EFFECTS OF BOVINE RESPIRATORY DISEASE ON IMMUNE RESPONSE, ANIMAL PERFORMANCE, NITROGEN BALANCE, AND BLOOD AND NUTRIENT FLUX ACROS TOTAL SPLANCHNIC IN BEEF STEERS

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EFFECTS OF BOVINE RESPIRATORY DISEASE ON IMMUNE RESPONSE, ANIMAL PERFORMANCE, NITROGEN BALANCE, AND BLOOD AND NUTRIENT FLUX ACROOS THE PORTAL DRINED VISCERA IN BEEF STEERS

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CHAPTER I

INTRODUCTION

Economically, bovine respiratory disease (**BRD**) is the most important disease affecting feedlot cattle (Martin et al., 1989; Edwards, 1996). Annual economic losses due to death, decreased feed efficiency, and medicine costs associated with BRD are estimated at $800-900 million (Chirase and Greene, 2001). Bovine respiratory disease accounts for approximately 75% of feedlot morbidity and from 50 to 80% of mortality (Edwards, 1996; Chirase and Greene, 2001). Although the medical costs attributable to the treatment of BRD are substantial (Martin et al., 1982; Perino, 1992), the economic impact of BRD on animal performance, carcass merit, and meat quality are likely even more devastating. McNeill et al. (1996) reported that "healthy" steers had greater daily gains and 12% more U.S. Choice carcasses than cattle identified as "sick" at some point during the finishing period. Gardner et al. (1999) showed that steers with lung lesions plus active lymph nodes had $73.78 less net return, of which 21% was due to medicine costs and 79% due to lower carcass weight (8.4% less) and lower quality grade (24.7% more U.S. Standards). This negative impact on carcass traits 200 d after receiving the cattle illustrates the importance of preventing BRD.

With demand for higher quality products and an increase in value-based marketing, beef producers are becoming more in-tune to health management practices that have the potential to increase profitability and beef product quality. Effects of BRD illustrate the

importance of pre-weaning health management to producers because of the associated economic risk (Speer et al., 2001). If feedlots were able to purchase calves that are more likely to remain healthy during the feeding period, their profits would increase through reduced costs and higher revenues. Previous studies have indicated that calves preconditioned prior to being placed in the feedlot remain healthier and perform better (Step et al., 2008); however, few studies have documented the value of preconditioning programs to the feedlot operator, cow-calf producer, or the public in general.

One of the greatest challenges with implementing programs and management strategies to prevent BRD is our lack of understanding of the multi-factorial etiology and predisposing factors involved with the disease (Duff and Galyean, 2007). Despite the availability of new generation vaccines and antimicrobials, mortality associated with BRD has increased in the last decade (Babcock et al., 2006). Booker et al. (2008) reported a wide variety of bacterial and viral pathogens in animals that succumbed to BRD. Thus, a better understanding of the impact that stress and viral and bacterial pathogens alone or in combination have on animal health and growth is essential to improving production efficiency, meat quality, and food safety in beef cattle. In non-ruminant species, recent research has implicated the fat cell as an interface between energy status of the animal and immune function, which may regulate muscle fiber growth through a variety of signals which influence fatty acid oxidation, glucose uptake, and insulin sensitivity (Jacobi et al., 2006). Intramuscular fat cells are strategically

positioned to act as immunological sensors to regulate direct and indirect responses of muscle cells to inflammatory signals. During an infectious process, immune cells release soluble proteins (i.e., "inflammatory signals") called cytokines (Bannerman, 2008). Cytokines have been demonstrated to redirect nutrient utilization from growth to support of an immune response (Humphrey and Klasing, 2004). Therefore, an acute phase response has the potential to have long-term effects on animal growth and development; however; little information is available in the bovine.

Objectives

Basic information regarding the underlying mechanisms of how stress associated with current cattle marketing strategies and BRD influence growth and development of beef cattle are largely lacking. Information in this regard is critical to improving livestock productivity and end product quality. The overall goal of the work presented herein was to identify biological links that exist between the BRD complex and decreased animal performance and carcass quality. Our objectives were to evaluate the effects of BRD challenge models on immune response, cattle performance, carcass characteristics, visceral organ mass, apparent nutrient digestibility, nutrient retention, and net flux of nutrients across the portal-drained viscera (**PDV**) and liver.

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CHAPTER II

LITERATURE REVIEW

Pathogens involved in Bovine Respiratory Disease (BRD)

Bovine viral diarrhea virus (BVDV) and its relationship with BRD. The presence of animals persistently infected (PI) with BVDV in feedlot pens has been associated with increased BRD morbidity and mortality during the finishing period (Loneragan et al., 2005). Bovine viral diarrhea viruses involved in BRD can cause clinical infections on their own (Potgieter et al., 1984), but have also been reported to make cattle more susceptible to secondary bacterial infections that are common inhabitants of the respiratory tract of healthy animals (Barbour et al., 1997). Therefore, increasing our understanding of the impact of BVDV and it's interaction with BRD on animal health is important.

Gånheim et al. (2003; 2005) evaluated the effects of BVDV and *M. haemolytica* challenge on immune response of calves. In their study calves ranged from 9 to 18 wk of age and the virus of interest was directly inoculated into the nostril. Clinical signs were more severe in animals challenged with both BVDV and *M. haemolytica* followed by the BVDV only group. Calves receiving only the bacterial challenge had very mild clinical signs. Rectal temperature and serum concentrations of serum amyloid A, fibrinogen, and

haptoglobin were increased in all experimental treatments. In addition, BVDV inoculation induced a significant decrease in total white blood cell counts and absolute counts of neutrophils and lymphocytes, whereas *M. haemolytica* inoculation increased total white blood cell counts and neutrophils, while decreasing the lymphocyte count. Fulton et al. (2006a) suggested that in order to study the pathogenesis and efficacy of vaccines the use of a challenge model that simulates natural disease exposure would be beneficial. Niskanen and Lindberg et al. (2003) demonstrated that the route of transmission of BVDV from a PI calf could occur by direct contact, ambient air, or by sharing the feed-bunk. Therefore, it appears a potential model to study the effects of BRD on animal growth and health could include exposure of naïve animals to animals PI with BVDV.

Mannheimia haemolytica and its relationship in BRD. Despite the development of new vaccines and antibiotics, *Mannheimia haemolytica* has been reported to be the most common pathogen isolated in animal's succumbing from BRD (Booker et al., 2008). The difficulty of implementing prevention strategies against this bacteria has been associated with its high virulence due to different pathogenic factors that it possesses, including outer membrane proteins (Pandher et al., 1999) and leukotoxin (Rice et al., 2007). Leukotoxin is secreted during the logarithmic growth phase of *M. haemolytica* (Czuprynski et al., 2004) and is considered to be one of components of *M. haemolytica* with detrimental effects on health of the host animal. Previous challenge studies with *M. haemolytica* have shown that iron-regulated outer membrane protein and leukotoxin are produced in vivo by *M. haemolytica* during pathogenesis associated with this bacteria (Confer et al. 1995). Therefore, increased serum antibody concentration against these

proteins in non-vaccinated and sero-negative animals is associated with clinical or subclinical disease.

Effects of BRD on white blood cell counts

When correlated with a clinical evaluation, leukograms can provide informative and low cost evaluation of disease processes (Cole et al., 1997). Gånheim et al. (2005) and Corrigan et al. (2007) reported that an intratracheal *M. haemolytica* challenge can produce an acute immune response during the first 24 h after the challenge. In those studies, the long-term effects of *M. haemolytica* challenge were not addressed due to the relatively short duration of the experiments (138 h, Corrigan et al., 2007; and 23 d, Gånheim et al., 2003). However, their experiments have provided valuable information in respect to the cellular immune response and describing physiological events which occur during the acute phase response to BRD. In both studies, animals challenged with *M. haemolytica* developed an increased concentration of circulating neutrophils, which have been recognized as the first line of defense of the immune system during bacterial infection. The type of cells of the immune system that respond to an immune challenge depends on the type of antigen involved. In general, viruses require the cellular machinery of the host animal to replicate (Janeway et al., 2005), and it has been reported that BVDV increased affinity for lymphocytes in order to replicate. Therefore, one might speculate that an infection with BVDV would cause a decrease in circulating lymphocytes (Lambot et al., 1998). Potgieter et al. (1995) reported lymphocyte depletion and decreased capacity for phagocytosis in calves infected with BVDV. Corrigan et al. (2007) performed an intratracheal *M. haemolytica* challenge in a group of "market age" heifers of unknown history and reported a decrease in lymphocyte counts during the 138

h post inoculation with *M. haemolytica*. In addition, Gånheim et al. (2005) reported decreased lymphocyte counts when animals were challenged with *M. haemolytica* alone. It is evident that BVDV or bacterial challenges can elicit an immune response, and that responses could be attributed to the type of challenge utilized. However, limited information has evaluated the effects a natural exposure to BVDV by contact with a PI-BVDV animal alone or in combination with a bacterial challenge on cellular, humoral and performance of beef steers.

Cytokines as immune modulators during a BRD event

Cytokines are soluble proteins synthesized by a variety of cells and tissues in the body and are involved in a wide variety of biological processes (Bannerman, 2008) influencing energy (Jacobi et al., 2006) and protein (Webel et al., 1997) metabolism. Increased cytokine concentrations are thought to result in repartitioning of energy and amino acids away from adipose and skeletal muscle growth by providing substrates for higher priority immunological functions (Jacobi et al., 2006; Waggoner et al., 2008). Although previous experiments have reported the concentration of cytokines in beef cattle (Reuter et al., 2008; Waggoner et al., 2008), these studies were conducted using intravenous infusion of lipopolysaccharide (**LPS**). Varied concentrations and length of infusion of LPS can result in different responses from the immune system (Webel et al., 1997). Gouwy et al. (2005) reported that the primary mechanism of action of cytokines is by up or down-regulation of genes involved in specific intracellular transduction pathways. A greater understanding of cytokine response to the most common pathogens could provide valuable information of the implications of these proteins for decreasing

performance and carcass characteristics in animals that suffer from BRD during the growing and finishing period (Larson, 2005).

Interferon gamma (IFNγ). Interferon gamma has been reported as the link between the innate and adaptive immune systems and is critical for an adequate response to intracellular pathogens (Bannerman, 2008). Schroder et al. (2004) suggested that the main source of IFNγ is lymphocytes. Ellis and Baeman (2004) and Schroder et al. (2004) have reported that the effect of IFNγ results from macrophages and neutrophils enhancing phagocytosis of extracellular pathogens (bacteria) and inducing a respiratory burst and nitric oxide production which has microbiocidal effects. Tatsufumi et al. (2007) concluded that increased concentration of IFNγ is a strong indicator of a cellular immune response.

Interleukin-1 (IL-1). Dinarello (1998) described IL-1 as one of the most potent endogenous inducers of fever and the induction of this cytokine has been associated with viral, bacterial, fungal and parasitic infections. In addition, Pruitt et al. (1995) reported that IL-1 is one of the principal inducers of acute phase protein synthesis. Although IL-1 is expressed as both IL-1α and IL-1β, Bannerman (2008) differentiated the proteins as either intracellular (IL-1α) or secreted by the cells (extracellular; IL-1β). Godson et al. (1995) administered different doses of recombinant IL-1β to beef calves and reported that the effect of IL-1β on rectal temperature was dose dependant. Only animals dosed with 333 and 1,000 ng/kg of IL-1β had a mean rectal temperature greater than 40.0^{o}C compared with animals dosed with 10, 33 and 100 ng/kg. Maximum rectal temperature was observed at 6 h for all treatments and returned to baseline by 24 h post IL-1β injection. In addition, the acute phase protein haptoglobin concentration was increased

above 0.50 mg/mL in animals receiving 1,000 ng/kg of recombinant IL-1β starting at 24 h post injection, and haptoglobin concentration remained elevated for 72 h. Serum concentrations of IL-1β were not reported (Godson et al., 1995). These results suggest that the pyrogenic and acute phase induction of IL-1β is dose dependant.

Interleukin-4 (IL-4). Limited information exists regarding the role of IL-4 in beef cattle. Elkenov et al. (2005) and Carroll and Forsberg (2007) described IL-4 as an anti-inflammatory cytokine which inhibits the production of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα). Reuter et al. (2008) reported decreased concentrations of IL-4 in animals fed a 30% concentrate diet compared with animals fed 70% concentrate when challenged with LPS and provided an antimicrobial injection. Reuter et al. (2008) observed higher concentrations of IFNγ, TNFα and IL-6 and concluded that the increased concentrations of these pro-inflammatory cytokines could be associated with the decreased concentrations of IL-4. An additional mechanism of action of IL-4 in the immune response has been described by Salak-Johnson and McGlone (2007), who proposed that the main effect of IL-4 is to disrupt T-helper cell 1 and 2 (TH1 and TH2) homeostasis. Bot et al. (2004) reported that when IL-4 is over expressed it can have detrimental effects on the immune system by decreasing the recruitment, expansion or activity of TH1 cells, resulting in a strong TH2 bias. The resulting increase of TH2 relative to TH1 might interfere with viral clearance by the host and suppress the innate immune response predisposing the host to secondary infections (Salak-Johnosn and McGlone, 2007). Therefore, increased IL-4 may decrease viral clearance and predispose animals to secondary infections resulting in increased morbidity and mortality when feedlot cattle are exposed to BVDV.

