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# Mitigation of Bovine Respiratory Disease Complex: Interactions of Physiology, Immunology, Nutrition and Management

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MITIGATION OF BOVINE RESPIRATORY DISEASE COMPLEX: INTERACTIONS  
OF PHYSIOLOGY, IMMUNOLOGY, NUTRITION AND MANAGEMENT

MITIGATION OF BOVINE RESPIRATORY DISEASE COMPLEX: INTERACTIONS  
OF PHYSIOLOGY, IMMUNOLOGY, NUTRITION AND MANAGEMENT

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy in Animal Science

By

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## **ABSTRACT**

A series of experiments were conducted to determine the effects of on-arrival vs. delayed respiratory vaccination and exposure to persistently infected bovine viral diarrhea virus challenge on health, gain performance, and physiological and immunological measurements in newly received stocker cattle. Two experiments evaluated timing (d 0 vs. 14) of respiratory, clostridial, or both vaccinations in newly received stocker calves during stress-induced immunosuppression. In Exp. 1, calves receiving 14-d delayed vaccination of a pentavalent modified-live respiratory virus (MLV) vaccine had greater ADG during the 42-d receiving period and antibody titers against infectious bovine rhinotracheitis on d 42; however, morbidity rate did not differ. In the second study, stress indicated by serum cortisol concentration and neutrophil:lymphocyte ratio was greatest on d 0; nevertheless, no differences in performance or morbidity were observed for vaccination treatments. Both studies provided evidence that on-arrival administration of a pentavalent MLV respiratory vaccine to immunocompromised cattle does not mitigate clinical bovine respiratory disease (BRD) or improve animal performance because the majority of clinical respiratory disease for both studies occurred during the first 14-d and morbidity rate was not different when calves were vaccinated on d 0 vs. 14.

A third experiment was performed to determine the effects of weaning management and subsequent exposure to persistently infected (PI) bovine viral diarrhea virus (BVDV) challenge on health, performance, bovine viral diarrhea virus titers, peripheral blood leukocytes, and inflammatory cytokines. The objective was to compare preconditioned (PC) or auction market (AM) origin cattle, with (PI) or without (CON)

continuous exposure to PI-BVDV type 1b challenge in a  $2 \times 2$  factorial arrangement to evaluate main effects of management, exposure, and their interaction on health parameters and growth performance during a 42-d receiving trial. Preconditioned calves that were vaccinated, castrated, and weaned at their origin ranch had greater gain performance and reduced BRD morbidity compared to AM calves with unknown history. Furthermore, PC cattle had greater antibody titers to BVDV type 1a on d 0, and neutrophil:lymphocyte ratio and platelet count were lower during the 42-d receiving period. Exposure to PI-BVDV challenge reduced gain from d 28 to 42, perhaps due to an additive effect of continuous immune stimulation resulting in nutrients being preferentially utilized for immune pathways rather than tissue deposition. A treatment interaction was observed for the percentage of chronically ill animals; AMPI had the greatest number of chronically ill calves (7.6%), AMCON was intermediate (1.1%), and PCCON and PCPI were least (0.4 and 0.3%, respectively). Exposure to PI-BVDV challenge increased serum TNF- $\alpha$  concentrations, and IFN- $\gamma$  concentrations on d 14 and were greatest for AMPI, intermediate for PCPI, and least for AMCON and PCCON. The increased cytokine concentrations associated with PI-BVDV exposure illustrate a more stimulated immune response which has implications for animal health due to taxation of the immune system and growth due to a homeorhetic response in which nutrients may be preferentially shifted towards immune function rather than tissue growth.

**This dissertation is approved for  
Recommendation to the  
Graduate Council**

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## **LIST OF PREVIOUSLY PUBLISHED PAPERS INCLUDED IN THIS DISSERTATION**

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# CHAPTER I

## INTRODUCTION

Bovine respiratory disease (BRD) is a multifaceted syndrome involving physiological stress, commingling, and several viral and bacterial pathogens. Despite recent advances in biological and pharmaceutical products designed to prevent and control the disease, BRD remains the leading cause of feedlot morbidity and mortality (Woolums et al., 2005) resulting in increased antibiotic usage, reduced animal well-being, and decreased growth performance and production efficiency (Holland et al., 2010). The treatment costs and production losses associated with BRD result in a significant economic loss to the beef industry (Irsik et al., 2006) estimated to be at least \$750 million annually (Griffin, 1997).

Several factors contribute to the susceptibility and pathogenesis of BRD, one of the most significant being management and marketing of cattle. As calves move through the beef production system, stress from handling, weaning, commingling, transport, environment, and diet transition can combine synergistically resulting in physiological stress and immunosuppression. Furthermore, the estimated adoption rate of management factors including weaning (50.2%) or respiratory vaccination (39.4%) in US beef cow-calf operations remains low (USDA, 2010) because logistical challenges exist, cost and risk are a hindrance, and market availability is limited. The physiological stress and compromised immune system commonly found in newly received calves may render initial vaccination incapable of stimulating an effective immune response (Edwards, 2010).

Commingling from the marketing process can increase exposure to one of the four viruses known to be associated with BRD: 1) infectious bovine rhinotracheitis, 2) bovine viral diarrhea virus (BVDV), 3) parainfluenza-3, and 4) bovine respiratory syncytial virus. Bovine viral diarrhea virus is among the most economically important (Griffin, 1997) of the respiratory viruses, causing severe acute disease (Carman et al., 1998) or indirect effects of immunosuppression (Welch, 1995). Immunosuppression caused by acute BVDV infection can allow otherwise commensal bacterial inhabitants of the upper respiratory tract such as *Manheimia haemolytica* or *Pasteurella multocida*, to colonize the lower respiratory tract and lungs becoming pathogenic. Calves born persistently infected (PI) with BVDV are a primary transmission source (Houe, 1995); however, reports on the effects of PI-BVDV exposure on health, production and economic parameters of feeder cattle are: 1) limited; perhaps due to the challenging logistics of coordinating these studies, and 2) conflicting; because the management and immune status of experimental animals may differ and the amount (organisms/mL) and virulence of the BVDV strain being continually shed by PI challenge animals is varied.

The purpose of this series of experiments was to examine the physiological and immunological status of newly received calves, determine the effects of on-arrival versus delayed respiratory vaccination in high-risk, immunosuppressed calves, and observe the health, performance, humoral and cell-mediated immune response of calves with different physiological and immunological circumstances and subsequent exposure to PI-BVDV challenge.

## **CHAPTER II**

### **REVIEW OF LITERATURE**

#### **Overview of Bovine Respiratory Disease in the US**

Bovine respiratory disease (BRD) is a multifaceted syndrome involving physiological stress, commingling, and several viral and bacterial pathogens. Despite recent advances in biological and pharmaceutical products designed to prevent and control the disease, BRD remains the leading cause of feedlot morbidity and mortality (Woolums et al., 2005) resulting in increased antibiotic usage, reduced animal well-being, and decreased growth performance and production efficiency (Holland et al., 2010). Gardner et al. (1999) was among the first studies to demonstrate carcass quality being negatively impacted by BRD, and more recently Schneider et al. (2009) reported that in cattle treated for BRD, 16% fewer graded USDA choice than non-treated animals. Although difficult to assess, the treatment costs and production losses associated with BRD result in a significant economic loss to the beef industry (Irsik et al., 2006) estimated to be at least \$750 million annually (Griffin, 1997).

Bovine respiratory disease can occur in cattle of all ages and during all beef production systems; however, the most susceptible animals are physiologically stressed, immature feeder cattle that are recently weaned from their dam, subjected to commingling with cattle from other herds, and transported by truck to a stocker or finishing program where a transition in diet typically occurs. The beef production system is separated into distinct production segments of cow-calf, stocker, feedlot, packer, retailer, and ultimately, the beef consumer. Ownership frequently changes with each segment; therefore, an informational disconnect exists regarding the performance,

efficiency and health of calves as they move from one segment to another. This fundamental segmentation of the beef production system increases the susceptibility to BRD in the beef industry because inherent stressors exist as calves progress from one production segment to another. With the traditional auction market system, sellers convey health risk of their calves to the buyer with little consequence because communication among buyer and seller is rare. Another marketing roadblock for mitigation of BRD is the limited value-added marketing opportunities available to small cow-calf producers that wish to invest labor and resources in vaccination and management practices that can greatly reduce BRD health risk. Furthermore, because the estimated average herd size in the US is small (average = 42.9; USDA, 2007), a logistical challenge exists. Most cow-calf producers do not annually produce enough calves needed to comprise a full truck-load lot of ~22,500 kg (i.e. 90 calves weighing 250 kg), which limits options for direct-marketing or retained-ownership. Therefore, the commodity-based auction market system is the most common method of feeder cattle sale in the US (USDA, 2010), and most calves are sold individually (Troxel et al., 2002). Selling feeder cattle individually contributes to commingling because cattle buyers must assemble a full truck-load lot with cattle from multiple cow-calf operations, and commingling results in increased stress and BRD morbidity (Step et al., 2008) because the social hierarchy of a group must be re-established and exposure to novel pathogens is more likely.

The BRD mortality rate in US feedlots has remained relatively unchanged over the past 3 decades (Miles, 2009). One cause of the feedlot mortality rate remaining unchanged despite significant improvements in vaccine efficacy and antimicrobial availability may be the low adoption rate by beef producers of preconditioning

management practices that reduce physiological stress and improve disease resistance. According to the USDA Beef 2007-08 survey of US cow-calf producers, the estimated adoption rate of several cow-calf health management factors including castration (49.5%), weaning (50.2%), and respiratory vaccination (39.4%) remains low (USDA, 2008; USDA, 2010) and increases in the adoption rate of these management practices were not observed from the previous decade (Beef '97; USDA, 1997).

The pathogenesis of BRD typically occurs in a distinctive step-wise manner. Initially, stress from weaning, marketing, and transport of feeder cattle results in immunosuppression during a time of extensive commingling when potential viral pathogen exposure is increased, and naïve, immunosuppressed calves are more susceptible to viral pathogens associated with BRD. The 4 primary viral pathogens associated with BRD include: 1) bovine herpesvirus-1 (also known as infectious bovine rhinotracheitis virus; IBRV), 2) bovine viral diarrhea virus (BVDV), 3) parainfluenza<sub>3</sub> virus (PI3V), and 4) bovine respiratory syncytial virus (BRSV). Immunosuppression resulting from concurrent management stress and viral infection in calves allow otherwise commensal bacteria inhabiting the respiratory tract to defeat a compromised immune response and proliferate extensively in the nasopharynx and bovine lung resulting in “shipping fever” pneumonia (Hodgson et al., 2005). Furthermore, co-infection of viral and bacterial respiratory pathogens results in viral-bacterial synergy (Ohmann and Babiuk, 1985). As many as 5 different bacterial species, both Gram-negative and Gram-positive, are described today in their involvement of BRD. The most common bacterial pathogen associated with BRD is *Manheimia haemolytica* serotype 1 (Pandher et al., 1998); however, *Pasteurella multocida*, and *Haemophilus somnus* are less

frequently isolated. The unique virulence factors of each bacterium, combined with the host immune response can cause significant inflammation resulting in moderate to severe lung damage, morbidity or mortality of the host.

Vaccination with an efficacious, multivalent respiratory vaccine containing antigen strains of IBRV, PI3V, BVDV, and BRSV can be highly effective at preventing BRD if administered to an immunocompetent animal (Perino, 1996). However, the efficacy of on-arrival vaccination in high-risk, newly received calves is questionable (Perino and Hunsaker, 1997) because these calves may have been previously exposed to viral pathogens during the marketing process and disease progression may be at a period where vaccination is unable to provide protection.

### **Effects of Physiological Stress on Stocker and Feedlot Cattle**

#### *Overview and Potential Biomarkers of Stress and Disease*

The concept of stress can be traced to Empedocles, a Greek philosopher during the fifth century B.C. In the late 1930s, an endocrinologist named Hans Selye is credited as the first person to use the term stress as it pertains to modern understanding. Selye defined stress as “the body’s nonspecific response to a demand placed on it”. In general, stress is any external or internal challenge that disrupts the internal environment (Sheridan et al., 1994). As our understanding of physiological stress has improved, its role in growth regulation, immunosuppression and disease susceptibility has as well.

The most significant impact of stress is the negative effect it has on feed intake and immunocompetency (Loerch and Fluharty, 1999), both of which may have a direct role in the development of BRD. Loerch and Fluharty (1999) identified 5 consequences when stressors are manifested in cattle: 1) transient endocrine responses are likely, 2)

products of energy and protein metabolism may be altered, 3) appetite and growth rate are affected, 4) digestion and rumen function may be compromised, and 5) an animal's immune system may be challenged. Furthermore, a review by Grandin (1997) postulated 2 broad categories of stress in cattle: 1) psychological stress caused by commingling, handling or fear, and 2) physical stress caused by hunger, disease or injury.

Stress stimuli associated with either category can result in physiological alteration by activating the hypothalamic-pituitary adrenal axis leading to increased serum or plasma levels of glucocorticoids. Glucocorticoids such as cortisol have been linked to immunosuppression (Roth, 1985) and thus, the potential for increased disease susceptibility in cattle. However, changes in cortisol concentrations in response to different stress stimuli commonly encountered during the beef production system are varied. Several studies report stress-induced increases in cortisol concentrations of cattle (Kegley et al., 1997; Tyler and Cummins, 2003; Gupta et al., 2005), and Anglen et al. (2003) observed animals that underwent restraint stress failed to mount a normal immune response, which resulted in increased susceptibility to a subsequent pathogen challenge. Crookshank et al. (1979) reported that corticosteroids were most responsive to transport stress; however, other trials reported no differences in cortisol concentration when subjected to weaning (Lefcourt and Elsasser, 1995) or transport (Galyean et al., 1981) stress.

More recently, products of the pro-inflammatory response have been identified as potential biomarkers that may respond to host stress or immune challenge. Acute phase proteins (APP) such as haptoglobin, fibrinogen, ceruloplasmin, or serum amyloid-A are produced and secreted by the liver and have been measured in the blood of animals

subjected to transport, commingling or weaning stress because the rate of synthesis of APP can increase several hundredfold when influenced by pro-inflammatory cytokines (Klasing and Korver, 1997). Arthington et al. (2003) investigated the effect of transportation and commingling on measures of the APP response of newly weaned beef calves and reported that transported calves had greater serum amyloid-A, fibrinogen, and ceruloplasmin concentrations, yet significantly lower haptoglobin concentrations than non-transported controls. Haptoglobin levels may also correlate with clinical respiratory disease; Wittum et al. (1996) reported that feedlot calves treated for BRD had decreased serum haptoglobin concentrations from initial to final examination compared to calves never receiving antibiotic treatment. Although these APP are commonly used as biomarkers to determine the incidence of stress in cattle, their effects on disease pathology is less clear.

Interferon- $\gamma$  is involved in innate immunity serving as a macrophage-activating cytokine when it is released by natural killer cells responding to infected or stressed host cells, and adaptive immunity when released by T-cells subsequent to antigen recognition (Abbas, 2007). Interferon- $\gamma$  levels are responsive to stressors such as castration (Fisher et al., 1997), weaning (Hickey et al., 2003), and endotoxin challenge (Carroll et al., 2009; Kahl and Elsasser, 2006).

Another biomarker for stress and disease is total and differential leukocyte cell concentrations of blood. These cells of the immune system include neutrophils, lymphocytes, monocytes, eosinophils, and basophils that each comprise a unique role in innate and adaptive immunity. Increases in the concentration of these cell types is indicated with the suffix “philia” (i.e. neutrophilia), and is generally attributed to

inflammation and immune stimulation (Merck, 1998). Conversely, decreases are indicated by the suffix “penia” (i.e. neutropenia) and this condition is often associated with endogenous stress because resulting corticosteroids and pro-inflammatory cytokines mediate lysis of leukocytes. Although perhaps less extensively researched in the bovine model, an increased neutrophil:lymphocyte (N:L) ratio is implicated as an indication of shipping stress in pigs (McGlone et al., 1993) and social stress in chicks (Gross and Siegel, 1983).

#### *Interaction of Stress and Immunity*

Produced primarily by LPS-stimulated macrophages and T-cells, TNF- $\alpha$  mediates inflammation and recruitment of immune cells to areas of infection, especially during endotoxin infection; thus TNF- $\alpha$  regulates components of both innate and adaptive immunity. Carroll et al. (2009) observed that both TNF- $\alpha$  and IL-6 concentrations increased following endotoxin challenge in calves, and the magnitude of increase was greater for calves weaned at 250 d of age versus early-weaned at 80 d of age and shipped to a receiving facility.

In a review of stress and immunity in swine and cattle, Salak-Johnson and McGlone (2007) proposed that hormonal and immunological products influenced by physiological stress such as cortisol, IFN- $\gamma$ , and IL-4, modulate T helper cell differentiation, thereby influencing the direction of the immune response (i.e. humoral vs. cell-mediated). Activated CD4<sup>+</sup> T lymphocytes differentiate into distinct subsets called T helper1 (Th1) and T helper2 (Th2) cells during priming of immature CD4<sup>+</sup> T lymphocytes, and IFN- $\gamma$  and IL-4 influence the differentiation process (Mosmann and Coffman, 1989). If a T lymphocyte differentiates into a Th1 effector cell, these cells both

secrete and are regulated by IFN- $\gamma$ , which activate CD8+ cytotoxic T cells (cell-mediated immunity); whereas, Th2 effector cells activate B cells to produce antibodies (humoral immunity) and are regulated by and secrete IL-4, which inhibits Th1 (Coffman, 2006). Furthermore, glucocorticoids inhibit many pro-inflammatory cytokines and may favor a Th2 or humoral immune response (Wiegers et al., 2005). Salak-Johnson and McGlone et al. (2007) concluded that stress may shift the balance between Th1 and Th2 immune response, and the conflicting reports of stress and immune interactions in animals may be partially explained by the type and duration of stressor, age, genetics, and social status of experimental animals used within and among studies.

Filion et al. (1984) investigated the role of stress in the induction of pneumonic pasteurellosis of calves challenged with respiratory virus (IBR) or bacteria (*Pastuerella haemolytica*). Calves subjected to weaning, transportation, and handling in a feedlot were more susceptible to IBR, but not *Pastuerella haemolytica* challenge. Nevertheless, interactions of stress and viral-bacterial synergy in BRD have been described by Hodgson et al. (2005). In their review, 2 unique mechanisms by which stress-induced corticosteroids may directly and indirectly modulate viral-bacterial synergy which contributes to BRD were identified. First, corticosteroids may directly inhibit the pro-inflammatory responses induced by increased toll-like receptor expression through inhibition of transcriptional regulators NF $\kappa$ B and AP-1. Additionally, corticosteroids may indirectly regulate the pro-inflammatory response because of their effects on T cell development and increased IL-10, an anti-inflammatory cytokine. The authors concluded that stress has a significant impact on prevalence and severity of respiratory disease in human and animal models.

Babiuk et al. (1987) treated newly received calves intranasally or intramuscularly with recombinant bovine interferon- $\alpha$ , an immunomodulator and inhibitor of viral replication, 48 h prior to challenge with IBR, followed 4 d later with *Pastuerella haemolytica* challenge. In this study, interferon- $\alpha$  administration greatly reduced clinical BRD, mortality, weight loss, and mean lung score indicated by pneumonic lesions upon necropsy. Furthermore, evidence of bacterial-viral synergy existed for control treatments not receiving interferon- $\alpha$ ; these animals exhibited increased clinical illness score and temperature following d 0 IBR challenge, and these indices were further increased immediately following *Pastuerella haemolytica* challenge on d 4.

The modulation of humoral and cell-mediated immune response resulting from stress-induced physiological alterations, and the impact on vaccine response and disease outcome in newly received beef cattle warrants further investigation.

#### *Interaction of Stress and Growth*

Stimulation of the immune system can also be considered a stressor and research indicates the immune response to endotoxin challenge can cause a metabolic shift to repartition nutrients away from tissue deposition in favor of immunity (Elsasser et al., 1995). Several pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and interleukin-6 (IL-6) have been shown to be responsive to stress stimuli and are critical products of the immune response during an infectious challenge, yet can impair growth either directly by acting on tissues or indirectly through their effects on the endocrine system (Klasing and Korver, 1997).

Moreover, growth hormone (GH) and insulin-like growth factor-I (IGF-I) are critical in regulating growth and tissue deposition (Spurlock, 1997), and these hormones

are inversely altered during endotoxin challenge. An endotoxin challenge study using a mouse model reported decreased levels of plasma GH returning to baseline within 24 h; however, IGF-I remained low at 24 h (Fan et al., 1995). Therefore, because blood concentrations of TNF- $\alpha$  are elevated during endotoxin challenge, this would suggest increased TNF- $\alpha$  levels being correlated with decreased GH and IGF-I. Johnson (1997) reviewed pro-inflammatory cytokine regulation of growth and proposed that anorectic and metabolic effects of immunological challenge occur because: 1) several bacterial and viral pathogens stimulate leukocytes to synthesize and secrete cytokines, 2) animals with either clinical or subclinical infections are anorectic, 3) exogenous inflammatory stimuli and recombinant cytokines, when injected into animals, have anorectic and metabolic properties that characterize immunological stress, 4) bona fide receptors for cytokines are present in disparate nonimmune tissues, and 5) the anorectic properties of IL-1 are blocked by a specific receptor antagonist.

Klasing and Korver (1997) reviewed the effects of leukocytic cytokine immune stimulation by both pathogens and experimentally-induced cytokine levels on growth rate, feed intake, and feed efficiency. The authors refer to direct evidence of pro-inflammatory cytokines as mediators of growth in experiments which closely duplicate the qualitative and quantitative aspects of cytokine release in vivo (Klasing et al., 1987). In this series of experiments (Klasing et al., 1987), cortisol and IL-1 concentrations in immunologically challenged chicks were greater than in non-challenged controls. Furthermore, to determine the influence of cortisol and IL-1 on protein degradation and synthesis, skeletal muscles were incubated in the presence of cortisol and IL-1 at concentrations similar to those seen after immune challenge. It was observed that cortisol

did not affect the rate of protein degradation; however, rate of protein synthesis was reduced compared to control. Conversely, IL-1 did not affect rate of protein synthesis but resulted in a 24% increase in protein degradation compared to control. In addition to modulation of protein synthesis and degradation, cytokines can impair growth through induction of anorexia (Plata-Salaman, 1996) and Yang et al. (1994) observed synergistic effects of TNF- $\alpha$  and IL-1 in inducing anorexia in rats. Direct and indirect effects of pro-inflammatory cytokines may decrease feed intake by more than 50% during acute disease challenge (Klasing and Korver, 1997).

### **Identified Stressors in Beef Cattle Management**

#### *Weaning*

Weaning is one of the most stressful events encountered in beef calves. Weaning can be described two-fold: 1) to accustom a young animal to take nourishment other than by suckling, and 2) to detach from that to which one is strongly habituated or devoted. As calves mature they transition from a diet consisting primarily of milk, to a diet consisting of forage, grain, or both. Although necessary to facilitate standard beef production practices, weaning results in psychological (Price et al., 2003) and physiological (Hickey et al., 2003) stress, and recently weaned, newly received calves must learn to consume feed and water from a bunk or trough. Hickey et al. (2003) evaluated N:L ratio, plasma cortisol and APP concentration and IFN- $\gamma$  production in abruptly weaned calves. The N:L ratio was increased and IFN- $\gamma$  response to an antigen, keyhole limpet haemocyanin, was reduced for weaned calves versus control; however, no differences were observed for cortisol, haptoglobin, or fibrinogen in this experiment. Arthington et al. (2008) evaluated measures of stress and performance in 96 steers over a 2-year period in 4 weaning

management treatments: 1) control: weaned on the day of shipment, 2) creep-fed: allowed free-choice access to concentrate before weaning and shipment, 3) preweaned: weaned and provided supplemental concentrate on pasture before shipment, and 4) early-weaned: weaned at 70 to 90 d of age and kept on pasture. Overall ADG was greater for early-weaned vs. control steers during the 29-d receiving period. Before shipment (d 0), plasma ceruloplasmin concentration was less for control vs. early-weaned steers; however, subsequent to shipment on d 15 and 22, ceruloplasmin was greater for control vs. early-weaned steers. Plasma haptoglobin concentrations tended to be greater for creep-fed vs. preweaned calves, but no other differences in haptoglobin concentrations were observed. In a subsequent experiment (Carroll et al., 2009), a more intensive investigation into the immunological response of early-weaned (80 d of age) vs. normal-weaned (250 d of age) calves was conducted after endotoxin challenge. Serum samples were analyzed for TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, cortisol, ceruloplasmin, and haptoglobin. A weaning  $\times$  time interaction was observed for TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and ceruloplasmin; these indices were greater for normal-weaned vs. early-weaned calves subsequent to endotoxin challenge. Furthermore, a weaning effect was reported for haptoglobin, with serum concentrations being greater for normal-weaned. The authors concluded that the immune system of early-weaned calves appears to be more competent in responding to pathogen challenge than normal-weaned calves.

### *Transportation*

Transportation of cattle by truck to and from the different cow-calf, stocker, and feedlot segments is yet another inherent stressor associated with the beef production system. Blecha et al. (1984) performed research on the effects of transportation stress and

the immune response in which steers were either shipped 700 km to a feedlot or remained at the origin ranch. Transported steers had greater total leukocytes and neutrophils; however, cortisol concentrations did not differ. Lymphocyte blastogenic responses were reduced after transport; whereas, monocyte phagocytosis and packed cell volume were not affected by transportation. Evidence also suggests that confinement associated with the transportation process may play a significant role in transit stress. Phillips (1984) observed increased concentrations of fibrinogen when steers were shipped 400 km and confined in novel surroundings for 15 h, yet this increase was not different from steers that were confined without transport. Buckham Sporer et al. (2008) observed several alterations in physiological blood parameters following a 9-h truck transportation of beef bulls. Blood was collected at -24, 0, 4.5, 9.75, 14.25, 24 and 48 h from initiation of transport (0 h). Plasma concentrations of haptoglobin and fibrinogen were decreased with the onset of transportation stress. Conversely, plasma cortisol increased sharply by 4.5 and 9.75 h after transport, returning to sub-baseline at 14.25 h. Furthermore, total leukocytes increased in response to transportation stress. Although effects on these measurements beyond 48-h were unknown, rapid, acute physiological changes were clearly evident and garner potential as biomarkers for stress and disease. Stanger et al. (2005) performed a similar transportation stress study; however, blood collection occurred 48 h before transport, immediately following a 72 h transport event, and 6 d thereafter (216 h). In this study, IFN- $\gamma$  production did not differ; whereas, total leukocyte concentration following transport decreased transiently at 72 h and returned to near baseline values by 216 h. To determine effects of weaning and transportation on APP response, Qiu et al. (2007) evaluated blood APP concentrations periodically in 3 breeds

of cattle. Blood samples were collected at weaning and at 24 and 72 h after weaning. Additionally, blood was collected immediately before shipment, upon arrival, and 24 and 72 h after arrival. Haptoglobin and fibrinogen concentrations were significantly increased at 72, but not 24 h after weaning. In response to transportation stress, haptoglobin concentrations were least prior to shipment, intermediate for on-arrival and 72 h after shipment, and greatest 24 h after shipment. Fibrinogen was increased on-arrival and 72 h post-shipment, but not 24 h after shipment, compared to pre-shipment fibrinogen concentrations.

