory products are licensed by the United States Department of Agriculture for use in food-producing animals and few have received approval from the Food and Drug Administration (Huenefeld, 1988; Blecha, 2001). In addition, an important factor that must always be considered when utilizing an immunostimulatory product is whether it is cost effective.

Imrestor

Pegbovigrastim injection contains PEGylated recombinant bovine granulocyte colony stimulating factor (PEG bG-CSF) in a buffered sodium acetate solution (US Food and Drug Administration, 2016). The brand name as marketed by Elanco Animal Health, is Imrestor and is approved for the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers (US Food and Drug Administration, 2016). The product is supplied in a 15 mg, 2.7 mL pre-dosed syringe and is labeled to administer a full syringe 7 days prior to the cow or heifer's anticipated calving date with a second administration of a full syringe within 24 hours after calving (US Food and Drug Administration, 2016). Cows or heifers treated with Imrestor have shown increased white blood cell count (WBC), absolute neutrophil counts and percentages, and absolute band cell counts and percentages when compared to controls after initial periparturient administration (US Food and Drug Administration, 2016).

Dairy cows suffer from decreased immune cell function around the time of calving due to decreases in polymorphonuclear neutrophil (PMN) and lymphocyte function 2 to 3 weeks before calving (Kimura et al., 2014). Several studies have investigated the effects of pegbovigrastim injection in clinical and non-clinical outcomes for use of the drug and potential to decrease mastitis incidence and increase performance among dairy herds (Kimura et al., 2014; Canning et al., 2017; McDougall et al., 2017; Ruiz et al., 2017). Whereas Kimura et al. (2014) observed increases in total PMN counts after initial pegbovigrastim dose was administered before and after calving, treated

animals and controls exhibited large day to day variation in phagocytosis activity and there was no observed effect on production of superoxide anion in stimulated or resting PMN. Similar results regarding increased PMN counts were observed by Canning et al. (2017) and while they did not measure neutrophil functionality, overall incidence of clinical mastitis was reduced by 35 percent among pegbovigrastim treated animals. This reduction in mastitis is a common trend among clinical studies evaluating the effects of pegbovigrastim (Hassfurther et al., 2015; Canning et al., 2017; Ruiz et al., 2017). In addition, McDougal et al. (2017) reported increased total WBC, PMN, lymphocyte, and monocyte counts in multiparous cows that received pegbovigrastim injection as early as one day after the initial injection 7 to 10 days before parturition, with continued elevated levels 7 days following the second injection that was administered within 24 hours of parturition. Decreased PMN phagocytosis reported by McDougall et al. (2017) declined over time throughout the sampling period after pegbovigrastim was administered; however, this was not affected by pegbovigrastim treatment and corresponds with inconsistent results reported by Kimura et al. (2014) where pegbovigrastim did not improve PMN function. It can be concluded that utilizing pegbovigrastim allows for reduction in clinical mastitis incidences which also reduces antimicrobial treatment for the disease by increasing total PMN counts in blood circulation.

Zelnate

The first commercial DNA immunostimulatory product in the veterinary market was released in 2015, marketed as Zelnate, (Ilg, 2017) and has been shown to reduce lung lesions and mortality due to BRD (Bayer, 2014). The product consists of bacterial

plasmid DNA rich in non-methylated CpG motifs, that is encased in a cationic liposome shell (Ilg, 2017). Zelnate is indicated for the use as an aid in the treatment of BRD due to *Manheimia haemolytica* in 4-month-old cattle or older, when administered at the time of, or within 24 hours after, a perceived stressful event (Nickell et al., 2016). Ilg (2017) studied the potential molecular mode of action of Zelnate by investigating cellular DNA recognition pathways in cell culture by a set of gene knockout cell lines and reporter gene assays. He utilized activating ligands for the first PRR discovered that recognizes DNA, type I transmembrane toll-like receptor 9 (TLR9), from humans and mice, and concluded that Zelnate may interact with and activate TLR9; however, this possibility was not fully demonstrated (Ilg, 2017). A video provided on the Bayer website for Zelnate claims the product mimics a pathogen infection and stimulates the innate immunity of calves to aid in prevention of disease and treat existing infections.

Rogers et al. (2017) carried out a field study with the DNA immunostimulant to evaluate health outcomes as the indication for administering the product on arrival was to activate the innate immune response to fight BRD pathogens at the time of stress and disease challenge. Whereas there were no differences in percentages reported in cattle that did or did not receive the product treated once for BRD, there tended to be a reduction in third antimicrobial treatment (Rogers et al., 2017). In addition, the inclusion of immunostimulant reduced the percentage of BRD-associated and overall mortality on day 60 (Rogers et al., 2017). These results, along with previously reported results by Bayer (2014), show a reduction in morbidity and mortality in cattle who receive DNA

immunostimulant and further suggests that the product has potential to positively influence the health outcome of feedlot calves.

Conclusions from the Literature

Bovine respiratory disease is a multifaceted disease highly influenced by stress-induced immunosuppression and pathogen exposure from the beef cattle marketing process and management practices among dairy producers. Viral pathogens gain potential to infect the host upon exposure and during times of immunosuppression caused by chronic stress. This results in greater replication of viral pathogens in the host with additional bacterial pathogen proliferation and infection in the lungs. Much research effort has been aimed to minimize the prevalence and severity of BRD; however, as new products are approved BRD-associated morbidity and mortality does not seem to be improving across the industry. This shows the impact of management strategies as well as traditional marketing practices have on animal outcomes. Vaccination provides immunity against viral infection, but it is important to understand the relationship between stress and viral antigen type. Modified-live virus and KV vaccines provide potential protection against viral infection if they are utilized appropriately. In addition, recently introduced immunostimulatory products may contribute in prevention of respiratory disease that may serve as an alternative control method and thereby decrease antimicrobial use in food-producing animals. Further research is warranted to improve understanding of the relationship between stress, immune status, disease, and vaccination to ensure that current practices are safe and effectively reduce the negative impacts of BRD.

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CHAPTER III

IMMUNE RESPONSES ARE INFLUENCED BY VACCINE ANTIGEN TYPE AND ACUTE OR CHRONIC STRESS MODEL IN BEEF CALVES

ABSTRACT

The study objective was to determine if acute or chronic stress models affected antibody, endocrine, or hematological responses to modified-live virus (MLV) or killed virus (KV) respiratory vaccination in beef steers. A total of 48 crossbred beef steers (d 0 BW = 226 ± 6.2 kg) from a single ranch origin were used in a 2 × 2 factorial design to evaluate main effects of stress model, vaccine type and their interaction; resulting in 4 treatments (n = 12) consisting of acute stress with killed virus vaccination (ACUKV), ACU with modified-live virus vaccination (ACUMLV), chronic stress with KV (CHRKV), and CHR with MLV (CHRMLV). The ACU treatments were weaned at their origin ranch on d -37 and transported 472 km to the study site near Canyon, TX on d -21 to allow acclimation. The CHR treatments were weaned on d -3, transported 460 km to a facility near Lubbock, TX on d -2, and again transported 164 km to the study site on d -1 to mimic the beef marketing process prior to feedlot arrival. Vaccine treatments were administered on d 0 and KV was revaccinated on d 14. Animal was experimental unit and dependent variables were analyzed using PROC MIXED with repeated measures (d). Binomial virus detection data generated from nasal swab collection was analyzed using Fisher's exact test via PROC FREQ. Haptoglobin (Hp) concentration was determined using ELISA from serum collected on d -2, 0, 1, 3, 5, 7, and 14. Cortisol concentration

was determined using enzyme immunoassay (EIA) from serum collected on d -2, 0, 1, 3, 5, and 7. Complete blood count was determined from whole blood collected on d -2, 0, 1, 3, 5, 7, 14, and 21 via automated hematology analyzer. Vaccination with KV elicited a vaccine type × d ($P < 0.01$) interaction with increased ($P \leq 0.01$) antibody titers against PI3V and IBRV on d 21; conversely, MLV calves had increased ($P \leq 0.01$) BVDV-specific antibody titers on d 14, 28, 35, 42, 49, and 56. Increased ($P \leq 0.05$) BRSV-specific antibody titers were observed in a stress model × d ($P < 0.01$) interaction for CHR calves on d 21, 28, 36, and 42; however, ACU exceeded CHR calves in BVDV-specific antibody concentration on d 21, 28, and 49. Over 50% of ACU calves were determined positive for BRSV in nasal specimens on d 0, suggesting wild-type exposure. In addition, of the CHRMLV calves, at least one calf was detected positive for IBRV, BVDV, BRSV, or PI3V in the naris on d 7. A d effect ($P < 0.01$) was observed for Hp with greatest ($P < 0.01$) concentration on d 3 (284,331 mg/dL), following vaccination on d 0. Serum cortisol concentration was greater ($P \leq 0.04$) for ACU than CHR calves on d -2, 0, 1, 3, and 5 and overall serum cortisol was increased ($P < 0.01$) for ACU (58.96 ng/mL) vs. CHR (41.06 ng/mL). Total leukocytes were not different on d -2 ($P = 0.66$) but decreased for CHR vs. ACU on d 0, 1, 3, 5, 7, 14 and 21 ($P \leq 0.02$). Vaccine type also affected total leukocytes such that they were decreased ($P \leq 0.04$) for MLV on d 5, 7, and 14 compared to KV. Neutrophils (2.01 vs. 3.83 K/µL) and neutrophil:lymphocyte (0.27 vs. 0.67) were markedly increased ($P \leq 0.01$) for CHR on d -2; conversely, neutrophils were decreased ($P \leq 0.01$) on d 1 and 21 for CHR. Monocytes were decreased on d 1, 5 and 7 for MLV ($P \leq 0.04$) and d -2 to 14 for CHR ($P \leq 0.03$). Eosinophils were

reduced ($P = 0.007$) for CHR (0.097 K/µL) vs. ACU (0.176 K/µL) on d -2, yet a rebound response ($P = 0.03$) was noted on d 0 such that eosinophils were 0.288 and 0.160 K/µL for CHR and ACU, respectively. Serum cortisol was greater ($P \leq 0.04$) for ACU on d -2 to 5, suggesting suppression of endocrine activity in CHR over time. Results indicate CHR stress model and MLV vaccination may have more profoundly induced immunosuppression in beef calves.

Introduction

Cattle may be vaccinated with an array of antigens and antigen types (i.e., killed or modified-live versions) with the goal of preventing disease; however, host-dependent vaccine failure may be attributable to parasitic infection, poor health or nutrition status, and physiological stress (Comerford, 2017). Acute stress is defined as a less intense event < 24 h (Richeson et al., 2016), and has been proposed to stimulate the immune system and increase resistance against infection (Dragos and Tanasescu, 2010; Hughes et al., 2013). Extended exposure to stress (> 24 h) and thus prolonged secretion of glucocorticoid and catecholamine can result in immunosuppression (Hughes et al., 2013; Hughes et al., 2017). Chronic stress manifests when an animal experiences a prolonged insult to its homeostatic state with potential to shift the stress response from one that is preparatory to one that is immunosuppressive (Roth 1985; Carroll and Burdick Sanchez, 2013; Hughes et al., 2013). Immunosuppression initiated by sustained glucocorticoid synthesis has been reported to result in transient neutrophilia due to diminished L-selectin-mediated neutrophil migration (Burton et al., 1995, 2005; Lynch et al., 2010), lymphopenia from likely inhibition of mitosis and redistribution to lymphoid tissue (Roth et al., 1982; Roth, 1985), eosinopenia due to cellular apoptosis (Chrousos, 1995), and anti-inflammatory effects by inhibiting synthesis and release of pro-inflammatory cytokines (IL-1, IL-2, IL-3, IL-4, IFN-γ, TNF-α) and inhibition of other mediators such as histamine (Sapolsky et al., 2000). There may be negative consequences when vaccinating chronically stressed cattle, yet the effects may differ for killed vs. modified-liv versions. The current study objective was to determine the effect of an acute (**ACU**) or

chronic (**CHR**) physiological stress model on immunological, endocrine, and acute phase protein (**APP**) response in beef calves administered pentavalent respiratory vaccines containing replicating modified-live viruses or non-replicating killed viruses. Our hypothesis was that immune, endocrine, and APP response are differentially altered by ACU and CHR stress model and vaccine antigen type.

Materials and Methods

This study was conducted from November 2016 to March 2017 at 2 research sites. The initial d -2 collection sample reported herein for the chronic stress model was conducted at the USDA-ARS Livestock Issues Research Unit near Lubbock, TX. Subsequent samples were collected at the West Texas A&M University (**WTAMU**) Research Feedlot in Canyon, TX. Animal procedures and experimental protocols were independently approved by the animal care and use committees at USDA-ARS (protocol number 1603S) and WTAMU (protocol number 2-11-16) specific to procedures conducted at each location.