Tumor necrosis factor-α (TNFα). TNFα is a proinflammatory cytokine produced by different immune cells and presents a wide variety of activities (Ordas et al., 2006). Sordillo and Peel (1992) reported increased concentrations of TNFα in milk and serum of cows suffering from mastitis, and the increased concentration of TNFα was associated with the severity of the clinical manifestation of the disease. In addition, when dogs and cats were infused with recombinant human TNFα, a similar endotoxic response was observed (Tracey et al., 1986). Interestingly, recombinant anti-TNF monoclonal antibodies prevented the induction of septic shock during acute bacteremia in baboons (Tracey et al., 1987). Therefore, increasing concentrations of TNFα might be associated with severity of disease and decreased growth rates (Levine et al., 2005).

Implications of BRD on antibody production

One factor which can influence antibody production to a pathogen is antigen load (Katare et al., 2005). In addition, higher antibody production to BVDV has been reported as an indicator of better protection induced by vaccines (Kelling et al., 2007). Zhang et al. (1997) suggested that exposure of healthy animals to a virus that produces an immunosuppression might not only predispose animals to secondary infections, but could also have a detrimental effect on antibody production to other pathogens (i.e., vaccines), which could subsequently have a negative effect on animal health and performance.

Rectal Temperature and its relationship with BRD

During a BRD event, animals exhibit distinct behavioral patterns caused in part by changes in body temperature (Johnson et al., 1998; Johnson, 2002). Confer et al. (2008) reported an increased rectal temperature in animals challenged with *M. haemolytica* when compared with control animals. Similarly, Corrigan et al. (2007)

reported that animals that were challenged with *M. haemolytica* had increased rectal temperature (>40^{o}C) during the time elapsed between 0 to 12 h and returned to normal by 24 h. Reuter et al. (2008) reported rectal temperatures above 40^{o}C for 1 to 2 h, 3 to 6 h post LPS challenge depending on the dietary treatment, whereas Gilliam et al. (2008) and Waggoner et al. (2008) reported the highest rectal temperature to be ~39.5 and ~39.2^{o}C at 2 and 4 h post LPS challenge, respectively, and returned to pre-challenge temperature 6 h post-challenge

Kelling et al. (2007) reported that non-vaccinated animals that were challenged with BVDV type 2 had higher rectal temperature from d 9 to 11 after intranasal challenge compared with a vaccinated group. In addition, Gånheim et al. (2005) reported that animals that were challenged with non-cytopathic type 1 BVDV had a mild fever (>39.5^{o}C) from d 1 through 5 after viral inoculation. These results suggest that increased rectal temperature can result from either direct inoculation of known amounts of BVDV virus into nostrils of calves or exposure of healthy calves to animals PI with BVDV.

Increased body temperature during a febrile process in humans and rats (Dantzer, 2002) has been reported to increase metabolic rate by 13% for every 1^{o}C increase in body temperature. The length and amount of increased rectal temperature can have a great impact on nutrient requirements of sick animals due to the increased energetic cost for this metabolic process that should be accounted for when formulating diets for animals suffering from BRD.

Haptoglobin production during a BRD event

Haptoglobin is an acute phase protein produced mainly by the liver in response to pro-inflammatory cytokines including TNFα, IL-1 and IL-6 secreted by bronchoalveolar

macrophages in response to bacterial infection (Yoo et al., 1995; Morsey et al., 1999). Haptoglobin has been reported to have antibacterial and anti-inflammatory functions during immunological stress (Wassel, 2000). In cattle, haptoglobin has been evaluated as a predictor of BRD (Burciaga-Robles et al., 2009a) and correlated with the number of antimicrobial treatments for BRD (Carter et al., 2002; Berry et al., 2004; Step et al., 2008). Increased haptoglobin concentrations was associated with clinical cases of BRD and number of antimicrobial treatments required for animals during the disease (Carter et al., 2002; Berry et al., 2004). In addition to bacterial infections, Gånheim et al. (2002) reported that animals that were challenged directly into the nostrils with a non-cytopathic type 1 BVDV had higher concentrations of haptoglobin than control animals.

Klasing (1998a) suggested a switch in amino acid utilization from growth to production of acute phase proteins occurs following an immune challenge in chicks. The synthesis of acute proteins by the liver in response to an immune challenge could change the amino acid requirements as well as energy required for maintenance in support of the acute phase response. In the absence of stress or infections, animals will assimilate nutrients into tissues according to their genetic potential and according to age and stage of production (Klassing et al., 1987; Elsasser, 1993). However, during infection, there is an increase in protein turnover (Jepson et al., 1986) due to anorexia induced by cytokine production in response to the pathogen (Klassing, 1998b; Johnson, 1997), increased amino acid requirements for acute phase protein synthesis by the liver (Reeds and Jahoor, 2001), and increased mobilization of protein stores from muscle either by decreased synthesis or increased catabolism (Ling et al, 1997) providing energy precursors for gluconeogenesis or amino acids for protein synthesis by the liver (Espat et al., 1994).

Effects of BRD on dry matter intake (DMI)

Pro-inflammatory cytokines secreted by activated cells of the immune system have been reported to decrease feed intake (Johnson, 1997; Klasing, 1988). According to Hutcheson and Cole (1986), when expressed as a percentage of BW, DMI may be decreased during the first wk after arrival to the feedlot and that depression in DMI can last up to 28 d post arrival. To account for potential differences that intake has on nutrient digestibility and metabolism, pair feeding has been suggested for use in experiments comparing animals challenged with a pathogen vs. control animals (Sandberg et al., 2007). In addition, because pathogens induce anorexia in host animals, potential effects of decreased intake should be accounted for in order to develop feeding strategies for morbid animals in production settings (Kyriazakis et al., 1998). In fact, according to most recent Beef Cattle NRC (1996), when formulating diets for high-stressed calves entering the feedlot, the principal consideration to account for is decreased dry matter intake (DMI; Hutchenson and Cole, 1986). This is due to decreased DMI in sick/stressed calves (0.90 ± 0.70% of BW) compared to healthy animals (1.55 ± 0.51% of BW) during the first week after arrival to a feedlot. Differences in DMI can remain for two wk, and therefore nutrient intake during this period might be limiting to achieve adequate immune function and/or maximum growth potential (Chirase et al., 1991)

Blood flow across total splanchnic tissues (TST)

Several studies exist that have addressed changes in blood flow and net PDV flux of amino acids in cattle due to different dietary protein sources and feeding management strategies (Lomax and Baird, 1983). In growing and lactating animals, the splanchnic bed provides the majority of nutrients required for growth and milk synthesis,

respectively (Lomax and Baird. 1983). Therefore, any change in the metabolic status of the animal that affects DMI or alterations in splanchnic metabolism can have a detrimental effect on growth and lactation in food producing animals. In Holstein calves, feed deprivation decreased portal blood flow when animals were fasted for 36 h (Koeln et al., 1993). In addition, Lomax and Baird (1983) reported that in lactating and non-lactating cows, portal blood flow was lower in feed deprived animals 2 d after feed deprivation occurred, and continued to decrease being lowest at the end of the fasting period (6 d). Hepatic blood flow decreased 2 d after feed deprivation occurred in lactating animals but remained unchanged in non-lactating cows (Lomax and Baird. 1983). Therefore, when animals are deprived of feed during transit or DMI is decreased due to morbidity, changes in blood flow and net nutrient flux through splanchnic tissues and might be expected.

Effect of BRD on N Balance

Cole et al. (1986) conducted a series of experiments to evaluate N metabolism in calves challenged with either bovine adenovirus-3 (PI-3) or infectious bovine rhinotracheitis (IBR) and reported that during the first 7 d after inoculation with IBR, calves that developed a febrile response had lower N digestion and N balance that non-febrile calves that were also challenged. Recently, Gilliam et al. (2008) reported N balance of steers with or without supplementation of branched-chain amino acids following (d 2 to 7) a bacterial LPS intravenous challenge. In their study, there was lower N retention by steers when LPS was administered, and these differences were due to lower N intake and increased urinary N excretion. In addition, Waggoner et al. (2008) reported a decrease in DMI and fecal N output during the first 6 d after LPS challenge.

As a result of these changes in protein metabolism and/or turnover, animals can experience a negative N balance (Ling et al., 1997) reflected in decreased growth (Le Floc'h et al., 2004).

Effect of BRD on short and long term performance in finishing cattle

In previous experiments, the number of antimicrobial treatments required for BRD has been associated with decreased performance during the feedlot phase (Gardner et al., 1999; Roeber et al., 2001). Roeber et al. (2001) showed that calves that required two or more antimicrobial treatments for BRD had lower ADG during the first 56 d on feed than calves that did not require an antimicrobial treatment, but there was no difference between animals that required one antimicrobial treatment compared with animals that did not require an antimicrobial treatment. This might suggest that calves receiving a single antimicrobial treatment were misdiagnosed or that severity or number of times a BRD event occurs plays a role in how the disease affects overall performance of beef cattle.

Hesseman (2006) concluded that the incidence of calves PI with BVDV arriving at the feedlot is approximately 0.3% of total cattle, and that PI and acutely infected cattle are the main sources of transmission of this disease to susceptible animals. However, the negative effects that PI calves have on health and performance has been debated. O'Connor et al. (2005) reported that in a population of 5,041 calves (40 pens), the presence of a PI calf had no negative effects on overall health of cattle in the pens. In contrast, Loneragan et al. (2005) attributed an increased risk of developing BRD to animals PI with BVDV present in pens of feedlot cattle. Stevens et al. (2007) reported that the presence of PI calves in a pen increased morbidity (18.8 vs. 29.6%) compared

with non-exposed cattle. In addition, these authors evaluated the epidemiological curve of BRD when a PI calf was present in a pen, and reported a twofold increase (15.3 vs. 31.7%) in the number of animals requiring an antimicrobial treatment during the first 7 d on feed. Booker et al. (2008a) evaluated the effects of the presence of a PI animal and also the type of BVDV involved on health and performance of non-exposed cattle. They concluded that the presence of a PI BVDV type 1 animal in a pen resulted in an increased number of BRD treatments and mortalities compared with non-exposed pens, but a PI BVDV type 2 animal had no negative effects on health of finishing cattle. Elam et al. (2008) reported that short-term (60 d) or long-term (215 d) exposure to calves PI with BVDV did not affect DMI or final BW at the end of the finishing period; however, they did observe a tendency for decreased ADG during the first 28 d in exposed cattle, which they attributed to the cost of developing immunity to the wild type strain of BVD. It is important to mention that in the study conducted by Elam et al. (2008), all animals had been vaccinated at least twice against BVDV before entering the feedlot, and at the time of entry to the feedlot received a metaphylactic antimicrobial treatment reducing the possibility of sickness not only to BVDV, but also to *M. haemolytica* and other microorganisms associated with BRD.

Olchowy et al. (2000) and Corrigan et al. (2007) have reported effects of *M. haemolytica* in challenge studies on animal performance; although the length of their experiments was relatively short (16 d and 136 h, respectively). Olchowy et al. (2000) reported decreased ADG of calves challenged with *M. haemolytica* which did not receive an antimicrobial treatment. In addition, Gånheim et al. (2003, 2005) reported the effects of an aerosolized challenge with BVDV with or without a subsequent *M. haemolytica*

challenge. In these studies authors characterized the production of acute phase proteins (Gånheim et al., 2003) and changes in white blood cell counts (Gånheim et al., 2005) due to the bacterial and/or viral challenges, and the length of the experiments were only 23 d. To our knowledge, no long-term effects of acute *M. haemolytica* challenge on performance and carcass characteristics in cattle have been reported.

Visceral Organ Mass

Reports in the literature exist showing effects that anabolic implants (Hutcheson et al., 1997), postruminal carbohydrate load (McLeod et al., 2007), different winter grazing strategies (Hersom et al., 2004), protein levels (Baldwin et al., 2000), and mineral content of the diet (Soto-Navarro et al., 2004). Hersom et al. (2004) observed differences in several components of the GIT due to different grazing strategies during the growing phase. In addition, Sainz and Bentley (1997) reported differences in visceral organ mass attributed to the utilization of different levels of concentrate or forage in diets. However, there are no previous reports that have addressed any possible changes in visceral organ mass due to BRD.

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CHAPTER III

EXPOSURE BY PERSISTENTLY INFECTED CALVES WITH BVDV1B AND SUBSEQUENT INFECTION WITH *MANNHEIMA HAEMOLYTICA* DEMONSTRATING EFFECTS ON CLINICAL SIGNS AND IMMUNE PARAMETERS: MODEL FOR BOVINE RESPIRATORY DISEASE VIA VIRAL AND BACTERIAL INTERACTION

ABSTRACT:

Objectives - The objective of this study was to determine effects of an intratracheal *Mannheimia haemolytica* challenge following short-term exposure (72 hours) to bovine viral diarrhea virus type 1b (BVDV1b) persistently infected (PI) calves on serum antibody production, total and differential white blood cell count (WBC), cytokine concentrations, and blood gases in feedlot steers.

Animals, Procedures and Experimental Design - Twenty-four steers (initial BW = 314 ± 31 kg) were randomly allocated to one of four treatments (six animals/treatment) arranged as a 2 × 2 factorial. Treatments were: 1) steers not exposed to BVDV nor challenged with *M. haemolytica* (Control); 2) steers exposed to two calves PI with BVDV1b for 72 hours (BVD); 3) steers intratracheally challenged with *M. haemolytica* on day 0 (MH); and 4) steers with 72 hour exposure to calves PI with BVDV1b and intratracheally challenged with *M. haemolytica* on day 0 (BVD+MH).