### *Castration*

Castration is necessary to reduce aggressive and sexual behavior and improve meat quality in male cattle, yet the practice causes pain and stress that temporarily reduces performance (Fisher et al., 1996), and the performance reduction due to castration is greater in older bulls that are also experiencing weaning stress (Peterson et al., 1989). Bretschneider (2005) reported that castration closer to birth resulted in less weight loss for the 30-day period following castration. The immune status may also be altered by castration, as evidenced by increased leukocytes in the blood for castrates vs. controls (Chase et al., 1995), and the incidence of BRD has been reported to be much greater in calves castrated on arrival at a feedlot (Daniels et al., 2000).

Fisher et al. (1997) determined the effects of castration on cortisol concentration, in vitro IFN- $\gamma$  production, leukocyte concentration, and plasma haptoglobin and fibrinogen concentrations. On d 1, surgically castrated animals had lower IFN- $\gamma$  production and greater neutrophil and N:L ratio compared to control. Plasma haptoglobin on d 1 and 3 and fibrinogen concentrations on d 3 and 7 were increased for castrated

animals. Growth performance was also affected by castration; ADG was less for the first 7 d in surgically castrated animals. Their results indicate significant physiological alterations in response to surgical castration; whereas, performance was transiently reduced in castrated animals.

### *Commingling*

A less investigated area of management research is the effect of commingling cattle on health and performance. To mitigate the effects of commingling and marketing stress, single-source cattle are purchased from larger cow-calf ranches through direct-marketing; however, commingling is inherent in the auction market process because cattle from small cow-calf farms are often sold one at a time, and an order buyer purchasing cattle for a stocker or feedlot operation must assemble larger group lots weighing ~22,727 kg to facilitate a full truckload. The commingling of cattle results in 2 primary issues: 1) potential exposure to un-encountered pathogens increases, and 2) the hierarchy of the newly assembled group must be established resulting in increased psychological and physiological stress. Because commingling during the marketing process both increases the potential of viral pathogen exposure and increases stress, acute infection with respiratory viruses is more likely because stress-induced immunosuppression allows viruses to evade host immune defense mechanisms (Sheridan et al., 1994). Step et al. (2008) evaluated effects of health and performance in beef calves from a single-source ranch (RANCH) either weaned and immediately shipped to a feedlot (WEAN), weaned and kept on the ranch for 45 d before shipment without receiving any vaccinations (WEAN45), weaned and kept on the ranch for 45 d before shipment and vaccinated with a MLV respiratory vaccine (WEANVAC45), procured and assembled

through several auction markets (MARKET), or a commingled group (COMM) containing a portion of RANCH and MARKET origin cattle. Regardless of weaning or vaccination status, calves originating from RANCH tended to have greater ADG than COMM or MARKET calves for the 42-d receiving period. Clinical morbidity was greatest for MARKET, intermediate for COMM, and least for RANCH. Gupta et al. (2005) observed increased plasma cortisol, albumin, urea, and nonesterified fatty acids in 14 mo-old Holstein-Friesian steers that were subjected to repeated regrouping and repenning. Arthington et al. (2003) observed effects of transport and commingling in newly weaned calves on plasma fibrinogen, ceruloplasmin, haptoglobin, and cortisol concentrations. No differences were observed for commingling in this study; however, commingling was not extensive, consisting of 2 outsourced steer calves being placed in the commingled-designated pens. Transportation effects were reported for fibrinogen in experiment 1 and haptoglobin in experiment 2; both of these APP increased after transport.

### **Effects of Preconditioning Management on Bovine Respiratory Disease and Growth Performance**

Preconditioning is a comprehensive management practice first identified in the 1960s designed to reduce the incidence and susceptibility to BRD during the stocker and finishing segments of the beef production system. The negative effects of stress are mitigated through preconditioning management; however, this must occur during a critical time period before marketing and transport to a stocker operation or feedlot occurs. Although the specific requirements of different preconditioning programs may vary, typical requirements include weaning calves on their origin ranch for a specified

time (i.e.  $\geq 45$  d), vaccinating against clostridial (*Clostridium chauvoei-septicum-novyi-sordellii perfringens* type C & D) and respiratory (IBR, BVDV type 1 & 2, PI3V, BRSV) antigens, treatment with anthelmintic, castration, dehorning, and training to consume feed from a bunk and water from a trough before being marketed or transported to a stocker or feedlot facility (Cole, 1985; Duff and Galyean, 2007). Each of these preconditioning requirements functions to reduce stress and disease risk in preparation for the stocker or feedlot environment. For example, in the preconditioned calf, weaning stress is reduced and overcome on the ranch of origin before shipping and commingling occurs. This mitigates the additive effect of multiple stressors by shifting stress occurrences earlier (ie. weaning stress on the ranch of origin rather than during transport to a feedlot with concurrent stressors). Justifiably so, preconditioned cattle are more valuable than non-preconditioned cohorts. Net return for preconditioned vs. non-preconditioned steers selling in a Kansas auction market from 1999 to 2004 was estimated between \$14.28 (winter) and \$31.84 (fall)/animal depending on market conditions, calf weight and condition (Dhuyvetter et al., 2005). Whereas, the estimated \$40 to \$60/animal value of preconditioned cattle in the feedlot is considerably greater than the estimated net return from marketing preconditioned calves (Dhuyvetter et al., 2005).

Although research is surprisingly limited, several studies demonstrate that preconditioning management reduces the incidence of BRD and increases gain performance. In a trial conducted by Clark et al. (2006), crossbred beef steers of low- (preconditioned) or high-risk for BRD were used to evaluate differences in health and performance. Preconditioned calves had greater average daily gain (ADG) during the

finishing period than high-risk auction market calves of unknown history; however, no differences in performance were observed during the 28-d receiving period. Antibiotic treatment cost was also reduced, averaging \$15.55 and \$0.26/steer for high- and low-risk cattle groups, respectively. Seeger et al. (2008) observed that auction market steers of unknown origin or health history gained 0.18 kg/d less during a finishing period than weaned beef calves receiving a health protocol on their origin ranch prior to marketing; however, an unequal number of calves testing positive for PI-BVDV remained in their treatment pens during the trial which may have confounded results. Another study (Macartney et al., 2003) reported that calves of unknown health history had greater morbidity compared with those administered health protocols prior to marketing. Furthermore, Roeber et al. (2001) observed that cattle originating from preconditioning programs had fewer hospital visits than cattle originating from auction markets. Earlier research conducted by Pritchard and Mendez (1990) suggested that non-preconditioned calves may fully compensate for initial performance loss by the end of the feeding period; however, the non-preconditioned cattle used in that experiment would be considered low-risk by current industry accepted standards because they were maintained as a single-source and were not subjected to stressors associated with the auction market system.

### **Overview of Bovine Viral Diarrhea Virus and its role in Bovine Respiratory Disease**

Bovine viral diarrhea virus is a critical viral pathogen that can adversely affect reproduction, performance and health of beef cattle from the point of conception through the finishing phase of the beef cattle industry. A member of the *pestivirus* genus within the Flavivirus family (Ridpath, 2008), BVDV is classified into genetically distinct genotype groups 1 and 2, and is further classified into subgenotypes 1a, 1b, 2a, and 2b

(Ridpath, 2005), among others. In addition to genetic differentiation, the biotype of a specific BVDV strain is classified as cytopathic or noncytopathic based upon its ability to cause cellular damage in cultured cells (Peterhans et al., 2010). Research would also indicate that antigenic differences may exist among BVDV subgenotypes because differences in cross-neutralization are observed (Bachofen et al., 2008). Furthermore, Burciaga-Robles et al. (2010) observed increased antibody titers to BVDV subgenotype 1b (strain TGAC 8HB), but not type 1a (Singer strain) or 2a (strain 125-C) when steers were exposed to 2 PI-BVDV type 1b challenge animals for 72 h. Ridpath et al. (2010) reported that the ratio of virus cross-neutralization among BVDV type 1a and 1b subgenotype strains is 36%. These findings identify a critical need for further research because currently no commercially licensed modified-live virus (MLV) respiratory vaccine in the US contains a BVDV type 1b isolate. Also noteworthy, BVDV type 1b is the predominant strain isolated from calves in the US with respiratory disease (Fulton et al., 2002).

Bovine viral diarrhea virus infection of susceptible cows during the second to fourth month of gestation (Peterhans et al., 2010) can result in the birth of a persistently infected (PI), immunotolerant calf (McClurkin et al., 1984) that will shed large amounts of the noncytopathic biotype of the BVDV subgenotype strain it was exposed to *in utero* throughout its entire lifetime (Kahrs, 2001). There may be a correlation between the majority of PI-BVDV calves being infected with BVDV subgenotype 1b (Fulton et al., 2005), and the fact that this specific BVDV strain is not included in commercial vaccines that could provide protection against BVDV emergence in the field. Nevertheless, PI-BVDV calves may contribute to one of the most critical consequences of BVDV

infection, BRD complex, by acting as a primary respiratory pathogen source or through immunosuppression of exposed cohorts (Welsh et al., 1995). Although the prevalence of PI-BVDV calves in the feedlot is estimated to be relatively low (0.3%; Loneragan et al., 2005), a single PI-BVDV animal has the potential to continuously expose an entire pen and adjoining pens to the virus. Moreover, the large population of confined cattle that exist for a typical commercial feedlot located in the high-plains region of the US suggests that more than 100 PI-BVDV animals may be sporadically located throughout a single facility (ie. 50,000 animals  $\times$  0.3 prevalence rate = 150 PI-BVDV animals).

Some consultants and veterinarians have promoted to stocker or feedlot clientele with limited success, testing upon arrival and removal of animals identified as PI-BVDV. A significant barrier to more widespread adoption of this practice is the conflicting research on effects of PI-BVDV exposure reported in the literature. Nevertheless, several commercial and university veterinary diagnostic laboratories offer PI-BVDV testing services. The latest, most efficient procedure to determine PI-BVDV status is to test an ear-notch tissue sample for the presence of BVDV using the antigen-capture, or competitive enzyme-linked immunosorbent assay (ACE) via a commercially available test kit (IDEXX HerdChek, IDEXX Laboratories, Westbrook, ME). At the present time, the commercial laboratory fee to perform an ACE test ranges from \$3.75 to \$5.00 per sample excluding next day parcel service fees. Because PI-BVDV calves often appear otherwise healthy with no visual symptoms of disease, it is impossible to visually identify and remove suspected PI-BVDV animals; therefore, a stocker or feedlot producer considering this management practice must test an entire population of newly received

calves to identify and remove PI calves, representing a significant cost to a producer considering this management strategy.

Research results on effects of exposure to PI-BVDV calves in the feedlot are both limited and conflicting; thus, the decision to invest in PI-BVDV testing and removal of PI-positive animals to minimize economic losses associated with BVDV exposure of newly received cattle remains controversial. Elam et al. (2008) evaluated the effects of long- (duration of study) or short-term (60 h) exposure to a calf identified as PI-BVDV in heifers that had been previously vaccinated for BVDV and received antibiotic metaphylaxis upon arrival. In this study, no differences in final body weight, dry matter intake, feed efficiency, or carcass characteristics were observed with PI-BVDV exposure. Furthermore, none of the experimental animals in this study required treatment for BRD, indicating that PI-BVDV exposure did not affect BRD morbidity; however, based upon the excellent health of experimental animals, physiological stress and immunosuppression was probably minimal for heifers in this study. Booker et al. (2008) performed a prospective study in 3 Alberta feedlots to observe differences in health and performance outcomes of pens with or without a PI-BVDV calf present. Although mortality attributed to BVDV was more common in pens containing a PI-BVDV animal, no differences in morbidity, overall mortality, ADG, or feed efficiency were observed between PI and non-PI pens. Both Elam et al. (2008) and Booker et al. (2008) concluded that the presence of a PI-BVDV calf had little or no effect on health or performance, and both authors suggested that PI-BVDV exposure may provide enhanced immunity against acute BVDV infection.

Loneragan et al. (2005) conducted a cross-sectional study to estimate prevalence of PI-BVDV cattle in the feedlot population, prevalence of chronically ill and dead PI-BVDV cattle, and the effects on BRD morbidity when exposed to a PI-BVDV calf. Prevalence of PI-BVDV cattle arriving in the feedlot was 0.3%, and it was reported that cattle exposed to a PI-BVDV animal had a 43% greater risk of developing BRD. Furthermore, a greater percentage of PI-BVDV animals were present in chronically ill (2.6%) and dead (2.5%) cattle. In a study evaluating the effects of PI-BVDV exposure of high-risk auction market calves of unknown history (Hessman et al., 2009), cattle directly exposed to PI-BVDV challenge had a greater percentage of first relapse, chronic illness, and fatalities; however, overall morbidity was not different compared to unexposed groups. It was determined through economic analysis that fatalities and performance losses due to PI-BVDV exposure accounted for economic losses of \$5.26 and \$88.26/animal, respectively. Furthermore, the authors provided comprehensive discussion on susceptibility of PI-BVDV exposure attributing factors of virulence and amount of virus particles being shed, frequency of contact, population density, and immunocompetence of exposed cohorts as factors that can affect the magnitude of health and performance outcome in studies evaluating PI-BVDV exposure.

O’Conner et al. (2005) evaluated the association between commingling and PI-BVDV exposure on feedlot pen morbidity. They reported that disease prevalence was least in pens containing single-source cattle with a PI-BVDV calf present, and exposure to PI-BVDV in commingled groups also reduced morbidity compared to commingled pens with no PI-BVDV calf. Nevertheless, commingling was associated with an increase in BRD morbidity. Fulton et al. (2006) suggested that calves previously vaccinated and

preconditioned prior to exposure to a PI-BVDV animal can provide protection against viremia compared to naïve calves. One inconsistency among PI-BVDV research is the use of experimental cattle with varied management and vaccination history, and peer-reviewed research evaluating effects of PI-BVDV exposure in different BRD risk-groups of cattle such as single source, preconditioned versus commingled, auction market calves is lacking.

Research on the effects of PI-BVDV exposure on APP and cytokine response is limited. Burciaga-Robles et al. (2010) examined effects of an intratracheal *Manheimia haemolytica* challenge after 72-h exposure to PI-BVDV subgenotype 1b calves on leukocyte and cytokine concentration of feedlot steers. Steers were randomly allocated to 1 of 4 treatments in a  $2 \times 2$  factorial arrangement: 1) not exposed to PI-BVDV challenge nor challenged with *M. haemolytica* (control), 2) exposed to PI-BVDV challenge for 72 h, 3) intratracheally challenged with *M. haemolytica*, and 4) exposed to PI-BVDV challenge for 72 h and challenged with *M. haemolytica*. A main effect of PI-BVDV exposure was observed, with PI exposure resulting in reduced numbers of total leukocytes, neutrophils, lymphocytes, eosinophils, and basophils. Haptoglobin concentrations were not different in steers exposed to PI-BVDV challenge; conversely, Ganheim et al. (2003) reported greater haptoglobin concentrations in steers challenged intranasally with BVDV type 1. Several pro-inflammatory cytokine levels were impacted in the Burciaga-Robles et al. (2010) study. Exposure to PI-BVDV challenge resulted in increased IL-1, IL-6, and TNF- $\alpha$  concentrations; whereas, tendencies were noted for IFN- $\gamma$  and IL-4. A treatment interaction was observed for IFN- $\gamma$ , with concentrations being greatest for steers exposed to both BVDV and *M. haemolytica* challenge models, and

least for control, BVDV only, and *M. haemolytica* only challenge. A similar treatment interaction was observed for TNF- $\alpha$ , with the greatest serum concentration observed for calves exposed to both disease challenge models, suggesting that exposure of naïve calves to PI-BVDV challenge increased the potential for secondary infection.

### **Conclusions from the Literature**

Implementation of management (or lack thereof) through the unique segments of the beef production system can tremendously impact the health, growth performance, and profitability of feeder cattle. As the scientific literature evolves regarding interactions of management, physiological stress, and immunosuppression, hopefully adoption of management and discovery of new products or methods for mitigation of bovine respiratory disease will increase, thereby reducing the industry's dependence on antibiotics to treat bovine respiratory disease. Several biomarkers that may indicate physiological stress and disease status, and the continually improving assays to detect such biomarkers, hold promise for future research and understanding. Improved understanding of physiological stress and its resulting modulation of immunity and disease will have profound effects on the health and well-being of cattle, profitability, and ultimately sustainability of the beef industry.

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### CHAPTER III

#### EFFECTS OF ON-ARRIVAL VERSUS DELAYED MODIFIED LIVE VIRUS VACCINATION ON HEALTH, PERFORMANCE AND SERUM INFECTIOUS BOVINE RHINOTRACHEITIS TITERS OF NEWLY RECEIVED BEEF CALVES<sup>1</sup>

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**ABSTRACT:** Stress commonly associated with weaning, marketing, and shipment of feeder cattle can temporarily compromise immune function; thereby, reducing the effective response to vaccination intended to control bovine respiratory disease (BRD). Two vaccination timing treatments were used to evaluate the effect of timing of a multivalent modified live virus (MLV) BRD vaccine on health, performance, and infectious bovine rhinotracheitis (IBR) antibody titer level of newly received stocker cattle. Crossbred bull and steer calves ( $n = 528$ ) were weighed ( $197 \pm 2.4$  kg) and randomly assigned to MLV vaccination treatment: 1) MLV vaccination upon arrival (AMLV), or 2) delayed (14 d) MLV vaccination (DMLV). All cattle were processed similarly according to routine procedures with the exception of initial MLV vaccination timing. Subsequently, BW were recorded on d 14, 28, and 42. Blood samples were collected on d 0, 14, 28, and 42 to determine differences in serum IBR titer levels, and comparisons were made between treatments on a receiving-day basis and an equivalent postvaccination day basis. Daily BW gains were greater ( $P \leq 0.05$ ) for DMLV calves from d 0 to 14 ( $1.16$  vs.  $0.88 \pm 0.22$  kg/d) and from d 0 to 42 ( $0.75$  vs.  $0.65 \pm 0.09$  kg/d). Days to first treatment, total treatment cost, percentage death loss, and pasture ADG after the 42-d receiving period did not differ ( $P \geq 0.15$ ). Morbidity rates for BRD were high for both AMLV and DMLV (71.5 and 63.5%, respectively), and did not differ ( $P = 0.12$ ). Positive IBR titer seroconversion was greater ( $P \leq 0.03$ ) for DMLV calves on d 42 of the study, and for the 28- and 42-d equivalent postvaccination basis. Delaying vaccination by 14 d may increase ADG during the receiving period compared with AMLV and seroconversion to IBR was greater in DMLV calves, indicating a possible improvement in acquired immune response when MLV vaccination is delayed.

## INTRODUCTION

Bovine respiratory disease (BRD) is the most economically important disease in newly received beef cattle (Edwards, 1996), and is the leading cause of morbidity and mortality according to a recent survey of US feedlots (Woolums et al., 2005). A common receiving management strategy includes classification of cattle groups into risk categories (high or low risk) for BRD; this is determined by factors including transportation stress, immune status, nutritional condition, environment, and the skill level of management personnel (Smith, 2004). Calves purchased at local auctions are typically classified as high risk for developing symptoms of BRD because these cattle are of unknown origin, commingled, and recently weaned from small cow-calf operations that seldom use vaccination or other BRD prevention strategies (National Animal Health Monitoring System, 1997). Stress and previous exposure to BRD pathogens may decrease vaccine efficacy (Blecha et al., 1984; Loerch and Fluharty, 1999). The transportation stress period can endure for as long as 15 d after arrival, based on serum hemolytic complement concentration of calves (Purdy et al., 2000). Perino and Hunsaker (1997) indicated that BRD vaccination on arrival at feedlots is equivocal at best. Other complications with on-arrival administration of a modified live virus (MLV) vaccine may include reduced gain performance caused by immunological challenges from antigens contained in a vaccine; however, Stokka and Edwards (1990) reported no detrimental effects on gain of stressed calves receiving a multiple polyvalent MLV vaccine. Current feedlot receiving protocols include multivalent vaccination against BRD viruses within 48 h of arrival for high-risk cattle, although the vaccine response and health benefit are questionable.

Thus, our objective was to evaluate the effect of delayed (14 d) MLV vaccination vs. on-arrival MLV vaccination on health, performance and serum infectious bovine rhinotracheitis (IBR) titer levels of newly received high-risk stocker calves.

## **MATERIALS AND METHODS**

Animal methods were approved by the University of Arkansas Animal Care and Use Committee.

A total of 528 crossbred bull (n = 364) and steer (n = 164) calves (initial BW =  $197 \pm 2.4$  kg) were purchased from a northern Arkansas auction barn and shipped approximately 9 km to the University of Arkansas, Livestock and Forestry Branch Station, near Batesville, AR. Cattle were received on 6 dates: September 9, 2004 (Block 1, n = 110), September 16, 2004 (Block 2, n = 98), January 10, 2005 (block 3, n = 80) January 13, 2005 (Block 4, n = 80), February 14, 2005 (block 5, n = 70), and February 17, 2005 (Block 6, n = 90). Pen was designated as the experimental unit. For each date block, 6 pens (3 treatments/pen) were used; therefore, each treatment was replicated a total of 18 times. Because the number of animals per block varied, the number of animals per pen ranged from 10 to 19, but within each block the number of animals assigned to each treatment were equivalent.

Upon arrival (d -1), cattle were weighed (unshrunk), assigned a unique ear identification tag, and arrival castrate status (bull or steer) was determined. Calves remained commingled overnight, with access to hay and water. The following day (d 0), bulls and steers were assigned to treatment based on castrate status, which was equally distributed to treatments by assigning a similar number of bull and steer calves to each treatment pen, with pens then assigned to treatment. Treatments included 1) initial

vaccination of a multivalent BRD MLV on arrival (d 0; AMLV), or 2) 14-d delayed initial vaccination of the BRD MLV (DMLV). On d 0, calves were reweighed, administered a clostridial bacterin with tetanus toxoid (Covexin-8; Schering-Plough Animal Health, Inc., Elkhorn, NE), treated for internal and external parasites (Ivomec; Merial, Iselin, NJ) and bull calves were castrated using the California banding method (InoSol Co. LLC, El Centro, CA). Calves were administered 2 injections of a MLV BRD vaccine 14 d apart. Arrival MLV calves were vaccinated with a 5-way MLV vaccine containing isolates of IBR, bovine virus diarrhea (BVD) types I and II, bovine respiratory syncytial virus (BRSV), and parainfluenza (PI<sub>3</sub>) in combination (Express 5; Boehringer-Ingelheim Vetmedica, Inc., St. Joseph, MO), whereas cattle assigned to the DMLV treatment did not receive their initial 5-way MLV vaccination until d 14 of study.

In addition, the rectal temperature of each calf was determined using a digital thermometer (Model No. M216, GLA Agricultural Electronics, San Luis Obispo, CA). Calves with rectal temperatures of  $\geq 40^{\circ}\text{C}$  during the initial processing were administered prophylactic treatment with tilmicosin phosphate (10mg/kg of BW, Micotil, Elanco Animal Health, Indianapolis, IN) as described by Galyean et al. (1995). The percentage of animals administered antibiotic on arrival (AMLV = 18.6%; DMLV = 23.4%) was not different ( $P = 0.19$ ), and these calves were excluded from subsequent morbidity analysis. After cattle were randomly sorted, they were moved to 0.4-ha pens separated by electrified fencing and provided a 16% CP supplement at 1% of BW (DM basis) and free-choice access to Bermudagrass hay (10% CP, 56% TDN) for the entire 42-d receiving study. The receiving supplement contained (as-fed basis) 67% corn, 19%

cottonseed meal, 12.5% corn gluten feed, 1.5% limestone, and 0.04% Rumensin 80 (Elanco Animal Health) and supplied 368 g of CP and 200 mg of monensin/d.

To determine differences in BW gain performance, cattle were weighed (unshrunk) at 14-d intervals during the trial (d 14, 28, and 42). On d 14, both AMLV and DMLV cattle received a booster vaccination of the clostridial bacterin with tetanus toxoid (Covexin-8), AMLV cattle received a booster vaccination of the 5-way MLV (Express 5), and DMLV cattle received their initial dosage of 5-way MLV (Express 5). Two weeks later (d 28), DMLV cattle received their respective booster vaccination of 5-way MLV (Express 5). At the conclusion of the 56-d receiving period, calves were implanted with 40 mg of trenbolone acetate and 8 mg of estradiol (Revalor G, Intervet Inc., Desoto, KS) and the pasture phase of the study began.

Calves were observed each morning (0800 h) by Livestock and Forestry Branch Station personnel for symptoms of respiratory illness (depression, lethargy, rapid breathing, nasal or ocular discharge), slowness in going to the feedbunk when supplement was provided, and a gaunt or emaciated appearance. Personnel were blinded to the treatment allotment of each pen. Cattle with observed visual symptoms of BRD were removed, restrained using a hydraulic squeeze chute (Flying W Inc., Watonga, OK) and considered morbid if their rectal temperature was  $\geq 40^{\circ}\text{C}$ . Morbid animals from both treatments were administered antibiotic therapy following the same predetermined antibiotic treatment protocol which included initial antibiotic therapy with tilmicosin phosphate (Micotil, 10 mg/kg of BW). Cattle were reevaluated 72 h later, and those with a rectal temperature of  $\geq 40^{\circ}\text{C}$  were considered morbid a second time and administered a second antibiotic treatment with enrofloxacin (Baytril, Bayer Animal Health, Shawnee

Mission, KS). After reevaluation, cattle requiring treatment a third time were administered florfenicol (Nuflor, Schering-Plough Animal Health, Summit, NJ). After 3 treatment events, cattle were considered non-responsive and no further antibiotic therapy was administered regardless of the symptoms. Cattle were returned to their respective home pen immediately following each antibiotic treatment. Treatment data recorded for individual animals included the treatment date and the amount, type and cost of antibiotic administered.

Blood samples were collected from 3 randomly selected animals in each pen to evaluate serum IBR titer levels. Blood was collected via jugular venipuncture from the same animals on d 0, 14, 28, and 42 for AMLV and d 0, 28, 42, and 56 for DMLV and stored in 15-mL Vacutainer tubes (BD Inc., Franklin Lakes, NJ). Blood was placed in a refrigerator at 4°C for 6 h after collection, and serum was separated by centrifugation at  $1,000 \times g$  for 30 min. Serum was then decanted and stored at -5°C for subsequent analysis, when all samples from the entire study were compiled. Once all serum samples were collected, they were packaged and shipped overnight to the diagnostic laboratory (Oklahoma Animal Disease Diagnostic Center, Stillwater, OK) for assay of IBR virus antibodies by the serum neutralization method, as described by Rosenbaum et al. (1970). Titers were reported as the reciprocal of the greatest dilution of serum that provided complete protection of the cells. The lowest dilution of serum tested was 1:4, whereas the greatest dilution of serum tested was 1:256. Serum that did not provide protection at the 1:4 level were reported as <4, and was considered negative for seroconversion to IBR. Samples with a reported serum neutralization value of  $\geq 4$  were considered positive for seroconversion to IBR. Titers were evaluated to determine differences in the percentage

of animals in each treatment with positive seroconversion to IBR. According to laboratory results (Oklahoma Animal Disease Diagnostic Center), samples with a reported serum neutralization value of  $<4$  were considered negative and those  $\geq 4$  were considered positive for IBR seroconversion. Titers  $<4$  were assigned a value of 0, whereas titers  $\geq 4$  were assigned a value of 1 and analyzed by  $\chi^2$  analysis to determine the percentage positive for differences in treatment by day. Percentage of IBR seroconversion was compared on both a receiving-day basis and a vaccination timing-equivalent basis. Receiving-day IBR titer comparisons were made from samples collected on d 0, 28, and 42. Vaccination timing-equivalent comparisons were made for IBR titers based on the number of days postinitial vaccination (d 0 AMLV vs. d 14 DMLV) and postbooster vaccination (d 14 AMLV vs. d 28 DMLV). Therefore, a blood sample was collected from DMLV calves after the receiving period had ended (d 56) to allow a final vaccine timing-equivalent comparison (d 42 AMLV vs. d 56 DMLV).