Animals and Housing

Forty-eight clinically heathy, previously unvaccinated Angus × Hereford beef steers (d 0 average BW = 226 ± 6.2 kg) that were seronegative on d -37 to infectious bovine rhinotracheitis (**IBRV**), bovine viral diarrhea virus (**BVDV**), parainfluenza-3 virus (**PI3V**), and bovine respiratory syncytial virus (**BRSV**) antibodies were acquired for use in the study from a single ranch located in central New Mexico. The calves were placed in an isolated pen at their ranch of origin prior to relocation to the study site. Blood was collected on d -37 via jugular venipuncture to confirm seronegative status to

IBRV, BVDV, PI3V, and BRSV. Also on d -37, ACU steers were weaned and placed in an isolated pen at the origin ranch until d -21 when they were transported in a sanitized trailer to the WTAMU Research Feedlot (Canyon, TX). The ACU steers were meant to serve as a control group; however, were classified as ACU due to handling during sampling. The CHR steers were not separated from their dams until d -3 and were transported on d -2 approximately 450 km in a sanitized trailer to the USDA-ARS Bovine Immunology Research and Development (BIRD) facility (Lubbock, TX). Upon arrival to BIRD, calves were allowed to rest overnight in dirt lot pens with ad libitum access to hay and water. The calves were transported approximately 177 km on d -1 in a sanitized trailer to the WTAMU Research Feedlot. Upon arrival to the WTAMU Research Feedlot, the CHR calves were held in soil-surfaced pens separate from the ACU calves. Simultaneous weaning, transportation, handling, and relocation imposed on CHR calves was intended to mimic the typical marketing process that occurs for high-risk beef calves and associated chronic stress (Carroll and Forsberg, 2007; Araujo et al., 2010; Cooke, 2017).

Treatments and Vaccination

On d 0, steers assigned to ACU and CHR were randomly and equally allocated to receive either a pentavalent modified-live virus (MLV) respiratory vaccine (Pyramid 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]) or pentavalent killed virus (KV) respiratory vaccine (Triangle 5; BIVI). Both vaccines were administered s.c. at 2 mL in the neck at 0600 h on d 0. On d 14, per label directions, the KV treatment was administered a secondary booster in the same manner as the primary dose. The handling

and administration of vaccines used in this study closely followed Beef Quality Assurance guidelines. This resulted in 4 experimental treatments arranged as a 2 × 2 factorial consisting of: 1) ACU with KV vaccination (**ACUKV**); 2) ACU with MLV vaccination (**ACUMLV**); 3) CHR with KV vaccination (**CHRKV**); 4) CHR with MLV vaccination (**CHRMLV**). Both vaccines consisted of IBRV, BVDV type 1 and 2, PI3V and BRSV antigens, but with replicating (MLV) or non-replicating (KV) properties. Treatment randomization was achieved by drawing labeled cards from a hat with treatment designation of KV or MLV resulting in 12 animals assigned to each of the 4 treatments.

After vaccine treatments were administered, steers were allotted to 1 of 8 pens. Each treatment was housed in 3 consecutive pens with 4 animals per pen. Between each third pen (i.e. between treatments), a pen was left empty to keep treatments separated. This biosecurity consideration was implemented to reduce the risk of transmission of viruses from animals that might have shed vaccine-origin virus between stress model or vaccine type. One steer assigned to CHRMLV treatment died on d 10; the cause of death was due to peritonitis and was not determined to be influenced by the experimental treatment.

Blood Collection and Serology

An anti-coagulated blood sample was collected on d -2 at the WTAMU Research Feedlot for ACU calves and at the USDA-ARS BIRD facility for CHR calves via jugular venipuncture into tubes containing 7.2 mg EDTA (Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ). The timing of d -2 blood collection began at 1800 h

and was coordinated between the 2 sites. Subsequent anti-coagulated blood samples were collected at the WTAMU Research Feedlot on d 0, 1, 3, 5, 7, 14, and 21 and analyzed using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) at the WTAMU Animal Health Laboratory to determine complete blood count.

Jugular blood was additionally collected into evacuated blood tubes with no additive (Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) to harvest serum used to determine haptoglobin and cortisol concentration. All blood samples were placed in an insulated cooler immediately after sample collection without ice to achieve storage temperature of approximately 20°C and immediately transported to the WTAMU Animal Health Laboratory. The samples were allowed to clot ≥30 minutes before centrifugation at 1,250 × *g* for 20 minutes at 4°C. After centrifugation, serum was harvested and stored in triplicate aliquots at -20° C until subsequent analyses were performed.

One aliquot of the frozen sera was packaged on ice and transported to the Texas A&M Veterinary Medical Diagnostic Laboratory (**TVMDL**) located in Amarillo, TX to determine IBRV-, BVDV-, PI3V-, and BRSV-specific antibody titers from d 0, 7, 14, 21, 28, 35, 42, 49, and 56. Antibody titers were determined using the virus neutralization assay previously described by Rosenbaum et al. (1970). Sera from d -2, 0, 1, 3, 5, 7, and 14 were used to determine haptoglobin (**Hp**) concentration at the WTAMU Animal Health Laboratory via a commercial, bovine-specific ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR) with intra- and inter-assay CV of 8.46 and

11.53%, respectively. Cortisol concentration was determined from sera on d -2, 0, 1, 3, 5, and 7 at the WTAMU Animal Health Laboratory via commercial EIA kit (Arbor Assays, LLC, Ann Arbor, MI) with intra- and inter-assay CV of 8.62 and 15.50 %, respectively.

Nasal Swab Collection and Virus Detection

Nasal swab specimens were collected on d 0, 7, and 14 by inserting 2 nylon-flocked swabs (Puritan Medical Products, Guilford, ME) in the mid-naris region proximate to the nasal concha and rotated until both swabs were completely saturated. The duplicate nasal swabs were clipped, placed into a sterile polystyrene tube (Falcon; Corning Inc., Corning, NY) without additive, sealed, and stored at -80° C until subsequent analyses was performed. Swabs were transported on ice to the TVMDL in Amarillo, TX to detect the presence of IBRV, BVDV, PI3V, and BRSV via real-time PCR (**RT-PCR**) analysis. The general procedures for extraction and amplification of IBRV (Wang et al., 2007), BVDV (Mahlum et al., 2002), BRSV (Boxus et al., 2005), and PI3V (Richeson et al., 2016) are previously described.

Statistical Analyses

This 2 × 2 between-subjects factorial experiment used animal within pen as the experimental unit for all analyses of dependent variables. Data derived from serum samples (antibody titer, Hp, and cortisol) and hematological variables were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with repeated measures. The model for these repeated variables included main effects of stress model, vaccine type, d, and the 2-way (stress model × d; vaccine type × d) and 3-way (stress model × vaccine type × d) interactions. The repeated statement was d, and the covariance structure

with the lowest Akaike information criterion for each dependent variable was used. Antibody titers were log_2-transformed prior to statistical analysis with consequent results shown. Virus specific- antibody titers, serum Hp, cortisol, and CBC 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. Statistical significance was established for main effects of stress model, vaccine type, d, and each possible interaction if the resulting *P*-value was ≤ 0.05 and statistical tendency was considered if the resulting *P*-value was ≤ 0.10 and ≥ 0.06. Binomial virus detection data generated from nasal swab collection was analyzed using Fisher's exact test via PROC FREQ in SAS. The frequency of virus positive and negative nasal swabs was determined within virus type and d with significance established if a *P*-value was ≤ 0.05.

Results and Discussion

Virus-Specific Antibody Response

All calves used in this study were seronegative to IBRV, BVDV, PI3V, and BRSV on d 0. There was a vaccine type × d interaction ($P < 0.01$) for PI3V-specific neutralizing antibodies detected in serum such that KV calves had greater ($P \leq 0.01$) PI3V antibody titer than MLV from d 21 to 49 (Figure 3.1). Likewise, a vaccine type × d interaction ($P < 0.01$) existed for IBRV-specific neutralizing antibodies such that KV calves exhibited greater ($P \leq 0.02$) IBRV antibody titer than MLV from d 21 to 42.

Efficacy studies have explored virus-neutralizing antibody titers in MLV versus KV vaccination. Grooms and Coe (2002) reported cattle who received at least one dose of a MLV vaccine had higher virus-neutralizing antibody titers to BVDV than calves that only received a KV vaccine. This corresponds with our results as MLV calves overall yielded higher BVDV-specific antibody titers throughout the sampling period. In addition, Fulton et al. (1995) tested several commercially available MLV and KV vaccines and while KV antibodies peaked on d 28 and were maintained until 84 d post vaccination, MLV provided antibodies 140 d post vaccination.

There was a vaccine type × d interaction ($P < 0.01$) observed for BVDV-specific antibody; in contrast with PI3V and IBRV observations, the MLV calves had increased ($P \leq 0.01$) BVDV antibody titer on d 14, 28, 35, 42, 49, and 56. The increased MLV antibody response for BVDV may be indicative adjuvants present in the vaccine that increased virulence for this virus. Temporal trends for all vaccine antigens were indicative of a typical vaccine response with antibody concentrations detectable between d 7 and 14 with peak levels observed d 28 to 42 after vaccine administration. A stress model × d interaction ($P < 0.01$) was noted for serum BRSV-specific antibody titer (Figure 3.2). While ACU had greater ($P \leq 0.01$) BRSV titer than CHR on d 7 and 14, CHR exceeded ($P \leq 0.05$) ACU BRSV titer on d 21, 28, 36, and 42 in response to vaccination. This increase in the CHR model may be indicative of greater antibody response in CHR calves. Richeson et al. (2016) reported an increase in IBRV and BVDV antibody response to MLV vaccine agents in animals that concurrently received a chronic stress challenge using exogenous dexamethasone (**DEX**). A stress model × d interaction

($P < 0.01$) also existed for BVDV-specific antibody titer. Unlike BRSV detection, ACU calves overall exceeded CHR calves with greater ($P \leq 0.03$) BVDV titer concentrations on d 21, 28, and 49. These results contradict results reported by Roth and Kaeberle (1983) in which the antibody response was enhanced to BVDV in cattle administered adrenocorticotropic hormone, and Richeson et al. (2016) in which the BVDV-specific antibody titer was increased for calves administered ACU or CHR dexamethasone treatment vs. control. These differences may be due to differences in natural vs. artificial stress model implementation; however, contradicting results necessitate further exploration of stress and effects on host-viral replication after MLV or KV vaccination. Unfortunately, our results only represent a 56-d sampling period and no antibody duration differences were detected.

Serum Haptoglobin

Synthesis of APP primarily by hepatocytes can occur in response to exposure of stressors that result in acute inflammation (Baumann and Gauldie, 1994). Therefore, alterations in APP have been recognized as indicators of cattle morbidity (Godson et al., 1996), stress (Deak et al., 1997; Nukina et al., 2001) and inflammation (Murata et al., 2004). Although no treatment × d interactions ($P = 0.68$) were observed for serum Hp activity, there was a d effect ($P < 0.01$) resulting in overall serum Hp concentration being increased on d 3 (284, 331 mg/dL), 5 (238, 255 mg/dL), and 7 (230, 993 mg/dL) and decreased on d -2 (7, 506.58 mg/dL), 0 (17, 665 mg/dL), and 14 (15, 455 mg/dL; Figure 3.3). This Hp response was likely additively influenced by vaccination of the calves on d 0 (Arthington et al., 2013) and the stress models imposed. However, serum Hp

concentration differed from results reported by Richeson et al. (2016); they observed ACU stress to yield greater synthesis of Hp when compared to CHR stress whereas we observed no differences in Hp between ACU and CHR. These differences may be due to dissimilar approaches to stress model implementation; Richeson et al. (2016) utilized dexamethasone as a stress challenge that likely induced a robust anti-inflammatory effect whereas we utilized natural stress models in attempt to induce physiological stress. Nevertheless, the current APP results demonstrated the typical transient acute phase response overall.

Serum Cortisol

The primary hormone released from physiologic stress exposure is cortisol and has historically been measured to indicate stress (Grandin, 1997; Queyras and Carosi, 2003) due to its easily accessible peripheral measure that provides reliable stress response indication (Baum and Grunber, 1997). A stress model × d interaction existed ($P < 0.01$) for serum cortisol such that ACU had increased ($P \leq 0.04$) serum cortisol concentration on d -2, 0, 1, 3, and 5 (Figure 3.4). The timing of the initial stress event and associated cortisol concentration is important. The reduced cortisol observed for CHR may be influenced by the negative feedback mechanism to the hypothalamus-pituitary-adrenal (**HPA**) axis such that cortisol concentration may have reached peak concentration in CHR calves when they were weaned and transported on d -3, prior to the initial sample collected on d -2. Although serum cortisol concentration was not measured on d -3, our interpretation is further supported by a marked increase in circulating neutrophil on d -2 for CHR. Previous research demonstrates that neutrophils in the blood are increased in

response to stress and HPA activation due to the negative effect of cortisol on L-selectin expression (Burton et al., 1995; 2005). Queyras and Carosi (2003) reported that during times of intense or chronic stress, cortisol levels can return to baseline concentration in as little as 90 min after induction of the stressor due to the negative feedback cortisol has on the HPA axis. Negative feedback occurs by inhibiting synthesis and/or secretion of corticotropin-releasing hormone (**CRH**), vasopressin (**VP**), and adrenocorticotropic hormone (**ACTH**), and perhaps expression of the CRH (Aguilera, 1998). This suggests that serum cortisol concentration does not clearly indicate the degree of stress during times of prolonged exposure to stressors, and corroborates our serum cortisol observations.