Results - Changes were observed in serum antibody production, total and differential white blood cell count, cytokine concentrations, and blood gases consistent with an immune challenge in beef cattle.

Conclusions - Our results suggest that development of a successful immune challenge model using exposure of naïve steers to steers PI with BVDV1b, intratracheal challenge with *M. haemolytica*, or their combination which will allow researchers to further characterize the detrimental effects that BRD pathogens have on food producing animals.

Clinical Relevance - Understanding the physiological changes in morbid animals will lead to improved strategies for decreasing severity and economic losses associated with BRD.

Key Words - Bovine respiratory tract disease, Bovine viral diarrhea virus, Immunity to bacteria, Cytokines

INTRODUCTION

Bovine viral diarrhea virus (BVDV) has been isolated alone or in combination with other viral and bacterial pathogens in animals diagnosed with bovine respiratory disease (BRD). Cattle persistently infected (PI) with BVDV have been suggested to be the main source of transmission of this disease in feedlot settings,[1] and the presence of an animal PI with BVDV in a feedlot pen has been reported to increase the risk of antimicrobial treatment for BRD by 43% compared with non-exposed cattle.[2] Because the economic losses associated with BRD are not just the direct cost associated with treatments, but also decreased performance and carcass quality,[3] a better understanding of the physiological changes associated with BRD are needed to improve management strategies for the prevention and treatment of the disease.

Based on BVDV studies to determine prevalence of BVDV subtypes in cattle, the BVDV1b subtype is more commonly identified than BVDV1a and BVDV2a based on a survey of BVDV isolates from diagnostic laboratory accessions and PI cattle entering a feedlot.[4,5] In addition, over the last 20 years *Mannheimia haemolytica* serotype 1 has been the most common bacterial pathogen isolated in calves suffering from BRD.[6] The objective of the present experiment was to develop a model that would allow us to study immune and metabolic changes associated with the acute response to BRD and the possible implications of this response on subsequent performance and carcass characteristics in feedlot cattle. The effects of exposure to steers PI with BVDV1b and intratracheal challenge with *M. haemolytica* serotype 1 on serum antibody production, total and differential white blood cell count (WBC), cytokine concentrations, and blood gas analysis in feedlot steers were determined.

MATERIALS AND METHODS

Animals

All procedures for the present experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Protocol# AG-06-16). A total of 24 Angus crossbred steers (initial body weight = 313 ± 31 kg) were housed at the Nutrition Physiology Research Center, Oklahoma State University, Stillwater to determine the effects of viral exposure and/or bacterial challenge with pathogens that have been associated with BRD on immune response of feedlot cattle. All animals were considered clinically healthy and were seronegative to all pathogens involved in the study as determined with paired serum samples collected 14 days apart prior to the start of the experiment.

Treatments

The 24 steers were randomly allocated to one of four treatments (six animals/treatment) arranged as a 2 × 2 factorial. Treatments were: 1) steers not exposed to BVDV nor challenged with *M. haemolytica* (Control); 2) steers exposed to two calves PI with BVDV1b for 72 hours (BVD); 3) steers intratracheally challenged with *M. haemolytica* on day 0 (MH); and 4) steers with 72 hour exposure to calves PI with BVDV1b and intratracheally challenged with *M. haemolytica* on day 0 (BVD+MH). Steers exposed to the calves PI with BVDV1b were transported approximately 3.2 km to the Willard Sparks Beef Research Center, Stillwater, OK where they were commingled in a 6 × 10.8 m pen with two steers previously confirmed as being PI with BVDV1b via immunohistochemistry and genotyping. The two PI with BVDV 1b calves were identified at 2575 and 3604. The PI subtype was determined by sequencing a region of the 5'-

UTR.[5] For both the BVD and BVD+MH groups, the length of exposure to the PI calves was 72 hours. After the time of BVDV exposure, calves were returned to the Nutrition Physiology Research Center where they remained for the remainder of the experiment. Steers challenged with *M. haemolytica* received 10 mL of a solution containing 6×10^9 CFU of *M. haemolytica* serotype 1 that was reconstituted and grown prior to the challenge following a previously published procedure.[7] Steers not challenged with *M. haemolytica* were intratracheally dosed with 10 mL of a phosphate–buffered saline[a] (PBS) as described[8] with some modifications. Briefly, steers were restrained and a broncheoalveolar lavage (BAL) tube[b] was inserted into the ventral meatus of a nostril, passed on into the trachea, and advanced to the tracheal bifurcation. At the tracheal bifurcation, the tube was removed approximately 2-3cm. The challenge material was then delivered such that material would be allowed to enter both lung fields. Calves were observed for any adverse effects of the challenge procedure itself. The broncheoalveolar lavage tube was sanitized with weak chlorhexidine diacetate[c] solution, rinsed with saline, and was re-used for animals on the same treatment only. Challenge with *M. haemolytica* occurred on the same day for all appropriate treatment groups beginning at 0800. To facilitate sample collection, steers were blocked by body weight into 2 groups of twelve, and the challenge procedures and sample collections for each weight block were staggered by a 2 week interval.

The experiment proceeded 28 days during which the animals were kept in individual pens (3.7×3.7 m) with the exception of days 0 to 4, 7 to 11 and 14 to 18. During those days, animals were placed in individual metabolic stanchions to allow for the collection of total urine and feces. During the experiment, steers were offered feed for ad libitum intake

with feed delivered twice daily. The diet contained 46% dry rolled corn, 9% corn dried distiller's grains, 40% alfalfa hay, 1% liquid supplement and 4% dry supplement, and was formulated to meet or exceed nutrient requirements.[9]

Data Collection

Rectal Temperature, Respiration Rate, and Subjective Clinical Score. Rectal temperatures were recorded using a digital veterinary thermometer[d] and respiration rates were measured by counting flank movements for 1 minute with a stopwatch.[10] In addition, all steers were monitored by trained personnel throughout the length of the experiment for clinical signs consistent with BRD. The visual evaluation utilized in this experiment was performed using the standard protocol for our research facility.[11] Briefly, the subjective criteria included depression (e.g., hanging head, sunken eyes, arched back and difficulty getting up from lying down), abnormal appetite, and respiratory signs (e.g., labored breathing, head and neck extended). Based on the severity of the signs, the evaluator assigned a numeric score ranging from 1 to 4, where 1 was assigned for mild, 2 for moderate, 3 for severe, and 4 for moribund (steer would not rise from recumbency or assistance was needed). Rectal temperature, respiration rate, and subjective clinical score were recorded prior to BVDV exposure and *M. haemolytica* challenge and at 4, 6, 12, 18, 24, 36, 48, 72 and 96 hours following the intratracheal bacterial challenge.

Serum Haptoglobin. Blood samples were collected via jugular venipuncture with an 18 gauge × 1 inch needle into an evacuated tube for serum harvest[e] on days -3, 0 (prior to and 2, 4, 6, 12, 18, 24, 36, 48, 72 and 96 hours following the 0 hour challenge), 7, 17 and 28. Samples collected were allowed to clot for 24 h at 4^{o}C. After the clotting time, chilled blood samples were centrifuged at 3,000 × g at 40^{o}C for 20 min. Serum was

harvested in 2 mL centrifuge tubes and stored at -20°C until further analyses were performed. Once all the serum samples were collected, a bovine haptoglobin ELISA test[f] was used to determine the haptoglobin concentration of each serum sample. Prior to the analysis, serum samples were diluted 1:10,000 in Tris-buffered saline.[g] The intra- and inter-assay coefficients of variation were below 5%.

Cytokines. Before cytokine analysis, all serum samples were diluted 1:1 in Tris-buffered saline.[g] After samples were diluted, cytokine [interferon γ (IFNγ), interleukin 1β (IL-1β), interleukin 4 (IL-4), interleukin 6 (IL-6), and tumor necrosis factor α (TNFα)] concentrations in serum were measured in duplicate with commercially available ELISA kits (IFNγ[h], IL-1β[i], IL-4[j], IL-6[k], and TNF-α[l]) with reagents provided by the manufacturer unless otherwise specified. Briefly, 96 well plates[m] were coated overnight at room temperature with the appropriate bovine specific coating antibody diluted in Tris-buffered saline.[g] After the incubation, the coating solution was aspirated followed by the addition of 300 μL of ELISA ultra block solution[n] to each well, and incubated at room temperature for 1 hour. This step was followed by aspiration of the blocking solution, addition of 100 μL of samples or standards to wells, and incubation at room temperature for 1 hour. Following sample incubation, standards and samples were aspirated and a three-step wash was performed using a manual plate washer.[o] Three hundred microliters of ELISA wash buffer[p] were added during each wash followed by aspiration. Following the initial three-step wash, the appropriate detection antibody was added to each well and incubated for 1 hour at room temperature, followed by the three-step wash as described before. Following the washing step, 100 μL of horse radish peroxidase-labeled streptavid

in Tris-buffered saline[g] were added to each well and incubated at room temperature for 30 min, followed by a final three-step wash.

For the colorimetric determination of cytokines, 100 μL of 3,3',5,5'-tetramethylbenzidine substrate solution[q] were added to each well and incubated for 20 min at room temperature followed by the addition of 100 μL of stop solution[r] and optical density was measured at 450 and 550 nm using a plate reader.[s] Detection limits for the cytokine procedures were: 2,000 to 31 pg/mL for IFNγ and IL-1β; 1,000 to 17 pg/mL for IL-4; 5,000 to 78 pg/mL for IL-6; and 8,500 to 133 pg/mL for TNFα based on maximum and minimum concentrations of the recombinant bovine cytokine standards, respectively. Concentrations of cytokines in serum samples were calculated by subtracting the 450 nm absorbance from the 550 nm absorbance to account for any optical imperfections in the plates. Subsequently readings were subtracted from the blank and a 4-point parameter logistic method standard curve was developed for each plate to calculate the concentrations of the cytokine in each sample. All intra-assay coefficients of variation were lower than 5% and inter-assay coefficient of variation below 7.5%.

Hemogram. Blood samples were collected via jugular venipuncture with an 18 gauge × 1 inch needle into an evacuated tube containing EDTA[t] on days -3 and at 0, 6, 18, 36, 96 and 168 hours following the *M. haemolytica* challenge. Samples were immediately submitted to a commercial laboratory[u] for total and differential white blood cell count determination, total red blood cells, total platelets, hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) concentration.

Blood gas, pH, Glucose and Lactate. Blood samples were collected via jugular venipuncture with an 18 gauge × 1 inch needle into an evacuated tube containing lithium

heparin[v] on days -3, 0 (prior to and 2, 4, 6, 12, 18, 24, 36, 48, 72 and 96 hours following the 0 hour challenge), 7, 17, and 28. These samples were immediately analyzed using a blood gas analyzer[w]. Response variables for blood gas analysis included partial pressure of carbon dioxide, sodium, potassium, calcium, glucose, lactate, hematocrit, bicarbonate ion, carbonic acid, carbon dioxide, sulfur dioxide, hemoglobin, and base excess of extracellular fluid. After the analysis, samples were centrifuged at 3,500 × g at 4^{o}C for 30 min and 1.5 mL of plasma were harvested and stored in plastic tubes at -20^{o}C.

Mannheimia haemolytica. Serum samples from days -3, 0, 4, 7, 14 and 28 were used to determine antibodies to whole bacterial cell and leukotoxin for a formalin killed *M. haemolytica* S1 by an ELISA test as described in previous studies.[12, 13] Antibody responses were expressed as nanograms of immunoglobulin binding based on a set of IgG standards on each plate. The intra- and inter-assay coefficients of variation were below 5%.

Bovine viral diarrhea virus antibodies. Serum samples from days -3, 0, 4, 7, 17 and 28 were submitted to a commercial veterinary diagnostic laboratory[x] for BVDV serology using a virus neutralization test (VNT) in Madin-Darby bovine kidney cell monolayers in 96-well microtiter plates. The viruses used as challenge viruses in the VNT were CP BVDV1a (Singer strain), CP BVDV1b (TGAC 8HB), and CP BVDV2a (125-C). A 1:4 dilution was the lowest tested, and titers of less than 1:4 were considered negative.

Statistical Analyses

The experiment was designed as a randomized complete block with a 2 × 2 factorial arrangement of treatments; animal served as the experimental unit. Data for BVDV antibody titers, *Mannheimia haemolytica* whole cell and leukotoxin antibody titers, rectal

temperatures, haptoglobin, cytokines, hemogram, blood gas, pH, glucose and lactate were analyzed using repeated measures analysis of the MIXED procedure of SAS[y] with a non-structured covariance structure and slice output option. The model for all variables included the main effects of BVD, MH, BVD × MH, and all possible interactions with time. When a BVD or MH × time interaction was significant ($P \leq 0.05$), the slice output option was used to determine the time points at which the time effect was different across treatments. In addition, when a BVD × MH interaction was significant ($P \leq 0.05$), Least squares means were separated using the pdiff statement of SAS.

RESULTS

There was a BVD × time interaction ($P < 0.0001$) for BVDV1b antibody titers (Figure 1a). Antibody titers increased across time for steers exposed to steers PI with BVDV, and on day 28, BVDV1b antibody titers were greater ($P < 0.05$) for steers exposed to BVDV compared with steers not exposed to BVDV. In addition, there was a MH × time interaction ($P < 0.0001$) for *M. haemolytica* whole cell antibodies (Figure 2a). Antibody titers for *M. haemolytica* whole cells increased across time for steers challenged with *M. haemolytica*, and were greater ($P < 0.05$) on days 7, 17, and 28 following *M. haemolytica* challenge compared with steers not challenged with *M. haemolytica*. Antibodies for *M. haemolytica* leukotoxin responded with a BVD × MH × time interaction ($P = 0.001$; Figure 2b). *Mannheimia haemolytica* leukotoxin antibodies increased over time, and on days 7, 14, and 28 were greatest ($P < 0.05$) for MH steers, lowest for steers not challenged with *M. haemolytica*, and intermediate for BVD+MH steers.