### *Statistical Analysis*

Treatment data were analyzed as a randomized complete block design. Pen was identified as the experimental unit. Date of shipment arrival was treated as the random block effect in the model. The treatment  $\times$  block interaction was used as the denominator mean square for the treatment effect test. Gain performance data, days to first pull, and treatment cost were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Initial BW was used as a covariate, and arrival castrate status (steer vs. bull) was used as a source of variation in the model to minimize the unwanted effects of arrival BW and castrate status on treatment outcome.

## RESULTS AND DISCUSSION

### *Performance and Health*

Body weight and ADG during receiving and grazing are presented in Table 1. Body weight of calves in the DMLV treatment was greater ( $P = 0.007$ ) on d 14 and tended to be greater ( $P = 0.07$ ) on d 42 than those in the AMLV treatment. Average daily gain was greater ( $P = 0.007$ ) for DMLV cattle compared with AMLV from d 0 to 14 of the study (1.16 vs.  $0.88 \pm 0.22$  kg/d). For the first 14 d of the study, AMLV had been administered an MLV vaccine, whereas DMLV had not yet received an initial MLV vaccine and could be considered a negative control. The difference in gain performance remained for the overall 42-d receiving period, with AMLV averaging 0.65 vs.  $0.75 (\pm 0.09)$  kg/d for DMLV ( $P = 0.05$ ). Average daily gain from d 14 to 28 was not different ( $P = 0.45$ ). The performance reduction in AMLV cattle from d 0 to 14, and in DMLV cattle from d 14 to 28, suggests that the initial physiological response to an MLV vaccine may elicit consequences that are temporarily detrimental to growth performance. No differences ( $P = 0.15$ ) were detected for pasture ADG during the subsequent grazing period.

Mass antibiotic metaphylaxis was not used in the current study; however, cattle were given prophylactic antibiotic treatment on arrival if the rectal temperature was  $\geq 40.0^{\circ}\text{C}$ . Galyean et al. (1995) reported that regardless of whether newly received calves were mass medicated with tilmicosin or received tilmicosin when the rectal temperature was  $\geq 39.7^{\circ}\text{C}$ , there was a similar reduction in percentage of calves treated for BRD compared with control calves. Based on our protocol, a similar ( $P = 0.19$ ) number of animals for AMLV (18.6%) and DMLV (23.4%) qualified for on-arrival antibiotic

treatment, and there was no difference ( $P = 0.83$ ) in rectal temperature on arrival (averaging 39.6°C; Table 2). Of the calves included in the morbidity analysis, 90.0% of initial BRD treatment episodes for AMLV and 88.8% of treatment episodes for DMLV had occurred by d 14 of the study (Figure 1). There were no differences ( $P \geq 0.16$ ) in the number of days to first treatment, mortality, or treatment cost for morbidity. The initial treatment rate for BRD was high (AMLV = 71.5%, DMLV = 63.5%) but not different ( $P = 0.12$ ; Table 2). The high rate of BRD morbidity is not uncommon in calves purchased at local auction barns and received at the Livestock and Forestry Branch Station. In this study, on-arrival MLV vaccination did not seem to be advantageous for either gain performance or disease prevention based on our results.

Duff et al. (2000) used 2 studies to evaluate the effects of viral vaccine route of administration and vaccine timing on the health and performance of newly received beef cattle. In the first study, ADG was greater for calves receiving an intranasal vaccine compared with an i.m. MLV IBR-PI<sub>3</sub> vaccine but was not greater than for unvaccinated control calves, yet the rate of morbidity did not differ. In the second study, Duff et al. (2000) reported no differences in BW gain among nonvaccinated calves, calves with 4-way MLV vaccination delayed until d 7, calves that received an intranasal IBR-PI<sub>3</sub> vaccine administered on d 0 with 4-way MLV vaccination delayed until d 7, or calves that received a 4-way MLV vaccination on both d 0 and 7. However, G:F tended to be improved for vaccinated calves vs. nonvaccinated calves. Kreikemeier et al. (1996b) found that during the initial 21 d of the feedlot receiving period, calves vaccinated with a killed virus on the farm before weaning and revaccinated with a killed virus at the time of commingling at a sale barn tended to gain faster than calves given an MLV upon arrival

at the feedyard and revaccinated 21 d after feedyard arrival. Preweaning BRD vaccination is certainly preferable; however, newly received calves of unknown origin that are allowed a period to adjust to their new environment and recover from previous stressors may be better suited to respond to MLV vaccination. Kreikemeier et al. (1996a) used a 2 × 2 factorial arrangement of treatments, which included mass medication with either tilmicosin phosphate or chlortetracycline or no mass medication, and routine processing on either d 1 or 21. Processing included a growth implant, an 8-way clostridial vaccine, a 4-way MLV vaccine, and an injectable dewormer. For the entire 56-d receiving period, calves that received processing on d 1 gained faster than calves with processing delayed until d 21. This is inconsistent with our results of increased ADG for DMLV; however, their study also included delayed administration of a growth implant and dewormer for 21 d, which could explain the difference in results.

No differences were detected ( $P > 0.11$ ) for the effects of MLV vaccination timing on initial treatment for BRD-associated morbidity, percentage of calves retreated for BRD, mortality, or treatment cost (Table 2). The overall consensus among animal health professionals and veterinarians seems to support arrival vaccination against BRD as an effective method of disease prevention, and a study by Hansen et al. (1992) supports this view when the morbidity rate is high. However, several groups have reported a neutral outcome when vaccinating newly received beef cattle for BRD (Bateman, 1988; Johnson et al., 1988; Duff et al., 2000). Martin et al. (1982) reported an increased risk of mortality when a respiratory vaccine was administered within 14 d of arrival. In that study, delaying the BRD vaccination in calves for 14 d from arrival decreased the mortality and treatment cost in cattle fed corn silage-based diets; however, no vaccination

timing differences were noted when cattle were fed dry hay-based diets. In the current study, the initial morbidity rate for BRD was high for both AMLV and DMLV (71.5 and 63.5%, respectively) but did not differ between treatments ( $P = 0.12$ ). Overall, 93% of BRD pulls occurred within the first 14 d of receiving (Figure 1). These data suggest little or no advantage of administering a 5-way MLV vaccine to high-risk stocker cattle on arrival. The high morbidity rate (Figure 1) observed before d 14 indicates that few changes in the receiving protocol could have affected morbidity rates.

### ***IBR Titers***

Serum titers are used as an indication of antibody protection against a specific pathogen, thereby providing an indication of vaccine efficacy (Callan, 2001). It is poorly understood how acquired antibody levels may be affected by the timing of vaccination before or during the receiving period; however, stress is known to compromise immune function (Chirase et al., 2004), and label guidelines according to the vaccine manufacturers recommend that vaccination of stressed cattle be avoided.

No differences ( $P = 0.94$ ) were detected for the percentage of positive IBR titers on d 0 (Figure 2), and positive seroconversion was low (AMLV = 8.8%; DMLV = 8.4%). On the basis of the vaccination timing-equivalent comparison, DMLV resulted in a greater percentage ( $P \leq 0.03$ ) of animals seropositive to IBR 28 and 42 d after the initial vaccination (Figure 2). When treatments were compared at the conclusion of the receiving period (d 42) the DMLV treatment exhibited greater ( $P = 0.01$ ) IBR virus antibody titer seroconversion. Results of IBR titer level comparisons suggest an improved vaccine response for DMLV. Natural disease exposure and subsequent host immune response may have contributed to the increased IBR antibody titers in this study;

however, the extent to which natural exposure affected IBR seroconversion is unknown. No difference in arrival (d 0) IBR titer levels were detected ( $P \geq 0.95$ ) in animals that became morbid once or twice compared with those never treated during the study (data not shown). Martin et al. (1999) reported seroconversion of BRSV and BVD viruses from d 0 to 28 in unvaccinated animals; however, mean IBR titers were low and were not different on either d 0 or 28 in unvaccinated calves both treated for BRD and not treated. Measurement of neutralizing antibody titers has been reported to provide an indication of the amount of BVD protection present in calves (Bolin and Ridpath, 1995) and has been positively correlated with disease prevention (Howard et al., 1989). This has not been studied as extensively in IBR; thus, further research on the role IBR titers may relate to BRD protection in the field is warranted.

Delaying initial MLV vaccination by 14 d improved the gain performance of high-risk, newly received cattle during the receiving period compared with MLV vaccination on d 0. Morbidity rate or cost associated with BRD was not different. Moreover, serum IBR titers were greater when initial MLV vaccination was delayed. Because no differences in morbidity or mortality were detected for the 2 treatments, and performance for DMLV cattle was slightly improved, results of the current study suggest an economic advantage to delaying initial MLV vaccination until 14 d after arrival.

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**Table 1. Effect of BRD vaccination timing on performance of newly received cattle.**

	On Arrival <sup>a</sup>	Delayed <sup>a</sup>	SE <sup>b</sup>	P-Value
<hr/>				
BW, kg <sup>d</sup>				
Day 0	197.7	195.9	2.42	0.33
Day 14	208.6	212.7	3.03	0.007
Day 28	217.4	219.9	2.93	0.16
Day 42	224.4	228.1	4.08	0.07
<hr/>				
ADG, kg/d <sup>c</sup>				
D 0 to 14	0.88	1.16	0.22	0.007
D 14 to 28	0.61	0.53	0.15	0.45
D 28 to 42	0.45	0.56	0.10	0.12
D 0 to 42	0.65	0.75	0.09	0.05
Pasture ADG, kg <sup>d</sup>	0.89	0.84	0.08	0.15

<sup>a</sup>Treatments were vaccination of incoming stocker cattle with Express® 5 (Boehringer Ingelheim) modified live IBR, PI3, BRSV and BVD type I and II vaccine either on arrival at initial processing (d-0) or on d-14. Cattle were re-vaccinated 14 d following initial vaccination.

<sup>b</sup>Standard Error of the mean (n = 524).

<sup>c</sup>All analysis (except d-0 BW) was conducted using BW and gender on d-0 as a covariate.

<sup>d</sup>Grazing performance calculated subsequent to the 42 d receiving period.

**Table 2. Effect of BRD vaccination timing on morbidity, mortality and treatment cost of newly received cattle.**

	On Arrival <sup>a</sup>	Delayed <sup>a</sup>	SE <sup>b</sup>	P-Value
Rectal Temperature on d-0, °C	39.61	39.56	0.23	0.83
BRD Treatment, %				
Initial <sup>c</sup>	75.3	70.2	0.10	0.21
Re-treat <sup>d</sup>	29.4	35.1	0.11	0.08
Days to 1 <sup>st</sup> treatment	5.7	5.1	1.10	0.44
Death loss, %	2.3	0.8	0.75	0.19
BRD Treatment Cost, \$ <sup>e</sup>	10.29	10.64	2.36	0.75

<sup>a</sup>Treatments were vaccination of incoming stocker cattle with Express® 5 (Boehringer-Ingelheim) modified live IBR, PI3, BRSV and BVD type I and II vaccine either on arrival at initial processing (d-0) or on d-14. Cattle were re-vaccinated 14 d following initial vaccination.

<sup>b</sup>Standard Error of the mean (n = 524).

<sup>c</sup>Initial, cattle with observed symptoms of BRD and temperature in excess of 40° C were injected with Micotil (Elanco) at 1.5 cc/ 45.5 kg BW.

<sup>d</sup>Re-treat, 72 h following initial, cattle with observed symptoms of BRD and temperature in excess of 40° C were injected with Baytril (Bayer) at 4.0 cc/ 45.5 kg BW.

<sup>e</sup>Treatment cost for BRD assuming value of Micotil \$1.10/cc and Baytril \$0.53/cc.

## CHAPTER IV

### EFFECTS OF ON-ARRIVAL VERSUS DELAYED CLOSTRIDIAL OR MODIFIED LIVE RESPIRATORY VACCINATIONS ON HEALTH, PERFORMANCE, BOVINE VIRAL DIARRHEA VIRUS TYPE I TITERS, AND STRESS AND IMMUNE MEASURES OF NEWLY RECEIVED BEEF CALVES<sup>2</sup>

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**ABSTRACT:** Stress, commonly associated with weaning, marketing, and shipment of feeder cattle can compromise immune function, and vaccine administration during immunosuppression may reduce vaccine efficacy and calf growth. Four treatments were compared in a  $2 \times 2$  factorial arrangement to evaluate the effect of on-arrival (d 0) vs. delayed (d 14) administration of clostridial (CLOS) and respiratory (RESP) vaccines on health, performance, bovine viral diarrhea virus (BVDV) antibody titers, and physiological immune measurements of high-risk, newly received calves. Crossbred bull and steer calves ( $n = 263$ ) were weighed ( $239 \pm 1.2$  kg), stratified by gender, and randomly assigned to vaccination treatment: 1) arrival CLOS, arrival RESP (ACAR); 2) arrival CLOS, delayed RESP (ACDR); 3) delayed CLOS, arrival RESP (DCAR); and 4) delayed CLOS, delayed RESP (DCDR). Body weight and blood samples were collected on d 0, 14, 28, 42, and 56. Average daily gain did not differ ( $P \geq 0.34$ ) averaging 0.98, 0.93, 0.95, and 0.91 kg/d for ACAR, ACDR, DCAR and DCDR, respectively, for the entire 56 d trial. Vaccination timing did not affect morbidity ( $P \geq 0.23$ ); however, there tended to be a CLOS timing effect ( $P = 0.07$ ) and RESP timing effect ( $P = 0.09$ ) on d to initial bovine respiratory disease (BRD) treatment. Average d to initial BRD treatment were less for ACAR ( $6 \pm 0.8$  d) compared to DCDR ( $8 \pm 0.8$  d;  $P = 0.01$ ). Greater white blood cell (WBC) counts were observed for DCDR than ACDR ( $P = 0.01$ ), with ACAR and DCAR being intermediate. Serum cortisol concentrations were greater on d 0 than d 14 ( $P < 0.01$ ) or d 28 ( $P = 0.01$ ) but no treatment  $\times$  day interaction ( $P = 0.21$ ) was observed. Timing of RESP administration affected ( $P = 0.001$ ) serum BVDV type I titer levels, with greater ( $P < 0.01$ ) levels in calves receiving RESP vaccine on arrival. Delaying CLOS or RESP vaccination did not affect gain or morbidity in high risk, newly

received stocker calves. Calves administered RESP vaccine on d 0 developed antibody titers to BVDV type I earlier than delayed RESP treatments. Total WBC count was greatest when RESP and CLOS vaccination were delayed (DCDR).

## **INTRODUCTION**

Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality according to a recent survey of US feedlots (Woolums et al., 2005), resulting in significant economic losses to the beef cattle industry. Calves purchased at local auction markets throughout the southeastern US are typically classified as high-risk for developing signs of BRD because these cattle are of unknown origin and recently weaned from small cow-calf operations that seldom utilize vaccination or other BRD prevention strategies (NAHMS, 1997). These high-risk cattle may be suffering stress effects resulting from a combination of commingling, transport, nutritional, weaning and environmental stressors that can compromise the immune system (Smith, 2004), and the transportation stress period can endure for as long as 15 d post-arrival based on serum hemolytic complement concentration (Purdy et al., 2000). Vaccination against viral and/or bacterial pathogens that contribute to BRD can be an effective preventative strategy; however, vaccine efficacy may be reduced if administered during immunosuppression (Callan, 2001), and a review of field vaccine efficacy by Perino and Hunsaker (1997) suggested that administering BRD vaccination at arrival in North American feedlots was generally not supported by published research. Other complications with on-arrival vaccination may include reduced gain performance (Chirase et al., 2001; Richeson et al., 2008), perhaps due to immunological challenge from vaccine antigens during a period when calves may be experiencing stress-induced

immunosuppression. Thus, our objective was to evaluate the effect of delaying respiratory and/or clostridial vaccination (d 14 vs. arrival) on health, performance, serum bovine viral diarrhea virus (**BVDV**) type I titers, and physiological stress and immune measures in high-risk, newly received calves.

## MATERIALS AND METHODS

Animal methods were approved by the University of Arkansas (**UA**) Animal Care and Use Committee.

Crossbred bull (n = 207) and steer (n = 57) calves (BW = 239 ± 1.2 kg) were procured by an order buyer from multiple auction markets located in western Arkansas and eastern Oklahoma and shipped to the UA Agricultural Experiment Station located near Savoy. These calves were likely commingled extensively from numerous herds and were experiencing other stressors described by Smith (2004) and thus would be considered high-risk for BRD. Three separate shipment dates representing each block in the experimental model were received on February 12 (Block 1, n = 90), March 1 (Block 2, n = 91), and April 17, 2007 (Block 3, n = 83). Calves in each block were divided into 8 pens (10 to 12 calves/pen); thus, each of the 4 treatments were replicated 6 times.

Upon arrival, cattle within each block were weighed, assigned a unique ear identification tag and gender was determined (bull or steer). Within gender (bull or steer), calves were randomly distributed to 1 of 8 pens. The 8 pens had been randomly assigned to 1 of 4 vaccination-timing treatments (Table 1). Treatments were arranged as a 2 × 2 factorial with *Clostridium chauvoei*, *septicum*, *novyi*, and *sordellii* and *perfringens* types C & D bacterin-toxoid in a special oil adjuvant (Alpha 7, Boehringer Ingelheim Vetmedica, St. Joseph, MO; CLOS) and infectious bovine rhinotracheitis, bovine viral

diarrhea virus type 1a (Singer strain) and type 2a (Bolin 296 strain), parainfluenza<sub>3</sub>, and bovine respiratory syncytial virus modified-live virus vaccine (Express 5, Boehringer Ingelheim Vetmedica, St. Joseph, MO; RESP) administered either on arrival (d 0) or delayed (d 14). This resulted in the 4 treatments: 1) arrival CLOS and RESP (ACAR), 2) arrival CLOS and delayed RESP (ACDR), 3) delayed CLOS, and arrival RESP (DCAR), and 4) delayed CLOS and RESP (DCDR). Treatments were not arranged spatially according to vaccination treatment; because of random treatment assignment there were instances where vaccinated and non-vaccinated calves had fence-line contact during the study. However, using the same RESP vaccine as in the current study, Kleiboeker et al. (2003) concluded that shedding of BVDV type I or II in calves after vaccination was either nonexistent or undetected and did not result in transmission of either BVDV genotype in pregnant contact control cows.

In addition to receiving their assigned vaccination treatment on d 0, calves were treated for internal and external parasites (Cydectin, Fort Dodge Animal Health, Fort Dodge, IA), branded, administered metaphylactic antibiotic treatment with tilmicosin (Micotil, Elanco Animal Health, Indianapolis, IN) according to BW, and bull calves were surgically castrated. An ear notch sample was collected from each animal to test for persistently infected BVDV (Cattle Stats, Oklahoma City, OK). One animal in Block 1 was identified as such and was removed from the study pen and quarantined on d 3. Furthermore on d 0, blood samples from 5 randomly selected animals in each pen were drawn intravenously from a jugular vein into untreated vacuum tubes (Vacutainer, BD Inc., Franklin Lakes, NJ) for subsequent analysis of serum cortisol concentrations and BVDV type I titer levels (plain tube). A second blood sample (10 mL) was collected into

vacuum tubes containing EDTA (BD, Inc.) for determination of white blood cell (WBC) total and differential counts. Cattle were then moved to their assigned 0.45-ha pens and provided 0.9 kg/calf (as-fed basis) of a receiving supplement (20.6% CP, DM basis; Table 2) and free-choice access to bermudagrass hay (14.5% CP, 33.6% ADF, 68.3% NDF, and 7.9% ash, DM basis). Supplement was offered daily at 0800 h and increased to a maximum of 1.8 kg/calf daily as calves began consuming the supplement.

A booster vaccination of RESP was administered 14 d following initial RESP vaccination according to treatment protocol (Table 1). Cattle were re-weighed, unshrunk, before offering supplement at 14-d intervals during the trial (d 14, 28, 42, and 56) to determine interim and overall differences in BW gain performance. The same 5 animals per pen selected for blood collection upon arrival were similarly sampled on d 14, 28, 42, and 56.

Blood in the plain tubes was centrifuged at  $2,100 \times g$  for 20 min at 20°C, and serum was stored frozen at -20°C until analyzed. Serum cortisol concentrations were used as an indication of overall physiological stress and were determined using commercially available RIA kits (Coat-A-Count, DPC, Los Angeles, CA) with an intra- and inter-assay CV of 1.04 and 23.0%, respectively. Sensitivity of the assay was 0.2 µg/100 mL. The use of this assay has been previously validated (Godfrey et al., 1991). Frozen serum samples were shipped on ice via overnight parcel service to the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater) for determination of serum neutralizing antibodies for BVDV type I. Whole blood collected in tubes containing EDTA was kept refrigerated and used within 24 h to determine concentrations (n cells/µL) of total WBC, concentrations and percentages of differential WBC (lymphocytes, neutrophils,

monocytes, eosinophils, and basophils), total red blood cells, hemoglobin, hematocrit, and platelets with an automated hematology analyzer (Cell-Dyn 3500 system, Abbott Laboratories, Abbot Park, IL) standardized for analysis of bovine blood.

After arrival metaphylaxis with tilmicosin (Micotil, Elanco Animal Health, Indianapolis, IN), a 48 h post-treatment interval (PTI) was implemented; therefore, calves were observed each morning beginning on d 3 of the study for clinical signs of respiratory illness by 2 experiment station personnel having a combined 20-yr experience evaluating cattle with BRD. These personnel were present during processing and were not blinded to treatment; however, no records left at the facility identified treatment assignments. Signs used to identify respiratory illness included depression, nasal and ocular discharge, cough, increased respiratory rate and poor appetite. Cattle with 2 or more visual signs of BRD were pulled and considered morbid if rectal temperature was  $\geq 40^{\circ}\text{C}$ . Morbid animals were given antibiotic therapy following a pre-determined antibiotic treatment protocol and returned to the home pen. A re-check temperature was taken 48 h following initial treatment with florfenicol (Nuflor, Schering-Plough Animal Health, Summit, NJ). If the re-check temperature was  $\geq 40^{\circ}\text{C}$ , a second antibiotic treatment with ceftiofur crystalline free acid (Excede, Pfizer Animal Health, New York, NY) was administered. A 72 h PTI was implemented for cattle administered ceftiofur crystalline free acid and rectal temperature was re-evaluated. If the re-check temperature was  $\geq 40^{\circ}\text{C}$ , a third and final antibiotic treatment with danofloxacin mesylate (A180, Pfizer Animal Health) was administered and repeated 48 h following the first injection. Cattle that continued to display BRD symptoms after the third treatment were considered chronically ill and no further antibiotic treatment was administered. If at any time a re-

check temperature was  $< 40^{\circ}\text{C}$ , the animal was left untreated unless further symptoms developed. Treatment data recorded for individual animal included treatment date, rectal temperature, and the amount and type of antibiotic administered.

Titers were evaluated to determine differences in the concentration of BVDV type I antibodies and the percentage of animals in each treatment with positive seroconversion to BVDV type I using the serum neutralization method as described by Rosenbaum et al. (1970). Titers were reported as the reciprocal of the greatest dilution of serum to provide complete protection of cells. The lowest dilution of serum tested was 1:4; whereas, the greatest dilution tested was 1:256. Serum that did not provide protection at the 1:4 level was reported as  $< 4$ , and was considered negative for seroconversion to BVDV. Samples with a reported serum neutralization value of  $\geq 4$  were considered positive for seroconversion to BVDV. For the titer level analysis, the reported values were  $\log_2$  transformed and statistically evaluated. To analyze for percentage seroconversion to BVDV type I, titer values  $< 4$  were assigned 0 (negative); whereas, titers  $\geq 4$  were assigned a value of 1 (positive). Receiving-day BVDV titer comparisons were made from samples collected on d 0, 14, 28, and 42. Vaccination timing-equivalent comparisons were made for BVDV titers based on the number of d post-initial RESP vaccination (d 0 ACAR, DCAR vs. d 14 ACDR, DCDR) and post-booster vaccination (d 14 ACAR, DCAR vs. d 28 ACDR, DCDR).

### ***Statistical Analysis***

Performance and morbidity data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Pen was considered the experimental unit. The class statement included block (date of arrival),

treatment, and replicate. Block and block  $\times$  replicate  $\times$  treatment were treated as random effects in the model. Single degree of freedom orthogonal contrasts evaluating effects of CLOS timing, RESP timing, and the interaction were used. If the interaction was significant ( $P \leq 0.10$ ), treatment means were separated with a t-test using the PDIFF option in SAS. Cortisol, total and differential WBC, total red blood cell, hemoglobin, hematocrit, platelets and BVDV type I titer data were analyzed using the PROC MIXED procedure with repeated measures. Pen was considered the experimental unit for these data. The model included treatment, day and treatment  $\times$  day. Block, replicate, treatment, and day were used in the class statement. Block was the random variable, and the repeated statement was day with block  $\times$  treatment within replicate as the subject. The covariance model structure used was AR(1). Contrasts for the repeated measures data included CLOS timing, RESP timing, their interaction, and linear, quadratic, cubic, and for some variables quartic effects of day.

## **RESULTS AND DISCUSSION**

### ***Performance***

No differences ( $P \geq 0.34$ ) in ADG were observed during the 56 d receiving period (Table 3). Experiments involving vaccination timing of newly received calves are conflicting. In one trial (Richeson et al., 2008), calves that received delayed (14 d) vaccination of a modified-live virus (MLV) respiratory vaccine (Express® 5) had greater ADG. Kreikemeier et al. (1996) observed that during the initial 21 d of the feedlot receiving period, calves vaccinated for BRD with a killed-virus at the farm prior to weaning and re-vaccinated with a killed virus at the time of commingling at a salebarn tended to gain faster than calves given a MLV upon arrival at the feedyard and

revaccinated 21 d after feedyard arrival. Conversely, Duff et al. (2000) reported no differences in BW gain among 4 vaccination treatments, including 1) no vaccine (control); 2) delayed 4-way [infectious bovine rhinotracheitis (**IBR**), BVDV, parainfluenza<sub>3</sub> (**PI3**), bovine respiratory syncytial virus] MLV vaccination until d 7; 3) intranasal IBR-PI3 administered on d 0 with delayed 4-way MLV vaccination until d 7; and 4) 4-way MLV vaccination on both d 0 and d 7. Clostridial vaccines contain a special and often proprietary adjuvant used to enhance the immune response to the killed antigens contained in the vaccine; however, the effect of different adjuvants on animal performance deserves consideration. Chirase et al. (2001) conducted 2 experiments to evaluate the effects of clostridial vaccines and injection site on performance. In Exp. 1, calves given an injection of saline subcutaneously prescapula (control) had greater ( $P < 0.01$ ) ADG than calves injected s.c. prescapula with Vision 7 (7-way clostridial, Intervet Inc. Millsboro, DE) or Ultra 7 (7-way clostridial, Pfizer Animal Health) but not Alpha 7 injected subcutaneously either prescapula or in the ear, which is the clostridial vaccine used in the current study. Spurlock (1997) reported that repeated immune stimulation can result in decreased growth of an animal because nutrients are preferentially utilized for immune and homeostatic pathways. Conflicting results of vaccination on receiving cattle performance may be due to a number of host factors such as vitamin/mineral status, vaccination history, and the stress level of a particular group. Further research is needed to evaluate the effects of vaccination on animal performance during the receiving period.