Virus Detection in Nasal Swabs

Prevalence of PI3V, BRSV, BVDV, and IBRV in nasal swab specimens collected from steers on d 0, 7, and 14 is displayed in Table 1. Immediately prior to vaccination on d 0, no IBRV, BVDV, or PI3V was detected while 9 ACUKV and 4 ACUMLV steers were determined positive for BRSV via RT-PCR. The detection of BRSV was unexpected as the calves were single sourced from an isolated location and biosecurity efforts were established for this study. Vaccines had yet to be administered on d 0 upon nasal specimen collection, so BRSV detection on d 0 was from wild-type exposure. A greater ($P = 0.03$) percentage of ACU calves were shedding BRSV on d 7 with 33, 67, 33 and 8% of ACUKV, ACUMLV, CHRKV, CHRMLV, respectively, detected positive. A treatment effect ($P < 0.01$) was observed for BVDV presence in nasal swabs on d 7 such that 83% of CHRKV and 67% CHRMLV, respectively, were shedding BVDV in nasal

secretions whereas, BVDV was not detected in nasal swab samples from any of the ACU calves. One CHRMLV was determined to be positive for IBRV and 1 ACUMLV as well as 1 CHRMLV were determined positive for PI3V on d 7. Furthermore, of the CHRMLV calves, at least one calf was detected positive for IBRV, BVDV, BRSV, or PI3V in the naris on d 7. On d 14, a treatment effect ($P < 0.01$) was observed for BRSV; CHRMLV had the greatest proportion of calves shedding BRSV in the naris (100%) compared with CHRKV (67%). Positive detection in CHRKV calves was unexpected. This may be the result of false positives during RT-PCR analysis in the lab, exposure through aerosol or contact when worked through processing facility, or sample contamination. No ACU calves were shedding BRSV in the naris on d 14; however, 25% of ACUMLV were positive for BVDV and 1 ACUMLV calf was determined positive for PI3V on d 14. Additionally, 1 CHRMLV was detected positive for IBRV and PI3V on d 14. Molecular detection of IBRV, BVDV, BRSV, and PI3V in the naris of steers after vaccination suggests antigen exposure from replicating vaccination on d 0 for MLV calves; however, it is important to note that virus genotyping is necessary to confirm if detected respiratory virus strains in nasal specimens are homogenous to MLV vaccine, and that was not performed in the current study. Despite this limitation, all calves were tested for IBRV-, BVDV-, PI3V-, and BRSV-specific antibody titers from a serum aliquot on d -37 and d 0, and biosecurity measures implemented to all calves upon weaning, transportation, and arrival to WTAMU were in place to mitigate excessive exposure to wild-type virus exposure. The steers were sourced from a single isolated herd and never encountered other cattle after relocation and the trailer and research facilities were sanitized before

every use. Despite cautious biosecurity protocol, it is evident that ACU steers were exposed to wild-type BRSV virus prior to respiratory vaccination on d 0. It has been reported that BRSV has great genetic stability (Gershwin, 2007) with much genetic heterogeneity due to frequent mutations in the G-coding region resulting in a quasispecies (Deplanche et al., 2007). Field studies have shown continuous evolution of BRSV protein isolates since 1967 (Valarcher et al., 2000). This validates the stability of BRSV in the environment and provides possible explanation to increased wild-type BRSV exposure risk.

Treatment differences noted for the prevalence of BVDV on d 7 in addition to IBRV, BRSV, and PI3V on d 7 and 14 in nasal secretions suggest that immunosuppression was induced by CHR. The CHR model was representative of the segmented beef marketing system and enhanced viral replication and shedding of the virus-specific MLV strains administered to MLV CHR calves on d 0. These observations are similar to results reported by Richeson et al. (2016) such that greater prevalence of respiratory viruses were detected in nasal specimens for CHR steers induced by DEX administration when compared to ACU steers on d 7 and 14 after a MLV vaccine was administered on d 0. In addition, despite probable wild-type BRSV exposure before vaccination on d 0, ACUMLV calves had more positive detections of BRSV and PI3V on d 7 and BVDV and PI3V on d 14 when compared to ACUKV calves. These results suggest vaccine-induced shedding of MLV vaccine in both ACU and CHR calves. Further research is warranted to determine the safety and efficacy of MLV vaccine when compared to KV vaccine in highly stressed, newly received cattle upon arrival to a

feedlot or stocker setting. While MLV vaccines require careful consideration, they have been shown to be safe and efficacious when administered at appropriate times when stress is minimal and before exposure. Platt et al. (2006) observed immunity induced by a MLV vaccine decreased respiratory virus replication and febrile responses up to 6 months after a single dose was administered. In addition, previously mentioned data reported by Fulton et al. (1995) clearly validates the efficacy of respiratory vaccination practices; however, vaccination should be implemented under appropriate conditions such as during preconditioning at the ranch of origin before stress and disease challenge occurs (Richeson 2015; Richeson et al., 2016). Due to current marketing practices, vaccination in the U.S. beef production system is not always implemented at appropriate times, which was the impetus of the current research.

Complete Blood Count

There was a stress model × d interaction ($P < 0.01$) for total leukocytes (**WBC**), neutrophils (**NEU**), lymphocytes (**LYM**), neutrophil to lymphocyte ratio (**N:L**), monocytes (**MONO**), eosinophils (**EOS**), percent neutrophils (**PERNEU**), percent lymphocytes (**PERLYM**), percent monocytes (**PERMONO**), percent eosinophils (**PEREOS**), hemoglobin (**HGB**), hematocrit (**HCT**), mean corpuscular volume (**MCV**), mean cell hemoglobin (**MCH**), mean cell hemoglobin concentration (**MCHC**), red cell distribution width (**RDW**), and platelets (**PLT**). Increased ($P \leq 0.02$) WBC concentrations were observed for ACU relative to CHR steers beginning on d 0 and continued through d 56 (Figure 3.5). A similar trend was observed for MONO and PERMONO such that MONO were increased ($P \leq 0.04$) for ACU calves from d -2 to 14

and PERMONO were increased ($P \leq 0.01$) for ACU from d -2 to 7. The greatest ($P < 0.01$) total WBC was observed on d 1 (11.22 K/µL) in ACU steers followed by a decrease until d 5 (8.45 K/µL). This corresponds with results reported by Hughes et al. (2017) in which increased WBC from 24 to 48 h post-vaccination followed by a decrease 72 h post-vaccination were reported for animals that were administered a single DEX injection intended to represent ACU stress. While it has been proposed that ACU induces an initial increase followed by a decrease in blood leukocyte concentrations (Dhabar and McEwen, 2001), the overall increase in WBC for ACU compared to CHR calves may be indicative of the greater serum cortisol concentration observed for ACU calves (Roth, 1985). The initial increase followed by a decrease in WBC for ACU calves was greatly influenced by NEU. An increase in circulating NEU was observed such that ACU calves exhibited greater ($P < 0.01$) NEU on d 1 (3.36 K/µL), CHR calves NEU were (2.00 K/µL) followed by a decrease on d 3 (1.64 K/µL). In addition, CHR calves exhibited a marked increase ($P < 0.01$) in NEU on d -2 (3.83 K/µL) followed by a decrease on d 0 (1.63 K/µL). This may be the result of a rebound effect on d 0 for CHR following the marked increase in NEU on d -2. Similar results were observed for PERNEU and N:L with the greatest ($P < 0.01$) N:L for CHR steers on d -2 (Figure 3.6). There was an additional PERNEU increase ($P < 0.01$) on d 1 for ACU and CHR steers. No further differences were reported for PERNEU or N:L until d 21 when increased ($P \leq 0.03$) PERNEU and N:L were observed for ACU calves. Decreased ($P \leq 0.04$) LYM were observed on d -2, 1, 5, and 7 for CHR calves whereas PERLYM was decreased ($P < 0.01$) on d -2 for CHR calves with increased PERLYM on d 1 ($P < 0.04$) and d 5 ($P <$

0.05). A marked increase ($P \leq 0.02$) was observed for EOS (Figure 3.7) and PEREOS (Figure 3.8) on d 0 following decreased ($P \leq 0.01$) concentrations on d -2 for CHR calves. Decreased ($P \leq 0.03$) EOS remained on d 1, 3, 5, and 7 and decreased ($P \leq 0.01$) PEREOS remained on d 3 and 7. Additionally, there was over a 50 percent decrease ($P \leq 0.01$) in EOS and PEREOS from d 0 to d 7 for both ACU and CHR calves. This corresponds with results reported by Kaufman et al. (2017) and may be suggestive of viral vaccination and glucocorticoid release from CHR model and handing of ACU calves as glucocorticoids are known to decrease EOS concentrations (Hughes et al., 2017). Dexamethasone administration has been reported to decrease LYM (Burton et al., 1995; Anderson et al., 1999; Hughes et al., 2017) and EOS (Roth, 1985; Anderson et al., 1999; Hughes et al., 2017) concentrations in the blood of cattle. This agrees with our results, as CHR calves exhibited decreased ($P \leq 0.04$) LYM and EOS than ACU calves which may be the result of redistribution of LYM to lymphatic tissue, apoptosis and decreased proliferation of LYM (Anderson et al., 1999), and speculated decrease in metabolic function for EOS (Roth, 1985) from intense endogenous glucocorticoid release. However, it is important to note that CHR calves did not exhibit classically defined lymphopenia or eosinopenia as the decreased LYM and EOS concentrations resided in the normal reference range for cattle (Saini et al., 2007). Increased ($P \leq 0.01$) HGB and HCT concentrations were observed on d -2 for CHR. In addition, CHR calves exhibited decreased ($P \leq 0.05$) HGB on d 7, 14, and 21 and HCT ($P < 0.01$) on d 14. Trends for MCV and MCH were comparable such that CHR calves consistently exhibited numerically less concentration of each variable than ACU calves. Decreased ($P \leq 0.03$)

concentrations were observed for MCHC in CHR calves on d -2 and d 1 and PLT were decreased ($P < 0.02$) for CHR calves on d 3. Stress model influenced RDW with decreased ($P \leq 0.01$) RDW for CHR calves from d -2 to d 14 of the sampling period with similar ($P > 0.72$) values on d 21. These RBC component responses were most likely indicative of dehydration and feed deprivation experienced by CHR calves from weaning on d-3 and transportation on d -2 and d -1. Similar results have been reported such that HCT and HGB (Kaufman et al., 2017; Schaefer et al., 1990) were increased with increased time of feed and water deprivation and although plasma volume reduction was reported by Schaefer at al. (1990), MCV and MCHC were not greatly affected by feed and water deprivation, and agrees with our results.

There was a vaccine type × d interaction ($P < 0.01$) on WBC, LYM, MONO, PERMONO, EOS, PEREOS, HGB, HCT, MCV, MCH, RDW, and PLT. Decreased ($P \leq 0.01$) WBC were observed from d 5 to 14 for MLV which was greatly influenced by the decreased ($P \leq 0.04$) LYM observed from d 3 to 14 for MLV calves (Figure 3.9). Viral replication is widely accepted to decrease circulating LYM concentrations and this was observed by Ellis et al. (1998) in cattle that were challenged with live BVDV; this response is most likely due to redistribution of LYM to lymphatic tissue in response to BVDV infection. The MLV calves had decreased ($P \leq 0.05$) MONO and PERMONO on d 1 and MONO was decreased ($P \leq 0.02$) on d 5 and 7, MLV had greater ($P < 0.01$) PERMONO than KV calves on d 14. Monocytopenia observed for MLV could be from viral replication that existed for MLV, but not KV calves until PERMONO was similar ($P > 0.80$) on d 21 for KV and MLV. Trends detected for EOS and PEREOS were similar

such that MLV had reduced ($P \leq 0.02$) EOS and PEREOS concentrations on d 5. These decreases in EOS and PEREOS in MLV calves are suggestive of EOS recruitment to infected sites from viral replication initiated by the replicating antigens in the vaccine. Gleich (2000) reported increased EOS recruitment to respiratory syncytial virus (RSV) infected lungs in mice and guinea pigs, suggesting decreased circulating EOS concentrations. Whereas results from MCV, MCH, RDW, and PLT revealed a vaccine type × d interaction, means within d were not different (data not shown).