Rectal temperatures of steers in response to exposure to BVDV and *M. haemolytica* challenge are shown in Figures 3a and 3b, respectively. Rectal temperatures were greater

for steers challenged with *M. haemolytica* from 2 to 24 hours following the challenge (MH × time interaction, $P < 0.0001$). In addition, there was a BVD × time interaction ($P = 0.02$). Rectal temperature was greater ($P < 0.05$) for steers exposed to steers PI with BVDV at 36, 48, and 72 hours after BVDV exposure compared with steers not exposed to BVDV.

Haptoglobin concentrations responded with a MH × time interaction ($P = 0.0007$; Figure 4a). Haptoglobin concentrations were greater ($P < 0.05$) for steers challenged with *M. haemolytica* from 18 to 96 hours following the challenge. There was no BVD × time interaction ($P = 0.93$) for serum haptoglobin concentrations (Figure 4b).

Total and differential white blood cell count data are shown in Table 1. There was a BVD × MH interaction ($P = 0.002$) for total white blood cells. This resulted from a decrease in total white blood cells for steers exposed to steers PI with BVDV but not challenged with *M. haemolytica*. Exposure to steers PI with BVDV ($P < 0.0001$) and not challenged with *M. haemolytica* ($P = 0.003$) decreased total white blood cells. In addition, there was a MH × time interaction ($P < 0.0001$; Figure 5a) for total white blood cells. Steers challenged with *M. haemolytica* had greater ($P < 0.05$) total white blood cells at 18 and 36 hours following *M. haemolytica* challenge. There was a BVD × MH interaction ($P = 0.01$) for neutrophils. Similar to total white blood cells, this resulted from a decrease in neutrophils (Table 1) for steers exposed to steers PI with BVDV but not challenged with *M. haemolytica*. Neutrophils were increased ($P < 0.0001$) when steers were challenged with *M. haemolytica* compared to control and BVD steers. In addition, neutrophils were greater ($P < 0.05$) for steers challenged with *M. haemolytica* compared with steers not challenged with *M. haemolytica* at 18, 36, and 168 hours after the *M.*

haemolytica challenge (MH × time interaction, $P < 0.0001$; Figure 5b). Both exposure to steers PI with BVDV ($P = 0.002$) and *M. haemolytica* challenge ($P < 0.0001$) decreased lymphocytes (Table 1). Lymphocytes were lower ($P < 0.05$) for steers challenged with *M. haemolytica* compared with steers not challenged with *M. haemolytica* at 18, 36, and 96 hours after the *M. haemolytica* challenge (MH × time interaction, $P < 0.05$; Figure 5c). Monocytes were not affected ($P \geq 0.50$) by exposure to BVDV or *M. haemolytica* (Table 1). However, exposure to steers PI with BVDV decreased ($P = 0.03$) and challenge with *M. haemolytica* increased ($P = 0.01$) eosinophils. Similarly, basophils were decreased ($P = 0.0007$) by exposure to BVDV, but were increased ($P = 0.001$) by *M. haemolytica*. Hematocrits tended ($P = 0.08$) to be decreased by challenge with *M. haemolytica* (Table 1), whereas hemoglobin was decreased ($P < 0.0001$) by *M. haemolytica*. A BVD × MH interaction ($P = 0.02$) resulted from a greater decrease in hemoglobin concentration when steers challenged with *M. haemolytica* were not exposed to steers PI with BVDV than when steers challenged with *M. haemolytica* were exposed to BVDV. Mean corpuscular volume and hemoglobin concentration were decreased ($P \leq 0.05$) by challenge with *M. haemolytica*. Similar to hemoglobin, a BVD × MH interaction ($P = 0.03$) resulted from a greater decrease in mean corpuscular hemoglobin concentration when steers challenged with *M. haemolytica* were not exposed to steers PI with BVDV than when steers challenged with *M. haemolytica* were exposed to BVDV. Concentration of platelets were not affected ($P \geq 0.28$) by exposure to steers PI with BVDV or challenge with *M. haemolytica*. However, red cells were decreased ($P = 0.04$) by *M. haemolytica*. Cytokine data are shown in Table 2. There was a BVD × MH interaction ($P = 0.02$) for IFNγ. This resulted from an approximately twofold increase in IFNγ concentration for

BVD+MH steers compared with steers on the remaining treatments. Exposure of steers to steers PI with BVDV tended ($P = 0.06$) to increase IFNγ and challenge with *M. haemolytica* increased ($P = 0.02$) IFNγ. Interleukin-1β was increased by exposure to steers PI with BVDV ($P = 0.04$) and challenge with *M. haemolytica* ($P = 0.0006$). In addition, IL-4 tended ($P = 0.08$) to be increased by exposure to steers PI with BVDV. There was a BVD × MH interaction ($P = 0.01$) for IL-6. Interleukin-6 was greatest ($P < 0.05$) for BVD steers, lowest for control steers, and intermediate for steers challenged with *M. haemolytica*. In addition, IL-6 was increased ($P = 0.04$) by exposure to steers PI with BVDV. Both exposure of steers to steers PI with BVDV ($P = 0.0005$) and challenge with *M. haemolytica* ($P = 0.0009$) increased serum concentrations of TNFα. In addition, TNFα concentration was greatest ($P < 0.05$) for BVD+MH steers, lowest for control steers, and intermediate for BVD and MH steers (BVD × MH interaction, $P = 0.01$). Clinical score, blood gas and metabolite data are shown in Table 3. Exposure to BVDV or *M. haemolytica* did not affect ($P \geq 0.18$) blood pH, calcium, potassium, glucose, and sulfur dioxide concentrations, or base excess in blood. Subjective clinical score was greater ($P < 0.0001$) in steers challenged with *M. haemolytica* than in steers not challenged with *M. haemolytica*. There was a BVD × MH interaction ($P = 0.01$) for respiration rate. Challenge with *M. haemolytica* decreased respiration rate when steers were not exposed to steers PI with BVDV and increased respiration rate when steers were exposed to steers PI with BVDV. In addition, there was a BVD × MH interaction ($P = 0.005$) for plasma sodium concentration. Challenge with *M. haemolytica* decreased sodium to a greater extent when steers were not exposed to steers PI with BVDV compared with steers exposed to BVDV. Plasma lactate concentration was decreased (*P*

= 0.02) when steers were exposed to steers PI with BVDV. Base excess in extracellular fluid was increased ($P = 0.01$) by exposure to BVDV, and was decreased ($P = 0.0002$) by challenge with *M. haemolytica*. There were BVD × MH interactions ($P \leq 0.04$) for plasma carbon dioxide, carbonic acid, and sodium bicarbonate concentrations. Challenge with *M. haemolytica* decreased carbon dioxide, carbonic acid, and sodium bicarbonate concentrations to a greater extent when steers were not exposed to steers PI with BVDV compared with steers exposed to BVDV. Carbon dioxide and sodium bicarbonate concentrations acid were decreased ($P \leq 0.04$) by exposure to steers PI with BVDV, whereas carbonic acid concentration was increased ($P = 0.02$). Challenge with *M. haemolytica* resulted in MH × time interactions ($P \leq 0.03$) for carbon dioxide (Figure 6a), carbonic acid (Figure 6b), and sodium bicarbonate (Figure 6c), generally resulting from decreased ($P < 0.05$) concentrations from 4 to 48 h after the challenge. There was a BVD × MH interaction ($P = 0.0006$) for partial pressure of carbon dioxide. Challenge with *M. haemolytica* decreased partial pressure of carbon dioxide to a greater extent when steers were not exposed to steers PI with BVDV compared with steers exposed to BVDV.

DISCUSSION

Although previous research has evaluated the effects of BVDV and *M. haemolytica* challenge on immune response of calves,[14,15] in those studies calves ranged from 9 to 18 weeks of age and the virus of interest was directly inoculated into the nostril. In order to better understand BVDV pathogenesis and to study efficacy of BVDV vaccines, a natural exposure model has been recommended as opposed to direct virus inoculation to the animals.[16] Therefore, in the present experiment, time exposure of healthy animals to

steers PI with BVDV was an attempt to simulate a commercial feedlot operation where animals from different sources might be commingled and then tested for BVDV at arrival. Generally, if an animal tests positive for being PI with BVDV it is removed after a 48-hour assay result time and potentially an additional 24 hours from receiving the results to removing the PI calf from the pen. In the present experiment, increased serum BVDV antibody concentrations 28 days after exposure to animals PI with BVDV compared with non-exposed animals confirmed that animals became infected with BVDV without direct inoculation of the virus. This BVDV infection in the present study could be either by direct contact with body fluids of a PI steer, ambient air, or by sharing the feedbunk.[17]

Mannheimia haemolytica has been shown to have several pathogenic factors including outer membrane proteins[18] and leukotoxin.[19] In the present experiment, total antibody production to *M. haemolytica* whole cell increased across time in challenged steers compared with steers that received the PBS solution only (Figure 1b). It has been previously reported that antigen load can influence antibody production.[20] Therefore, the lack of a BVD × MH interaction in the present experiment might suggest that all steers challenged with *M. haemolytica* received the same amount of bacteria during the challenge. Leukotoxin is secreted during the logarithmic growth phase of *M. haemolytica*.[21] In the present experiment, the BVD × MH × time interaction for *M. haemolytica* leukotoxin (Figure 1c) suggests that animals exposed to steers PI with BVDV had decreased antibody production to *M. haemolytica* leukotoxin. Our results are in agreement with a previous study[22] in which animals that were challenged with bovine immunodeficiency virus had a decreased and delayed post-vaccinal antibody production

to BVDV and bovine herpes virus-1. Higher antibody production has been reported as an indication of better protection induced by vaccines.[23] The present and previously published data[22,23] suggests that exposure of healthy animals to a virus that produces an immunosuppression might predispose animals to secondary bacterial infections due to decreased antibody production. Decreased antibody production may also have a detrimental effect on the efficacy of vaccines, which could subsequently have a negative effect on animal health and performance.[22]

Morbid animals exhibit distinct behavioral patterns caused in part by changes in body temperature.[24,25] In the present experiment, animals challenged with *M. haemolytica* developed an acute increase in rectal temperature during the first 24 hours post *M. haemolytica* challenge compared with animals that were not challenged. Rectal temperature (Figure 2a) was significant reaching a maximum of 41.5^{o}C 6 hours after the challenge. Our results are in agreement with previous results[26] in which animals that were challenged with *M. haemolytica* had an increased rectal temperature compared with control animals. Similarly, it was reported that animals challenged with *M. haemolytica* had increased rectal temperature (>40^{o}C) during the time elapsed between 0 to 12 hours and returned to normal by 24 hours.[27] In the present experiment, there was no effect of exposure to steers PI with BVDV on rectal temperature during the first 24 hours following the intratracheal challenge. However, from 36 to 72 hours animals that had been exposed to steers PI with BVDV had a higher rectal temperature (Figure 2b) compared with animals that were not exposed. The rectal temperature during this period ranged from 39.3 to 39.5^{o}C for the steers exposed to BVDV. In a previous study, a higher rectal temperature was reported in non-vaccinated animals challenged with BVDV

type 2 from days 9 to 11 after intranasal challenge compared with a vaccinated group.[23] In addition, animals challenged with non-cytopathic type 1 BVDV had a mild fever (>39.5^{o}C) from days 1 to 5 after viral inoculation when compared to non-challenged animals.[15] These results suggest that increased rectal temperature can result from either direct inoculation of known amounts of BVDV virus into nostrils of calves or from exposure of healthy calves to animals PI with BVDV.

Haptoglobin is an acute phase protein produced mainly by the liver in response to pro-inflammatory cytokines including TNFα, IL-1 and IL-6 secreted by bronchoalveolar macrophages in response to bacterial infection.[28,29] In the present experiment, haptoglobin concentrations in animals that were exposed to PI steers were no different when compared with animals that were not exposed. In a previous study, calves that were challenged directly into the nostrils with a non-cytopathic type 1 BVDV had higher haptoglobin concentrations than control animals.[14] These conflicting results could be attributed to differences in the challenge model used in the previous experiment[14] compared with the natural exposure model used for the present experiment. For animals that were challenged with *M. haemolytica* in the present study, the increase in haptoglobin concentration was acute starting at 18 hours post challenge and continued to be greater until returning to baseline concentrations after 96 hours of the bacterial inoculation. These results are in agreement with haptoglobin concentrations in response to *M. haemolytica* challenge in cattle.[14,27] Increased haptoglobin concentrations have been associated with clinical cases of BRD and number of antimicrobial treatments required for animals during the disease.[30, 31]

The presence of PI BVDV animals in feedlot pens has been associated with increased BRD morbidity and mortality during the finishing period.[2] Bovine viral diarrhea viruses involved in BRD can cause clinical infections by themselves,[32] but also have been reported to increase cattle's susceptibility to secondary bacterial infections from common inhabitants of the respiratory tract of healthy animals.[33] When correlated with a clinical evaluation, leukograms can provide informative evaluation of disease processes.[34] In the present study, animals exposed to steers PI with BVDV had decreased total WBC counts compared with non-exposed animals. After the *M. haemolytica* challenge, animals that had been exposed to PI steers had a decreased total WBC count at 18, 36 and 96 hours post challenge compared with steers that were not exposed to PI animals but were challenged with *M. haemolytica*. In addition, steers that were challenged with *M. haemolytica* had a marked increase in neutrophil counts during the first 36 h post inoculation compared with animals that were not challenged. These results are in agreement with previous observations[15, 27] and show that an intratracheal *M. haemolytica* challenge can produce an acute immune response during the first 24 hours after the challenge.