### ***Health***

The majority of the cattle became sick with 69% of all calves being treated at least one time for BRD (Table 4), but morbidity rates were not different among

treatments ( $P \geq 0.23$ ). Likewise, no differences were observed in the number of 2nd ( $P \geq 0.18$ ) or 3rd ( $P \geq 0.37$ ) BRD treatments. Combined death loss (1.9%) did not differ ( $P \geq 0.64$ ) among treatments. There was a vaccine type  $\times$  timing interaction ( $P = 0.05$ ) for the percentage of chronic animals, ACDR had a greater percentage of chronic animals than ACAR ( $P = 0.04$ ) and DCDR ( $P = 0.08$ ), but not DCAR ( $P = 0.26$ ).

There was a tendency for CLOS ( $P = 0.07$ ) and RESP ( $P = 0.09$ ) timing effects on days to initial BRD treatment. Days to initial BRD treatment were fewer ( $P = 0.01$ ) for ACAR ( $6 \pm 0.76$  d) than DCDR ( $8 \pm 0.76$  d). This difference may be a result of additive effects of on-arrival vaccination on the visual symptoms of BRD in cattle receiving both CLOS and RESP on d 0 (ACAR) vs. cattle receiving no vaccine until d 14 (DCDR). The increase of early visual BRD symptoms in cattle receiving vaccination on arrival did not translate to increased ( $P \geq 0.23$ ) morbidity rates. However, there was a numerical difference; 73.4% of cattle receiving RESP on-arrival required treatment for BRD compared to 65.1% of cattle that received delayed RESP vaccination. Furthermore, CLOS ( $P = 0.01$ ) and RESP ( $P = 0.05$ ) timing affected rectal temperature at the time of initial antibiotic treatment. Delaying either vaccine resulted in a greater rectal temperature at the time of initial antibiotic treatment. Because DCDR calves were pulled later than the other treatments, discrepancy in disease stage may explain the greater rectal temperature at the time of initial BRD treatment. There was also an impact (vaccine type  $\times$  timing interaction,  $P = 0.05$ ) on the d to treatment with the second antibiotic; DCDR calves were treated later (d  $13 \pm 1.3$ ) than calves on the other treatments (d  $9 \pm 1.3$ ).

### ***BVDV Type I Antibody Titers***

Two separate comparison methods were used to evaluate differences in BVDV type I titers: 1) trial-d basis and 2) equivalent-d post-RESP vaccination basis.

Furthermore, results were analyzed and reported as either antibody concentration or percent positive seroconversion as described previously in the Materials and Methods.

***Trial-Day Basis Comparison.*** As anticipated there was a treatment  $\times$  day interaction ( $P \leq 0.04$ ) for BVDV type I titers for both analyses. On d 14, BVDV type I titer levels for ACAR were greater than ACDR ( $P = 0.001$ ) or DCDR ( $P = 0.01$ , Figure 1). On d 28, both treatments administered RESP on-arrival (ACAR and DCAR) had greater ( $P \leq 0.005$ ) BVDV type I titers than treatments administered delayed RESP vaccination (ACDR and DCDR). No treatment differences ( $P \geq 0.10$ ) existed on d 42. Results were similar when trial-day basis titer data were analyzed as percent seroconversion to BVDV type I (Figure 2). Positive seroconversion to BVDV type I was faster ( $P = 0.01$ ) when RESP was administered on d 0. On d 14 and 28, ACAR and DCAR had greater percentage seroconversion than ACDR or DCDR; however, by d 42 of the trial essentially all animals had positive seroconversion to BVDV type I.

***Equivalent-Day, Post-RESP Comparison.*** There was no treatment  $\times$  day interaction ( $P \geq 0.82$ ) for the equivalent-day post-RESP comparison method. The overall  $\log_2$  concentration of BVDV type I antibody was 5.2, 4.9, 5.0, and 4.7 for ACAR, ACDR, DCAR and DCDR treatments, respectively, but not different ( $P = 0.68$ ). Likewise, no differences ( $P = 0.56$ ) were detected when analyzed as percent seroconversion by the equivalent-day post-RESP comparison.

The BVDV titer results in this study suggest that high-risk, newly received calves administered RESP vaccination on-arrival are able to respond adequately to RESP

vaccination despite the typical stress and immune challenges present during the initial 14 d of receiving. Natural disease exposure and subsequent host immune response may have affected BVDV type I antibody titers; however, the extent to which this may have occurred is unknown. Measurement of BVDV neutralizing antibody titers has been reported to provide an indication of the amount of BVDV protection present in calves (Bolin and Ridpath, 1995) and has been positively correlated with disease prevention (Howard et al., 1989). Although calves administered RESP on arrival developed titers to BVDV type I faster, there was not a decrease ( $P \geq 0.23$ ) in morbidity or increase in protection as was reported in other studies (Bolin and Ridpath, 1995; Howard et al., 1989). However, Bolin and Ridpath (1995) and Howard et al. (1989) were both BVDV challenge studies where calves were only exposed to BVDV; whereas in our study the specific agent causing morbidity was not tested and therefore unknown. In the current study it is likely that more than 1 viral or bacterial pathogen, or both, was causing morbidity, which may or may not have been vaccinated against.

#### ***Total and Differential WBC Count***

There was no treatment  $\times$  day interaction for total WBC count ( $P = 0.97$ ). There tended to be a main effect of CLOS timing ( $P = 0.08$ ) on total WBC count (Table 5). Overall, delaying CLOS resulted in greater WBC counts. There was also a vaccine type  $\times$  timing interaction ( $P = 0.06$ ); DCDR had greater WBC counts than ACDR ( $P = 0.01$ ), and ACAR and DCAR were intermediate. The greater WBC levels recorded for DCDR may indicate that these cattle had greater occurrence of pathogenic infection. However, greater total WBC count may also indicate that an animal has increased ability to mount an innate or adaptive immune response to a foreign antigen, which is a key goal of

vaccination. There were effects of day of sampling on differential percentages (Figure 3), concentrations (data not shown), and total WBC count (Figure 4). The percentage of lymphocytes (quartic,  $P < 0.001$ ), monocytes (quartic,  $P = 0.02$ ), eosinophils (linear,  $P < 0.001$ ), and the WBC count (quartic,  $P < 0.001$ ) increased as the study progressed; while the percentage of neutrophils decreased (quartic,  $P < 0.001$ ) over time. Concentrations of differential WBC reacted similarly with number of neutrophils decreasing (quadratic,  $P < 0.001$ ), and numbers of lymphocytes (quadratic,  $P < 0.001$ ), monocytes (quadratic,  $P = 0.001$ ), eosinophils (linear,  $P < 0.001$ ), and basophils (linear,  $P < 0.001$ ) increasing as the study progressed. Previous studies measuring total leukocytes before and after transport observed different results. Blecha et al. (1984) reported greater total leukocyte concentration at unloading and 7 d after receiving in transported steers vs. non-transported controls. In contrast to our results, Stanger et al. (2005) observed a transient decrease in total leukocyte count when measured 48 h prior to, 72 h following and 216 h following transport of *Bos indicus* steers. The neutrophil:lymphocyte ratio (N:L), reported as an indicator of shipping stress in pigs (McGlone et al., 1993) and social stress in chickens (Gross and Siegel, 1983), was also affected by day of sampling (quartic,  $P \leq 0.05$ ). As the study progressed, the N:L decreased; therefore, physiological stress early in the receiving period seemingly elevated the N:L ratio of calves across all treatments.

There were no treatment  $\times$  day interactions ( $P \geq 0.18$ ) for differential percentages or concentrations of WBC. Overall, CLOS timing affected some differential percentages and concentrations. Delaying CLOS increased ( $P \leq 0.02$ ) the percentage and concentration of lymphocytes and decreased ( $P \leq 0.05$ ) the percentage of monocytes, and the percentage and concentration of eosinophils. The N:L tended to be affected by a

vaccine type  $\times$  timing ( $P = 0.10$ ) interaction; DCDR had the smallest N:L. Therefore, postponing both vaccines 14 d may have reduced stress.

Red blood cells and platelets were greatest ( $P = 0.02$ ) in calves administered delayed RESP vaccination (Figure 4). Red blood cells (quadratic, cubic and quartic effects of day,  $P \leq 0.05$ ), hemoglobin (linear, quadratic, cubic and quartic effects of day,  $P \leq 0.05$ ), and hematocrit (quadratic and cubic effects of day,  $P < 0.001$ ) were least on d 14. Platelet concentrations were the least on d 56 (quadratic,  $P = 0.01$ ).

### ***Serum Cortisol***

There was no treatment  $\times$  day interaction ( $P = 0.21$ ) for serum cortisol concentrations (Figure 5), nor were there any treatment differences ( $P \geq 0.53$ ); however, there was a significant day effect ( $P = 0.002$ ) on cortisol concentrations. Cortisol concentrations averaged 3.04  $\mu\text{g}/100\text{ mL}$  on d 0 and declined on d 14 (2.43  $\mu\text{g}/100\text{ mL}$ ,  $P < 0.001$ ) and d 28 (2.57  $\mu\text{g}/100\text{ mL}$ ,  $P = 0.01$ ), suggesting that physiological stress was greatest during the first 14 d of the receiving period. Several studies report stress induced increases in cortisol concentrations (Kegley et al., 1997; Tyler and Cummins, 2003; Gupta et al., 2005), and Anglen et al. (2003) observed animals that underwent restraint stress failed to mount a normal immune response which resulted in increased susceptibility to a subsequent pathogen challenge. Crookshank et al. (1979) reported that corticosteroids were most responsive to transport stress; however, other trials reported no differences in cortisol related to weaning (Lefcourt and Elsasser, 1995) or transport (Galyean et al., 1981) stress. According to results of the current study, the early physiological stress during receiving indicated by serum cortisol concentrations, did not impact the ability to respond to vaccination because calves administered RESP on-arrival

(ACAR, DCAR) had adequate and earlier titer response to BVDV type I compared to the delayed RESP treatments (ACDR, DCDR). Likewise, Blecha et al. (1984) reported that elevated cortisol did not correlate with a suppressed immune response.

In conclusion, timing of vaccination did not affect ADG or health in high-risk, newly received calves. Antibody titers to BVDV type I developed earlier when cattle were administered a respiratory vaccine on d 0 vs. 14. Several differential WBC measures were greater when both clostridial and respiratory vaccines were delayed. This observation may correlate to either the occurrence of pathogenic infection or differences in immune status. Serum cortisol concentration was greatest during the first 14 d of receiving; however, cortisol level did not affect BVDV type I titer response and vaccination timing did not affect cortisol level.

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**Table 1.** Vaccination schedule for experimental treatments

Treatment	d 0	d 14	d 28
ACAR <sup>1</sup>	Alpha 7 <sup>2</sup> / Express 5 <sup>3</sup>	Express 5	
ACDR <sup>4</sup>	Alpha 7	Express 5	Express 5
DCAR <sup>5</sup>	Express 5	Alpha 7 / Express 5	
DCDR <sup>6</sup>		Alpha 7 / Express 5	Express 5

<sup>1</sup>ACAR = arrival (d 0) clostridial and respiratory vaccination.

<sup>2</sup>Clostridium chauvoei-septicum-novyi-sordellii-perfringens types C & D bacterin-toxoid, Boehringer-Ingelheim Vetmedica.

<sup>3</sup>Bovine rhinotracheitis-virus diarrhea-parainfluenza<sub>3</sub>-respiratory syncytial modified-live virus vaccine, Boehringer-Ingelheim Vetmedica.

<sup>4</sup>ACDR = arrival clostridial, and delayed (d 14) respiratory vaccination.

<sup>5</sup>DCAR = delayed clostridial, and arrival respiratory vaccination.

<sup>6</sup>DCDR = delayed clostridial and respiratory vaccination.

**Table 2.** Ingredient composition of grain supplement (as-fed basis)

Ingredient	%
Corn, cracked	73.385
Soybean meal	20
Molasses	2
Fat	1
Dicalcium phosphate	0.18
Limestone	1.8
Salt, white	1
Vitamin ADE premix <sup>1</sup>	0.1
Vitamin E <sup>2</sup>	0.05
Trace mineral premix <sup>3</sup>	0.085
Corn / Rumensin premix <sup>4</sup>	0.4

<sup>1</sup>Contained 8,800,000 IU Vitamin A, 1,760,000 IU Vitamin D, and 1,100 IU Vitamin E /kg.

<sup>2</sup>Contained 44,000 IU/kg.

<sup>3</sup>Contained 12% Zn, 8% Mn, 4% Cu, 500 mg Co, 2,000 mg I, and 600 mg Se/kg.

<sup>4</sup>Provided 88 mg monensin (Elanco Animal Health, Indianapolis, IN)/kg of supplement.

**Table 3.** Effect of clostridial and bovine respiratory disease vaccination timing on performance of newly received cattle

	ACAR <sup>2</sup>	ACDR <sup>3</sup>	DCAR <sup>4</sup>	DCDR <sup>5</sup>	SEM	Contrasts <sup>1</sup> , <i>P</i> =		
						CLOS	RESP	Interaction
Initial wt., kg	241	239	238	238	7.7	0.42	0.51	0.62
Final wt., kg	295	290	291	289	7.3	0.36	0.27	0.60
ADG, kg								
d 0 to 14	1.10	0.94	0.96	0.94	0.20	0.64	0.54	0.65
d 14 to 28	1.02	0.92	0.96	0.93	0.21	0.81	0.59	0.74
d 28 to 42	1.02	1.04	0.92	0.96	0.22	0.42	0.80	0.95
d 42 to 56	0.74	0.80	0.93	0.80	0.19	0.41	0.75	0.46
d 0 to 56	0.98	0.93	0.95	0.91	0.07	0.62	0.34	0.87

<sup>1</sup>CLOS = main effect of timing of clostridial vaccination (d 0 vs. 14), RESP = main effect of timing of respiratory virus vaccination, Interaction = vaccine type × timing.

<sup>2</sup>ACAR = arrival (d 0) clostridial and respiratory vaccination.

<sup>3</sup>ACDR = arrival clostridial, and delayed (d 14) respiratory vaccination.

<sup>4</sup>DCAR = delayed clostridial, and arrival respiratory vaccination.

<sup>5</sup>DCDR = delayed clostridial and respiratory vaccination

**Table 4.** Effect of clostridial and bovine respiratory disease vaccination timing on health of newly received cattle

	ACAR <sup>2</sup>	ACDR <sup>3</sup>	DCAR <sup>4</sup>	DCDR <sup>5</sup>	SEM	Contrasts <sup>1</sup> , <i>P</i> =		
						CLOS	RESP	Interaction
Morbidity, %	71.4	60.7	75.3	69.5	9.6	0.36	0.23	0.72
Day 1 <sup>st</sup> treated	6	7.3	7.3	8	0.76	0.07	0.09	0.56
Rectal temperature at treatment, °C	40.3	40.4	40.4	40.6	0.11	0.01	0.05	0.47
Rectal temperature 48 h after treatment, °C	39.8 <sup>a</sup>	39.9 <sup>a</sup>	39.8 <sup>a</sup>	39.5 <sup>b</sup>	0.24	0.05	0.36	0.04
Treated with 2 <sup>nd</sup> antibiotic, % <sup>6</sup>	38.5	39.5	36.1	25.5	8.7	0.18	0.43	0.34
Day 2 <sup>nd</sup> treated	8.8 <sup>b</sup>	10 <sup>b</sup>	8.7 <sup>b</sup>	13.1 <sup>a</sup>	1.3	0.07	0.002	0.05
Treated with 3 <sup>rd</sup> antibiotic, % <sup>6</sup>	20.5	22.4	19.8	10.8	9.4	0.37	0.61	0.43
Dead, %	1.4	1.6	3	1.5	2.4	0.67	0.7	0.64
Chronic, %	1.5 <sup>b</sup>	11.4 <sup>a</sup>	6.3 <sup>ab</sup>	3.2 <sup>ab</sup>	4.7	0.59	0.29	0.05

<sup>a,b</sup>Means within a row without a common superscript are different ( $P < 0.05$ ).

<sup>1</sup>CLOS = main effect of timing of clostridial vaccination (d 0 vs. 14), RESP = main effect of timing of respiratory virus vaccination, Interaction = vaccine type × timing.

<sup>2</sup>ACAR = arrival (d 0) clostridial and respiratory vaccination.

<sup>3</sup>ACDR = arrival clostridial, and delayed (d 14) respiratory vaccination.

<sup>4</sup>DCAR = delayed clostridial, and arrival respiratory vaccination.

<sup>5</sup>DCDR = delayed clostridial and respiratory vaccination.

<sup>6</sup>Percentage of total population.

**Table 5.** Effect of clostridial and bovine respiratory disease vaccination timing on cellular blood constituents

	ACAR <sup>2</sup>	ACDR <sup>3</sup>	DCAR <sup>4</sup>	DCDR <sup>5</sup>	SEM	Contrasts <sup>1</sup> , <i>P</i> =		
						CLOS	RESP	Interaction
White blood cells, n × 10 <sup>3</sup> /μL	10.3 <sup>ab</sup>	9.9 <sup>b</sup>	10.2 <sup>ab</sup>	10.9 <sup>a</sup>	0.37	0.08	0.62	0.06
Neutrophils, %	25	26	25	22	2.6	0.08	0.51	0.18
Lymphocytes, %	61	61	63	65	2.3	0.02	0.38	0.31
Monocytes, %	10.7	10.4	9.8	9.9	0.61	0.04	0.79	0.58
Eosinophils, %	2.3	1.9	1.7	1.6	0.34	0.05	0.30	0.59
Neutrophil:lymphocyte ratio	0.55 <sup>a</sup>	0.58 <sup>a</sup>	0.54 <sup>ab</sup>	0.45 <sup>b</sup>	0.06	0.06	0.39	0.10
Red blood cells, n × 10 <sup>6</sup> /μL	9.7	10.3	10.0	10.4	0.25	0.34	0.02	0.51
Platelets, K/μL	516	662	589	663	55	0.43	0.02	0.44

<sup>a,b</sup>Means within a row without a common superscript are different ( $P \leq 0.05$ ).

<sup>1</sup>CLOS = main effect of timing of clostridial vaccination (d 0 vs. 14), RESP = main effect of timing of respiratory virus vaccination, Interaction = vaccine type × timing.

<sup>2</sup>ACAR = arrival (d 0) clostridial and respiratory vaccination.

<sup>3</sup>ACDR = arrival clostridial, and delayed (d 14) respiratory vaccination.

<sup>4</sup>DCAR = delayed clostridial, and arrival respiratory vaccination.

<sup>5</sup>DCDR = delayed clostridial and respiratory vaccination.

## CHAPTER V

WEANING MANAGEMENT OF NEWLY RECEIVED BEEF CALVES WITH OR  
WITHOUT CONTINUOUS EXPOSURE TO A PERSISTENTLY INFECTED BOVINE  
VIRAL DIARRHEA VIRUS CALF: EFFECTS ON HEALTH, PERFORMANCE,  
BOVINE VIRAL DIARRHEA VIRUS TITERS AND PERIPHERAL BLOOD  
LEUKOCYTES<sup>1</sup>

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**ABSTRACT:** Calves persistently infected (PI) with bovine viral diarrhea virus (BVDV) are a major source of the virus; however, consequences of exposure to a PI-BVDV calf in low-risk, single-source, preconditioned (PC) vs. high-risk, commingled, auction market (AM) cattle may differ. Our objective was to compare treatments of PC or AM origin, with (PI) or without (CON) exposure to a PI-BVDV calf in a  $2 \times 2$  factorial arrangement to evaluate effects on health and performance. Four sets (block) of crossbred PC steers ( $n = 236$ ) from 3 ranch-origins were selected randomly, weaned, dewormed, vaccinated, tested for PI-BVDV status, and kept on the ranch for  $\geq 42$  d. Subsequently, PC steers were transported to a stocker receiving unit (RU), weighed ( $251 \pm 2$  kg), bled, stratified by d -1 BW, and assigned randomly to treatment (PCPI or PCCON) with no additional processing. Simultaneously, 4 sets of crossbred AM calves ( $n = 292$ ) were assembled from regional auction markets for delivery to the RU  $\pm 36$  h from PC arrival. The AM calves were weighed ( $245 \pm 1.3$  kg) and administered the same processing procedures as PC received at their origin ranch; however, bull calves were stratified by gender and d -1 BW, castrated surgically, then AM calves were assigned randomly to treatment (AMPI or AMCON). Treatment pens (0.45 ha) were arranged spatially so that PI did not have fence-line contact with CON. Calves were fed identically and followed the same antibiotic treatment protocol. Daily gain for the entire 42-d receiving trial was greater ( $P < 0.001$ ) for PC (1.2 kg) than AM (0.85 kg). There was an exposure effect ( $P = 0.002$ ) on ADG from d 28 to 42; CON gained 1.12 kg vs. 0.90 kg for PI. Morbidity rate was greater ( $P < 0.001$ ) in AM (70%) than PC (7%). Treatment with a third antibiotic occurred more often ( $P = 0.04$ ) for PI, likewise antibiotic treatment cost ( $P = 0.05$ ) and the percentage of chronic cattle ( $P = 0.06$ ) were greatest for AMPI. The BVDV type 1a titer levels were

greater on d 0 for PC (treatment  $\times$  day,  $P < 0.001$ ), and seroconversion to BVDV type 1a on d 0 was 100% for PC vs. 23% in AM. Circulating leukocytes were greater ( $P < 0.001$ ) for PC on d 0, 14, and 28. The neutrophil:lymphocyte ratio was greater ( $P < 0.001$ ) for AM on d 14 and 28. Platelet count increased transiently ( $P < 0.001$ ), with greater platelets observed in AM ( $P < 0.001$ ). Results suggest that PC calves gain faster and require fewer antibiotic treatments; whereas, PI-BVDV exposure reduced gain from d 28 to 42 and increased the number of calves requiring a third antibiotic treatment.

## INTRODUCTION

Bovine respiratory disease (BRD) is a multifaceted disease involving stress, commingling, and several viral and bacterial pathogens. Despite advances in prevention and control, BRD remains the leading cause of feedlot morbidity and mortality (Woolums et al., 2005) resulting in significant economic loss (Irsik et al., 2006) and reduced animal well-being. Preconditioning is a comprehensive management practice to prepare young calves for stocker or feedlot entry by reducing stress and improving disease protection through pre-arrival vaccination against BRD pathogens (Cole, 1985; Duff and Galvayan, 2007). Preconditioned calves have reduced morbidity and improved gain performance compared to high-risk calves originating from auction markets (Clark et al., 2006; Seeger et al., 2008). Unfortunately, the estimated adoption rate of management factors including weaning (50.2%) or respiratory vaccination (39.4%) in US beef cow-calf operations remains low (USDA, 2010).

Bovine viral diarrhea virus (BVDV) is a major culprit in the development of BRD either directly via acute clinical disease or through indirect effects of immunosuppression (Welsh et al., 1995). Furthermore, calves born persistently infected (PI) with BVDV are a

key source of BVDV transmission (Fulton et al., 2005), and although prevalence of PI-BVDV calves in the feedlot is estimated to be relatively low (0.3%; Loneragan et al., 2005), a single PI-BVDV animal has the potential to continuously expose an entire pen and adjoining pens to the virus. Research results on effects of exposure to PI-BVDV calves in the feedlot are conflicting; thus, the decision to invest in PI-BVDV testing of newly received cattle remains controversial. One inconsistency among the literature is the use of cattle with varied management and health history, and research evaluating effects of PI-BVDV exposure in low-risk, single-source, preconditioned versus high-risk, commingled, auction market calves is needed to determine if outcomes differ for these different management systems.

## **MATERIALS AND METHODS**

### ***Cattle***

Animal methods and experimental procedures were approved by the University of Arkansas Animal Care and Use Committee.

***Experimental Cattle.*** A total of 528 crossbred, male beef calves were used to determine effects of weaning management and PI-BVDV exposure. Calves from 2 different weaning management systems were utilized for this receiving trial; 1) low-risk, single-source, preconditioned (PC) crossbred steer calves ( $n = 236$ ; initial BW =  $251 \pm 2$  kg) arrived in 4 shipment blocks from 3 Arkansas cow-calf ranches, and 2) high-risk, commingled, auction market (AM) crossbred bull ( $n = 210$ ) and steer ( $n = 82$ ) calves (initial BW =  $245 \pm 1.3$  kg) that arrived in 4 shipment blocks acquired from multiple Arkansas auction markets. The 4 shipment blocks of PC calves originated from cow-calf herds located in Izard (block 1 and 4), Hempstead (block 2), and Pulaski (block 3),

County, AR and arrived at the University of Arkansas Agricultural Experiment Station located near Savoy (receiving unit; RU) on October 27, 2008, January 19, August 11, or December 6, 2009, respectively. The PC cattle were considered to be low-risk for developing BRD because they were weaned and vaccinated against BRD pathogens  $\geq 42$  d prior to shipment and maintained as a single-source without commingling. Each shipment block of AM calves were procured and assembled by an order buyer from 2 to 3 public auction markets located in Northwest or North Central, AR and arrived at the RU on October 25, 2008, January 20, August 10, or December 5, 2009 for the 4 shipment blocks, respectively. Order buyers were instructed to purchase AM cattle of similar BW and phenotype as the incoming PC block. The AM cattle were considered to be high-risk for developing BRD because they did not have known health or vaccination history and were commingled extensively resulting in greater probability of increased stress and exposure to BRD pathogens.

The main effects of management (AM or PC) and PI-BVDV exposure (not exposed = CON or exposed = PI) resulted in 4 treatments in a  $2 \times 2$  factorial arrangement. For PC treatments, 42 to 91 d prior to trial initiation depending on block, randomly selected steers were weaned and ear-notched to test for PI-BVDV status at a commercial laboratory (Cattle Stats, LLC, Oklahoma City, OK) using the antigen-capture ELISA (ACE) method (Idexx Laboratories, Inc., Westbrook, ME). Also on the day of weaning, PC calves were administered: 1) a 5-way modified-live virus (MLV) respiratory vaccine containing infectious bovine rhinotracheitis, bovine viral diarrhea virus type 1a and 2a, parainfluenza3, and bovine respiratory syncytial virus isolates [Express 5, Boehringer Ingelheim Vetmedica (BIVI), St. Joseph, MO], 2) *Manheimia haemolytica*-

*Pastuerella multocida* bacterin-toxoid (Pulmo-guard PHM-1, BIVI), and 3) pour-on or injectable anthelmintic (Cydectin, BIVI). Approximately 14 d later, PC calves were administered a 7-way clostridial bacterin-toxoid (Alpha 7, BIVI) and re-vaccinated with 5-way MLV respiratory vaccine. Preconditioned calves were isolated from other cattle, fed hay or pasture along with a supplement, and remained on their origin ranch during the preconditioning phase until approximately d -2 when they were shipped to the RU. Upon arrival to the RU, PC calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, PC calves were weighed and returned to their isolated holding pen. The following d (d 0), PC calves were weighed, bled via jugular venipuncture into evacuated tubes (Vacutainer; BD Inc, Franklin Lakes, NJ) to determine total and differential leukocytes (EDTA tube) and BVDV type 1a antibody titers (plain tube), stratified by d -1 BW, then assigned randomly to treatment (PCCON or PCPI).