A stress model × vaccine type × d interaction existed ($P < 0.04$) for RBC (Figure 3.10). A difference was observed between ACUKV and ACUMLV calves such that ACUMLV revealed greater ($P \leq 0.05$) RBC from d 0 to 7 and d 21. Additionally, CHRMLV and CHRKV calves had increased ($P \leq 0.05$) RBC compared to ACUKV calves on d -2, d 0, and d 1. These results could indicate more dehydration among treatments with elevated RBC because, as previously mentioned, RBC are known to increase during water deprivation in cattle (Schaefer et al., 1990; Kaufman et al., 2017). The increases in RBC for CHRMLV and CHRKV were indicative of our stress model implementation with induced water deprivation immediately prior to vaccination because calves were transported from the origin ranch to USDA-ARS on d -2 and to WTAMU on d -1.

Conclusion

Our data revealed alterations in antibody response, APP, endocrine, nasal viral detection, and hematological variables from implementation of ACU or CHR stress model and vaccination with KV or MLV vaccine. Antigen-specific antibody titer results

suggest that KV had a greater antibody response to PI3V and IBRV, whereas MLV had a greater antibody response against BVDV. Stress model implementation yielded inconsistent results to titer concentrations such that BRSV-specific antibody response was delayed in CHR and BVDV-specific antibody response was greater in ACU. The CHR model appeared to cause greater stress-induced immunosuppression because LYM and EOS were reduced, despite overall greater cortisol detected for ACU. Further research investigating the interaction between stress-induced immunosuppression and concurrent use of different vaccine types is needed to better define the safe and efficient use of respiratory vaccines in cattle.

Table 1. Prevalence of infectious bovine rhinotracheotits virus (IBRV), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and parainfluenza-3 virus (PI3V) in nasal swab specimens collected on d 0, 7, and 14

	Acute[1]		Chronic[1]		*P*-value[2]
	KV[3]	MLV[3]	KV[3]	MLV[3]	
Item					
Day 0					
IBRV	0.00	0.00	0.00	0.00	-
BVDV	0.00	0.00	0.00	0.00	-
BRSV	0.75	0.33	0.00	0.00	<0.01
PI3V	0.00	0.00	0.00	0.00	-
Day 7					
IBRV	0.00	0.00	0.00	0.08	1.00
BVDV	0.00	0.00	0.83	0.67	<0.01
BRSV	0.33	0.67	0.33	0.08	0.03
PI3V	0.00	0.08	0.00	0.08	1.00
Day 14					
IBRV	0.00	0.00	0.00	0.08	0.23
BVDV	0.00	0.25	0.00	0.00	0.05
BRSV	0.00	0.00	0.67	1.00	<0.01
PI3V	0.00	0.08	0.00	0.08	0.60

[1]ACU = acute stress model (weaned on d -37); CHR = chronic stress model (weaned on d -3)

[2]Fisher's exact test was used to determine the probability of treatment effect within d and virus type

[3]KV = killed virus vaccine (Triangle 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]); MLV = modified-live virus vaccine (Pyramid 5; BIVI)

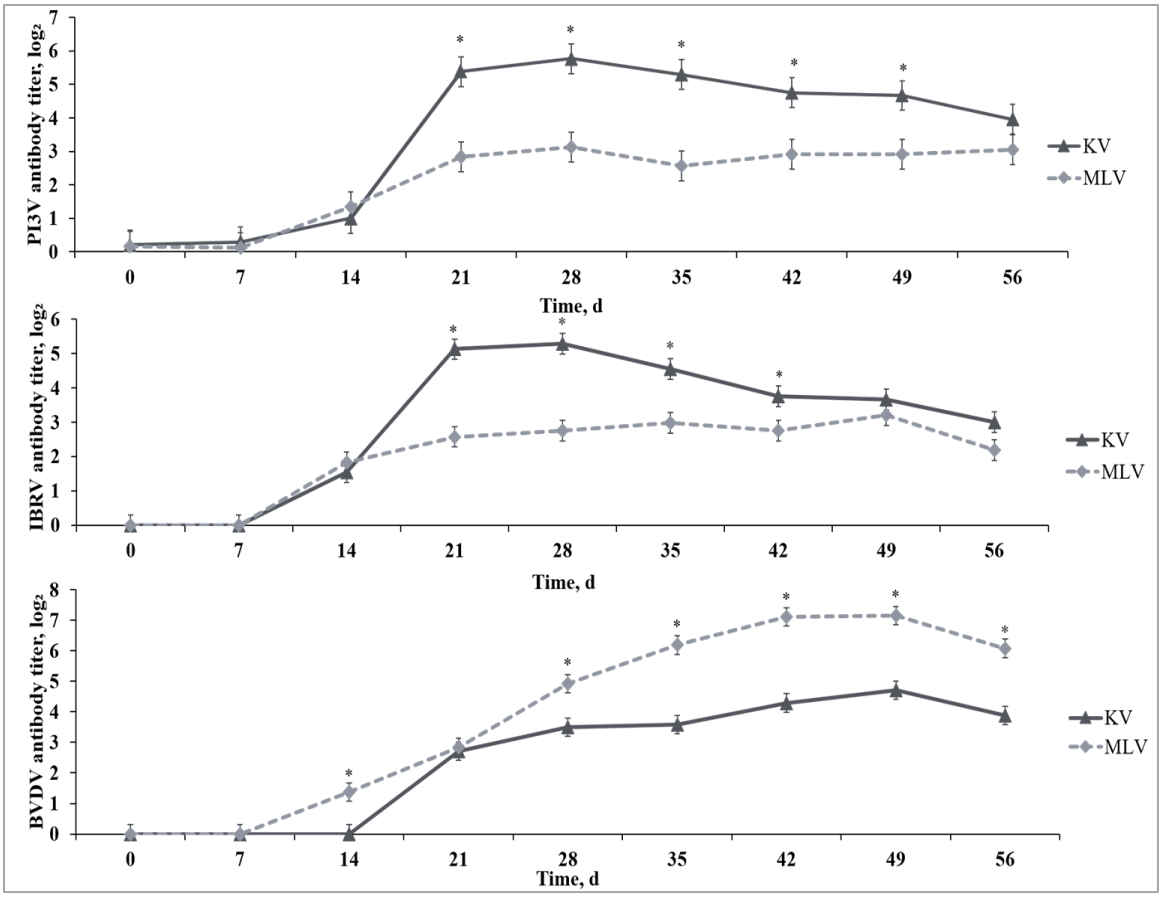

Figure 3.1. Effect of killed virus (KV; Triangle 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]) and modified-live virus vaccination (MLV; Pyramid 5; BIVI) on serum antibody concentration against parainfluenza-3 virus (PI3V), infectious bovine rhinotracheitits virus (IBRV), and bovine viral diarrhea virus (BVDV) after subcutaneous injection of multivalent respiratory vaccine on d 0 and KV booster on d 14. Effect of vaccination on serum PI3V-specific antibody titer concentration (vaccine treatment × d effect; $P < 0.01$); serum IBRV-specific antibody titer concentration (vaccine treatment × d effect; $P < 0.01$); serum BVDV-specific antibody titer concentration (vaccine treatment × d effect; $P < 0.01$). * KV differs from MLV within d, $P \leq 0.02$.

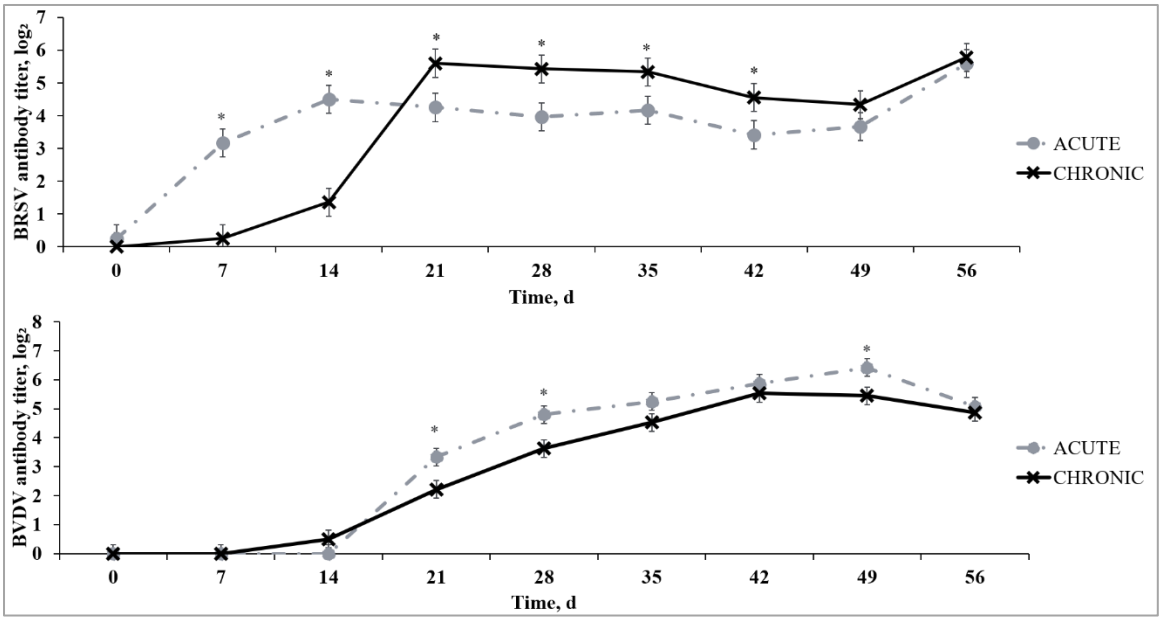

Figure 3.2. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University [WTAMU] research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on serum antibody concentration against bovine respiratory syncytial virus (BRSV) and bovine viral diarrhea virus (BVDV). Effect of vaccination and stress model implementation on serum BRSV-specific antibody titer concentration (stress model × d effect; $P < 0.01$); serum BVDV-specific antibody titer concentration (stress model × d tendency; $P < 0.09$). * ACU differs from CHR within d, $P \leq 0.02$.

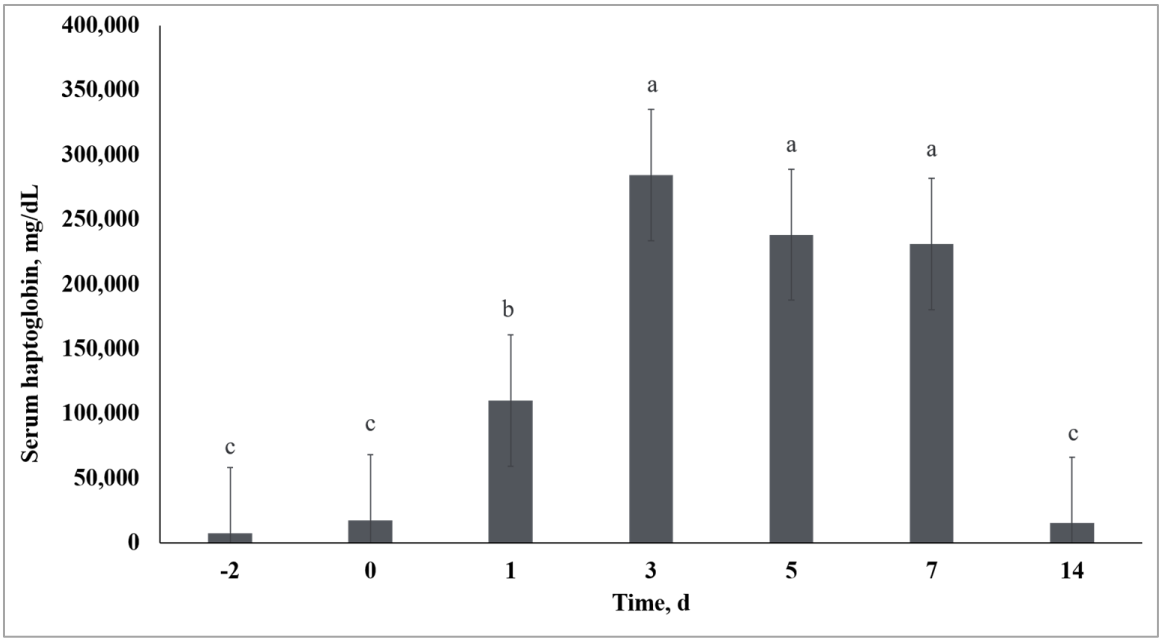

Figure 3.3. Effect of day on serum haptoglobin (Hp) concentration. Serum Hp concentration (d effect; $P < 0.01$). Across d, superscript letters indicate a difference ($P \leq 0.01$) in mean comparisons.