Both exposure to BVDV and *M. haemolytica* challenge resulted in steers having decreased lymphocytes. These results are in contrast with a previous report[27] in which decreased lymphocyte counts during 138 hours post inoculation with *M. haemolytica* were observed. However, decreased lymphocyte counts have been reported when animals were challenged with *M. haemolytica* alone.[15] Bovine viral diarrhea viruses have been reported to have high affinity to cells of the immune system[35] and a transient decrease in the total number of lymphocytes has been reported in animals with BVDV

infection.[36,37] This decrease in lymphocyte counts could be an explanation for the decreased *M. haemolytica* leukotoxin antibody concentrations observed in our experiment when animals were exposed to PI steers before the *M. haemolytica* challenge compared with animals not exposed to BVDV. Eosinophils and basophils were affected by exposure to steers PI with BVDV and challenge with *M. haemolytica*. These results are in agreement with a previous study;[27] however, the clinical relevance of the response is unknown.

Cytokines are soluble proteins involved in a wide variety of biological processes[38] influencing energy and protein metabolism.[39] Increased cytokine concentrations are thought to result in repartitioning of energy and amino acids away from adipose and skeletal muscle growth by providing substrates for higher priority immunological functions.[39,40] The primary mechanism of action of cytokines appears to be up or down-regulation of genes involved in specific intracellular transduction pathways.[41] A greater understanding of cytokine response to the most common pathogens could provide valuable information of the implications of these proteins for decreasing performance and carcass characteristics in animals that suffer from BRD during the growing and finishing period.[42,43] Interferon gamma has been reported as the link between the innate and adaptive immune systems and is critical for an adequate response to intracellular pathogens.[38] The main source of IFNγ is thought to be lymphocytes.[44] In the present experiment, serum concentrations of IFNγ tended to increase in response to exposure to steers PI with BVDV, although the total number of lymphocytes decreased during the first 18 hours after *M. haemolytica* challenge. Although unknown, the lower lymphocyte count might have affected the absolute concentration of IFNγ during the length of the

experiment. Challenge with *M. haemolytica* increased serum concentrations of IFNγ in the present experiment. It has been suggested that the effect of IFNγ results from macrophages and neutrophils enhancing phagocytosis of extracellular pathogens (bacteria) and inducing a respiratory burst and nitric oxide production which has microbiocidal effects.[44,45] Therefore, the increased concentration of IFNγ in the *M. haemolytica* treatment group might have been due to the increased number of circulating neutrophils in response to the bacterial infection 6 hours after *M. haemolytica* challenge in the present experiment. The greater IFNγ concentration for the BVD+MH group might be explained as an additive effect of increased IFNγ secretion from major cells affected in response to the BVDV and *M. haemolytica* challenge.

Interleukin-1 is one of the most potent endogenous inducers of fever and the induction of this cytokine has been associated with viral, bacterial, fungal and parasitic infections.[46] In addition, IL-1 has been reported as one of the principal inducers of acute phase protein synthesis.[47] Although IL-1 is expressed as both IL-1α and IL-1β, they have been differentiated as either intracellular (IL-1α) or secreted by the cells (extracellular; IL-1β).[38] Therefore, IL-1β was measured in the present experiment. In the present experiment, steers that were exposed to PI calves for 72 hours had increased concentrations of serum IL-1β compared with non-exposed animals. However, in contrast to previous reports,[46, 47] the increased concentration of serum IL-1β in BVD steers was not associated with rectal temperatures greater than 40.0^{o}C or greater concentrations of haptoglobin.[46,47] These conflicting results could be explained by different concentrations of circulating IL-1β. Recombinant IL-1β administered to beef calves suggested that the effect of IL-1β on rectal temperature was dose dependant.[48]

Only animals dosed with 333 and 1,000 ng/kg of IL-1β had a mean rectal temperature greater than 40.0°C compared with animals dosed with 10, 33 and 100 ng/kg. Maximum rectal temperature was observed at 6 hours for all treatments and returned to baseline by 24 hours post IL-1β injection. In addition, haptoglobin concentration was increased above 0.50 mg/mL in animals receiving 1,000 ng/kg of recombinant IL-1β starting at 24 hours post injection, and haptoglobin concentration remained elevated for 72 hours. Serum concentrations of IL-1β were not reported in that study.[48] Results suggest that the pyrogenic and acute phase induction of IL-1β is dose dependant. In the present experiment, although animals experiencing infection with BVDV due to exposure to PI calves had increased rectal temperature when compared with non-exposed animals, the increase in IL-1β may not have been enough to result in an increase in rectal temperature above 40.0°C or to stimulate hepatocytes to increase haptoglobin synthesis. Challenge with *M. haemolytica* increased IL-1β, haptoglobin, and rectal temperature in the present experiment. Interestingly, the tendency for an interaction between BVD and MH may indicate the possibility of an additive effect in the intensity of the immune response when both pathogens were utilized in the challenge.

Limited information exists regarding the role of IL-4 in beef cattle.[49] This cytokine has been described as an anti-inflammatory cytokine which inhibits the production of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα).[50,51] In a recent report,[43] decreased concentrations of IL-4 were detected in animals fed a 30% concentrate diet compared with animals fed 70% concentrate when challenged with LPS and provided an antimicrobial injection. The same study[43] observed higher concentrations of IFNγ, TNFα and IL-6 and concluded that the increased concentrations of these pro-inflammatory

cytokines could be associated with the decreased concentrations of IL-4. In the present experiment, serum concentrations of IL-4 tended to be increased along with pro-inflammatory cytokines in steers exposed to BVDV compared with control steers, which is in contrast to previous reports. It has also been suggested that when IL-4 is over expressed it can have detrimental effects on the immune system by decreasing the recruitment, expansion or activity of TH1 cells, resulting in a strong TH2 bias.[52] This increased TH2 relative to TH1 might interfere with viral clearance by the host and suppress the innate immune response predisposing the host to secondary infections.[52] Therefore, increased IL-4 may decrease viral clearance and predispose animals to secondary infections resulting in increased morbidity and mortality when feedlot cattle are exposed to BVDV.

In pre- and post-partum dairy cows, an increase in IL-6 has been associated with an increase in the TH2 population.[53] Steers exposed to BVDV or challenged with *M. haemolytica* in the present experiment had greater IL-6 concentrations than control animals, although the greatest IL-6 concentration occurred for animals exposed to BVDV but not challenged with *M. haemolytica*. Our data is in agreement with results showing increased concentrations of IL-6 when steers were challenged with LPS starting at 2 hours post LPS infusion and returning to baseline within the first 24 hours of the LPS challenge.[43] Interleukin-6 has been reported to induce fever and stimulate hepatocytes to synthesize acute phase proteins.[54,55] In the present experiment, haptoglobin concentrations were increased for steers challenged with *M. haemolytica*, but not for steers exposed to BVDV. Therefore, additional mechanisms that induce fever and synthesis of acute phase proteins likely exist. For example, it has been reported that the

injection of IL-6 in IL-6 knockout mice had no or a very mild pyrogenic effect compared with injecting the same mice with IL-1β suggesting that other neuromodulators (e.g., prostaglandin E2) are involved in modulating fever.[56]

Increased concentrations of TNFα in milk and serum of cows suffering from mastitis has been reported, and the increased concentration of TNFα was associated with the severity of the clinical manifestation of the disease.[57] In the present experiment, TNFα concentrations in serum were greatest in animals exposed to BVDV and challenged with *M. haemolytica*, although subjective clinical scores did not respond with the same interaction and were higher in steers challenged with *M. haemolytica* regardless of exposure to BVDV. During LPS challenge, septic shock has been associated with endotoxemia[40] which has resulted in death of challenged animals.[43] In addition, when dogs and cats were infused with recombinant human TNFα, a similar endotoxic response was observed; however, recombinant anti-TNF monoclonal antibodies prevented the induction of septic shock during acute bacteremia in baboons.[58] Increased concentrations of TNFα might partially explain the increased mortality in calves with multiple BRD pathogens isolated from lungs.[59] In addition, due to the systemic effects of TNFα[60] detrimental effects on meat quality could be observed with increased severity of BRD. Decreasing the number or severity of pro-inflammatory cytokine responses might be helpful for decreasing economic losses associated with decreased carcass quality in feedlot cattle treated for BRD.[3]

In contrast to a previous report where increased plasma lactate concentrations were correlated with BRD morbidity,[61] plasma lactate was decreased in animals that were exposed to steers PI with BVDV in the present experiment. In the earlier study,[61] no

changes in plasma lactate in animals receiving one treatment for BRD were reported, but increased plasma lactate was measured in animals suffering from BRD 24 hours prior to death. The rationale for these results was an increased anaerobic metabolism by animals during a BRD event due to decreased lung capacity, and the decreased O_2/CO_2 exchange changing the metabolism of glucose at the muscle level toward lactate production as the end product of glycolysis in the absence of O_2. In addition, *M. haemolytica* challenge was reported to decrease plasma lactate for 72 hours post *M. haemolytica* challenge,[27] whereas in the present study, *M. haemolytica* challenge had no effect on plasma lactate concentrations. To our knowledge, there are no previous studies in the literature that provide greater insight into the possible effects of BVDV or *M. haemolytica* on plasma lactate concentrations in feeder calves. However, it was reported that animals that received at least one antimicrobial treatment against BRD during a 36-day preconditioning program had lower plasma lactate concentrations at arrival.[62] Pathogens involved in BRD cases were not identified and no direct comparisons with our results can be made. Plasma lactate can be increased during cases of ruminal acidosis.[63] Both ruminal acidosis and BRD have been associated with decreased or altered DMI. Due to similar clinical signs, ruminal acidosis should be considered in the list of differential diagnosis of BRD in field conditions.[64] However, ruminal pH measured in calves pulled or pulled and treated against BRD showed no differences between experimental groups.[65] Increased respiration rate is an important thermoregulatory mechanism for preventing heat overload in cattle via evaporative cooling.[66] In the present experiment, respiration rate of control steers was greatest compared with steers on the remaining treatments. In cattle exposed to heat stress, the initial response was an increased respiration rate, which

changed to panting midway through the heat stress period followed by a deep open mouth respiration at a reduced rate.[67] Interestingly, decreased respiration rate in our challenge model was present for steers on all experimental treatments that had an increased rectal temperature compared with control steers. Changes in frequency and dynamics of respiration in exposed and/or challenged steers also corresponded with changes in blood gases for steers in the present experiment. Respiratory alkalosis has been described as increased alveolar ventilation resulting in excretion of carbon dioxide at a rate exceeding production,[68] which results in decreased partial pressure of carbon dioxide, increased blood pH, and decreased concentration of bicarbonate. Cattle exposed to heat stress have been reported to have an increased respiration rate, decreased partial pressure of carbon dioxide and bicarbonate concentration, with no change in blood pH.[69] In the present experiment, *M. haemolytica* challenge decreased partial pressure of carbon dioxide and carbon dioxide and bicarbonate concentrations during the first 48 hours post *M. haemolytica* challenge. The lack of an effect on blood gases due to BVDV exposure might be due to the lack of a direct effect of this virus on the host animal's respiratory capacity and gas exchange, especially when compared to other viral or bacterial infections associated with BRD. Interestingly, despite changes in blood gases there was no effect of our infectious challenge or heat stress[69] on blood pH, reflecting the host animals' capacity to maintain homeostasis.

In conclusion, we observed changes in serum antibody production, total and differential white blood cell count, cytokine concentrations, and blood gases consistent with an immune challenge in beef cattle. Our results suggest that we were able to successfully develop an immune challenge model using exposure of naïve steers to steers PI with

BVDV, intratracheal challenge with *M. haemolytica*, or their combination which will allow us to further characterize the detrimental effects that BRD pathogens have on food producing animals. In addition, exposure to calves PI with BVDV and intratracheal challenge with *M. haemolytica* resulted in decreased antibody production to *M. haemolytica* leukotoxin and increased serum concentrations of IFNγ, IL-1β and TNFα, suggesting that exposure of naïve calves to PI calves increases potential for secondary infections and detriment to animal health and performance.