To coincide with PC groups, AM calves were assembled from regional markets and delivered to the RU  $\pm$  36 h from PC arrival. Upon arrival to the RU, AM calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, AM calves were weighed, identified with a unique ear identification tag, ear notched to test for PI-BVDV status at a commercial laboratory (Cattle Stats) using the ACE method (Idexx Laboratories), and returned to their isolated holding pen. One AM animal in block 3 was identified as PI-BVDV and removed from the study pen and quarantined on d 0 of the trial. On d 0, AM cattle received the same vaccinations and processing procedures as described for PC on their origin ranch; therefore, the first known processing for AM occurred on d 0 rather than 42 to 91 d

previously on an origin ranch for PC. For AM calves only, re-vaccination of the 5-way MLV respiratory vaccine (Express 5, BIVI) occurred on d 14 in addition to administration of a 7-way clostridial bacterin-toxoid (Alpha 7, BIVI). Additionally on d 0, bull calves were castrated surgically, stratified by gender and d -1 BW, then AM calves were assigned randomly to treatment (AMCON or AMPI). Cattle were moved to their randomly assigned 0.45-ha pens and provided 0.91 kg/d (as-fed basis) of a receiving supplement (Table 1; 15.3% CP, DM basis) and ad libitum access to bermudagrass hay (13.1% CP, 64% NDF, 42% ADF, DM basis) and water. Supplement offered was step-wise increased to a maximum of 2.73 kg/d as each pen completely consumed the supplement offered for 2 consecutive d.

All BW measurements were obtained individually without withholding feed or water on 2 consecutive days at the beginning (d -1 and 0) and end (d 42 and 43) of the receiving trial using a chute equipped with electronic load cells to determine overall differences in gain performance. To determine interim differences in gain performance, individual BW was recorded at 14-d intervals during the trial (d 14 and 28). Furthermore, calves were bled on d 14 and 28 to determine interim total and differential leukocytes and BVDV type 1a titers. Anticoagulated whole blood was collected from each animal into EDTA tubes, kept refrigerated (5° C), and analyzed within 24 h to determine percentage and concentrations (n cells/ $\mu$ L) of total and differential (lymphocytes, neutrophils, monocytes, eosinophils, and basophils) peripheral blood leukocytes (PBL), platelets, red blood cells, hemoglobin, and hematocrit with an automated hemacytometer (Cell-Dyn 3500 system, Abbott Laboratories, Abbott Park, IL) standardized for analysis of bovine blood.

Blood collected from each animal in plain evacuated tubes was centrifuged at  $2,100 \times g$  for 20 min at  $20^{\circ} \text{C}$ , and serum was decanted into duplicate aliquots and stored frozen at  $-20^{\circ} \text{C}$ . Subsequently, 5 sample aliquots were selected randomly from each treatment pen from d 0 and 28 and shipped on ice via overnight parcel service to the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL, Ames, IA) for determination of serum neutralizing antibody titers against BVDV type 1a (Singer strain) using the virus neutralization (VN) assay. A second set of serum aliquot samples were pooled within day (0 and 28 only) and treatment pen, and shipped on ice via overnight parcel service to the Texas Veterinary Medical Diagnostic Laboratory – Amarillo (TVMDL, Amarillo, TX) for determination of serum neutralizing antibody titers against BVDV type 1a (strain NADL), 1b (strain TGAC), and 2a (strain 125). All titers were reported as the reciprocal of the greatest dilution of serum to provide complete protection of cells. For the ISUVDL analysis, the least dilution of serum tested was 1:2, whereas the greatest dilution tested was 1:4096. Serum that did not provide complete protection at the 1:2 level was reported as  $< 2$  and was considered negative for seroconversion to BVDV type 1a. Samples with a reported serum neutralization value of  $\geq 2$  were considered positive for seroconversion to BVDV type 1a. For the TVMDL analysis, the least dilution of serum tested was 1:4, whereas the greatest dilution tested was 1:1024. For both titer level analyses, the reported values were  $\log_2$  transformed prior to statistical analysis.

***Persistently Infected Cattle.*** Two groups of calves that had been previously ear-notched, tested at a commercial laboratory (Cattle Stats), and identified as PI-BVDV according to ACE method were acquired from a stocker cattle operation in Washington County, OK to be utilized as PI-BVDV exposure sources. Upon arrival to the RU, each PI-BVDV calf

was ear-notched a second time, and samples were shipped via overnight parcel service to a different laboratory [Oklahoma Animal Disease Diagnostic Lab, Stillwater, OK or USDA, ARS, National Animal Disease Center (NADC), Ames, IA] for rapid re-affirmation of positive PI-BVDV status using the same ACE procedure. Group 1 (n = 10) was assembled prior to trial initiation and utilized for shipment block 1 and 2; whereas, group 2 (n = 9) was assembled prior to beginning block 3, and utilized for block 3 and 4 of the trial. Depending on block, 4 to 8 PI-BVDV challenge calves were assigned randomly to a PI-designated pen. An appropriate number of PI-BVDV calves were assembled for each group to allow for available alternates if an originally designated PI-BVDV animal died. Each PI-BVDV calf that died during the trial (Group 1, n = 2; Group 2, n = 1) was replaced immediately with a confirmed PI-positive alternate. Additionally, anticoagulated blood was collected from each PI-BVDV challenge animal and shipped on ice via overnight parcel service to NADC for subsequent differentiation of BVDV subgenotype strain using virus isolation of buffy coat cells and reverse-transcriptase PCR analysis previously described by Ridpath and Bolin (1998). The subgenotype strain of the 2 PI-positive alternates in Group 1 was unknown. Of the PI-BVDV calves acquired for use as exposure sources in the current study and subgenotype confirmed, 94.7% (18 of 19) were identified as subgenotype 1b. One PI-BVDV animal from Group 2 was identified as subgenotype 1a, and the pen in which this animal was assigned was removed from all statistical analyses. It is important to note that BVDV 1b, the subgenotype strain in which the PI-BVDV calves used in this experiment were identified, is the predominant BVDV subgenotype strain isolated from cattle in the US (Fulton et al., 2002; Ridpath et

al., 2010); however, currently no commercial licensed MLV respiratory vaccine in the US contains a BVDV 1b isolate.

### ***Pen Assignment and Arrangement***

To avoid unwanted PI-BVDV fence-line contact with CON, 0.45-ha mixed-grass receiving pens were arranged spatially prior to treatment allocation (Figure 1). Pens measured  $30.5 \times 152$  m, contained 5 m of feed bunk-line and a fence-line water source shared with an adjacent pen of the same treatment. Furthermore, the treatment pens were configured so that unlike treatment pens were separated by either a 3 m drovers, or 5 m feed alley. Within management group, calves were stratified by gender (AM only) and d - 1 BW, then assigned randomly to 1 of 2 or 1 of 4 PI or CON pens depending on block (8 to 11 calves/pen) resulting in total experimental unit (pen) replication of 14, 14, 12, and 11 for AMCON, AMPI, PCCON, and PCPI, respectively. For PI treatments, a PI-BVDV type 1b challenge animal was assigned randomly to each PI-designated treatment pen such that pens within block had an equivalent number of cattle. During all weighing and BRD evaluation procedures, CON treatments were evaluated first, followed by PI treatments to avoid unwanted CON contact with PI challenge animals or experimental cattle in the PI treatments, and to reduce possible exposure to fomites contaminated with secretory fluid or fecal material containing BVDV.

### ***Evaluation and Treatment of BRD***

Calves were observed daily for clinical signs of respiratory illness (depression, nasal discharge, ocular discharge, cough, gaunt appearance) by 2 experiment station personnel with a combined 35-yr experience evaluating cattle with BRD. If  $\geq 2$  visual signs existed, calves were brought to the restraining chute, weighed, and rectal

temperature was recorded via a digital thermometer (GLA Agricultural Electronics; San Luis Obispo, CA; readability =  $\pm 0.1^{\circ}\text{C}$ ). If rectal temperature was  $\geq 40^{\circ}\text{C}$ , cattle were considered morbid, administered antibiotic therapy with enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS) at a dosage rate of 10 mg/kg of BW, and immediately returned to their home pen. A 48 h post-treatment interval (PTI) was implemented following administration of enrofloxacin, and a second temperature was recorded upon expiration of the initial antibiotic PTI. If the second temperature was  $\geq 40^{\circ}\text{C}$ , a second antibiotic treatment with florfenicol (Nuflor, Schering-Plough Animal Health, Summit, NJ) was administered at a dosage rate of 40 mg/kg of BW. A 48 h PTI was also implemented for cattle administered florfenicol, and rectal temperature was evaluated upon expiration of the second antibiotic PTI. If the temperature was  $\geq 40^{\circ}\text{C}$ , a third and final antibiotic treatment with ceftiofur HCl (Excenel RTU, Pfizer Animal Health, New York, NY) was administered at a dosage rate of 2.2 mg/kg of BW and repeated for 2 consecutive days following the initial injection of ceftiofur HCl. If at any time rectal temperature was  $< 40^{\circ}\text{C}$ , the animal was left untreated and returned immediately to the treatment pen until further symptoms warranted re-examination. Treatment data were recorded for individual animal including treatment date, rectal temperature, and the amount (mL) of each antibiotic administered.

### ***Calculations and Statistical Analyses***

***Calculations.*** Average antibiotic treatment cost was calculated using a fixed cost of \$0.65/mL for enrofloxacin, \$0.47/mL for florfenicol, and \$0.65/mL for ceftiofur HCl. To determine percentage chronic, an animal was classified as chronically ill if 3 antibiotic treatments were administered coupled with  $\leq 0.45$  kg ADG for the entire 42-d trial. The

BRD relapse rate was determined by dividing the number of calves within each treatment requiring a 2<sup>nd</sup> antibiotic by the total number of calves in each treatment treated for BRD.

**Statistical Analyses.** Performance and morbidity data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Pen was considered the experimental unit. The class statement included block (date of arrival), treatment and replicate. Block was considered a random effect in the statistical model. Single degree of freedom orthogonal contrasts evaluating effects of management (PC or AM), exposure (PI or CON), and their interaction were used. If the interaction was significant ( $P \leq 0.10$ ), treatment means were separated with a t-test using the PDIFF option in SAS. For main effects of management and exposure, a  $P$ -value  $\leq 0.05$  was considered statistically significant. For repeated measures data, the model included treatment, day, and treatment  $\times$  day. Block, treatment, day and pen were used in the class statement. Block was the random variable, pen within block was the subject, and the repeated statement was day. The covariance model structure used was AR(1). Contrasts for the repeated measures data included management, exposure, management  $\times$  exposure interaction, and the linear and quadratic effect of day.

## **RESULTS AND DISCUSSION**

### ***Animal Performance***

No treatment interactions ( $P \geq 0.10$ ) were observed for animal performance; therefore, only main effects of management and exposure are presented (Table 2). Single-source PC calves had greater ( $P < 0.001$ ) ADG than commingled AM calves from d 0 to 14 (1.65 vs. 0.66 kg/d), d 0 to 28 (1.28 vs. 0.77 kg/d) and d 0 to 42 (1.20 vs. 0.85 kg/d). Results suggest clearly that prior vaccination against BRD pathogens and weaning

management that reduces psychological and physiological stress of single-source beef calves results in greater gain performance during a 42-d stocker receiving period. Step et al. (2008) evaluated effects of health and performance in beef calves from a single-source ranch (RANCH) either weaned and immediately shipped to a feedlot (WEAN), weaned and kept on the ranch for 45 d before shipment without receiving any vaccinations (WEAN45), weaned and kept on the ranch for 45 d before shipment and vaccinated with a MLV respiratory vaccine (WEANVAC45), procured and assembled through several auction markets (MARKET), or a commingled group (COMM) containing a portion of RANCH and MARKET origin cattle. Regardless of weaning or vaccination status, calves originating from RANCH tended to have greater ADG than COMM or MARKET calves for the 42-d receiving period. In a trial conducted by Clark et al. (2006), low-risk preconditioned calves had greater ADG during the finishing period than high-risk auction market calves of unknown history; however, no differences in performance were observed during the 28-d receiving period. Seeger et al. (2008) observed that auction market steers of unknown origin or health history gained 0.18 kg/d less during a finishing period than weaned beef calves receiving a health protocol on their origin ranch prior to marketing; however, an unequal number of calves testing positive for PI-BVDV remained in their treatment pens during the trial which may have confounded results. Research conducted by Pritchard and Mendez (1990) suggests that non-preconditioned calves may fully compensate for initial performance loss by the end of the feeding period; however, the non-preconditioned cattle used in that experiment would be considered low-risk because they were maintained as a single-source and were not subjected to typical stressors associated with the auction market system. Further research with careful

consideration of experimental design is needed to determine differences in health, performance and the economic value of single-source PC vs. commingled AM cattle.

Exposure to a PI-BVDV calf in the pen resulted in inconsistent, yet interesting differences in performance during the 42-d receiving period. From d 0 to 28, PI-BVDV exposure tended ( $P = 0.10$ ) to increase performance with PI-exposed calves averaging 1.06 kg/d versus 1.00 kg/d for non-PI exposed calves. Conversely, Elam et al. (2008) observed a tendency for PI-BVDV exposed cattle to gain less from d 0 to 28, although overall ADG was not affected by PI-BVDV exposure in that study. During the final interim period (d 28 to 42) of our receiving trial, PI-BVDV exposure resulted in a 0.22 kg/d decrease ( $P = 0.003$ ) in ADG suggesting that negative performance consequences of low-virulence PI-BVDV exposure in newly received beef calves may be delayed several weeks. The performance loss observed from d 28 to 42 agrees with the findings of Hessman et al. (2009), in which cattle directly exposed to a PI-BVDV animal gained 0.15 kg/d less than non-exposed cattle during a 66 d feedlot starter period. Reasons for our observation of a tendency for an increase, followed by a significant decrease in performance from PI-BVDV exposure are unknown; however, complex epidemiological and behavioral factors may have contributed. During the initial weeks of our study, PI-BVDV calves may have unintentionally served as a trainer animal because they had been previously acclimated to the RU facilities. In a series of 4 trials, Loerch and Fluharty (2000) observed increases in eating behavior and variable ADG for newly received calves with a trainer cow present in the pen during the initial 14 d; however, overall (28-d) differences in growth were undetectable in 3 of 4 trials. Another consideration is that abbreviated exposure to a low-virulent BVDV strain from PI-calves could simply mimic

an unattenuated autogenous vaccination (Elam et al., 2008) resulting in undetectable, or slight performance effects during the initial weeks of PI challenge. However, as the trial progressed and PI-BVDV exposure continued, an additive effect on host immune activity may have resulted in the performance loss observed from d 28 to 42 because repeated immune stimulation results in nutrients being preferentially utilized for immune and homeostatic pathways rather than tissue deposition (Klasing and Korver, 1997; Spurlock, 1997). Therefore, our ADG results would indicate that there may be a substantial metabolic cost associated with continuous exposure to a PI animal shedding a low-virulence BVDV strain and the resulting performance loss is not immediate but rather several weeks subsequent when nutrients utilized for humoral and cell-mediated immunity associated with repeated BVDV exposure presumably reach a threshold level. Because BW measurements were not recorded beyond the 42-d receiving period, the duration of performance loss and whether there was compensatory gain during the finishing phase is unknown. Performance from d 0 to 42 was not affected ( $P = 0.29$ ) by PI-BVDV exposure; however, the degree to which the slight increase in gain for PI-exposed calves from d 0 to 28 followed by the reduction in gain of PI-exposed calves for the final weigh period (d 28 to 42) would have confounded the d 0 to 42 ADG results should be considered. Research evaluating PI-BVDV exposure effects on performance is both limited and conflicting; therefore, further efforts with careful consideration of management and health history of experimental cattle, differences in the virulence and subgenotype strain of PI-BVDV challenge animals, and epidemiological factors are needed to determine effects of PI-BVDV exposure on performance throughout the various stages of the beef production system. Furthermore, a better understanding of the

metabolic cost associated with physiological and immunological products in response to stress and pathogen challenge is needed.

### ***Animal Health***

The BRD morbidity rate was markedly greater ( $P < 0.001$ ) for AM than PC with 70.4 and 6.7% of calves, respectively requiring treatment for BRD (Table 3).

Furthermore, a greater number of AM calves required treatment with a second ( $P < 0.001$ ), and third ( $P = 0.001$ ) antibiotic. Our BRD morbidity results were similar to Clark et al. (2006) in which BRD morbidity rates were 64.4 and 2.0% in steers considered either high- or low-risk for developing signs of BRD, respectively. Other studies (Macartney et al., 2003; Seeger et al., 2008), reported that calves of unknown health history had greater morbidity compared with those administered health protocols prior to marketing. Roeber et al. (2001) observed that cattle originating from preconditioning programs had fewer hospital visits than cattle originating from auction markets. In the Step et al. (2008) study described previously, morbidity was 11.1% for RANCH and 41.9% for MARKET calves. Among the 3 RANCH-origin treatments, those weaned and shipped directly to the feedlot (WEAN) had greater morbidity compared to WEAN45 and WEANVAC45, but vaccination with a MLV respiratory vaccine at weaning did not reduce morbidity during the receiving period among the 2 treatments held on the origin ranch for 45 d. Their results would provide evidence that weaning management intended to reduce physiological stress had a more profound effect on subsequent morbidity than did administration of a MLV respiratory vaccine.

In the present study, antibiotic treatment cost during the 42-d receiving period was markedly greater ( $P < 0.001$ ) for AM calves (\$20.52/animal) than PC (\$2.48/animal).

Moreover, the percentage of chronically ill cattle was increased ( $P = 0.03$ ) for AM. The large difference observed for health parameters among the 2 cattle management sources, supported by other peer-reviewed research, would suggest clearly that single-source PC calves have fewer BRD-related health problems, improved animal well-being, and reduced antibiotic usage compared to commingled AM calves of unknown management and health history.

The overall BRD morbidity rate was not affected ( $P = 0.50$ ) by PI-BVDV exposure. However, administration of a third antibiotic occurred more often ( $P = 0.04$ ) with PI exposure and a treatment interaction ( $P = 0.06$ ) was observed for the percentage of chronically ill animals; AMPI had the greatest number of chronically ill calves (7.6%), AMCON was intermediate (1.1%), and PCCON and PCPI were least (0.4 and 0.3%, respectively). A trend ( $P = 0.10$ ) was observed for PI-exposed calves having an increased antibiotic treatment cost which averaged \$12.59 and \$10.40/animal for PI and CON treatments, respectively. Within AM calves only, PI exposure resulted in an antibiotic treatment cost of \$4.06/animal more than CON; this numerical difference being similar to our PI-BVDV testing cost (\$4.25/sample including next day parcel service fees). Our results would agree with a large-scale study using high-risk calves of unknown history (Hessman et al., 2009) in that overall morbidity was not different for direct PI-exposed or unexposed groups; however, percentage of first relapse, chronic illness and fatalities were all greater for the direct PI-exposed groups. In that trial, treatment cost/animal was not statistically different ( $P = 0.24$ ), but a numerical increase of \$1.19/animal was observed for direct PI-exposed groups. Loneragan et al. (2005) reported cattle exposed to a PI-BVDV animal had a 43% greater risk of initial BRD treatment and in cattle that became

ill, those exposed to a PI-BVDV animal were administered more antibiotic treatments than non-exposed groups (1.76 vs. 1.46). Conversely, others have observed no differences in BRD health among PI- and non-PI-exposed pens (Booker et al., 2008; Elam et al., 2008); whereas, O’Conner et al. (2005) observed that disease prevalence was reduced in pens containing single-source cattle with a PI-BVDV calf present. Reasons for conflicting observations among the literature may be explained two-fold: 1) previous management and health history of cattle used within or among scientific experiments is varied; thus, susceptibility of experimental calves to PI-BVDV exposure may differ, 2) the virulence of BVDV strain and amount of BVDV particles shed by PI challenge animals varies among or within experiments.

Our observation of negative BRD-related health outcomes being most prevalent for AMPI, with few animal health differences observed among PCPI and PCCON treatments suggests that previous management and vaccination against BVDV and other respiratory pathogens may enhance protection against PI-BVDV challenge. Therefore, the degree to which PI-BVDV exposure affects economically important traits related to BRD morbidity in a stocker program or feedlot is influenced by previous management and health history in newly received calves, and the decision to test and remove PI-BVDV animals from an isolated group of calves may be based in part upon the perceived BRD risk level.

### ***BVDV Antibody Titers***

***Individual Analysis.*** For individual samples analyzed at ISUVDL, d 0 BVDV type 1a (Singer strain) antibody titers were profoundly increased (treatment  $\times$  day,  $P < 0.001$ ; Figure 2) for PC, and seroconversion to BVDV type 1a on d 0 was 100 and 23%

for PC and AM, respectively. The difference in antibody titers against BVDV type 1a on d 0 would indicate that AM calves were relatively naïve and PC calves had greater humoral immunity against either acute BVDV infection or PI-BVDV challenge. Serological data from previous studies indicate that greater serum antibody titers against BVDV upon arrival at the feedlot correspond with a subsequent reduction in risk for clinical BRD (Martin et al., 1999; O'Connor et al., 2001). Bolin and Ridpath (1995) observed that BVDV challenged calves with greater titer levels of passively acquired viral neutralizing antibody in serum had reduced duration and severity of clinical disease. In another trial (Fulton et al., 2006), vaccination with 2 doses of MLV respiratory vaccine containing both BVDV type 1a and 2a isolates provided protection against viremia in calves exposed to PI-BVDV type 2a challenge animals during a 35-d observation period. However, it has also been suggested that pre-existing antibodies, particularly those heterologous to the subgenotype strain shed by PI challenge animals may not be sufficient to provide complete protection (Fulton et al., 2005).

By d 28, BVDV type 1a titers levels were similar for all treatments; however, the incidence of first, second, and third BRD episode for all calves was d 2.3, 5, and 8.8, respectively, with few BRD episodes occurring after d 28. It should be considered that AM received identical vaccinations and processing procedures as PC, only the timing of administration differed, suggesting that MLV respiratory vaccination prior to market-associated stress and pathogen exposure is critical to achieve sufficient antibody titer concentrations before the initial 14 d of receiving when BRD is most often prevalent. Furthermore, arrival MLV respiratory vaccination of known single-source, recently vaccinated PC calves may be redundant because in our experiment PC calves were not re-

vaccinated on arrival, yet 100% were seropositive for BVDV type 1a on d 0 and BRD morbidity for PC calves during the 42-d receiving period was low (7.2 %).

**Pooled Analysis.** To provide field study insight on cross-reactivity of BVDV strains after PI-BVDV type 1b exposure, serum samples (pooled within treatment pen) were tested for titers against BVDV type 1a (NADL strain), BVDV type 1b (TGAC strain), and BVDV type 2a (125 strain) at TVMDL-Amarillo. A management effect ( $P < 0.001$ ) was observed for BVDV type 1a (Figure 3), BVDV type 1b (Figure 4), and BVDV type 2a (Figure 5) titers; thus, on d 0 PC had markedly greater titers compared to AM for all 3 BVDV subgenotypes tested. Furthermore, interactions were observed for BVDV 1b ( $P = 0.08$ ) and BVDV type 2a ( $P = 0.01$ ) antibody titers. For both subgenotypes, PCPI, which had not been vaccinated on d 0 of the trial, had greater serum antibodies on d 28 which suggests that continuous PI-BVDV challenge stimulated the humoral immune response in PC calves. A titer increase on d 28 was not observed for AMPI; however, the MLV respiratory vaccine administered on d 0 and 14 to AM calves, which contained BVDV 1a and 2a isolates, likely confounded the titer response to PI-BVDV exposure. Because no interaction or exposure effect was observed for BVDV type 1a, yet BVDV 1b titers were greatest on d 28 for PCPI calves exposed to PI-BVDV type 1b challenge, our data would provide limited evidence that some difference in cross-protection existed. Burciaga-Robles et al. (2010) reported increased antibody titers to BVDV type 1b (TGAC 8HB), but not type 1a (Singer strain) or 2a (125-C) when steers were exposed to 2 PI-BVDV type 1b challenge animals for 72 h and Ridpath et al. (2010) reported that the ratio of virus cross-neutralization among BVDV type 1a and 1b subgenotype strains is 36%. Therefore, increases in serum antibody titers associated with

PI-BVDV exposure may be dependent upon the specific subgenotype strain of PI-BVDV challenge.

### ***Hematology***

Effects of management ( $P < 0.001$ ; Table 4) and linear effects of day ( $P < 0.001$ ) were observed for the total concentration of PBL in whole blood samples. Total PBL averaged 10.9 and  $8.6 \times 10^3/\mu\text{L}$  for PC and AM treatments, respectively, and PBL concentration increased overall with time. Treatment  $\times$  day interactions were observed for percentage neutrophils ( $P = 0.002$ ), percentage lymphocytes ( $P = 0.004$ ), lymphocyte count ( $P = 0.02$ ), and neutrophil:lymphocyte ratio ( $P < 0.001$ ; Figure 6). Data indicate that neutrophils increased for AM, but not for PC calves (management effect,  $P < 0.001$ ); whereas, lymphocyte count and percentage increased for PC, but remained constant for AM as the study progressed (management effect,  $P < 0.001$ ). This resulted in the overall neutrophil:lymphocyte ratio (N:L) being greater ( $P < 0.001$ ) for AM calves; as the trial progressed, N:L increased for AM, but decreased for PC (treatment  $\times$  day,  $P < 0.001$ ). Other studies (Kegley et al., 1997; Ishizaki and Kariya, 2010) report transient increases in N:L for cattle experiencing transport stress; however, an additive effect from additional stressors also shown to increase N:L such as weaning (Hickey et al., 2003) and commingling (McGlone et al., 1993) may explain the extended duration of increased N:L observed for the AM calves in our study. Microbial pathogen challenge can also impact total and differential PBL in cattle; challenge with BVDV, *Manheimia haemolytica*, or both were shown to affect several PBL measurements (Burciaga-Robles et al., 2010). Further research is needed to determine the capability of PBL analysis to indicate stress and disease status of cattle.

A treatment  $\times$  day interaction was observed for concentrations of red blood cells ( $P < 0.001$ ), hemoglobin ( $P < 0.001$ ), platelets ( $P = 0.03$ ) and percentage hematocrit ( $P < 0.001$ ; Figure 7). A tendency for an exposure effect occurred for red blood cells ( $P = 0.10$ ), hemoglobin ( $P = 0.07$ ), and hematocrit ( $P = 0.09$ ); mean values for each of these measurements were greater for PI-exposed calves. A management effect ( $P < 0.001$ ) was observed for platelet concentration; AM had greater platelets on d 14 and 28, which may indicate the presence of increased inflammation or a more stimulated cell-mediated immune response because platelets are an abundant source of CD154, a signaling molecule for T- and B-cell activation (Sowa et al., 2009). Furthermore, there was anecdotal evidence suggesting the association among platelet level and cell-mediated immune stimulation because the majority of clinical BRD symptoms occurred by d 14, which correlates with peak platelet levels observed on d 14.