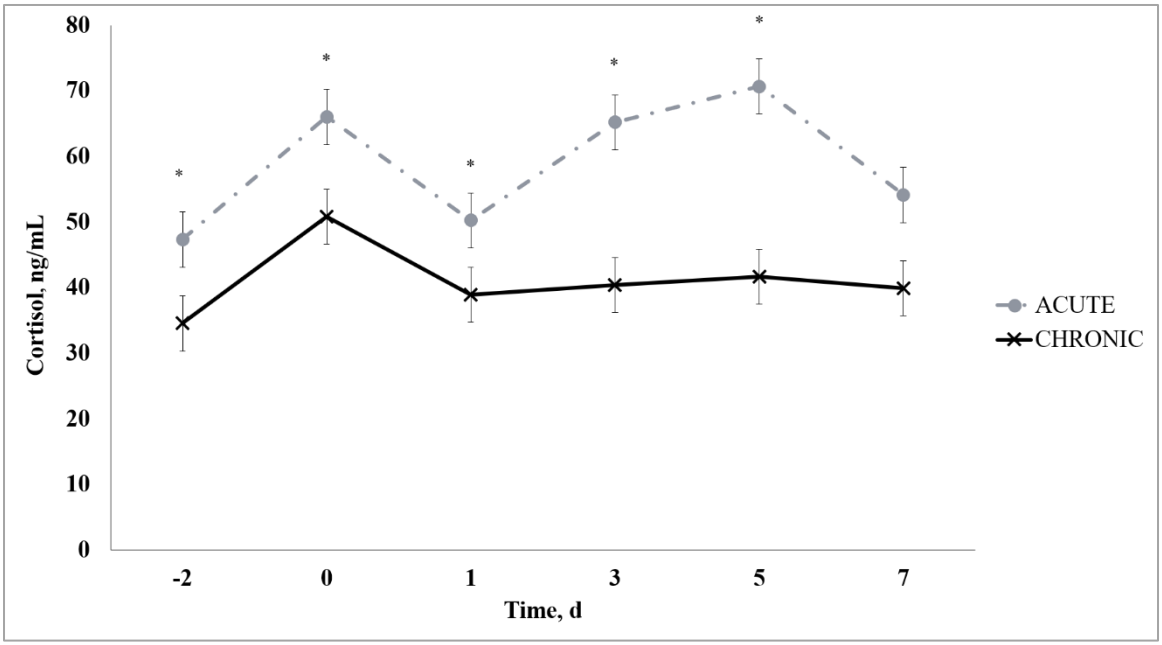

Figure 3.4. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on serum cortisol concentration. Serum cortisol concentration ACU vs. CHR (stress model × d interaction; $P < 0.01$). * ACU differs from CHR within d, $P \leq 0.05$.

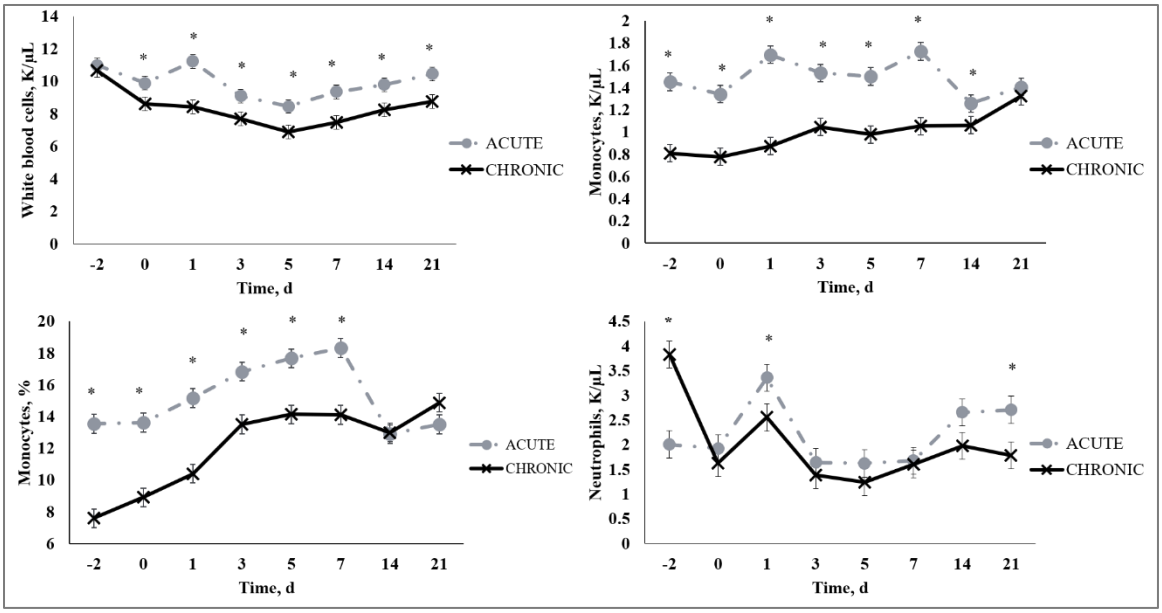

Figure 3.5. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on white blood cells (WBC), monocytes (MONO), percent monocytes (PERMONO), and neutrophils (NEU). Effect of stress model implementation on WBC (stress model × d effect; $P < 0.01$); MONO (stress model × d effect; $P < 0.01$); PERMONO (stress model × d effect; $P < 0.01$); NEU (stress model × d effect; $P < 0.01$). * ACU differs from CHR within d, $P \leq 0.02$.

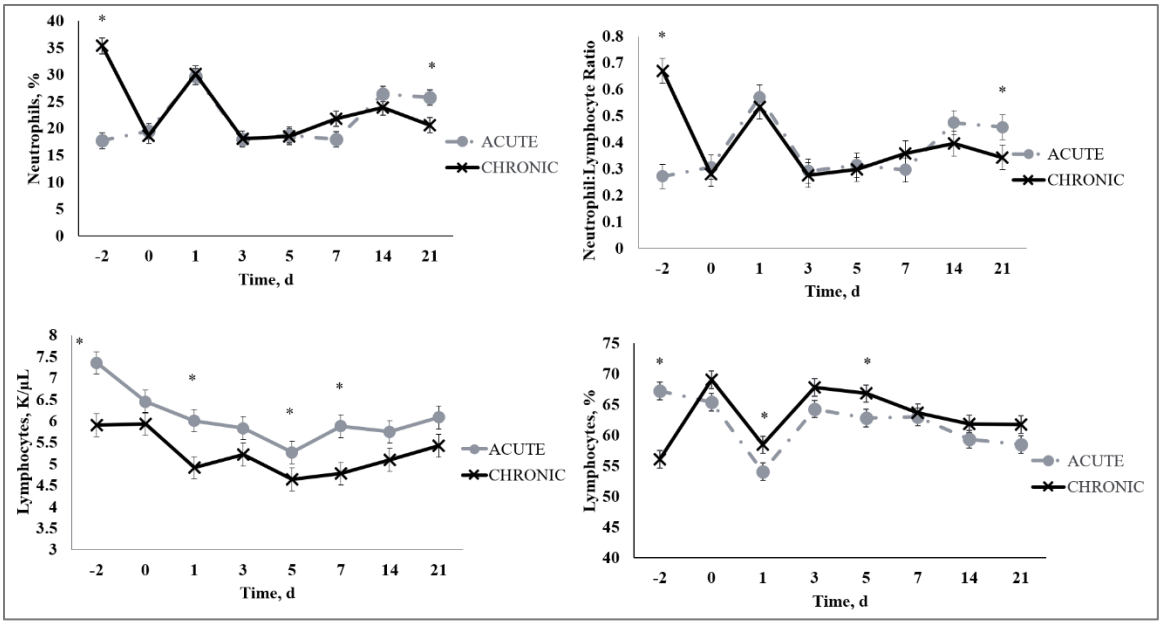

Figure 3.6. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on percent neutrophils (PERNEU), neutrophil:lymphocyte ratio (N:L), lymphocytes (LYM), and percent lymphocytes (PERLYM). Effect of stress model implementation on PERNEU (stress model × d effect; $P < 0.01$); N:L (stress model × d effect; $P < 0.01$); LYM (stress model × d effect; $P < 0.01$); PERLYM (stress model × d effect; $P < 0.01$). * ACU differs from CHR within d, $P \leq 0.02$.

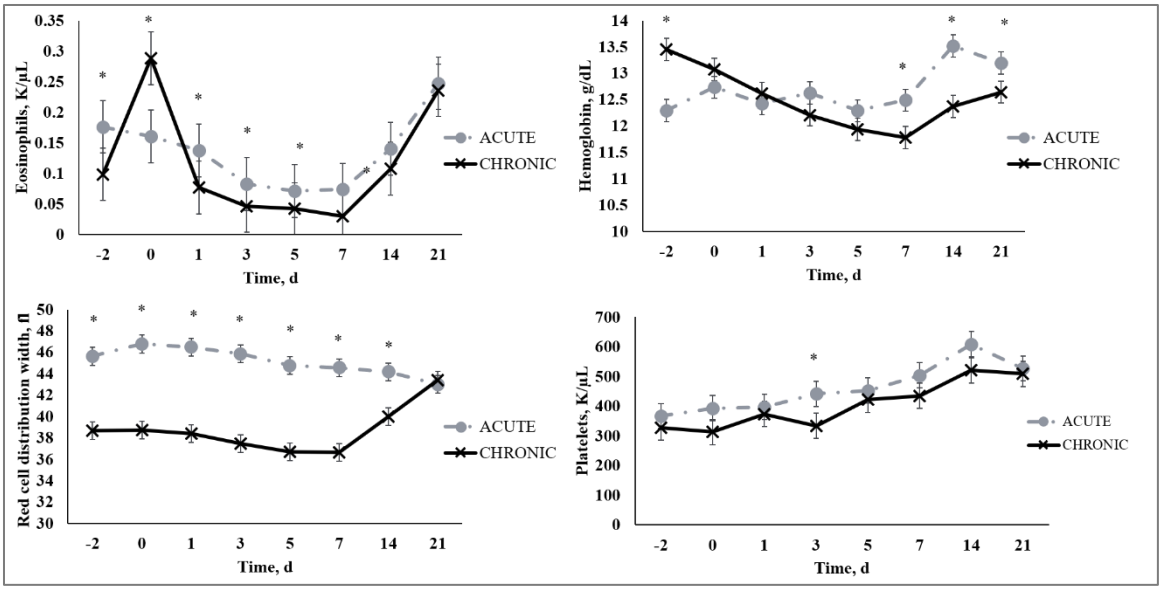

Figure 3.7. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on eosinophils (EOS), hemoglobin (HGB), red cell distribution width (RDW), and platelets (PLT). Effect of stress model implementation on EOS (stress model × d effect; $P < 0.01$); HGB (stress model × d effect; $P < 0.01$); RDW (stress model × d effect; $P < 0.01$); PLT (stress model × d effect; $P < 0.02$). * ACU differs from CHR within d, $P \leq 0.05$.

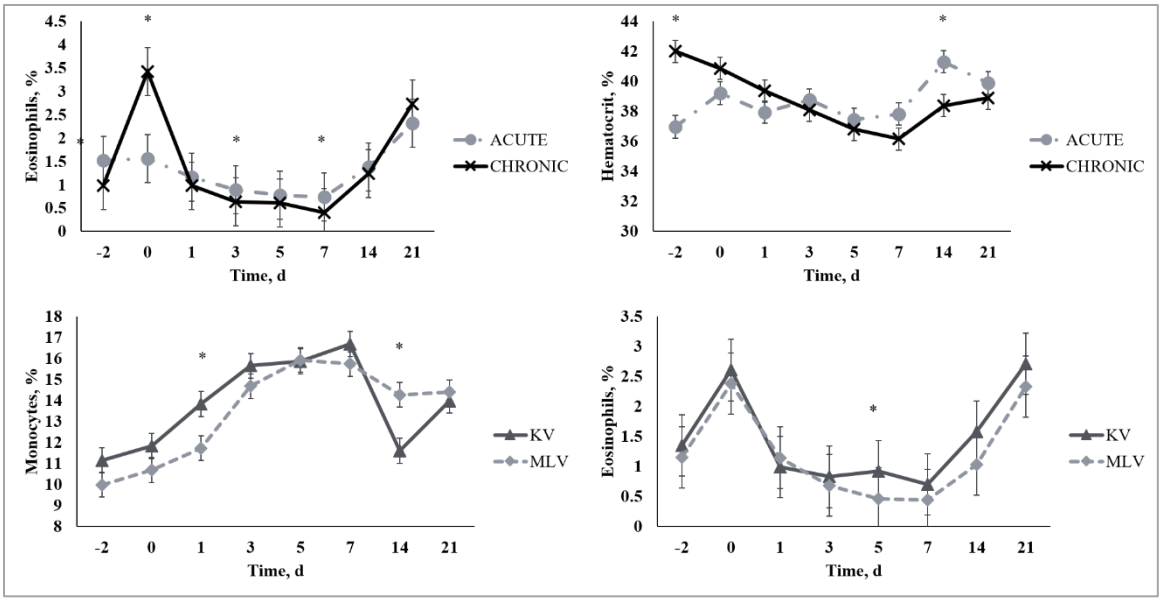

Figure 3.8. Effect of acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) on percent eosinophils (PEREOS) and hematocrit (HCT). Effect of stress model on PEREOS (stress model × d effect; $P < 0.01$); HCT (stress model × d effect; $P < 0.01$) and effect of killed virus (KV; Triangle 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]) and modified-live virus vaccination (MLV; Pyramid 5; BIVI) treatment on percent monocytes (PERMONO) and percent eosinophils (PEREOS) after subcutaneous injection of multivalent respiratory vaccine on d 0. Effect of vaccination on PERMONO (vaccine treatment × d effect; $P < 0.01$); PEREOS (vaccine treatment × day effect; $P < 0.01$). * ACU differs from CHR within d, $P \leq 0.02$; * KV differs from MLV within d, $P \leq 0.02$.