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PRODUCT SUPERSCRIPTS

a. Phosphate buffered saline pH 7.4, Sigma Aldrich.

b. Bivona Medical Technology, Gary, IN.

c. Nolvasan, Fort Dodge Animal Health, Overland Park, KS.

d. GLA M-500, GLA Agricultural Electronics, San Luis Obispo, CA.

e. Clott activator, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ.

f. Immunology Consultants Lab, Portland OR.

g. Tris-buffered saline with Tween 20 pH 4.0, Sigma-Aldrich, St Louis, MO.

h. Bovine IFNI gamma Screening Set, Pierce Protein Research Products, Thermo Scientific, Rockford, IL.

i. Bovine IL-1 beta ELISA Set, Pierce Protein Research Products, Thermo Scientific, Rockford, IL.

j. Bovine IL-4 Screening Set, Pierce Protein Research Products, Thermo Scientific, Rockford, IL.

k. Bovine IL-6 ELISA Set, Pierce Protein Research Products, Thermo Scientific, Rockford, IL.

l. Bovine TNF-alpha / TNFSF1A ELISA Development Kit, DuoSet, R&D Systems, Minneapolis, MN.

m. Microtiter 96-well plates, Thermo Scientific, Waltham, MA.

n. ELISA ultra block solution, AbD Serotec, Raleigh, NC.

o. Nunc Immuno Plate 12, Thermo Fisher Scientific, Rochester, NY.

p. ELISA Wash Buffer, AbD Serotec, Raleigh, NC.

q. TMB Solution, AbD Serotec, Raleigh, NC.

r. ELISA Stop Soution, AbD Serotec, Raleigh, NC.

s. Multiskan Spectrum, Thermo Scientific, Waltham, MA.

t. EDTA, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ.

u. Antech Diagnostics, Stillwater, OK.

v. Lithium heparin, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ.

w. GEM Premiere 3000, Instrumentation Laboratory, Lexington, MA.

x. Oklahoma Animal Disease Diagnostic Lab, Stillwater, OK.

y. SAS, Mixed Procedure, version 9.1, SAS Institute, Inc., Cary, NC.

Table 1. Effect of 72 h exposure to a PI BVDV calf with or without an intratracheal challenge with *M. haemolytica* on hemogram of steers

Item	No BVD		BVD		SEM	*P*-value		
	No MH	MH	No MH	MH		BVD	MH	BVD×MH
Total white blood cells, 10^3/µL	9.86[a]	9.83[a]	7.93[b]	9.48[a]	0.60	<0.0001	0.003	0.002
Neutrophils/µL	2,941[a]	3,977[b]	2,080[c]	4,045[b]	306	0.03	<0.0001	0.01
Lymphocytes/µL	6,341	5,215	5,340	4,827	338	0.002	<0.0001	0.09
Monocytes/µL	354	362	342	401	51.1	0.78	0.50	0.61
Eosionphils/µL	139	185	119	146	14.1	0.03	0.01	0.50
Basophils/µL	91	124	66	88	8.9	0.0007	0.001	0.53
Hematocrit, %	30.5	28.4	29.9	29.8	0.79	0.50	0.08	0.10
Hemoglobin, g/100 mL	11.4[a]	10.4[c]	11.1[ab]	10.7[bc]	0.12	0.55	<0.0001	0.02
Mean corpuscular volume, fL	38.7	36.7	38.2	37.6	0.51	0.67	0.01	0.20
Mean corpuscular hemoglobin, %	14.3[a]	13.5[b]	14.3[a]	14.1[a]	0.16	0.09	0.05	0.03
Platlets, 10^3/µL	487	506	517	524	33.1	0.28	0.56	0.78
Red cells, 10^6/µL	7.97	7.74	7.87	7.60	0.12	0.33	0.04	0.86

[a,b,c]Within a row means with different superscripts are different ($P < 0.05$).

Table 2. Effect of 72 h exposure to a PI BVDV calf with or without an intratracheal challenge with *M. haemolytica* on serum cytokine concentrations of steers

	No BVD		BVD			*P*-value		
Item	No MH	MH	No MH	MH	SEM	BVD	MH	BVD×MH
Interferon-γ, pg/mL	90[a]	86[a]	78[a]	181[b]	54.6	0.06	0.02	0.02
Interleukin 1β, pg/mL	75	157	90	329	89.2	0.04	0.0006	0.11
Interleukin 4, pg/mL	67	75	119	139	34.3	0.08	0.68	0.86
Interleukin 6, pg/mL	1,442[a]	1,966[b]	2,591[c]	2,003[b]	645	0.04	0.87	0.01
Tumor necrosis factor-α, pg/mL	367[a]	540[b]	583[b]	1,984[c]	319	0.0005	0.0009	0.01

[a,b,c]Within a row means with different superscripts are different ($P < 0.05$).

Table 3. Effect of 72 h exposure to a PI BVDV calf with or without an intratracheal challenge with *M. haemolytica* on clinical score, respiration rate, blood pH and gas and metabolite concentrations in steers

	No BVD		BVD			*P*-value		
Item	No MH	MH	No MH	MH	SEM	BVD	MH	BVD×MH
Subjective clinical score	0.00	0.34	0.05	0.45	0.04	0.99	<0.0001	0.57
Respirations per minute	58.0[a]	55.8[b]	54.8[c]	56.0[b]	3.50	0.16	0.02	0.01
pH	7.46	7.44	7.44	7.44	0.02	0.59	0.70	0.46
Calcium, m*M*	1.24	1.23	2.97	1.23	0.87	0.32	0.31	0.32
Potassium, m*M*	4.09	4.02	4.22	4.10	0.11	0.35	0.38	0.80
Sodium, m*M*	141[a]	139[b]	141[a]	140[ab]	0.70	0.002	<0.0001	0.005
Glucose, mg/100 mL	71.1	69.9	72.3	71.2	1.11	0.18	0.25	0.93
Lactate, m*M*	1.25	1.25	0.81	1.04	0.10	0.02	0.29	0.29
Sulfur dioxide, m*M*	69.9	71.8	70.7	70.5	1.90	0.74	0.36	0.30
Base excess in extracellular fluid, m*M*	6.51	4.88	6.98	6.10	0.48	0.01	0.0002	0.25
Carbon dioxide, m*M*[1]	32.1[a]	30.2[b]	31.8[a]	31.4[ab]	0.30	0.04	<0.0001	0.001
Carbonic acid, m*M*[1]	28.9[a]	27.9[b]	29.0[a]	28.7[a]	0.26	0.02	0.0007	0.04
Sodium bicarbonate, m*M*[1]	30.7[a]	28.9[b]	30.6[a]	30.1[a]	0.29	0.01	<0.0001	0.003
Partial pressure of carbon dioxide, mm/Hg	45.9[a]	42.1[b]	44.6[ac]	43.6[c]	0.41	0.77	<0.0001	0.0006
Base excess in blood, m*M*	5.78	5.44	5.85	5.50	0.52	0.90	0.50	0.99

[1]A BVD × MH × time interaction was detected ($P < 0.05$).

[a,b,c]Within a row means with different superscripts are different ($P < 0.05$).

Figures

Figure 1. Serum concentration of bovine viral diarrhea virus 1b (BVDV1b) neutralization antibody titers in calves exposed during 72 hours to two steers PI with BVDV (BVD) compared with non-exposed controls (No BVD). There was a BVD × time interaction ($P < 0.0001$; SEM = 275). Values plotted represent Least squares means ± standard error of the mean, calculated for 12 animals per experimental group. [a,b]Within day, Least squares means with different letters are different ($P < 0.05$).

Figure 2. Serum concentration of *M. haemolytica* whole cell antibodies (Figure 2a) and *M. haemolytica* leukotoxin antibodies (Figure 2b) in calves challenged intratracheally on d 0 with 6×10^9 CFU of *M. haemolytica* serotye 1 (MH) compared with non-challenged controls (No MH). There was a MH × time interaction ($P < 0.0001$; SEM = 0.36; Figure 2a) for *M. haemolytica* whole cell antibodies, and a BVD × MH × time interaction ($P < 0.0001$; SEM = 0.15; Figure 2b) for *M. haemolytica* leukotoxin antibodies. Values plotted represent Least squares means ± standard error of the mean, calculated for 12 animals per experimental group for *M. haemolytica* whole cell antibodies, and 6 animals per experimental group for *M. haemolytica* leukotoxin antibodies. [a,b,c]Within day, Least squares means with different letters are different ($P < 0.05$).

Figure 3. Rectal temperature of calves challenged intratracheally with 6×10^9 CFU of *M. haemolytica* serotye 1 (MH) compared with non-challenged controls (No MH; Figure 3a) or exposed during 72 hours to two steers PI with BVDV 1b (BVD) compared

with non-exposed controls (No BVD; Figure 3b). There was a MH × time interaction ($P < 0.0001$; SEM = 0.11; Figure 3a) and a BVD × time interaction ($P = 0.02$; SEM 0.11; Figure 3b). Values plotted represent Least squares means ± standard error of the mean, calculated for 12 animals per experimental group. [a,b]Within a day, Least squares means with different letters are different ($P < 0.05$).

Figure 4. Serum haptoglobin concentrations of calves challenged intratracheally with 6×10^9 CFU of *M. haemolytica* serotye 1 (MH) compared with non-challenged controls (No MH; Figure 4a) or exposed during 72 hours to two steers PI with BVDV 1b (BVD) compared with non-exposed controls (No BVD; Figure 4b). There was a MH × time interaction ($P < 0.0007$; SEM = 0.32; Figure 4a), and the BVD × time interaction was not significant ($P = 0.93$; SEM = 0.32; Figure 4b). Values plotted represent Least squares means ± standard error of the mean, calculated for 12 animals per experimental group. [a,b]Within a day, Least squares means with different letters are different ($P < 0.05$).

Figure 5. Total white blood cell (Figure 5a), neutrophil (Figure 5b) and lymphocyte (Figure 5c) counts of calves challenged intratracheally with 6×10^9 CFU of *M. haemolytica* serotye 1 (MH) compared with non-challenged controls (No MH; Figures 5a and 5b) or exposed during 72 hours to two steers PI with BVDV (BVD) compared with non-exposed controls (No BVD; Figure 5c). There were MH × time interactions ($P < 0.0001$, SEM = 0.71, Figure 5a; $P < 0.0001$, SEM = 417, Figures 5b) and a BVD × time interaction ($P < 0.01$; SEM = 445; Figure 5c). Values plotted represent Least squares

means ± standard error of the mean, calculated for 12 animals per experimental group. [a,b]Within a day, Least squares means with different letters are different ($P < 0.05$).

Figure 6. Carbon dioxide (Figure 6a), carbonic acid (Figure 6b), and sodium bicarbonate (Figure 6c) concentrations in whole blood of calves challenged intratracheally with 6×10^9 CFU of *M. haemolytica* serotye 1 (MH) compared with non-challenged controls (No MH). There were MH × time interactions ($P = 0.005$, SEM = 0.60, Figure 6a; $P = 0.03$, SEM = 0.50, Figure 6b; $P < 0.002$; SEM = 0.60, Figure 6c). Values plotted represent Least squares means ± standard error of the mean, calculated for 12 animals per experimental group. [a,b]Within a day, Least squares means with different letters are different ($P < 0.05$).

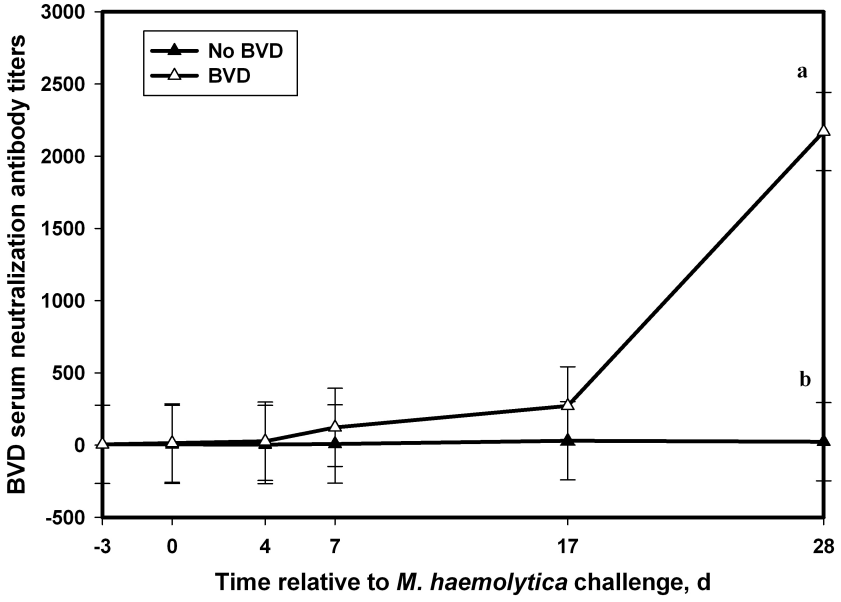
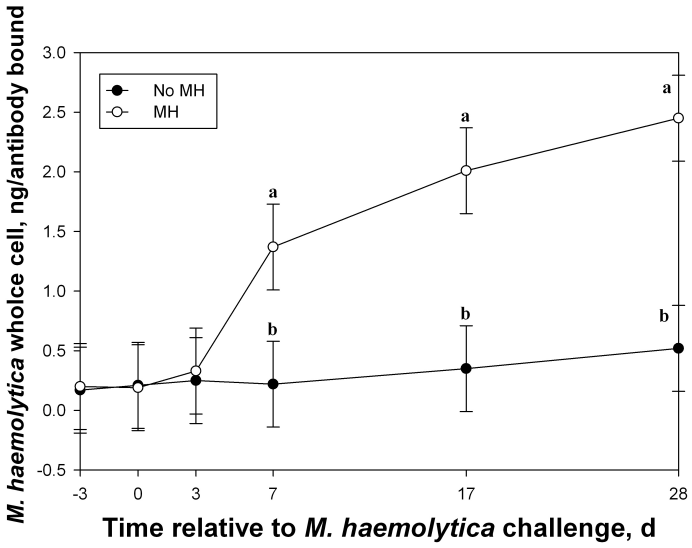

No BVD
BVD
a
b
BVD serum neutralization antibody titers
3000
2500
2000
1500
1000
500
0
-500
-3
0
4
7
17
28
Time relative to M. haemolytica challenge, d