In conclusion, differences in the subgenotype strain, virulence, and amount of BVDV particles shed by PI challenge calves, coupled with the vaccination and management history of a particular pen or group are key aspects that determine susceptibility and consequences of PI-BVDV exposure to non-PI cohorts. Study to study variation of these factors may explain the conflicting health and performance results in the published literature. Additional research considering these issues is needed to determine if PI-BVDV testing and removal in newly received calves is economically justified. Our results would suggest that costs incurred for labor, shipping, laboratory testing of ear-notch samples, and removal of animals identified as PI-BVDV may not overcome production losses for single-source, PC calves during the receiving period. Nevertheless, high-risk, commingled, AM cattle that are naïve and experiencing

physiological stress and immune modulation may warrant testing and removal of PI-BVDV animals; however, additional research from both large-scale and randomized controlled trials is needed. Continuous exposure to PI-BVDV challenge during the 42-d receiving period reduced gain from d 28 to 42; however, it was not known the duration of performance loss and whether compensatory gain occurred after the 42-d receiving period ended.

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**Table 1.** Ingredient composition of supplement (% , as-fed basis) for newly received cattle

Ingredient	Maximum feeding rate	
	1.82 kg/d	2.73 kg/d
Corn, cracked	68.365	68.595
Dried distillers grain with solubles	26	26
Salt, white	1	1
Limestone	2	2
Molasses	2	2
Vitamin A, D, E, premix <sup>1</sup>	0.15	0.09
Trace mineral premix <sup>2</sup>	0.085	0.055
Corn/Rumensin premix <sup>3</sup>	0.4	0.26

<sup>1</sup>Contained 5,896,000 IU vitamin A, 1,179,200 IU vitamin D, and 15,257 IU vitamin E/kg.

<sup>2</sup>Trace mineral premix contained 12% Zn, 8% Mn, 4% Cu, 500 mg of Co, 2,000 mg of I, and 600 mg Se/kg.

<sup>3</sup>Provided 88 mg of monensin (Elanco Animal Health, Indianapolis, IN)/kg of supplement.

**Table 2.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on performance of newly received beef calves

Item	Auction market		Preconditioned		SEM	Contrast, <sup>1</sup> <i>P</i> =		
	Control	PI-BVDV	Control	PI-BVDV		Management	Exposure	Interaction
BW, kg								
Initial	249	248	250	249	9.26	0.36	0.43	0.58
d 14	257	258	273	273	9.75	0.0001	0.60	0.72
d 28	270	270	285	286	9.09	0.0001	0.57	0.73
Final	286	283	301	299	7.51	0.0001	0.19	0.71
ADG, kg								
d 0 to 14	0.54	0.77	1.64	1.66	0.19	0.0001	0.16	0.20
d 0 to 28	0.74	0.81	1.25	1.32	0.15	0.0001	0.10	0.97
d 28 to 42	1.10	0.89	1.13	0.90	0.15	0.77	0.003	0.87

d 0 to 42	0.86	0.84	1.21	1.18	0.10	0.0001	0.29	0.99
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<sup>1</sup>Management = main effect of weaning management (auction market vs. preconditioned); Exposure = main effect of PI-BVDV

challenge (control vs. persistently infected bovine viral diarrhea virus challenge); Interaction = management × exposure.

**Table 3.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on health of newly received beef calves

Item	Auction market		Preconditioned		SEM	Contrasts, <sup>1</sup> <i>P</i> =		
	Control	PI-BVDV	Control	PI-BVDV		Management	Exposure	Interaction
Morbidity, %	67.2	73.7	7.7	5.7	4.30	0.0001	0.50	0.22
Treated once, %	33.1	30.2	3.2	0	4.00	0.0001	0.40	0.96
Day of first treatment	3.0	2.7	2.5	0.9	1.47	0.23	0.33	0.49
Treated twice, %	26.0	25.9	1.1	0.6	3.78	0.0001	0.94	0.95
Day of second treatment	7.3	6.2	4.0	2.6	1.65	0.007	0.30	0.92
Treated thrice, %	8.0	17.5	3.2	4.9	3.08	0.002	0.04	0.15
Day of third treatment	10.7	11.1	7.1	6.1	3.07	0.08	0.90	0.76
Relapse <sup>2</sup> , %	50.6	58.5	60.3	100	20.78	0.07	0.09	0.26
Chronic <sup>3</sup> , %	1.1 <sup>b</sup>	7.6 <sup>a</sup>	0.4 <sup>b</sup>	0.3 <sup>b</sup>	2.26	0.03	0.07	0.06

Medication cost, \$/calf	18.49	22.55	2.31	2.65	2.01	0.0001	0.10	0.16
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<sup>a,b</sup>Means within a row without a common superscript are different ( $P < 0.05$ ).

<sup>1</sup>Management = main effect of weaning management (auction market vs. preconditioned); Exposure = main effect of PI-BVDV challenge (control vs. persistently infected bovine viral diarrhea virus challenge); Interaction = management  $\times$  exposure.

<sup>2</sup>Relapse percentage calculated by dividing the number of calves within each treatment requiring a second antibiotic by the total number of calves in each treatment treated for BRD.

<sup>3</sup>Cattle were classified as chronic if 3 antibiotic treatments were administered and  $ADG \leq 0.45$  kg for the entire 42-d trial.

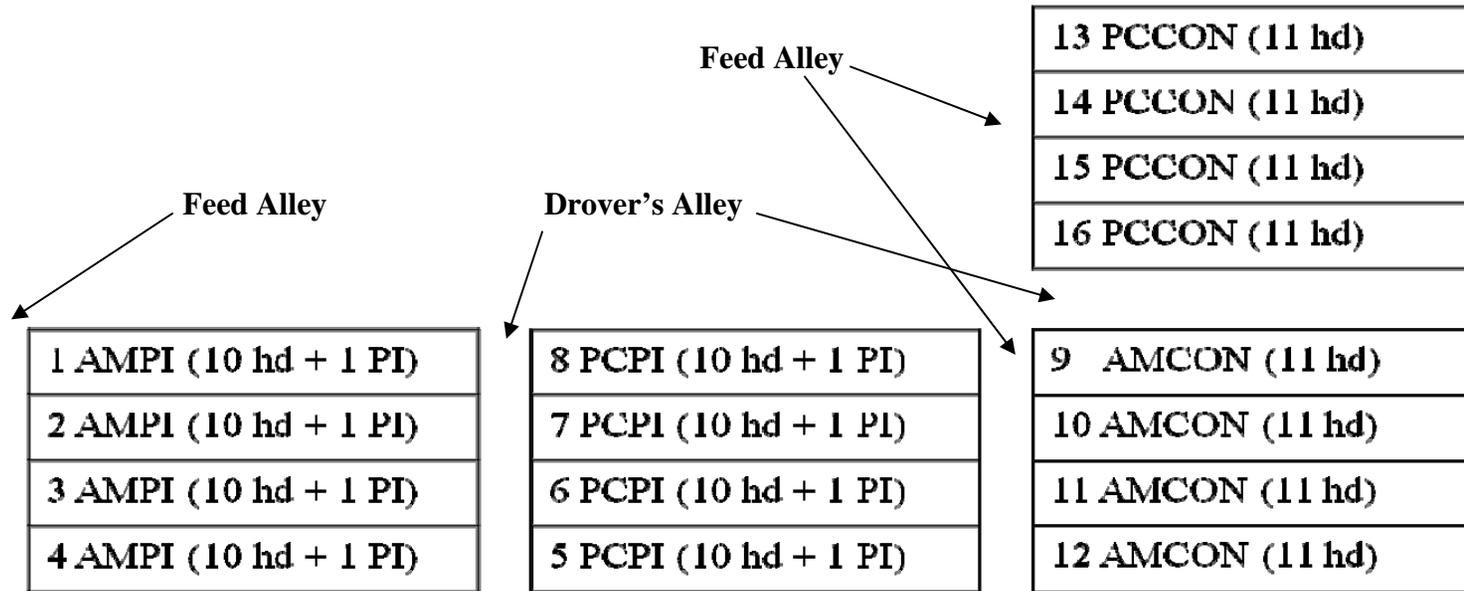
**Table 4.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on hematology of newly received beef calves

Item	Auction market		Preconditioned		SEM	Contrasts, <sup>1</sup> <i>P</i> =		
	Control	PI-BVDV	Control	PI-BVDV		Management	Exposure	Interaction
Total leukocytes, n × 10 <sup>3</sup> /μL	8.53	8.68	10.77	10.98	0.39	0.0001	0.62	0.94
Neutrophils, n/μL	2,344	2,407	2,599	2,403	161	0.41	0.66	0.39
Neutrophils, %	28.4	28.5	26.1	23.4	1.22	0.002	0.27	0.24
Lymphocytes, n/μL	4,886	5,040	6,784	7,255	358	0.0001	0.35	0.64
Lymphocytes, %	55.3	56.1	60.2	63.6	1.60	0.0001	0.18	0.38
Neutrophil:lymphocyte	0.64	0.67	0.53	0.45	0.04	0.0001	0.47	0.13
Monocytes, n/μL	1,071	1,021	1,100	1,030	64.2	0.75	0.32	0.87
Monocytes, %	13.3	12.8	10.8	10.1	0.57	0.0001	0.28	0.89
Eosinophils, n/μL	141	120	178	185	24.0	0.03	0.75	0.52

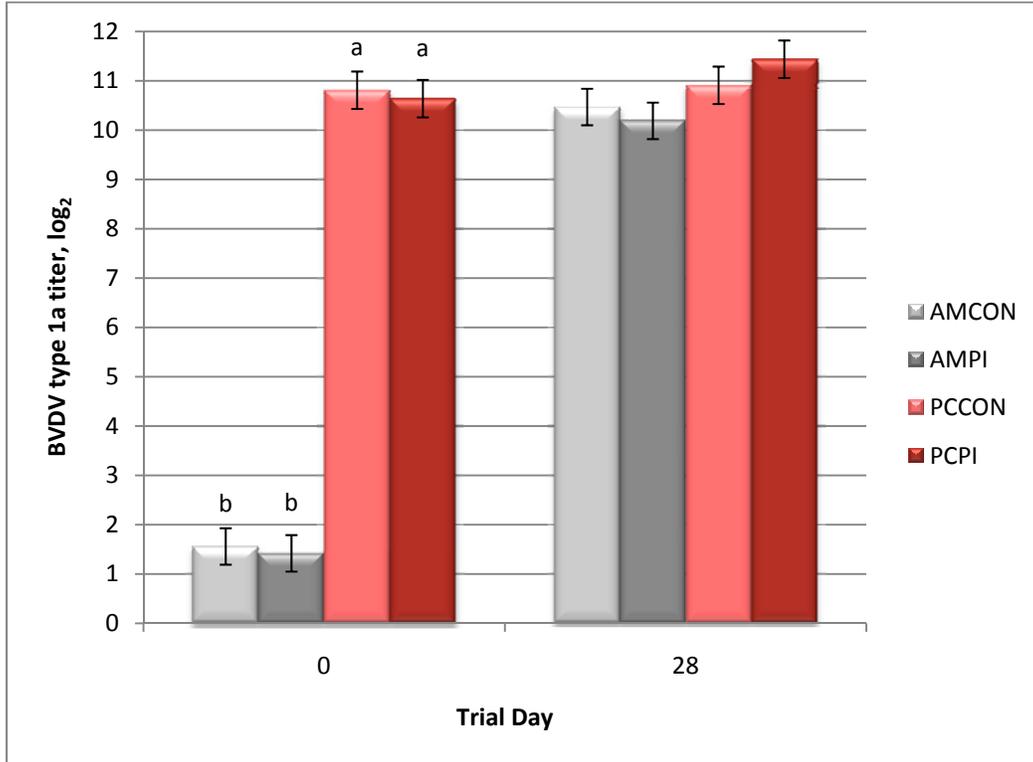
Eosinophils, %	1.7	1.4	1.7	1.8	0.23	0.38	0.58	0.39
Red blood cells, $n \times 10^6/\mu\text{L}$	9.60	9.86	9.75	9.92	0.14	0.40	0.10	0.71
Hemoglobin, g/100 mL	11.64	11.95	11.88	12.08	0.15	0.18	0.07	0.69
Hematocrit, %	34.9	35.9	35.5	36.0	0.44	0.36	0.09	0.59
Platelets, $n \times 10^3/\mu\text{L}$	555.6	542.1	459.3	461.6	29.0	0.002	0.84	0.77

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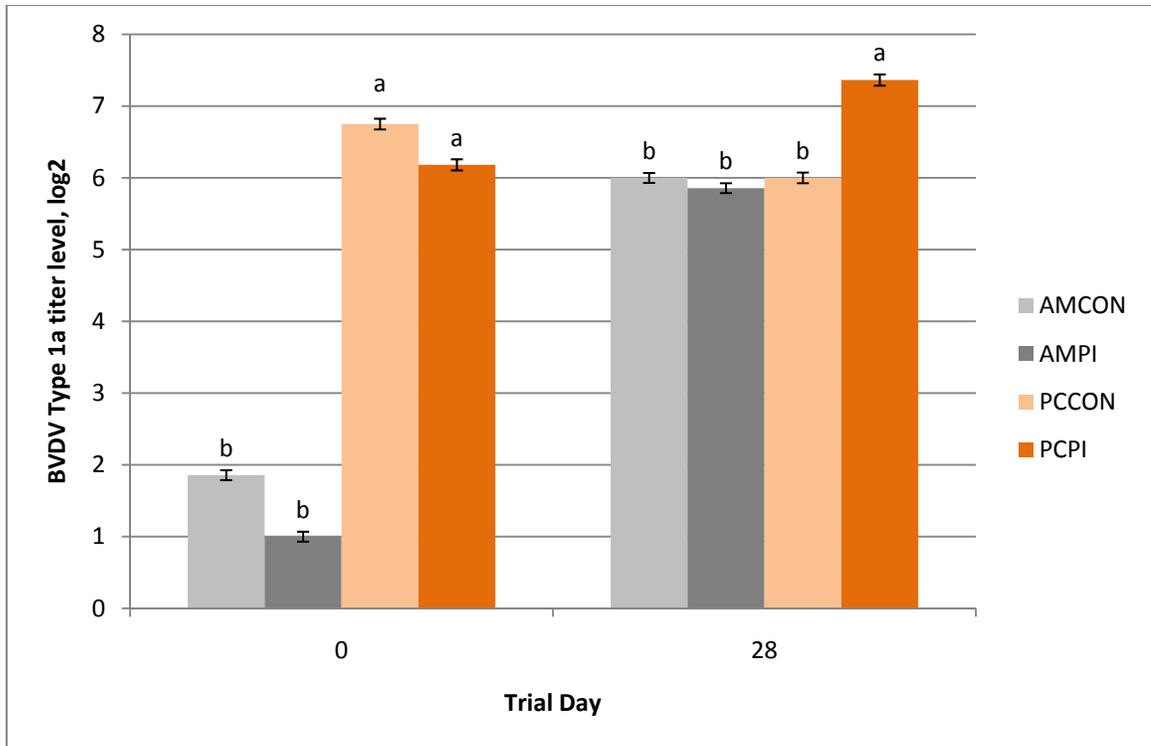
<sup>1</sup>Management = main effect of weaning management (auction market vs. preconditioned); Exposure = main effect of PI-BVDV challenge (control vs. persistently infected bovine viral diarrhea virus challenge); Interaction = management  $\times$  exposure.



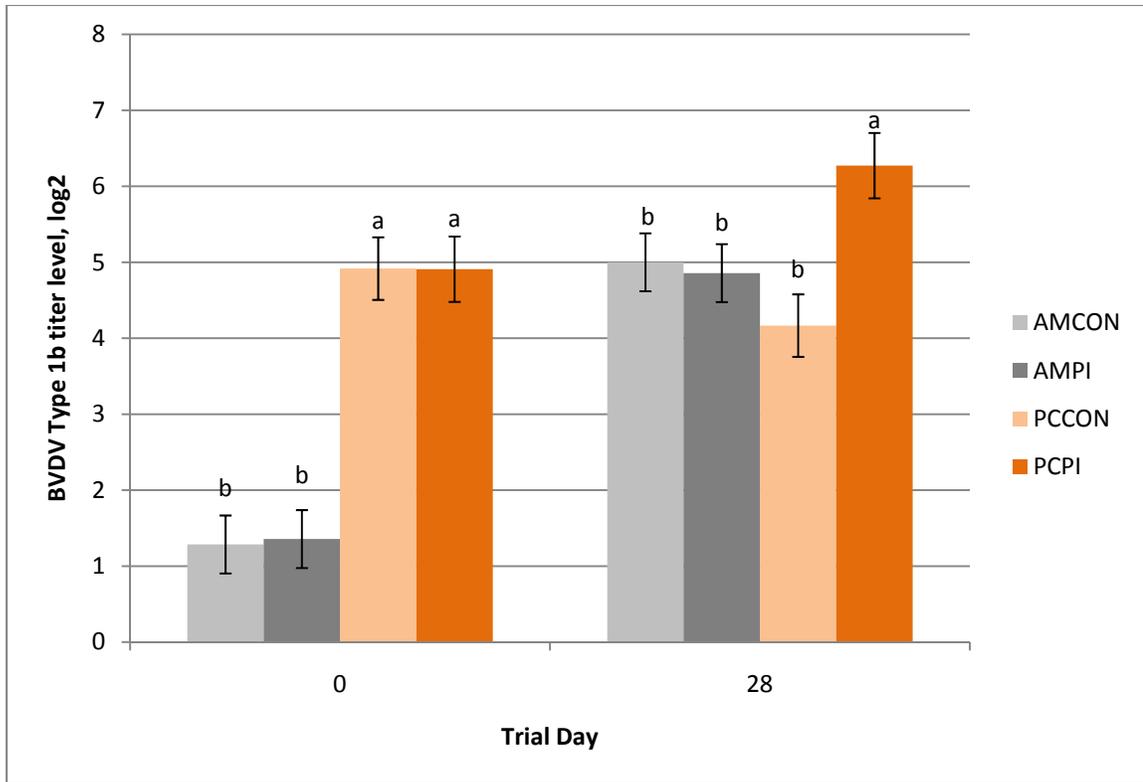
**Figure 1.** Example (not to scale) of spatial treatment arrangement of newly received calves located at UA Experiment Station (RU).



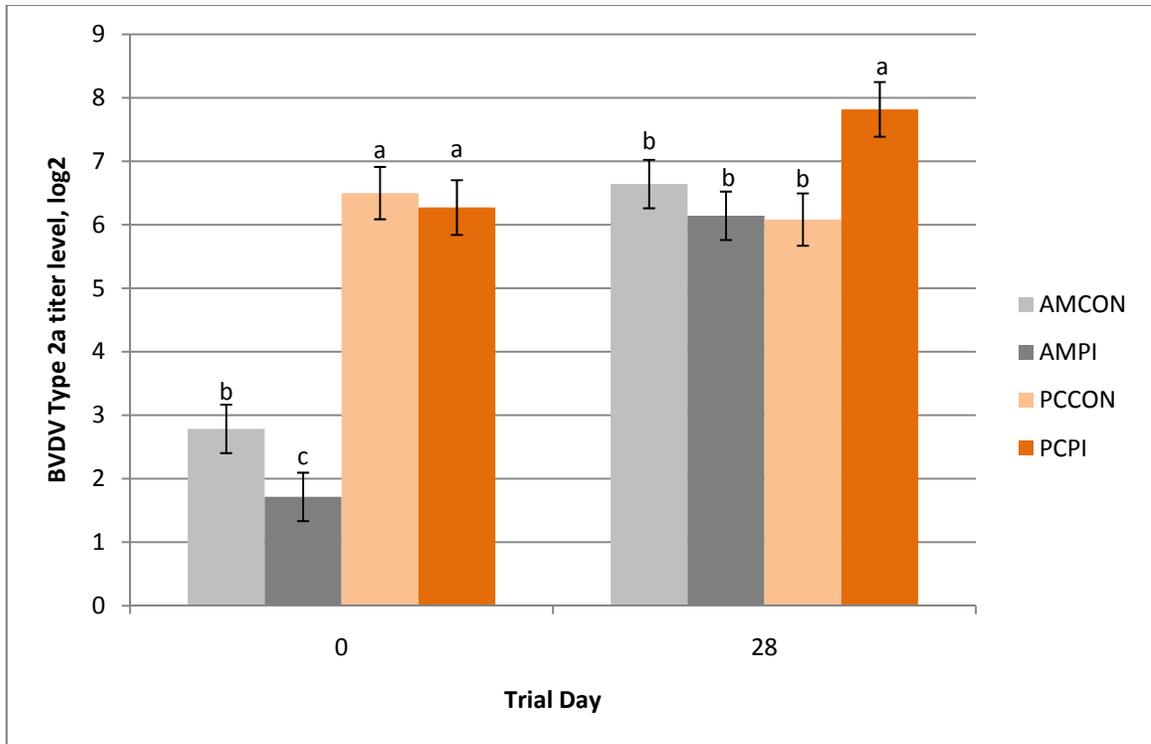
**Figure 2.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum antibody concentrations against bovine viral diarrhea virus (BVDV) type 1a (Singer strain) using individual animal analysis. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P < 0.001$ ). Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



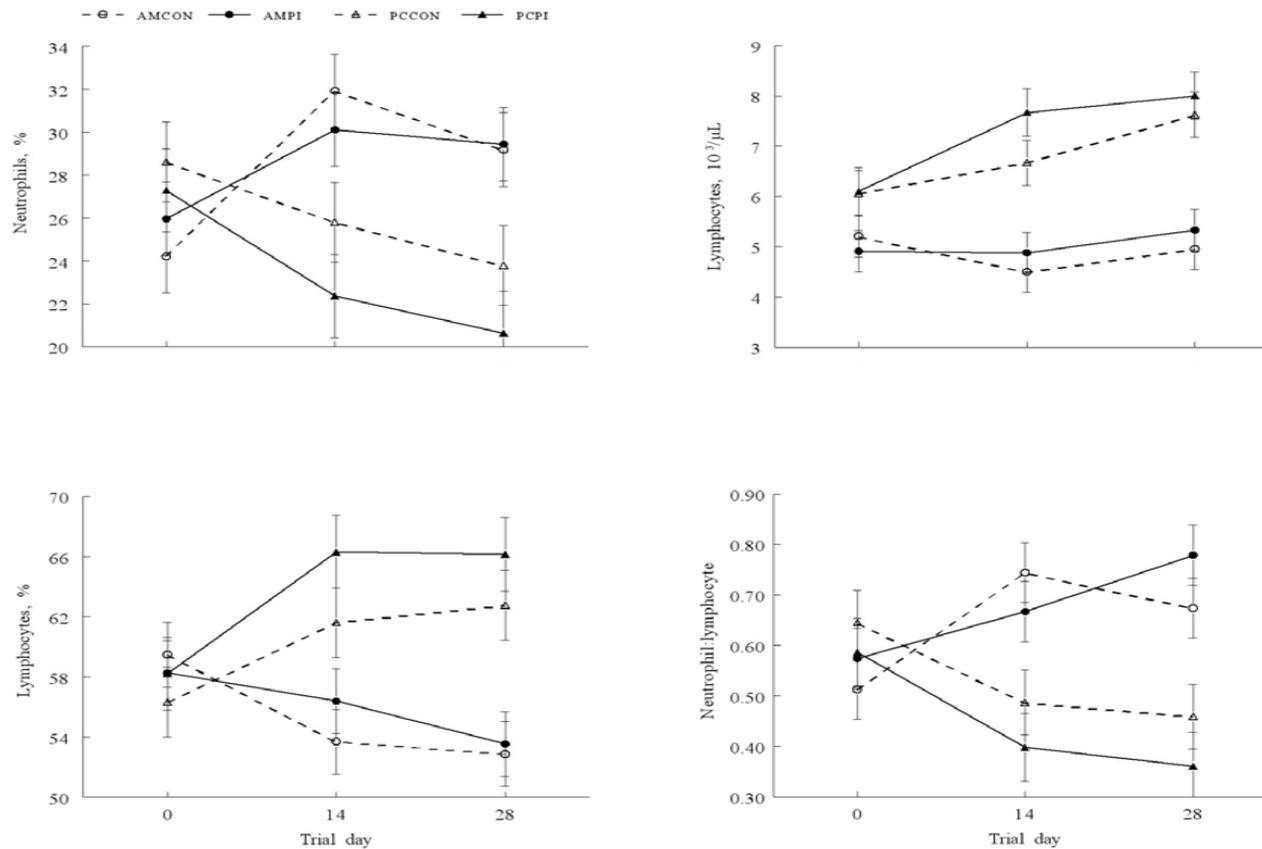
**Figure 3.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum antibody concentrations against bovine viral diarrhea virus (BVDV) type 1a (NADL strain) using pooled analysis. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P < 0.001$ ). Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



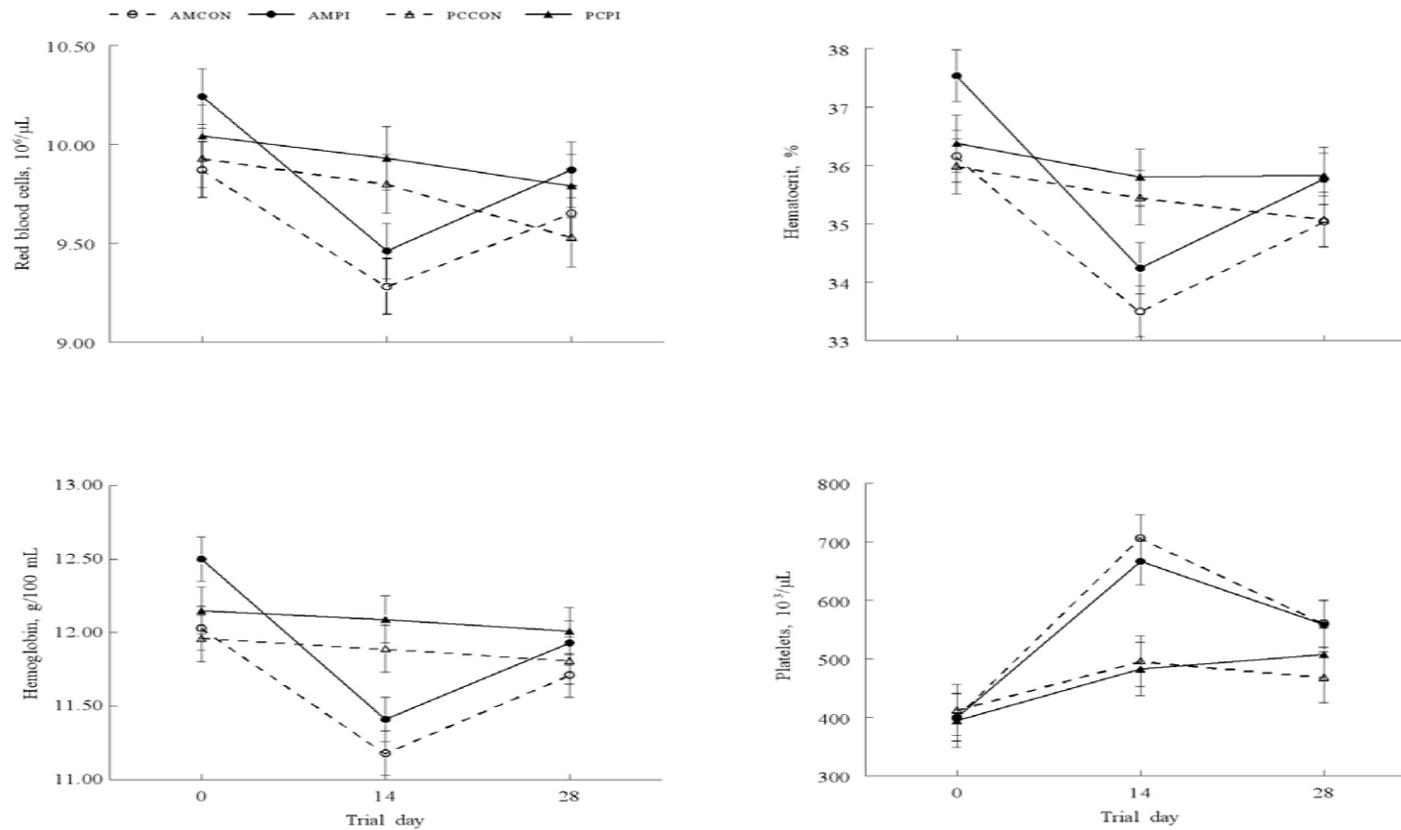
**Figure 4.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum antibody concentrations against bovine viral diarrhea virus (BVDV) type 1b using pooled analysis. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P < 0.001$ ). A tendency ( $P = 0.11$ ) was observed for exposure effect. Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 5.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum antibody concentrations against bovine viral diarrhea virus (BVDV) type 2 using pooled analysis. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P < 0.001$ ). Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 6.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on hematology of newly received beef calves. AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 7.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on hematology of newly received beef calves. AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.