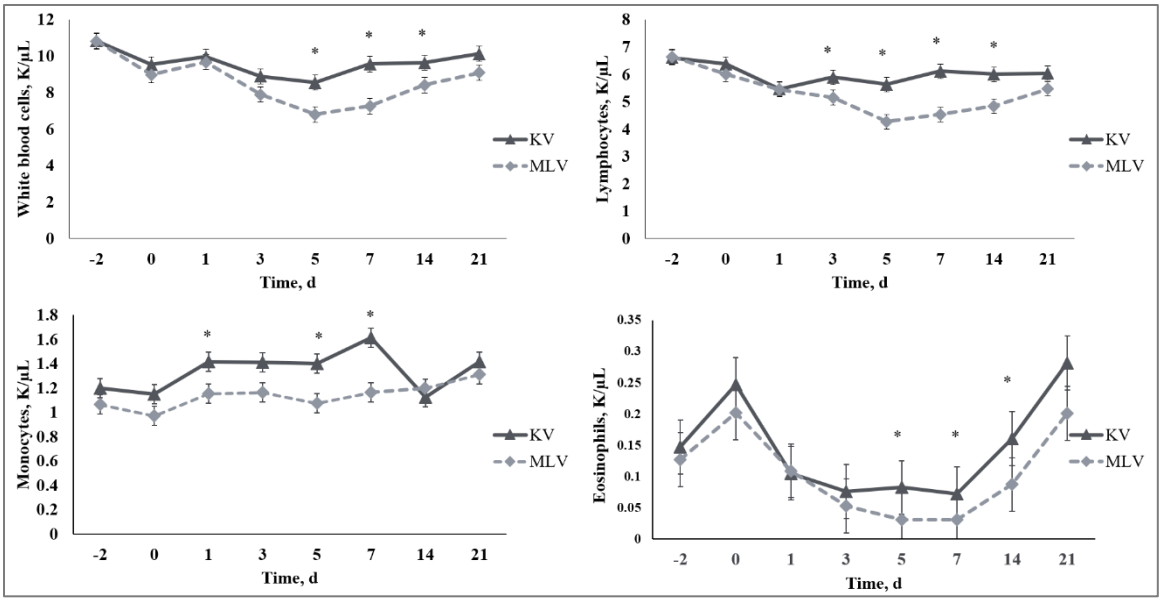

Figure 3.9. Effect of killed virus (KV; Triangle 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]) and modified-live virus vaccination (MLV; Pyramid 5; BIVI) on white blood cells (WBC), lymphocytes (LYM), monocytes (MONO) and eosinophils (EOS) after subcutaneous injection of multivalent respiratory vaccine on d 0. Effect of vaccination on WBC (vaccine treatment × d effect; $P < 0.01$); LYM (vaccine treatment × d effect; $P < 0.01$); MONO (vaccine treatment × d effect; $P < 0.01$); EOS (vaccine treatment × d effect; $P < 0.01$). * KV differs from MLV within d, $P \leq 0.04$.

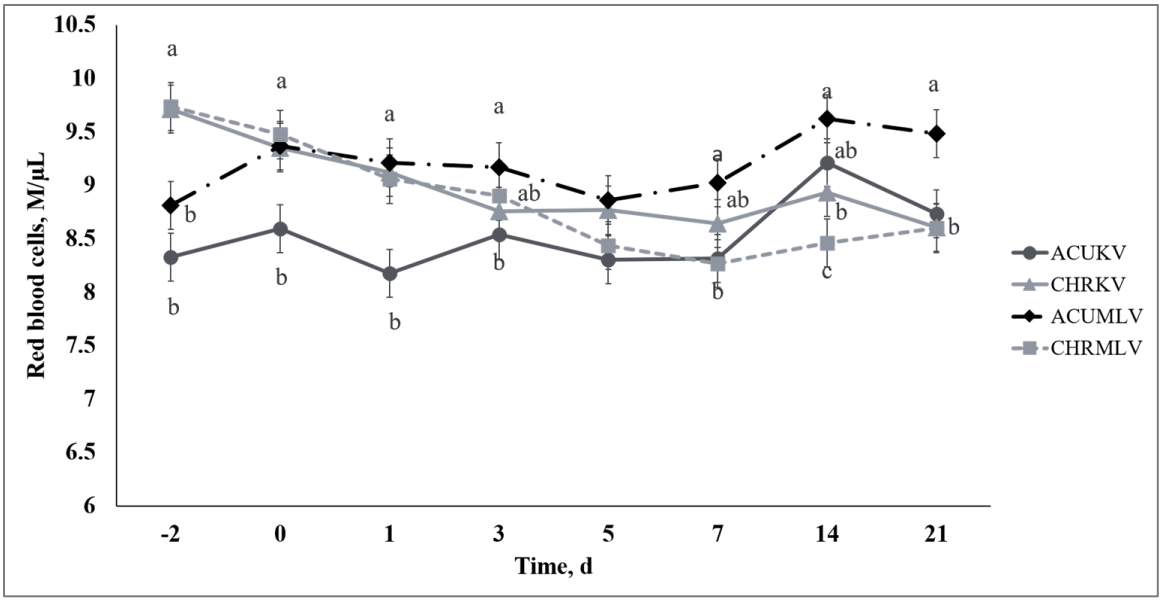

Figure 3.10. Effect of stress model and vaccination on red blood cells (RBC) after acute stress model (ACU; weaned on d -37; transported to West Texas A&M University research facility on d -21; vaccination on d 0) and chronic stress model (CHR: weaned on d -3; transported to USDA-ARS Livestock Issues Research Unit on d -2; relocated to WTAMU on d -1; vaccination on d 0) implementation and killed virus (KV; Triangle 5; Boehringer Ingelheim Vetmedica, Inc. [BIVI]) and modified-live virus vaccination (MLV; Pyramid 5; BIVI) on d 0. Red blood cells (stress model × vaccine treatment × d effect; $P < 0.04$). Across d, uncommon superscript letters between means indicate a difference ($P \leq 0.05$).

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CHAPTER IV

EFFECT OF PEGBOVIGRASTIM ON RECTAL TEMPERATURE, HEMATOLOGICAL VARIABLES, AND FUNCTIONAL CAPACITIES OF NEUTROPHILS IN WEANED JERSEY CALVES

ABSTRACT

The study objective was to determine the safety and efficacy of different dosage of injectable bovine polyethylene glycol-conjugated granulocyte colony-stimulating factor (pegbovigrastim; Imrestor, Elanco Animal Health, Greenfield, IN) weaned Jersey calves. A total of 33 Jersey bull calves (d 14 BW= 138 ± 34 kg) from a single dairy origin were used in a completely randomized design to evaluate effects of treatment, d, and treatment × d interaction; resulting in 3 treatments (n=11) consisting of administration of 1.11 mg (PEGA), 2.22 mg (PEGB), and 4.44 mg (PEGC) of pegbovigrastim injection. Treatments were allocated evenly among 6 pens. A 28-d acclimation period was allowed before pegbovigrastim treatments were administered on d 0. Animal was experimental unit and dependent variables were analyzed using PROC MIXED with repeated measures (d). Rectal temperature (RT) was measured on d 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14 using a digital thermometer. Complete blood count was determined from whole blood collected on d -2, 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14 via automated hemocytometer. Additional blood samples were used to analyze functional capacities of neutrophils on d 0, 1, 2, 4, 6, 10, and 14. A d effect ($P < 0.01$) was observed for RT with decreased ($P \leq 0.01$) RT values on d 2, 3, 4, 10, and 12 and the least ($P < 0.01$) RT on d 14. A treatment × d interaction ($P < 0.03$) was observed for total white blood cells (WBC), neutrophils (NEU), lymphocytes, and monocytes. Specifically, WBC was increased ($P < 0.05$) in PEGC

(40.50 K/µL) calves on d 2 compared to PEGA (27.56 K/µL) and was greater than PEGA and PEGB on d 3, 4, 12, and 14. In addition, PEGC had the greatest ($P \leq 0.01$) monocyte count on d 3, 4, 12, and 14. Increased ($P \leq 0.02$) NEU was observed for PEGC vs. PEGB on d 3 (31.19 vs. 21.17 K/µL), 4 (22.72 vs. 16.32 K/µL), and 14 (21.83 vs. 15.71 K/µL). A more pronounced difference ($P \leq 0.02$) in NEU was detected for PEGC vs. PEGA on d 3 (31.19 vs. 20.42 K/µL), 4 (22.72 vs. 15.79 K/µL), 12 (19.86 vs. 16.43 K/µL), and 14 (21.83 vs. 15.48 K/µL). A decrease ($P \leq 0.04$) in lymphocytes for PEGB and PEGC was detected on d 6 and 8. A treatment effect ($P < 0.02$) was noted for neutrophil:lymphocyte ratio (N:L), basophils, percent neutrophils, and percent lymphocytes. Increased ($P < 0.02$) overall basophil concentration was observed for PEGC. Also, PEGB and PEGC exhibited greater ($P \leq 0.04$) N:L and percent neutrophils than PEGA; however, PEGB and PEGC had less ($P \leq 0.02$) percent lymphocytes than PEGA. There was a d effect ($P < 0.01$) on L-selectin (CD62L), percent activated neutrophils, and phagocytosis and oxidative burst intensity. Suppression ($P \leq 0.01$) of geometric mean fluorescence intensity (MFI) of neutrophil L-selectin was observed on d 1 and 2 while increased ($P < 0.01$) L-selectin MFI occurred on d 4. L-selectin MFI decreased ($P \leq 0.01$) on d 6 until d 14 following the peak on d 4. Observed phagocytosis intensity was greatest ($P < 0.01$) on d 0 while oxidative burst intensity was greatest ($P < 0.01$) on d 2 subsequent to pegbovigrastim injection. Phagocytosis intensity followed a similar trend to L-selectin MFI with suppression ($P < 0.01$) on d 1 followed by an increase ($P < 0.01$) on d 6. All 3 of pegbovigrastim doses resulted in marked increase in the number of circulating leukocytes with transient reduction in neutrophil functional capacities.

Introduction

Mastitis is an endemic disease that is considered to be the most costly disease among the dairy industry (Halasa et al., 2007). While mastitis poses a major concern in the lactating cow, post-weaning bovine respiratory disease (**BRD**) is the predominant cause of mortality in weaned heifers (USDA-APHIS, 2010). In cattle, BRD is a complex disease with development typically initiated by an environmental stressor and viral infection that weaken the resistance mechanisms of the lungs and allow bacterial colonization by various microorgansims including *Pateurella multocida, Mannheimia haemolytica,* and *Mycoplasma spp.* (Confer, 2009; Stanton et al., 2012). Like mastitis, BRD causes an inflammatory response initiated by acute phase proteins (**APP**; Orro et al., 2011) and causes economic losses due to increased medical cost and mortality. While dairy cattle of all ages can be affected, yearlings and weaned calves are most susceptible to BRD (Radostits et al., 1994). This may be due to the physical, psychological and nutritional stressors imposed on calves throughout weaning or housing management practices. There is evidence that weaning combined with alterations in housing environments may elucidate transient neutrophilia and impaired neutrophil phagocytic function (Lynch et al., 2010). These outcomes are common occurrences during times of stress due to a reduction in surface expression of L-selectin on blood neutrophils. Previous studies have reported large increases in neutrophil counts following injection of recombinant bovine granulocyte colony-stimulating factor (**rbG-CSF**) (Kimura et al., 2014; Canning et al., 2017; McDougall et al., 2017). Commercial rbG-CSF is currently available as injectable pegbovigrastim (Imrestor; Elanco Animal Health) for the reduction

in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers (US Food and Drug Administration, 2016). Due to previous reports of increased neutrophil count and function from rbG-CSF administration, the current study objective was to determine an effective pegbovigrastim dose in weaned dairy calves to explore safety and efficacy for use in lighter weight animals as a potential method to control BRD. Our hypothesis was that pegbovigrastim dose would differentially increase neutrophil count, neutrophil phagocytic (**PG**) and oxidative burst (**OB)** capacity, and L-selectin in circulating neutrophils.