Figure 1

3.2a



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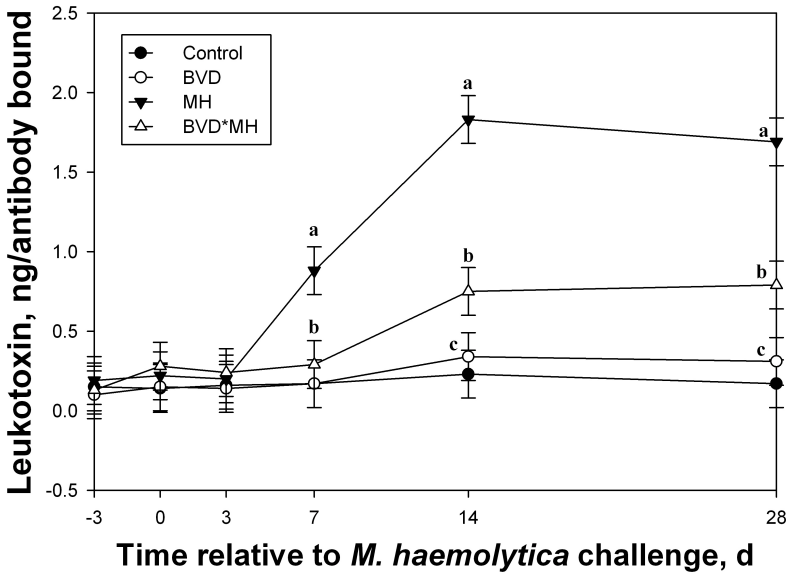
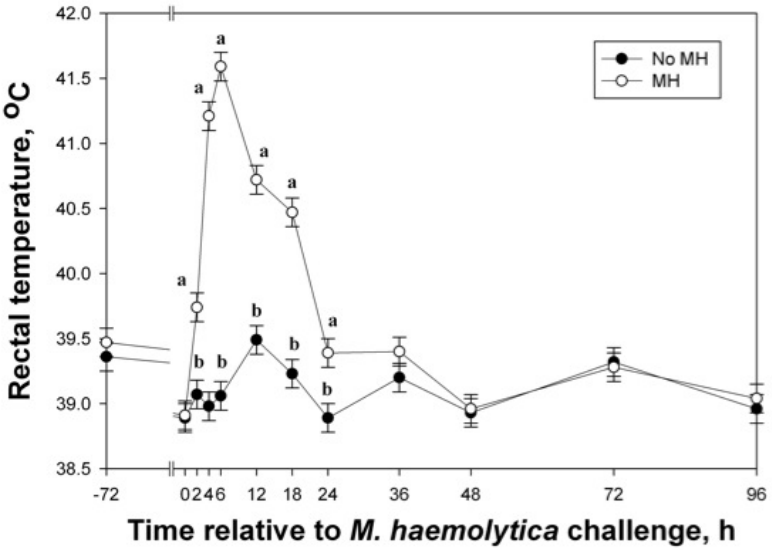


Figure 3.2

3.3a



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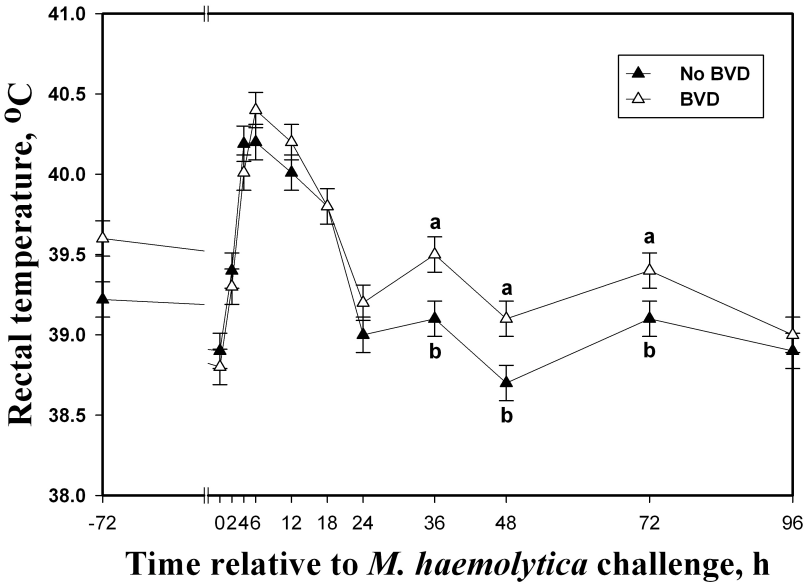
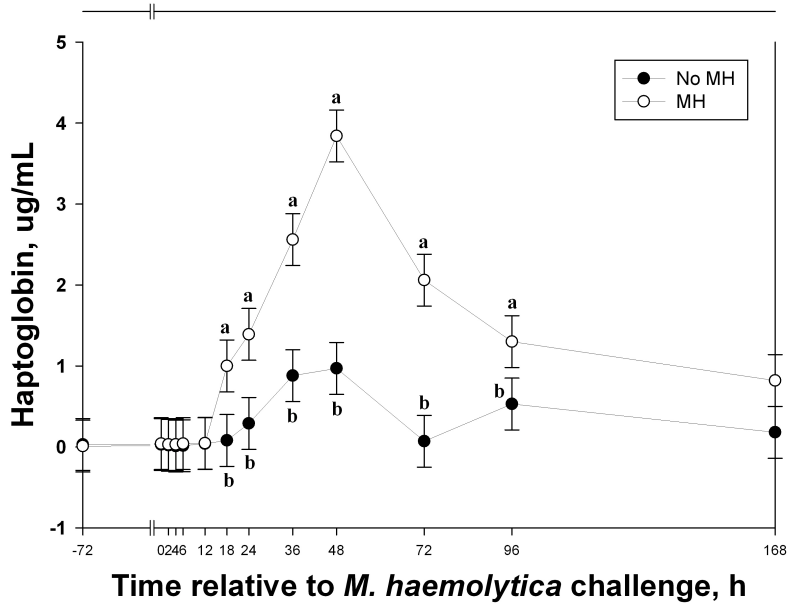


Figure 3.3

3.4a



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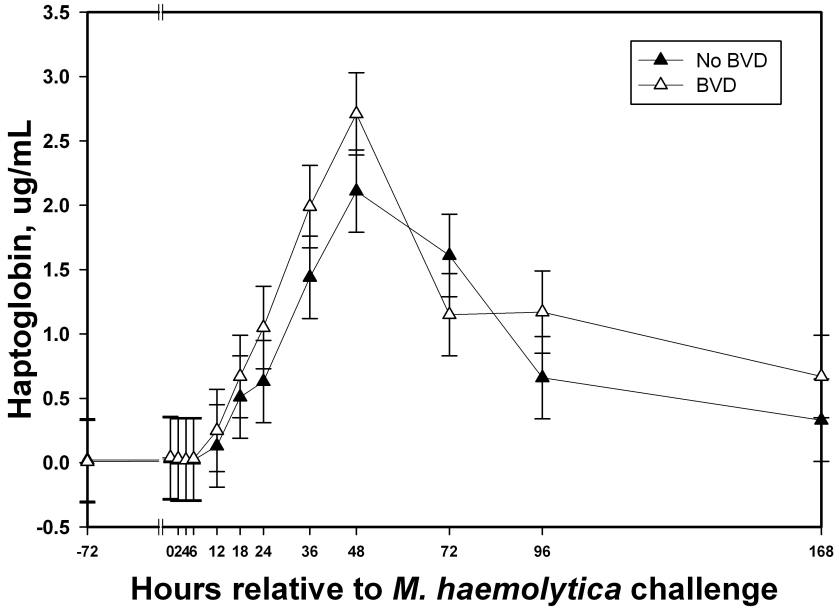
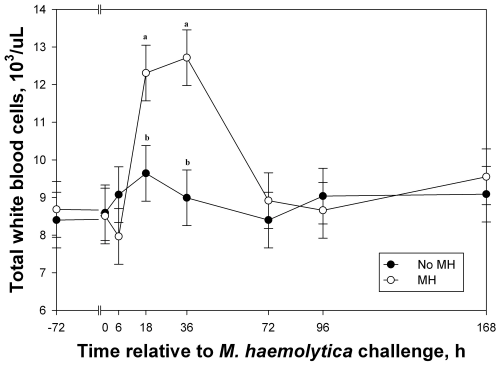


Figure 3.4

3.5a



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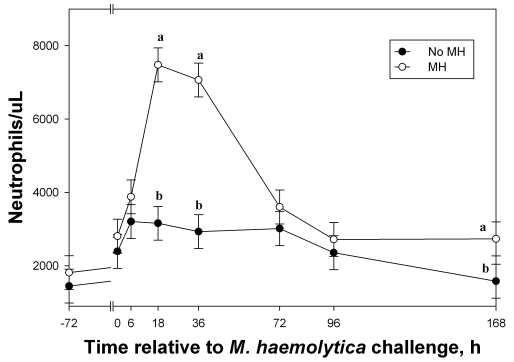

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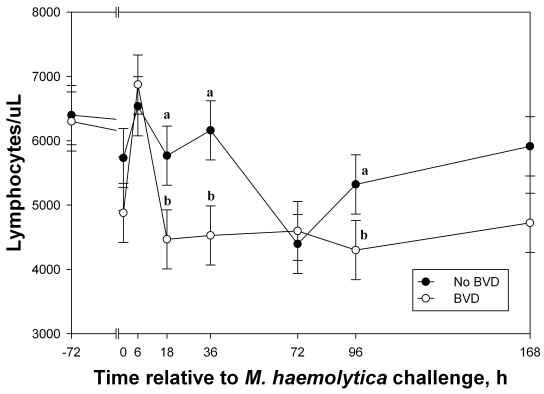
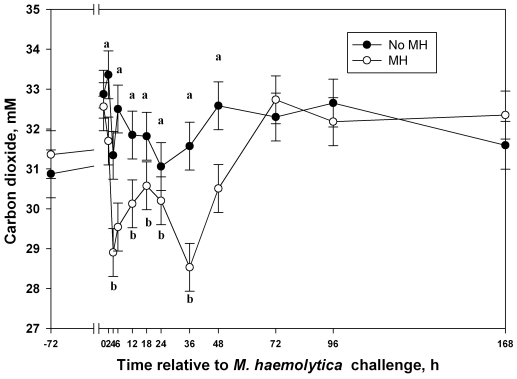
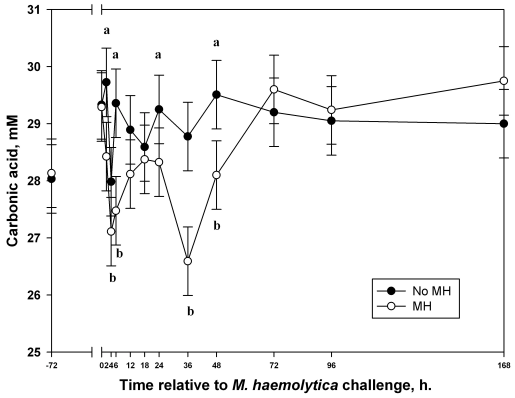


Figure 3.5

3.6a



3.6b



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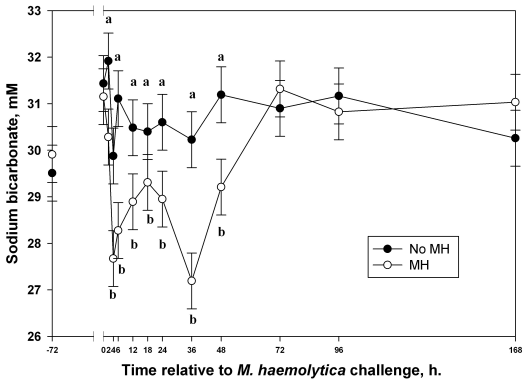

Figure 3.6

CHAPTER IV

EFFECTS OF EXPOSURE TO CALVES PERSISTENTLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS TYPE 1B AND *MANNHEIMIA HAEMOLYTICA* CHALLENGE ON ANIMAL PERFORMANCE, N BALANCE, AND VISCERAL ORGAN MASS IN BEEF STEERS

ABSTRACT: Bovine Viral Diarrhea viruses (BVDV) have been isolated alone or in combination with other viral and bacterial pathogens in animals diagnosed with Bovine Respiratory Disease (BRD), a disease causing major economic loss to the feedlot industry. The objective of this experiment was to determine the effects of *Mannheimia haemolytica* following short-term (72 h) exposure to BVDV persistently infected (PI) calves on performance, N balance and organ mass in finishing cattle. Treatments included: 1) steers not challenged with BVDV or *M. haemolytica* (Control); 2) steers challenged with 72 h exposure to steers PI with BVDV (BVD); 3) steers intratracheally challenged with *M. haemolytica* on d 0 (MH); and 4) steers challenged with 72 h exposure to steers PI with BVDV and intratracheally with *M. haemolytica* on d 0 (BVD+MH). Six steers/treatment (initial BW = 314 ± 31 kg) were used for the experiment. Steers were weighed every 28 d and were housed in metabolism crates during the first 5 d following the *M. haemolytica* challenge, the 2nd wk after the *M. haemolytica* challenge, and on d 28 to 32, d 56 to 60, and 7 to 10 d prior to slaughter

to determine N balance. At slaughter, carcass and organ mass data were collected. Data were analyzed as a randomized complete block design with a 2 × 2 factorial arrangement of treatments and steer as the experimental unit. From d -3 to 4, steers challenged with *M. haemolytica* had lower (P = 0.04) ADG than steers not challenged with *M. haemolytica*. In addition, across the entire finishing period, steers exposed to steers PI with BVDV tended (P = 0.09) to have lower ADG and G:F than steers not exposed to BVDV. Prior to slaughter, retained N expressed as g/d (P = 0.03) and as a % of N intake (P = 0.04) was lower in BVD steers compared with steers not exposed to steers PI with BVDV. There were no effects (P > 0.10) of BVDV exposure or *M. haemolytica* challenge on carcass characteristics or empty BW (EBW). Expressed as a percent of EBW, HCW was lower (P = 0.02) and blood plus total offal weight was greater (P = 0.02) for steers challenged with *M. haemolytica* compared with steers not challenged. Results are in agreement with those reported in larger scale finishing studies and suggest that acute exposure to BRD-related pathogens can have long-term effects on animal performance.