## CHAPTER VI

### WEANING MANAGEMENT OF NEWLY RECEIVED BEEF CALVES WITH OR WITHOUT CONTINUOUS EXPOSURE TO A PERSISTENTLY INFECTED BOVINE VIRAL DIARRHEA VIRUS CALF: EFFECTS ON SERUM PROINFLAMMATORY CYTOKINE CONCENTRATIONS AND PERIPHERAL BLOOD LEUKOCYTES

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**ABSTRACT:** Calves persistently infected (PI) with bovine viral diarrhea virus (BVDV) are a major transmission source of the virus; however, physiological alterations resulting from exposure to this natural BVDV challenge model are not well understood, and the magnitude may differ for single-source, preconditioned (PC) vs. commingled, auction market (AM) cattle. Our objective was to compare treatments of PC or AM origin, with (PI) or without (CON) 14-d continuous exposure to a PI-BVDV calf in a  $2 \times 2$  factorial arrangement to evaluate effects on total and differential leukocyte concentrations, serum TNF- $\alpha$ , IFN- $\gamma$ , IL-4, IL-6 concentrations and rectal temperature (RT). Crossbred PC steers ( $n = 27$ ) from a single ranch-origin were selected randomly, weaned, dewormed, vaccinated, tested for PI-BVDV status, and kept on the ranch for 61 d. Subsequently, PC steers were transported to a stocker receiving unit (RU), weighed ( $282 \pm 1.6$  kg), stratified by d -1 BW, and assigned randomly to treatment (PCPI or PCCON) with no additional processing. Simultaneously, a group of crossbred AM calves ( $n = 27$ ) were assembled from regional auction markets and delivered to the RU 24 h before PC arrival. The AM calves were weighed ( $268 \pm 2.3$  kg) and administered the same processing procedures as PC received at their origin ranch; however, bull calves were stratified by gender and d -1 BW, castrated surgically, then AM calves were assigned randomly to treatment (AMPI or AMCON). Treatment pens ( $50 \text{ m} \times 15 \text{ m}$ ) were arranged spatially so that PI did not have fence-line contact with CON. For cytokine and hemagram analyses, whole blood or serum was analyzed on d 0, 1, 3, 5 (hemagram only), 7, and 14. In AM calves, RT increased ( $P < 0.001$ ) sharply on d 1, and remained elevated until d 7. Exposure to PI-BVDV challenge decreased ( $P = 0.01$ ) the percentage of neutrophils, and increased ( $P = 0.02$ ) percentage lymphocytes resulting in a tendency ( $P = 0.07$ ) for a

decreased neutrophil:lymphocyte ratio. Concentrations of TNF- $\alpha$  tended to increase ( $P = 0.09$ ) for PI-BVDV exposed calves. Interferon- $\gamma$  concentrations on d 7 and 14, IL-6 concentrations on d 14, and platelets on d 7 were greatest for AMPI ( $P \leq 0.05$ ). Overall, these results indicate effects of weaning management and PI-BVDV exposure in altering the immune status of newly received calves, and these effects may be additive because physiological alterations resulting from stress, pathogens, or both were greatest for AMPI.

## INTRODUCTION

Bovine respiratory disease (BRD) is a multifaceted syndrome involving physiological stress, commingling, and several viral and bacterial pathogens. Physiological alterations in response to individual stressors such as weaning (Hickey et al., 2003; Carroll et al., 2009), transportation (Blecha et al., 1984; Buckham Sporer et al., 2008; Stanger et al., 2005), castration (Fisher et al., 1997) and commingling (Step et al., 2009) have been observed; however, high-risk, newly received stocker cattle are often subjected to these stressors concurrently. Whereas, the negative effects of these stressors are mitigated in cattle preconditioned on their origin ranch before marketing occurs (Duff and Galyean, 2007). The most significant impact of physiological stress is the negative effect it has on feed intake and immunocompetency (Loerch and Fluharty, 1999), both of which may have a direct role in the development of BRD. Furthermore, acute infection with respiratory viruses is more likely because stress-induced immunosuppression allows viruses to evade host immune defense mechanisms (Sheridan et al., 1994).

Bovine viral diarrhea virus (BVDV) is a major culprit in the development of BRD either directly via acute clinical disease or through indirect effects of immunosuppression

(Welsh et al., 1995). Furthermore, calves born persistently infected (PI) with BVDV are a key source of BVDV transmission (Fulton et al., 2005), and although prevalence of PI-BVDV calves in the feedlot is estimated to be relatively low (0.3%; Loneragan et al., 2005), a single PI-BVDV animal has the potential to continuously expose an entire pen and adjoining pens to the virus. Although some have observed no overall differences in health or performance of cohorts exposed to a PI-BVDV calf (Booker et al., 2008; Elam et al., 2008), continuous exposure to PI-BVDV challenge may reduce growth performance (Hessman et al., 2009) because repeated immune stimulation results in nutrients being preferentially utilized for immune and homeostatic pathways rather than tissue deposition (Klasing and Korver, 1997; Spurlock, 1997). We hypothesized that the proinflammatory cytokine response would be altered by PI-BVDV exposure; however, the magnitude of physiological alteration would be impacted by previous management. The objective of our study was to determine the serum proinflammatory cytokine concentration, total and differential blood leukocyte concentration, and rectal temperature in commingled auction market vs. single-source preconditioned calves with or without 14-d continuous exposure to PI-BVDV challenge.

## **MATERIALS AND METHODS**

### ***Experimental Cattle***

Animal methods and experimental procedures were approved by the University of Arkansas Animal Care and Use Committee.

Two different cattle management sources were utilized for this 14-d evaluation period; 1) a low-risk, single-source, preconditioned (PC) group of crossbred steer calves (n = 27; initial BW = 282 ± 1.6 kg) from a single origin ranch located in IZARD County,

Arkansas and 2) a high-risk, commingled, auction market (AM) group of crossbred bull (n = 15) and steer (n = 12) calves (initial BW = 268 ± 2.3 kg) acquired from an Arkansas auction market. The PC steers arrived at the University of Arkansas Agricultural Experiment Station located near Savoy (receiving unit; RU) on December 6, 2009 and were considered to be low-risk for developing BRD because they had been previously vaccinated against BRD pathogens, were weaned on their origin ranch for 61 d, and maintained as a single-source without commingling. The AM calves were procured and assembled by an order buyer from a public auction market located in North Central, AR and arrived at the RU on December 5, 2009. The order buyer was instructed to purchase AM cattle of similar BW and phenotype as the incoming PC calves. The AM cattle were considered to be high-risk for developing BRD because they did not have known health or vaccination history and were commingled extensively resulting in greater probability of increased stress and exposure to BRD pathogens.

The main effects of cattle management (AM or PC) and PI-BVDV exposure (not exposed = CON or exposed = PI) were tested in a 2 × 2 factorial arrangement resulting in 4 treatments. For PC treatments, 62 d prior to trial initiation, randomly selected steers were weaned and confirmed free of PI-BVDV via ear-notch skin samples tested for the presence of BVDV using the antigen-capture ELISA (ACE) method (Idexx Laboratories, Inc., Westbrook, ME) at a commercial laboratory (Cattle Stats, LLC, Oklahoma City, OK). Also on the day of weaning, PC calves were administered: 1) a 5-way modified-live virus (MLV) respiratory vaccine containing infectious bovine rhinotracheitis, bovine viral diarrhea virus type 1a and 2a, parainfluenza3, and bovine respiratory syncytial virus isolates [Express 5, Boehringer Ingelheim Vetmedica (BIVI), St. Joseph, MO], 2)

*Manheimia haemolytica*-*Pastuerella multocida* bacterin-toxoid (Pulmo-guard PHM-1, BIVI), and 3) pour-on anthelmintic (Cydectin, BIVI). Fourteen-d later (d -48), PC calves were administered a 7-way clostridial bacterin-toxoid (Alpha 7, BIVI) and re-vaccinated with 5-way MLV respiratory vaccine. Preconditioned calves were isolated from other cattle, fed hay or pasture along with a supplement, and remained on their origin ranch during the preconditioning phase until d -1 when they were shipped to the RU.

Upon arrival to the RU, PC calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, PC calves were weighed and returned to their isolated holding pen. The following d (d 0), PC calves were weighed, bled via jugular venipuncture into evacuated tubes (Vacutainer; BD Inc, Franklin Lakes, NJ) to determine total and differential leukocytes (EDTA tube) and serum proinflammatory cytokine concentrations (plain tube), stratified by d -1 BW, then assigned randomly to treatment (PCCON or PCPI).

To coincide with PC, AM calves were assembled from a regional market and delivered to the RU on d -2. Upon arrival to the RU, AM calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d -1, AM calves were weighed, identified with a unique ear identification tag, ear notched to test for PI-BVDV status at a commercial laboratory (Cattle Stats) using the ACE method (Idexx Laboratories), and returned to their isolated holding pen. On d 0, AM cattle received the same vaccinations and processing procedures as described for PC on their origin ranch; therefore, the first known processing for AM occurred on d 0 rather than 62 d previously for PC. For AM calves only, re-vaccination of the 5-way MLV respiratory vaccine (Express 5, BIVI) occurred on d 14 in addition to administration of a

7-way clostridial bacterin-toxoid (Alpha 7, BIVI). Additionally on d 0, bull calves were castrated surgically, stratified by gender and d -1 BW, then AM calves were assigned randomly to treatment (AMCON or AMPI). Cattle were then moved to their randomly assigned 3.7 × 29 m pens and provided 0.91 kg/d (as-fed basis) of a receiving supplement (15.1% CP, DM basis) and ad libitum access to bermudagrass hay (13.1% CP, 52.3% NDF, 40.6% ADF, DM basis) and water. Supplement offered was step-wise increased to a maximum of 2.73 kg/d as each pen completely consumed the supplement offered for 2 consecutive d. All BW measurements were obtained individually without withholding feed or water on 2 consecutive days at the beginning (d -1 and 0) and end (d 14 and 15) of the receiving trial using a chute equipped with electronic load cells.

#### ***Leukocyte and Cytokine Analyses***

Calves were bled on d 0, 1, 3, 5, 7, and 14 to determine interim total and differential leukocytes. For each d, anticoagulated whole blood was collected from each animal into EDTA tubes, kept refrigerated (5° C), and analyzed within 24 h to determine percentage and concentrations (n cells/μL) of total and differential leukocytes (lymphocytes, neutrophils, monocytes, eosinophils, and basophils), platelets, red blood cells, hemoglobin, and hematocrit using an automated hemacytometer (Cell-Dyn 3500 system, Abbott Laboratories, Abbott Park, IL) standardized for analysis of bovine blood. Equipment malfunction prohibited hematocrit and platelets from being analyzed on d 14.

Blood collected from each animal in plain evacuated tubes was centrifuged at 2,100 × g for 20 min at 20° C, and serum was decanted into duplicate aliquots and stored frozen at -20° C. Subsequently, 5 sample aliquots were selected randomly on d 0, 1, 3, 7, and 14 from each treatment pen and transported on ice to the USDA, ARS, National

Animal Disease Center (NADC) in Ames, IA for determination of serum proinflammatory cytokines TNF- $\alpha$ , IFN- $\gamma$ , IL-4, and IL-6 using a custom bovine-specific multiplex ELISA assay (SearchLight, Aushon Biosystems Inc., Billerica, MA) with an intra- and interassay CV of 10% and 15%, respectively.

### ***Rectal Temperature Evaluation and Treatment of BRD***

Rectal temperature (RT) was recorded concurrent with blood sampling via a digital thermometer (GLA Agricultural Electronics; San Luis Obispo, CA; readability =  $\pm$  0.1°C) and calves were considered morbid and treated with an antibiotic based solely on RT. If RT was  $\geq$  40°C, calves were administered antibiotic therapy with enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS) at a dosage rate of 10 mg/kg of BW, and immediately returned to their home pen. A 48 h post-treatment interval (PTI) was implemented following administration of enrofloxacin, and a second RT was recorded upon expiration of the initial antibiotic PTI. If the second RT was  $\geq$  40°C, a second antibiotic treatment with florfenicol (Nuflor, Schering-Plough Animal Health, Summit, NJ) was administered at a dosage rate of 40 mg/kg of BW. A 48 h PTI was also implemented for cattle administered florfenicol, and RT was evaluated upon expiration of the second antibiotic PTI. If the temperature was  $\geq$  40°C, a third and final antibiotic treatment with ceftiofur HCl (Excenel RTU, Pfizer Animal Health, New York, NY) was administered at a dosage rate of 2.2 mg/kg of BW and repeated for 2 consecutive days following the initial injection of ceftiofur HCl. Treatment data were recorded for individual animal including treatment date, RT, and the amount (mL) of each antibiotic administered.

### ***Persistently Infected Cattle***

Calves that had been previously ear-notched, tested at a commercial laboratory (Cattle Stats), and identified as PI-BVDV according to ACE method were acquired from a stocker cattle operation in Washington County, OK to be utilized as PI-BVDV exposure sources. Upon arrival to the RU, each PI-BVDV calf was ear-notched a second time, and samples were shipped via overnight parcel service to a different laboratory (NADC) for rapid re-affirmation of positive PI-BVDV status using the same ACE procedure. The PI-BVDV challenge calves were assigned randomly to 6 PI-designated pens. Additionally, anticoagulated blood was collected from each PI-BVDV challenge animal and shipped on ice via overnight parcel service to NADC for subsequent differentiation of BVDV subgenotype strain using virus isolation of buffy coat cells and reverse-transcriptase PCR analysis previously described by Ridpath and Bolin (1998). One PI-BVDV animal was identified as subgenotype 1a, and the 5 remaining were subgenotype 1b. It is important to note that BVDV 1b, the subgenotype strain in which the PI-BVDV calves used in this experiment were identified, is the predominant BVDV subgenotype strain isolated from cattle in the US (Fulton et al., 2002; Ridpath et al., 2010); however, currently no commercial licensed MLV respiratory vaccine in the US contains a BVDV 1b isolate.

#### ***Pen Assignment and Arrangement***

To avoid unwanted PI-BVDV fence-line contact with CON, receiving pens were arranged spatially prior to treatment allocation. Pens measured  $3.7 \times 29$  m, contained 3 m of feed bunk-line and a fence-line water source shared with an adjacent pen of the same treatment. Furthermore, the treatment pens were configured so that unlike treatment pens were separated by at least 1 unoccupied pen. Within management source, calves were stratified by gender (AM only) and d -1 BW, then assigned randomly to 1 of 6 PI or CON

pens. For PI treatments, a PI-BVDV challenge animal was assigned randomly to each PI-designated treatment pen such that pens had an equivalent number of cattle. During all bleeding procedures, CON treatments were evaluated first, followed by PI treatments to avoid unwanted CON contact with PI challenge animals or experimental cattle in the PI treatments, and to reduce possible exposure to fomites contaminated with secretory fluid or fecal material containing BVDV.

### *Statistical Analyses*

Performance data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Pen was considered the experimental unit. The class statement included treatment and replicate. Replicate was considered a random effect in the statistical model. Single degree of freedom orthogonal contrasts evaluating effects of management (PC or AM), exposure (PI or CON), and their interaction were used. If the interaction was significant ( $P \leq 0.10$ ), treatment means were separated with a t-test using the PDIFF option in SAS. For main effects of management and exposure, a  $P$ -value  $\leq 0.05$  was considered statistically significant. For morbidity data, the class statement included treatment and pen. Pen was considered a random effect. For repeated measures data, the model included treatment, day, and treatment  $\times$  day. Kenward Roger was specified as the degrees of freedom selection method. Treatment, day, replicate, pen and animal were used in the class statement. Replicate was the random variable, animal was the subject, and the repeated statement was day. The covariance model structure used was SP(POW). Contrasts for the repeated measures data included management, exposure, and management  $\times$  exposure interaction.

## RESULTS AND DISCUSSION

### *Rectal Temperature*

Mean RT for AM calves increased sharply (treatment  $\times$  day interaction;  $P < 0.001$ ) upon trial initiation and remained elevated until reaching baseline on d 14 such that a main effect of management ( $P < 0.001$ ) was evident (Figure 1). Febrile RT responses have been consistently reported in experimental models of viral (Chirase et al., 1991; Muller-Doblies et al., 2004), endotoxin (Reuter et al., 2008) and corticotrophin-release hormone (Cooke and Bohnert, 2011) challenge. Furthermore,  $RT \geq 40^{\circ}\text{C}$  is a standard objective index used to determine clinical BRD morbidity of cattle both in the production setting (Edwards, 2010) and in the current study. Peak RT was observed on d 1, averaging 40.3 and 39.1 $^{\circ}\text{C}$  for AM and PC, respectively. The elevated RT in AM corresponded with markedly greater (management effect;  $P < 0.001$ ) morbidity for AM (86%) vs. PC (4%) during the 14-d evaluation period; consequently, antibiotic treatment cost was \$23.25 vs. \$1.53/animal for AM and PC, respectively.

The greater RT observed for AM may be in response to physiological stress, disease pathogens, or both; however differentiation of the febrile response is difficult to determine. Nevertheless, PI-BVDV challenge did not affect ( $P = 0.95$ ) RT in our study; thus, the increased rectal temperature observed for AM but not PC treatments was likely due to greater physiological stress or acute effects of viral or bacterial pathogens encountered by AM cattle during the marketing process. Others reported RT being increased subsequent to PI-BVDV challenge (Burciaga Robles et al., 2010) or oral inoculation of BVDV (Muller-Doblies et al., 2004); however, the sampling intervals were

earlier and more concerted in these studies and differences in RT that may have occurred between sampling intervals in the current study are unknown.

### ***Total and Differential Leukocytes***

A main effect of management ( $P = 0.01$ ) and a treatment  $\times$  day interaction ( $P < 0.001$ ) was evident for total leukocytes (Figure 2). Total leukocytes were less for AM cattle (management effect;  $P = 0.01$ ) whereas, AMCON had the lowest concentration of circulating leukocytes on d 5, 7, and 14 (trt  $\times$  day;  $P \leq 0.05$ ). Total leukocytes did not differ ( $P = 0.17$ ) for PI compared to CON. In a previous study, total leukocytes were reduced in steers exposed to PI-BVDV challenge only, yet total leukocytes were increased when concurrently challenged with *M. haemolytica* (Burciaga-Robles et al., 2010). A frequent outcome of stress- or viral-induced immunosuppression is severe or fatal bacterial pneumonias (Panciera and Confer, 2010). However, the degree to which experimental animals in the current study were infected with pathogenic bacteria such as *M. haemolytica*, and potentially confounding affects on leukocyte concentrations was unknown.

The percentage of neutrophils were less ( $P = 0.01$ ; Figure 3), and the percentage of lymphocytes greater ( $P = 0.02$ ; Figure 4) in calves exposed to PI-BVDV challenge than CON. Consequently, a trend ( $P = 0.07$ ) was observed for neutrophil:lymphocyte (N:L) ratio (Figure 5) being less in calves exposed to a PI-BVDV calf. Similarly, Burciaga-Robles (2010) reported neutrophils were less in steers exposed to PI-BVDV challenge only; however, when PI-exposed steers were challenged with *M. haemolytica*, neutrophils were increased. In our study, cattle management also affected ( $P = 0.02$ ) N:L ratio; AM calves had greater N:L ratio than PC. Although neutrophils were not reported,

Gupta et al. (2005) observed decreased lymphocytes after steers were subjected to 6 regrouping and repenning events intended to induce physiological stress. Other studies reported transient increases in N:L for cattle experiencing transport (Kegley et al., 1997; Ishizaki and Kariya, 2010), weaning (Hickey et al., 2003), and commingling (McGlone et al., 1993) stress. Therefore, the increased N:L ratio would indicate greater stress for AM compared to PC.

On d 3, the percentage of monocytes was greatest for AMCON, intermediate for AMPI, and least for PCCON and PCPI (Trt  $\times$  day;  $P = 0.01$ ). A treatment  $\times$  day interaction ( $P < 0.001$ ) was observed for platelet concentrations. On d 1, platelets were greatest for PCPI, intermediate for PCCON and AMPI, and least for AMCON ( $P \leq 0.05$ ; Figure 6). However, on d 7 this pattern was inverted; AMPI had the greatest platelets, AMCON was intermediate, and PCPI and PCCON were least ( $P \leq 0.05$ ). Platelets are well known for their role in inflammation and hemostasis (George, 2000); however, their function and response to disease challenge or physiological stress are less clear. Sowa et al. (2009) proposed platelets as a potential modulator of adaptive immunity because they may enhance antigen presentation. Further investigation on the platelet response to physiological stress and disease in beef cattle seems warranted.

### ***Serum Cytokines***

Serum TNF- $\alpha$  concentration tended ( $P = 0.09$ ) to be greater in calves exposed to PI-BVDV challenge (Figure 7). There were no main effects of cattle management on serum proinflammatory cytokine concentrations in the current study. Day effects ( $P \leq 0.001$ ) were observed for TNF- $\alpha$ , IFN- $\gamma$ , IL-4 and IL-6; concentrations increased

transiently with time. Other than a day effect, no differences were observed for serum IL-4 concentration (Figure 9).

A treatment  $\times$  day interaction was observed for concentrations of IFN- $\gamma$  ( $P = 0.05$ ) and IL-6 ( $P = 0.006$ ; Figure 8 and 10, respectively). Peak IFN- $\gamma$  concentrations occurred on d 7 and were 3.3-fold greater in AMPI than PCCON ( $P = 0.004$ ). By d 14, overall IFN- $\gamma$  concentrations had decreased (day effect;  $P = 0.001$ ); nevertheless, AMPI was greatest, PCPI was intermediate, and AMCON and PCCON were least.

Peak concentrations of IL-6 were observed on d 1 and rapidly returned to near baseline levels by d 3; nevertheless, a treatment  $\times$  day interaction ( $P = 0.006$ ) was evident. On d 1, IL-6 concentrations were greatest for AMCON, intermediate for AMPI and PCPI, and least in PCCON. By d 14, AMPI had greater serum concentrations of IL-6 compared to AMCON.

Similar to our results of increased TNF- $\alpha$ , IFN- $\gamma$  and IL-6 for PI-exposed treatments, Burciaga-Robles et al. (2010) observed increases in TNF- $\alpha$ , IFN- $\gamma$ , and IL-6 when steers were exposed 72 h to 2 PI-BVDV calves. Carroll et al. (2009) observed that both TNF- $\alpha$  and IL-6 concentrations increased following endotoxin challenge in calves, and the magnitude of increase was greater for calves weaned at 250 d of age versus early-weaned at 80 d of age and shipped simultaneously to a receiving facility. Proinflammatory cytokine concentrations being greater for AMPI and PCPI suggests a more stimulated immune response via continuous PI-BVDV exposure that may consequently affect growth performance (Spurlock, 1997). Although differences in growth were not observed in this 14-d trial, evidence from a companion study (Chapter V) suggests that effects of PI-BVDV exposure are often sub-acute, and consequences on

growth performance may be delayed for several weeks, beyond the 14-d evaluation period herein.

In conclusion, continuous PI-BVDV exposure may decrease growth performance because the repeated immune stimulation increases proinflammatory cytokines IFN- $\gamma$ , TNF- $\alpha$ , and IL-6 that can impair growth either directly by acting on tissues, or indirectly through their effects on the endocrine system (Klasing and Korver, 1997). Differences in hematological variables, particularly neutrophil:lymphocyte ratio, may also indicate differences in physiological stress and disease in newly received beef calves.