Materials and Methods

This study was conducted from June 2017 to August 2017 at the West Texas A&M University (**WTAMU**) Animal Health Research facility in Canyon, TX. Animal procedures and experimental protocols (protocol number 03-06-17) were approved by the WTAMU animal care and use committee.

Animals and Housing

Thirty-three weaned Jersey bull calves were selected from a single commercial dairy facility located near Plainview, TX and were transported 106 km and housed at the WTAMU Animal Health Research facility for use in this study. Upon arrival, calves were allotted to 1 of 6 sand bedded pens where they remained for the duration of the 28-d acclimation period and 14-d study period. Calves were provided ad libitum access to feed and water.

Treatments and Vaccination

Calves received metaphylaxis with tilmicosin (Micotil, Elanco Animal Health, Greenfield, IN) on d -7 to reduce risk of respiratory infection prior to initiation of the study. On d 0, calves were randomly assigned to receive 1 of 3 treatments with treatment equivalently represented in each of 6 pens. The treatments consisted of: 1) administration of 1.11 mg/animal pegbovigrastim in 0.20 mL solution (**PEGA**); 2) administration of 2.22 mg/animal pegbovigrastim in 0.40 mL solution (**PEGB**); 3) 4.44 mg/animal pegbovigrastim in 0.80 mL solution (**PEGC**). Pegbovigrastim (Imrestor, Elanco Animal Health, Greenfield, IN) treatments were administered s.c. proximate to the sacral vertebrae at approximately 0700 on d 0. Treatment randomization was achieved by drawing labeled cards from a hat with the treatment designation of PEGA, PEGB, or PEGC. This resulted in 11 animals per treatment, respectively. One calf assigned to treatment PEGB treatment died on d 14 of the study with cause of death due to respiratory disease.

Blood Collection and Clinical Analysis

To facilitate sample collection, animals were briefly restrained in their home pen via halter on d -2, 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14. An anti-coagulated blood sample was collected via jugular venipuncture into tubes containing 7.2 mg EDTA (Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) on all sample collection days and analyzed using an automated hemocytometer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) at the WTAMU Animal Health Laboratory to determine complete blood count. An additional anti-coagulated blood sample was collected via jugular venipuncture

into tubes containing 7.2 mg EDTA on d 0, 1, 2, 4, 6, 10, and 14 and immediately placed on ice in a subsequent cooler for neutrophil L-selectin expression. Blood samples were collected on d 0, 1, 2, 4, 6, 10, and 14 into tubes containing 56 USP lithium heparin (Vacutainer SST; Becton, Dickinson and Company, Franklin Lakes, NJ) for neutrophil PG and OB capacity. Rectal temperature was measured and a calf respiratory score (data not shown) was assigned daily at approximately 0730 using the Wisconsin Scoring System (Calf respiratory scoring chart, CRSC). No clinical morbidity was observed.

Ex Vivo Immunological Responses

The ex vivo innate immune responses were evaluated at Texas Tech University and are previously described by Hulbet et al. (2011). Briefly, the simultaneous PG and OB capacities of neutrophils in response to an enteropathogenic *Escherichia coli* were analyzed by dual-color flow cytometry. Data are reported as the percentage of neutrophils phagocytizing and producing an oxidative burst (PG+OB+) as well as the geometric mean fluorescence intensity (MFI) of the FL-1 (oxidative burst) and FL-3 (phagocytosis). Additionally, L-selectin on circulating neutrophils was quantified with IgG ant-bovine (CD62L) primary antibody (Washington State University Monoclonal Antibody Center, Pullman, WA) with dilution of a secondary goat F(ab')2 anti-mouse IgG tagged with fluorescein isothiocyanate (SouthernBiotech, Birmingham, AL) after red blood cell lysis. Subsequent quantification of L-selectin was determined by mean fluoresce intensity (MFI) using flow cytometry (BD Biosciences, San Jose, CA).

Statistical Analyses

This completely randomized design experiment used animal within pen as the experimental until for all analyses of dependent variables. Data derived from RT, hematological variables, and neutrophil function assays were analyzed using the MIXED procedure of SAS (SAS Inst. In., Cary, NC) with repeated measures. The model for these variables included effects of treatment, d, and treatment × d interaction. The repeated statement was d, and the covariance structure with the lowest Akaike information criterion for each dependent variable was implemented. Dependent 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 data were analyzed and back-transformed means were subsequently generated and shown. Differences of least square means were determine using the PDIFF option in SAS. Significance was established for treatment, d and treatment × d if consequential *P*-value was ≤0.05 and a statistical tendency was considered if a *P*-value was ≤0.10 and >0.05.

Results and Discussion

Rectal Temperature

No treatment effect ($P = 0.28$) or treatment × d interaction ($P = 0.21$) was observed for RT (Figure 4.1). A day effect ($P < 0.01$) existed such that RT was decreased ($P \leq 0.01$) on d 2, 3, 4, 10, and 12 with the overall least ($P < 0.01$) RT observed on d 14. Average ambient temperatures are also reported in Figure 4.1 to illustrate the relationship between RT and ambient temperature. Changes in RT over time was most likely caused

by ambient temperature fluctuations as RT was observed to decrease on d 2 and 3, ambient temperature simultaneously decreased. In addition, ambient temperature decreased more than 50% on d 10, 12, and 14 compared to ambient temperature at initiation of the study on d 0. Therefore, change in RT in this study was likely a function of environmental conditions rather than pegbovigrastim injection.

Complete Blood Count

No treatment × d interactions ($P \geq 0.49$) were observed for red blood cells (**RBC**), hemoglobin, hematocrit (**HCT**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration, red cell distribution width (**RDW**), or platelets (data not shown). These results were expected as pegbovigrastim injection is intended to stimulate the innate immune system, with direct effects on blood neutrophils (US Food and Drug Administration, 2016). However, a treatment effect and d effect did exist for HCT ($P \leq 0.02$). Overall, PEGC exhibited lower ($P < 0.02$) HCT percentage (Figure 4.2) and HCT was decreased ($P < 0.02$) on d 8 after pegbovigrastim administration. While no previous studies that investigated the effects of pegbovigrastim on hematological variables have reported impacts on erythrocytes, all erythrocyte variables were within normal reference ranges for cattle (George et al., 2010) except MCV, in which we observed overall lower values, and RBC and RDW, in which we observed overall higher values. Nonetheless, the treatment effect for HCT may be influenced by the greater number of leukocytes elicited by PEGC after pegbovigrastim injection.

There was a treatment × d interaction ($P \leq 0.05$) observed for white blood cells (**WBC**), neutrophils (**NEU**), lymphocytes (**LYM**), monocytes (**MONO**), and percent monocytes (**PERMONO**). The WBC were greater ($P \leq 0.05$) for PEGC vs. PEGA on d 2 and greater than both PEGA and PEGB on d 3, 4, 12, and 14 (Figure 4.3). The differences in WBC were influenced by MONO and NEU such that PEGC had greater ($P \leq 0.01$) MONO than PEGA and PEGB on d 2, 3, 4, 12, and 14; NEU were greater ($P \leq 0.03$) for PEGC than PEGA and PEGB on d 3, 4, and 14. While no differences were reported for WBC on d 0 and 1, MONO were greatest ($P \leq 0.05$) for PEGC d 2, 3, 4, 12, and 14. Differences existed for PERMONO with greater ($P < 0.01$) values being observed for PEGA vs. PEGC on d 1 and greater ($P \leq 0.05$) PERMONO for PEGB and PEGC than PEGA on d 8. Whereas LYM was decreased ($P \leq 0.05$) for PEGB and PEGC on d 0, increased LYM were observed ($P \leq 0.03$) for PEGC on d 2 when versus PEGA and on d 3 versus PEGB. Increased ($P \leq 0.04$) LYM were detected for PEGA than PEGB and PEGC on d 6 and 8. In addition, LYM were increased ($P \leq 0.04$) for PEGA than PEGB on d 12. Individual treatment effects existed for neutrophil:lymphocyte ratio (**N:L**) ($P < 0.01$), basophils (**BASO**) ($P < 0.01$), percent neutrophils (**PERNEU**) ($P < 0.02$), percent lymphocytes (**PERLYM**) ($P < 0.01$), and percent basophils (**PERBASO**) ($P < 0.01$). Similar trends were reported for BASO and PERBASO such that PEGC had increased ($P \leq 0.02$) BASO and PERBASO when compared to PEGA and PEGB. Whereas PERNEU and N:L exhibited greater ($P \leq 0.04$) values for PEGB and PEGC when compared to PEGA; PEGB and PEGC had less ($P \leq 0.02$) PERLYM than PEGA.

According to Saini et al. (2007) all leukocyte variables were above normal reference ranges for cattle following pegbovigrastim administration on d 0. In addition, baseline variables observed on d -2 and 0 served as a baseline reference before pegbovigrastim was administered. This suggests PEGA, PEGB, and PEGC were all capable of inducing leukocyte responses, particularly NEU. This corresponds to results reported by Kimura et al. (2014) as they observed an increase in NEU for animals that received rbG-CSF injection and results by McDougall et al. (2017) such that increased WBC were reported. While trends in WBC variables were similar to previously reported results, it is important to note that increases in WBC, NEU, LYM, and MONO were greater for our study than McDougall et al. (2017). These differences are most likely indicative of sex differences, age, and parity status alterations between studies.

Functional Capacities of Blood Neutrophils

A d effect ($P < 0.01$) existed for L-selectin (CD62L) on the surface of neutrophils (Figure 4.4). L-selectin is an adhesion molecule that aids in leukocyte recruitment by selectin-mediated rolling and increases opsonization to sites of localized inflammation for migration into infected tissue to target antigen. Therefore, leukocyte migration into lymphatic tissues or inflammatory sites are highly regulated by L-selectin expression (Springer, 1994). An overall decrease ($P \leq 0.01$) in L-selectin on d 1 and 2 after pegbovigrastim injection suggests that while NEU concentrations were increased for all treatments, these NEU were likely immature and lacked abundant L-selectin; however, after L-selectin decreased on d 2, a marked increase ($P < 0.01$) occurred on d 4 with greatest L-selectin MFI at this time. It is important to note that this increase in L-selectin

exceeded ($P < 0.01$) baseline L-selectin expression, suggesting that pegbovigrastim injection has potential to increase L-selection expression after newly generated NEU are able to mature and express adhesion molecules. Decreases ($P \leq 0.01$) in L-selectin MFI were observed on d 6, 10, and 14 when compared to the peak on d 4 and baseline levels reported on d -2 and 0. While there is potential for this to be an effect of pegbovigrastim injection as a representation of L-selectin returning to concentration less than baseline, decreased MFI may have been due to adverse weather events. Significant rainfall (data not shown) occurred on d 8, 9, and 13 of the study. The rain resulted in wet pen conditions and likely caused physiological stress. While cortisol was not measured in the current study, Burton et al. (1995, 2005) have reported decreases in L-selectin expression in the presence of a glucocorticoid. If the decrease in L-selectin expression were an artifact of pegbovigrastim injection, it is thought that levels would not return to levels below baseline that were observed on d 0. Therefore, it is plausible that observed decreases with time were due to the presence of increased cortisol stimulated from environmental factors.

The percentages of NEU phagocytizing and producing an oxidative burst to *E. Coli* resulted in a d effect ($P < 0.01$) with the least ($P < 0.02$) activity observed on d 10 and greatest ($P < 0.01$) activity observed on d 14 after pegbovigrastim injection. Percent active NEU observed on all other sample days were not different from detected activity on d 0 when pegbovigrastim was administered. In addition, d effects ($P < 0.01$) existed for oxidative burst and phagocytic intensity with the least ($P \leq 0.02$) intensities observed on d 14. While percentages of active NEU were increased on d 14, total capability to

achieve oxidative burst or phagocytic processes were suppressed at this time. Variable results were observed for phagocytosis and oxidative burst intensities. Observed baseline phagocytosis intensity was greatest ($P < 0.01$) on d 0 while oxidative burst intensity was greatest ($P < 0.01$) on d 2 following pegbovigrastim injection. After pegbovigrastim administration, phagocytosis intensity immediately decreased ($P < 0.01$) on d 1 with an increase ($P < 0.01$) on d 6 before subsequent decreases ($P \leq 0.01$) on d 10 and 14. It is important to note that while results were variable, the increase in oxidative burst activity reported on d 2 was greater than baseline intensity reported on d 0 and is plausible to be an effect from pegbovigrastim injection. Our results correspond with results reported by Kimura et al. (2014) and McDougall et al. (2017) with no time effects observed for oxidative burst; McDougall et al. (2017) reported suppressed phagocytosis with time following pegbovigrastim injection while Kimura et al. (2014) reported no time effect for phagocytosis. Differences in phagocytic activity may be due to variances in age, parity status, and dose modifications utilized between studies.

Phagocytosis and oxidative burst are important contributions to the nonspecific defense mechanisms of the host by engulfing and destroying bacteria via production of toxic oxygen radicals and hydrogen peroxide (Böhmer et al., 1992). Pegbovigrastim injection has been reported to increase circulating NEU with varying results on NEU function (Kimura et al., 2014; Canning et al., 2017; McDougall et al., 2017). While NEU function is important for innate immune responses, it is important to assure increases in NEU do not lead to overstimulation of typically beneficial leukocytosis (Hughes et al., 2017). A paradoxical phenomenon may occur when excessive NEU activity causes host

tissue damage during microbial killing by amplified release of cytotoxic molecules into the extracellular milieu (Smith, 1994). While NEU function results did not elicit treatment differences and results with time were variable, the NEU paradox may be of greater concern with PEGC with compared to PEGA or PEGB. Further research is warranted to further investigate clinical efficacy in prevention of BRD and possible adverse effects associated with dose modification of pegbovigrastim injection.

Conclusion

Our data shows that pegbovigrastim injection influenced hematological responses for all 3 doses evaluated. Increases in WBC, NEU, LYM, and MONO were overall greatest for PEGC and overall neutrophil functionality was not improved. While this dose titration elicited an increase in leukocytes, further research is warranted to observe clinal differences and performance alterations between treatments to confirm a dose validation in lighter weight calves.

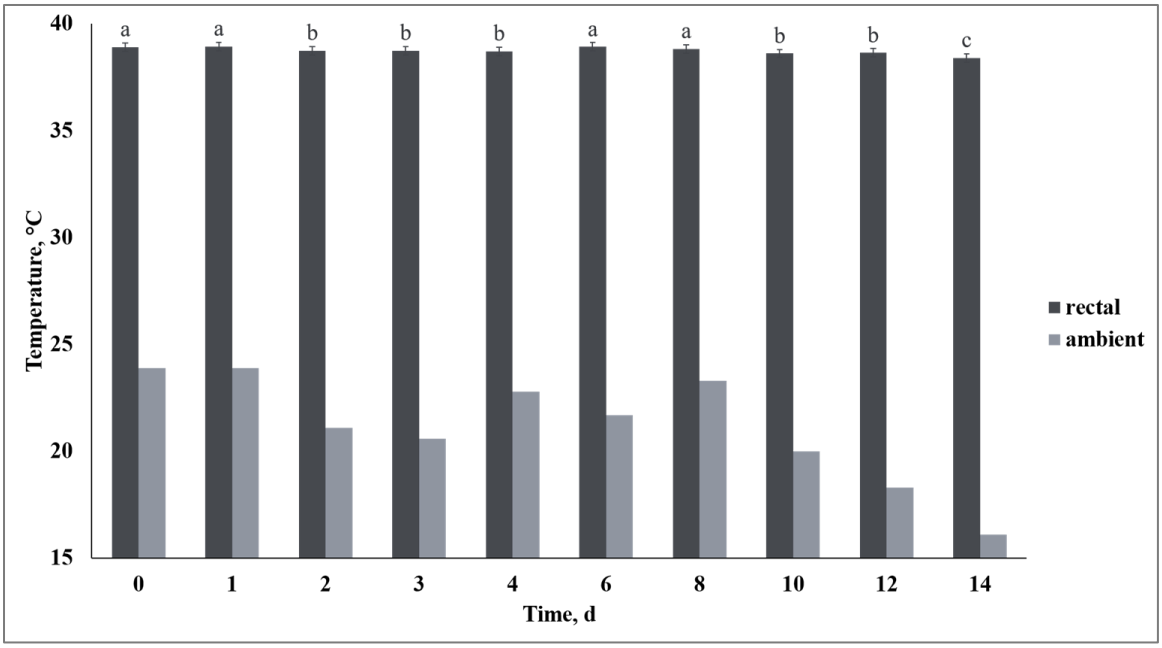

Figure 4.1. Effect of d on rectal temperature (RT) after vaccination of pegbovigrastim (PEGA = 1.11mg; PEGB = 2.22 mg; PEGC = 4.44 mg) on d 0 with subsequent ambient temperature values for each sample d. Daily RT measurement (d effect; $P < 0.01$). Across d, means with uncommon letter superscripts differ, $P \leq 0.03$.

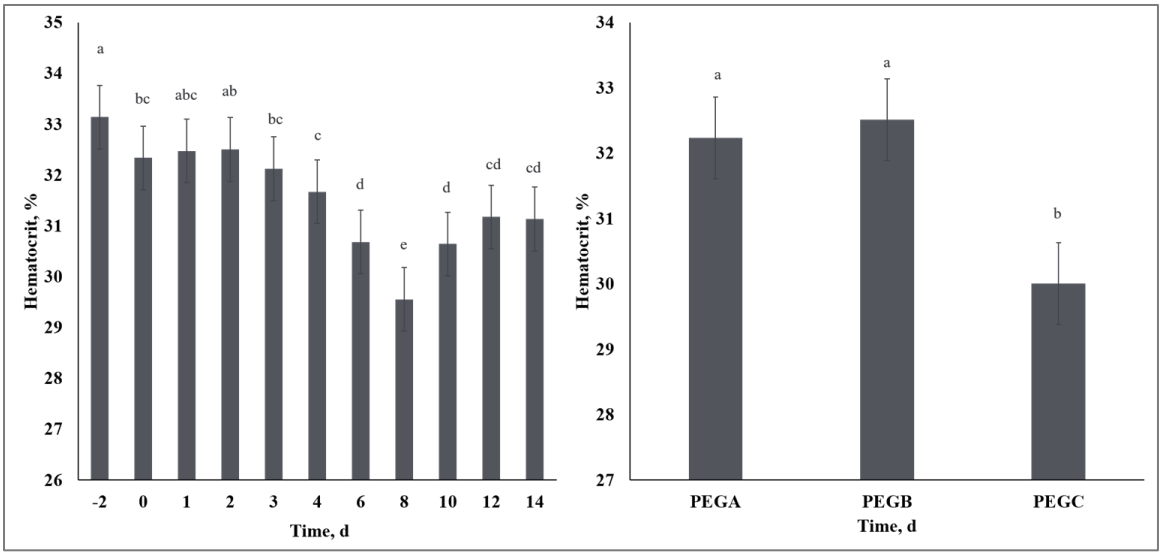

Figure 4.2. Effect of d and effect of treatment on hematocrit (HCT) after vaccination of pegbovigrastim (PEGA = 1.11mg; PEGB = 2.22 mg; PEGC = 4.44 mg) on d 0. Daily HCT measurement (d effect; $P < 0.01$). Treatment HCT measurement (treatment effect; $P < 0.02$). Unlike superscript letters indicate a difference ($P \leq 0.04$) in mean comparisons.

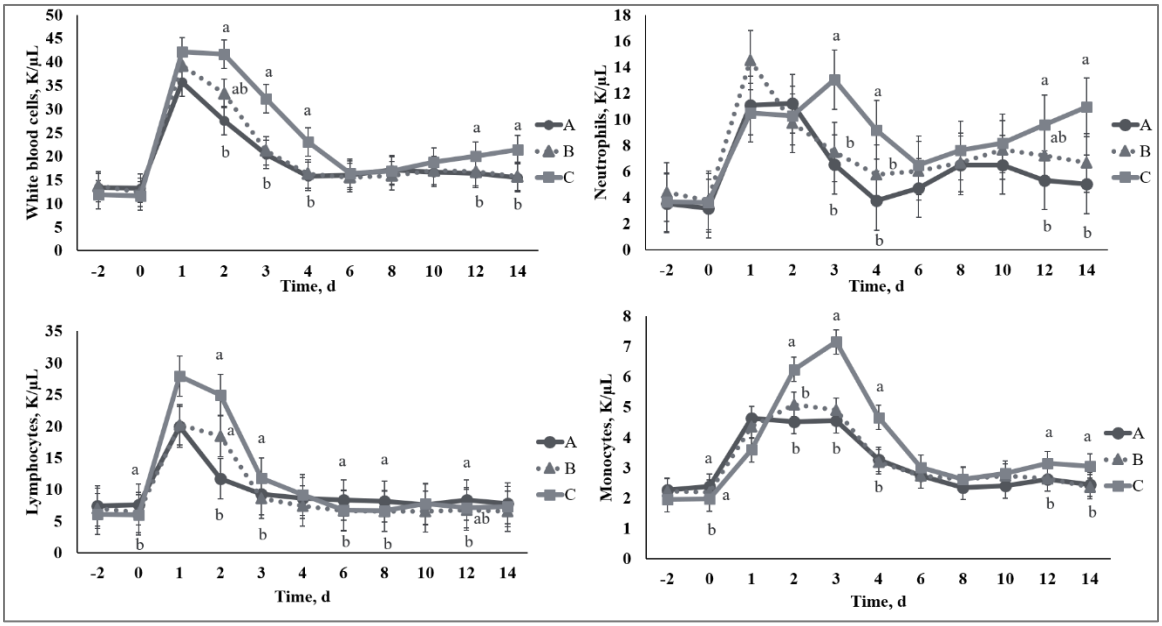

Figure 4.3. Effect of pegbovigrastim (PEGA = 1.11mg; PEGB = 2.22 mg; PEGC = 4.44 mg) treatment on white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), and monocytes (MONO) after subcutaneous injection on d 0 over time. Vaccination on WBC (treatment × d effect; $P < 0.01$); NEU (treatment × d effect; $P < 0.01$); LYM (treatment × d effect; $P < 0.01$); MONO (treatment × d effect; $P < 0.01$). Across d, means with uncommon letter superscripts differ, $P \leq 0.05$.

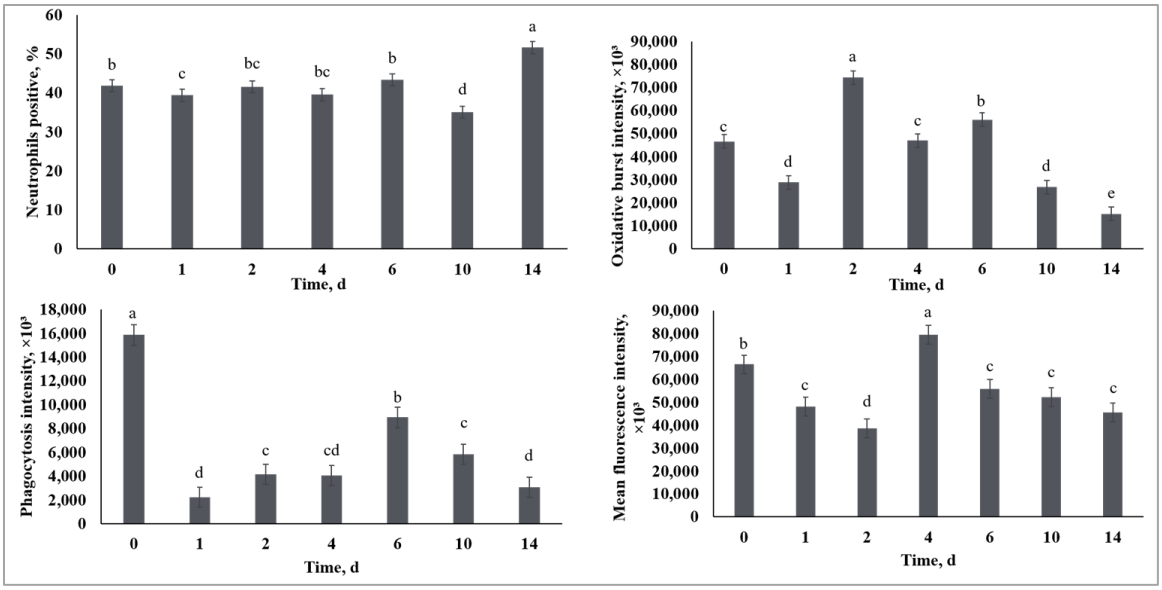

Figure 4.4. Effect of pegbovigrastim (PEGA = 1.11mg; PEGB = 2.22 mg; PEGC = 4.44 mg) treatment on total activated neutrophils, oxidative burst intensity, phagocytic activity intensity, and surface expression of L-selectin (CD62L). Pegbovigrastim administration on total activated neutrophils (d effect; $P < 0.01$); oxidative burst intensity (d effect; $P < 0.01$); phagocytic activity intensity (d effect; $P < 0.01$); surface expression of L-selectin (d effect; $P < 0.01$). Unlike superscript letters indicate a difference ($P < 0.03$) in mean comparisons.

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