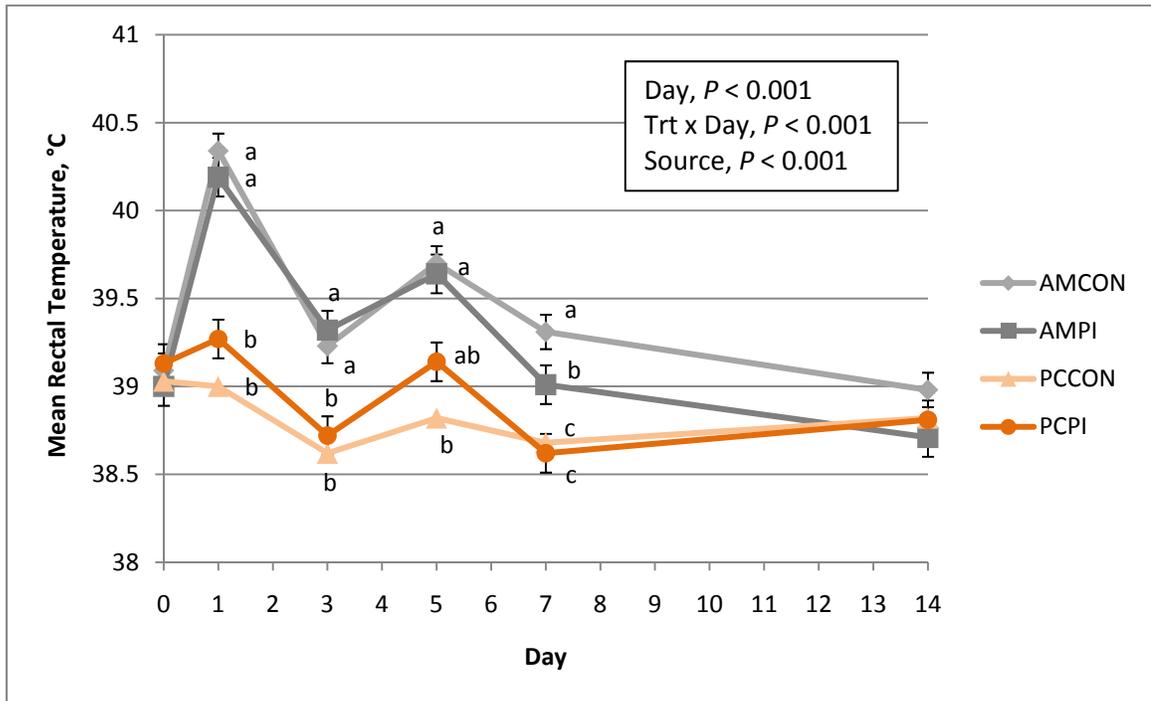
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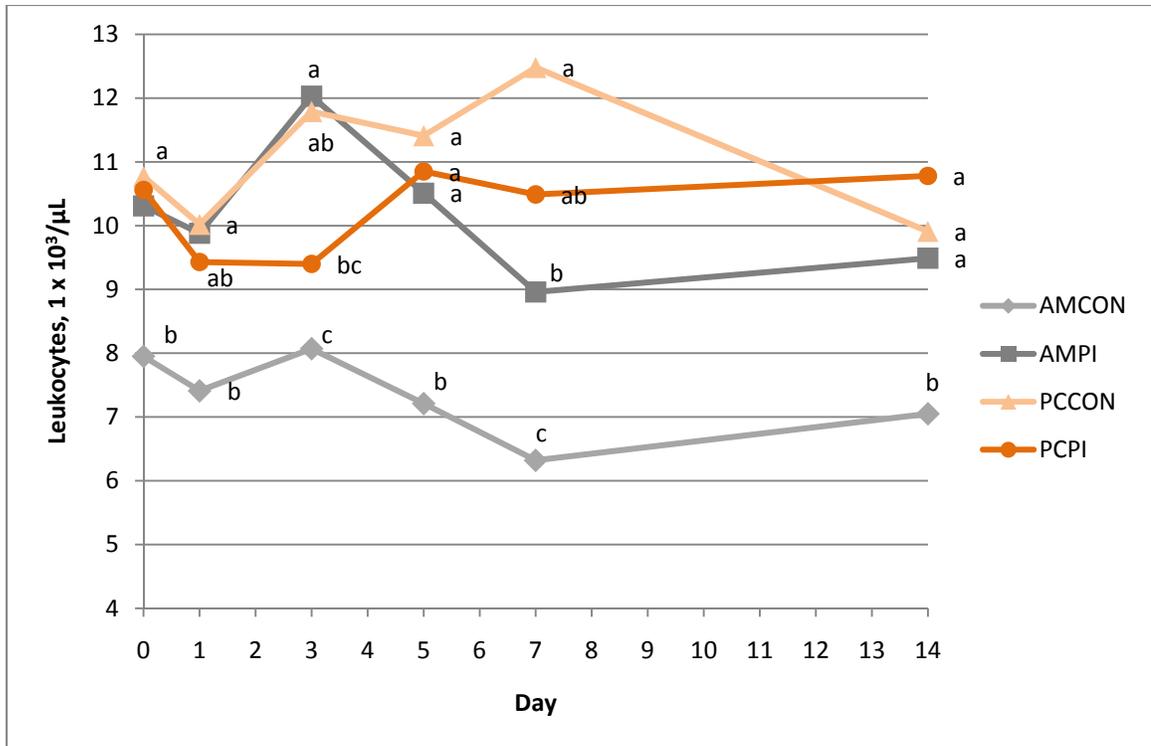
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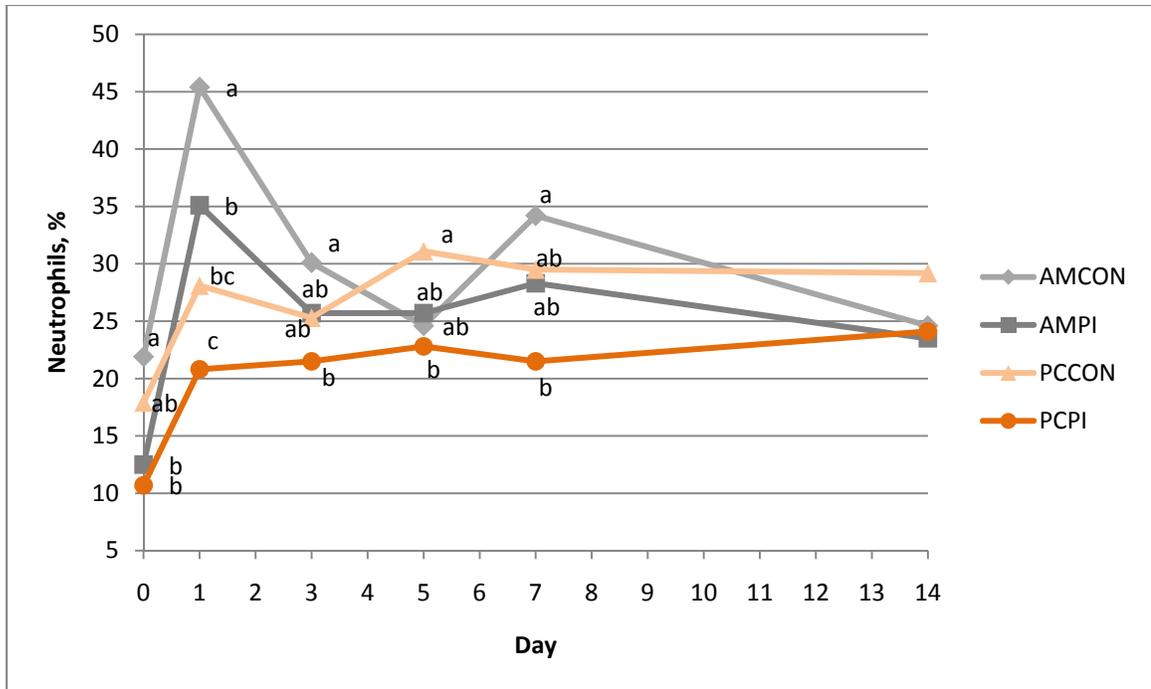
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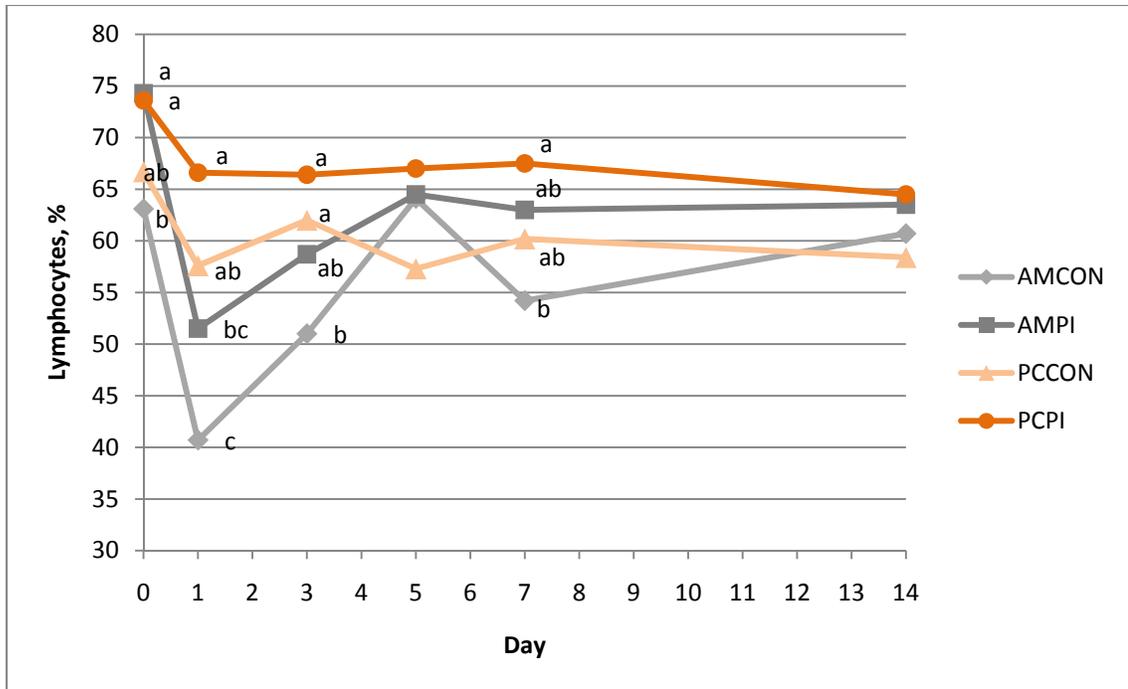
**Figure 1.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on mean rectal temperature of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P < 0.001$ ). Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



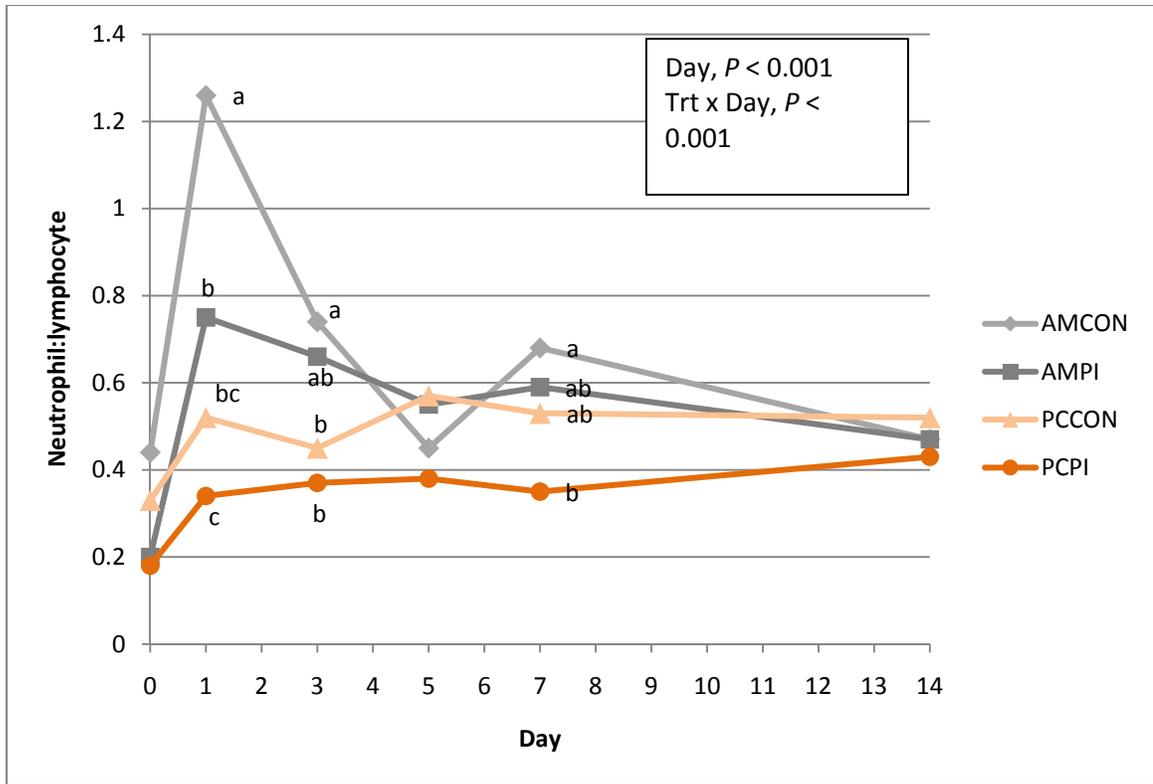
**Figure 2.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on blood leukocyte concentrations of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P = 0.005$ ), and management ( $P = 0.01$ ). Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



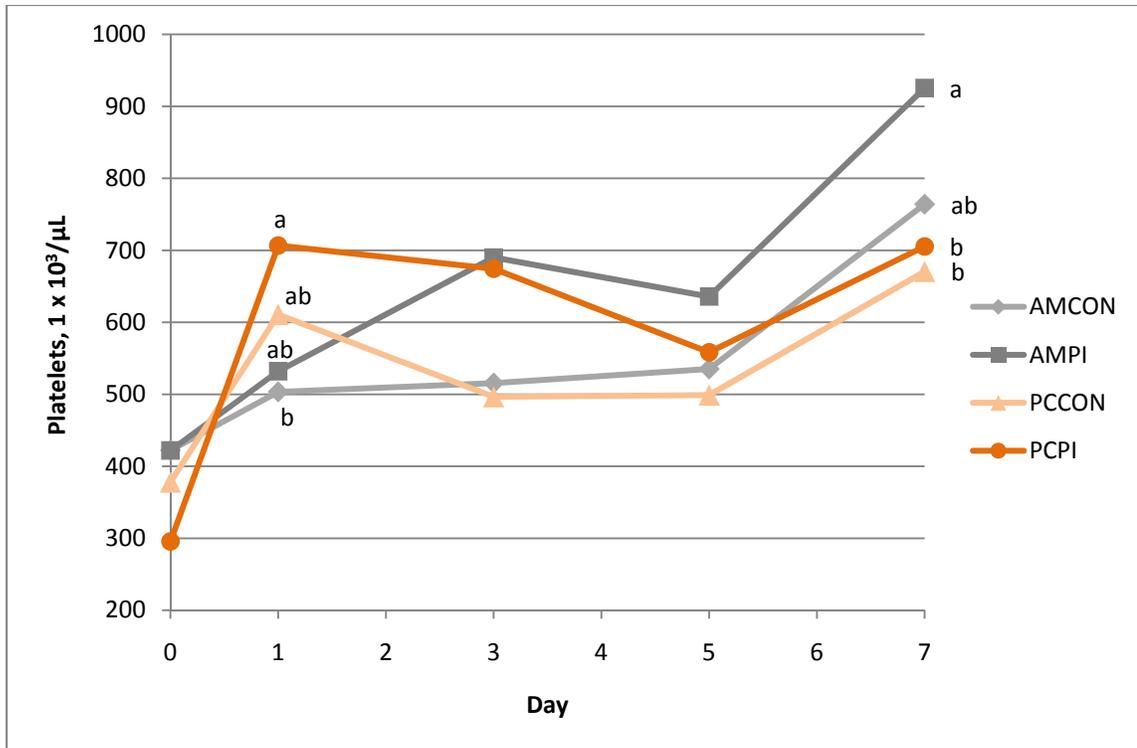
**Figure 3.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on percent neutrophils in blood of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and exposure ( $P = 0.01$ ). A tendency ( $P = 0.07$ ) was observed for management effect. Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



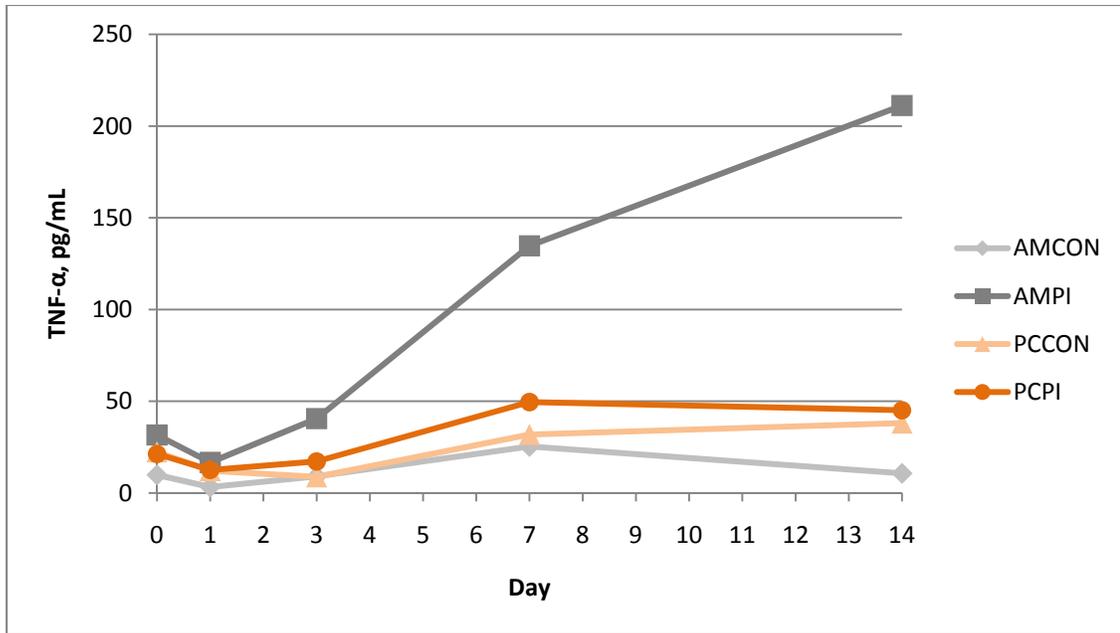
**Figure 4.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on percent lymphocytes in blood of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and exposure ( $P = 0.02$ ). A tendency ( $P = 0.10$ ) was observed for management effect. Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



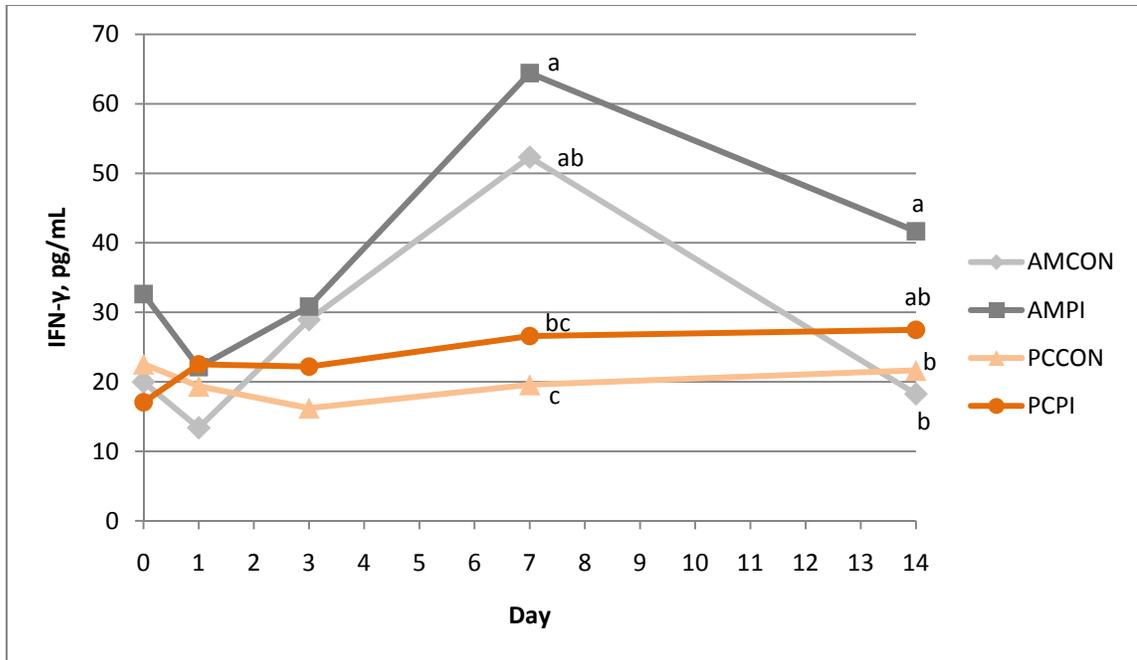
**Figure 5.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on blood neutrophil:lymphocyte ratio of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ), day ( $P < 0.001$ ), and management ( $P = 0.02$ ). A tendency ( $P = 0.07$ ) was observed for management effect. Means within a day without a common superscript differ ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



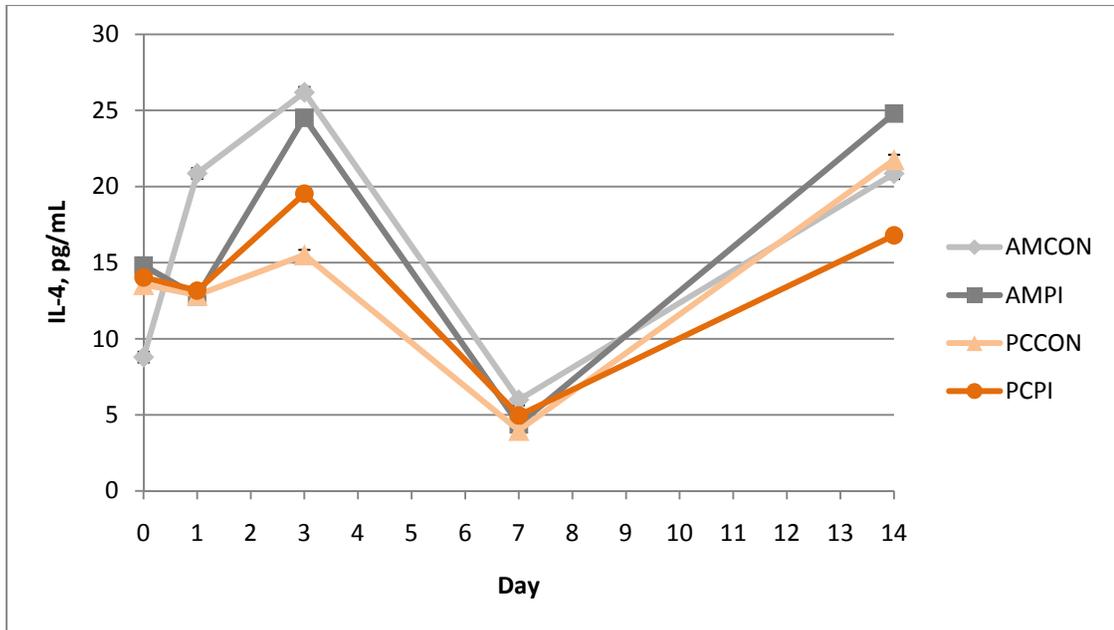
**Figure 6.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on blood platelet concentration of newly received beef calves. Effect of treatment  $\times$  day ( $P < 0.001$ ) and day ( $P < 0.001$ ). Means within a day without a common superscript are different ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



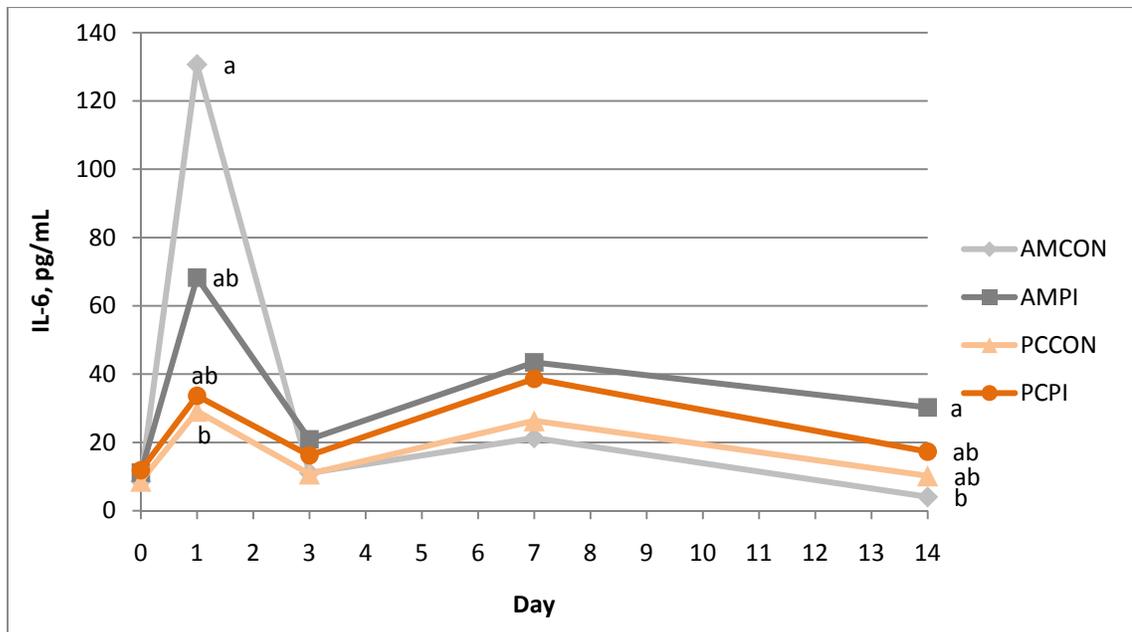
**Figure 7.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum tumor necrosis factor- $\alpha$  concentration of newly received beef calves. Day effect ( $P < 0.001$ ). A tendency ( $P = 0.09$ ) was observed for an exposure effect. AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 8.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum interferon- $\gamma$  concentration of newly received beef calves. Effect of treatment  $\times$  day ( $P = 0.05$ ) and day ( $P = 0.001$ ). Means within a day without a common superscript are different ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 9.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum interleukin-4 concentration of newly received beef calves. Day effect ( $P < 0.001$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.



**Figure 10.** Effects of weaning management and persistently infected bovine viral diarrhea virus challenge on serum interleukin-6 concentration of newly received beef calves. Effect of treatment  $\times$  day ( $P = 0.006$ ) and day ( $P < 0.001$ ). Means within a day without a common superscript are different ( $P \leq 0.05$ ). AMCON = auction market, control; AMPI = auction market, exposed to continuous PI-BVDV challenge; PCCON = preconditioned, control; PCPI = preconditioned, exposed to continuous PI-BVDV exposure.

## CHAPTER VII

### CONCLUSION

A series of experiments were conducted to determine the effects of on-arrival vs. delayed respiratory vaccination and exposure to persistently infected bovine viral diarrhea virus challenge on health, gain performance, and physiological and immunological measurements in newly received stocker cattle. Two experiments evaluated timing (d 0 vs. 14) of respiratory, clostridial, or both vaccinations in newly received stocker calves during stress-induced immunosuppression. In Exp. 1, calves receiving 14-d delayed vaccination of a pentavalent modified-live respiratory virus (MLV) vaccine had greater ADG during the 42-d receiving period and antibody titers against infectious bovine rhinotracheitis on d 42; however, morbidity rate did not differ. In the second study, stress indicated by serum cortisol concentration and neutrophil:lymphocyte ratio was greatest on d 0; nevertheless, no differences in performance or morbidity were observed for vaccination treatments. Both studies provided evidence that on-arrival administration of a pentavalent MLV respiratory vaccine to immunocompromised cattle does not mitigate clinical bovine respiratory disease (BRD) or improve animal performance because the majority of clinical respiratory disease for both studies occurred during the first 14-d and morbidity rate was not different when calves were vaccinated on d 0 vs. 14.

A third trial was performed to determine the effects of weaning management and subsequent exposure to persistently infected (PI) bovine viral diarrhea virus (BVDV) challenge on health, performance, bovine viral diarrhea virus titers, peripheral blood

leukocytes, and inflammatory cytokines. The objective was to compare treatments of preconditioned (PC) or auction market (AM) origin, with (PI) or without (CON) continuous exposure to PI-BVDV type 1b challenge in a  $2 \times 2$  factorial arrangement to evaluate main effects of management, exposure, and their interaction on health parameters and growth performance during a 42-d receiving trial. Preconditioned calves that were vaccinated, castrated, and weaned at their origin ranch had greater gain performance and reduced BRD morbidity compared to AM calves with unknown history. Furthermore, PC cattle had greater antibody titers to BVDV type 1a on d 0, and neutrophil:lymphocyte ratio and platelet count were lower during the 42-d receiving period. Exposure to PI-BVDV challenge reduced gain from d 28 to 42, perhaps due to an additive effect of continuous immune stimulation resulting in nutrients being preferentially utilized for immune pathways rather than tissue deposition. A treatment interaction was observed for the percentage of chronically ill animals; AMPI had the greatest number of chronically ill calves (7.6%), AMCON was intermediate (1.1%), and PCCON and PCPI were least (0.4 and 0.3%, respectively). These results would suggest that costs incurred for labor, shipping, laboratory testing of ear-notch samples, and removal of animals identified as PI-BVDV may not overcome losses in health or performance of single-source, PC calves during the receiving period. However, high-risk, commingled, AM cattle that are naïve and experiencing physiological stress and immunosuppression may warrant testing and removal of PI-BVDV animals.

During a 14-d evaluation period (trial 4), exposure to PI-BVDV challenge increased serum TNF- $\alpha$  concentrations, and IFN- $\gamma$  concentrations on d 14 were greatest for AMPI, intermediate for PCPI, and least for AMCON and PCCON. The increased

cytokine concentrations associated with PI-BVDV exposure illustrate a more stimulated immune response which has implications for animal health due to taxation of the immune system and growth due to a homeorhetic response in which nutrients may be preferentially shifted towards immune function rather than tissue growth.