Key words: bovine, bovine respiratory disease, bovine viral diarrhea, carcass merit, growth, nitrogen balance

INTRODUCTION

Bovine respiratory disease (**BRD**) involves the complex interaction between stress, bacteria, viruses and the environment (Duff and Galyean, 2007). Booker et al. (2008a) reported that *Mannheimia haemolytica* was the most common pathogen isolated at necropsy from animals that died from BRD. In addition, Haines et al. (2001, 2004) and Shahriar et al. (2002) reported a high incidence of bovine viral diarrhea viruses (**BVDV**) in animals that died due to BRD. These studies were conducted on individual animals, and indicated a negative effect of these pathogens on the economics of cattle production due to mortalities. However, in experiments using feedlot pens, contradictory information regarding the effects of animals persistently infected (**PI**) with BVDV on animal health, performance and carcass quality have been reported (Loneragan et al., 2005; O'Connor et al., 2005; Booker et al., 2008a, 2008b). Loneragan et al. (2005) reported a negative effect on health and performance of cattle when a PI animal was present in a pen or adjacent pens. O'Connor et al. (2005) and Booker et al. (2008b) reported no difference in performance but observed an increased morbidity in pens containing a PI animal. Despite differences in animal health and performance of feedlot cattle in response to individual pathogens, strong evidence exists (Gardner et al 1999; Thompson et al., 2006) which indicates that animals treated against BRD have decreased performance and lower carcass quality compared with animals never treated against BRD during the finishing period. An improved understanding of the etiologic agents involved in BRD and their relationship with animal performance and carcass quality is essential for the development of preventive and therapeutic strategies. The objective of this experiment was to evaluate the effects of short-term (72 h) exposure to steers PI with

BVDV with or without an intratracheal *M. haemolytica* challenge on performance, N balance, organ mass and carcass characteristics of beef steers.

MATERIALS AND METHODS

Twenty-four Angus crossbred steers (initial BW = 313 ± 31 kg) were individually housed at the Nutrition Physiology Research Center (NPRC), Oklahoma State University, Stillwater during the experiment. All steers were considered clinically healthy and were seronegative to all pathogens involved in the study as determined by paired serum samples collected 14 d apart prior to the start of the experiment. Steers were randomly allocated to 1 of 4 treatments (6 animals/treatment) arranged as a 2 × 2 factorial. Treatments were: 1) steers not challenged with BVDV or *M. haemolytica* (**Control**); 2) steers exposed for 72 h to two steers PI with BVDV (**BVD**); 3) steers intratracheally challenged with *M. haemolytica* on d 0 (**MH**); and 4) steers exposed for 72 h to two steers PI with BVDV immediately followed by intratracheal challenge with *M. haemolytica* on d 0 (**BVD+MH**). Steers exposed to calves PI with BVDV were transported 3.22 km to the Willard Sparks Beef Research Center, Stillwater, OK and were commingled in a 6 × 10.8 m pen with 2 steers previously diagnosed via immunohistochemistry as being PI with BVDV Type 1b (Fulton et al., 2006). Following exposure to BVDV Type 1b, steers were returned to the NPRC for the remainder of the experiment. Steers challenged with *M. haemolytica* received 10 mL of a solution containing 6×10^9 CFU of *M. haemolytica* serotye A-1 which was reconstituted and grown prior to the challenge as described by Mosier et al. (1998). Challenge with *M. haemolytica* occurred immediately following return of the BVDV-exposed steers to the NPRC and on the same d for all treatment groups beginning at 0800. Steers not

challenged with *M. haemolytica* were intratracheally dosed with 10 mL of a sterile phosphate-buffered saline (**PBS**) solution. Inoculation of the PBS solution for Control and BVD groups and the solution containing the *M. haemolytica* culture for the MH and BVD+MH groups was performed as described by Burciaga-Robles et al. (2009).

During the length of the experiment, steers were offered feed for ad libitum consumption. Feed was delivered twice daily based on the previous day's intake. Feed refusals were collected daily and any feed remaining was dried at 60^{o}C for determination of DMI. Steers were adapted to a high-concentrate finishing diet using adaptation diets shown in Table 1. Steers were fed Diet 1 for 2 wk prior to the beginning of the experiment and remained on this diet during the first 15 d following *M. haemolytica* challenge. Beginning on d 16, animals were fed Diet 2 for 3 consecutive d; on d 19, animals were fed Diet 3 for an additional 3 d (d 19 to 22 post *M. haemolytica* challenge). On d 23 steers were fed the finisher diet and remained on this diet until harvest. Animals were weighed on d -3, 0, 7, 14, 28, 56, 84 and 112 (heavy block) or 126 (light block). Average daily gain was calculated using BW and days on feed and G:F was calculated using DMI for the corresponding periods.

Nitrogen Balance

To facilitate sample collection, steers were blocked by BW into 2 groups of twelve, and the challenge procedures and sample collections were staggered by a 2 wk interval between periods to allow for total urine and fecal collections in metabolism stanchions. Steers were housed in individual 3.7 × 3.7 m pens with the exception of d 0 to 4, 7 to 11, 28 to 32, and on d 101 to 105 for the heavy block and d 111 to 116 for the light block (prior to slaughter), when animals were placed in metabolic stanchions for

collection of total feces and urine. Fecal and urinary excreta were collected for each steer and weighed to determine total daily output. Representative sub-samples of total feces (10%) and total urine (1%) were collected, composited by period, and frozen (-20ºC) for later analyses. To prevent the loss of NH_3, daily urine output was collected into buckets containing 400 mL of 6 *N* HCl to decrease pH to < 3.0. Composite samples of diet and feces were analyzed for DM and OM (AOAC, 1990) to calculate total tract digestibility. In addition, diet, feces, and urine were analyzed for total N (Leco® FP-528, Leco Corporation, St. Joseph, MI) to calculate N digestibility and retention.

Carcass and Organ Mass Collection

Approximately 16 h prior to harvest (d 112 and 126 for heavy and light block, respectively) steers were transported to the Oklahoma Food and Agricultural Products Research and Technology Center abattoir. On the morning of harvest, steers were stunned with a captive bolt and exsanguinated. Weight of blood, feet and ears, hide, head, heart, lungs, kidneys, emptied gastrointestinal tract (**GIT**; reticulorumen, omasum, abomasum, small and large intestine, and cecum), mesenteric fat trimmed from GIT organs, omental fat, pancreas, spleen, liver, and hot carcass were recorded. Total offal mass was calculated as the sum of blood, feet and ears, hide, trim (tail, spinal cord, and carcass trim), all organs, and mesenteric and omental fat. The reticulorumen, omasum, and abomasum were cut open, contents removed, and organs were rinsed free of remaining feed particles before weighing. Intestinal contents were removed by gently squeezing contents through the length of the organ. Empty-body weight was calculated as hot carcass mass plus total offal mass, and total splanchnic tissue (**TST**) mass was calculated as GIT plus liver, spleen, pancreas, and mesenteric/omental fat. In addition,

carcasses were evaluated for marbling score, fat thickness at the 12th rib, adjusted preliminary yield grade, LM area, and percentage of kidney, pelvic, and heart fat (**KPH**; USDA, 1997). Dressing percent and yield grades were calculated.

Statistical Analyses

The experiment was designed as a randomized complete block with a 2 × 2 factorial arrangement of treatments and animal served as the experimental unit. Animal performance (BW, ADG, DMI and G:F), total tract digestibility, N balance, organ mass, and carcass quality were analyzed with the MIXED procedure of SAS (SAS Inst. Version 9.1, Inc., Cary, NC). The model for all variables included the main effects of BVD, MH and the possible interaction between BVD and MH. Weight block (heavy or light) was included as a random effect in the model. Results are discussed as being significant if $P \leq 0.05$ and as a tendency if $P > 0.05$ to $P \leq 0.10$.

RESULTS

Effects of *M. haemolytica* challenge following short-term (72 h) exposure to steers PI with BVDV on animal performance is shown in Table 2. Body weight was not affected ($P \geq 0.35$) by BVD, MH, or the BVD × MH interaction. From d -3 to 4, steers challenged with *M. haemolytica* had lower ($P = 0.04$) ADG than steers not challenged with *M. haemolytica*. In addition, across the entire finishing period, steers exposed to steers PI with BVDV tended ($P = 0.09$) to have lower ADG than steers not exposed to BVDV. There was a tendency ($P = 0.10$) for a BVD × MH interaction for DMI from d -3 to 4. This resulted from steers with exposure to steers PI with BVDV and challenged with *M. haemolytica* having the lowest DMI, whereas steers with exposure to steers PI

with BVDV but not challenged with *M. haemolytica* had DMI similar to controls. This trend occurred throughout the entire finishing period (d -3 to finish; $P = 0.10$). In addition, from d -3 to 4, steers exposed to steers PI with BVDV tended ($P = 0.08$) to have lower DMI than steers not exposed to BVDV, and from d 84 to finish, steers challenged with *M. haemolytica* had lower DMI than steers not challenged with *M. haemolytica*. Exposure to steers PI with BVDV for 72 h decreased ($P = 0.05$) DMI as a percent of BW from d -3 to 4, and tended ($P = 0.08$) to result in a BVD × MH interaction, similar to DMI (kg/d). From d -3 to 4, G:F tended ($P = 0.08$) to be lower for steers exposed to steers PI with BVDV, and was lower ($P = 0.04$) for steers challenged with *M. haemolytica*. Across the entire finishing period, steers exposed to steers PI with BVDV had lower ($P = 0.04$) G:F than steers not exposed to animals PI with BVDV.

From d 1 to 4, DMI tended ($P = 0.09$) to be lower and fecal DM output was lower ($P = 0.04$) for steers challenged with *M. haemolytica* (Table 3). Apparent DM digestibility was lower ($P = 0.004$) for steers exposed to steers PI with BVDV compared with steers not exposed to BVDV. Similar to DMI, N intake tended ($P = 0.09$) to be lower and fecal N output was lower ($P = 0.04$) for steers challenged with *M. haemolytica*, and apparent N digestibility was lower ($P = 0.01$) for steers exposed to steers PI with BVDV compared with steers not exposed to PI steers. Urinary N output tended ($P = 0.09$) to be greater for steers challenged with *M. haemolytica*. Nitrogen retained expressed as g/d tended ($P = 0.09$) for steers challenged with *M. haemolytica.* In addition, Nitrogen retained as a % of N intake tended ($P = 0.07$) to be lower for steers exposed to steers PI with BVDV compared with steers not exposed to steers PI with BVDV whereas

steers challenged with *M. haemolytica* had lower ($P = 0.01$) Nitrogen retained as a % of N intake compared with non-challenged animals.

From d 7 to 11 and d 28 to 32, there were no effects ($P \geq 0.12$) of BVDV exposure or *M. haemolytica* challenge on DM or N intake or apparent total tract digestibility (Table 3). Steers challenged with *M. haemolytica* had lower ($P = 0.03$) urinary N output on d 7 to 11 than steers not challenged with *M. haemolytica*. In addition, there was a tendency for a BVD × MH interaction on d 7 to 11 ($P = 0.07$) and d 28 to 32 ($P = 0.06$). This resulted from the steers exposed to steers PI with BVDV and challenged with *M. haemolytica* having lower urinary N output than steers exposed to steers PI with BVDV but not challenged with *M. haemolytica*, whereas urinary N output for treatments with steers not exposed to BVDV was similar. Although urinary output tended to be different among treatments, there was no effect ($P \geq 0.12$) of BVDV exposure or *M. haemolytica* challenge on N retention on d 7 to 11 or d 28 to 32.

Prior to slaughter, there was a BVD × MH interaction ($P = 0.02$) for DM and N intake (Table 3). For steers not exposed to steers PI with BVDV, DM and N intake were lower for control steers than for steers challenged with *M. haemolytica*. In contrast, for BVD steers, DM and N intake were lower for steers challenged with *M. haemolytica* than for steers not challenged with *M. haemolytica*. Retained N expressed as g/d was lower ($P = 0.05$) and as a percent of N intake tended ($P = 0.09$) to be lower lower in BVD steers compared with steers not exposed to steers PI with BVDV.

There were no effects of BVDV exposure or *M. haemolytica* challenge on carcass characteristics (Table 4) or empty BW (**EBW**; Table 5). Expressed as a percent of EBW, HCW was lower ($P = 0.02$) and total offal weight was greater ($P = 0.02$) in steers

challenged with *M. haemolytica* compared with steers not challenged (Table 5). There was BVD × MH interaction ($P = 0.05$) for total offal weight. For steers not exposed to steers PI with BVDV, total offal weight was lower for control steers than for steers challenged with *M. haemolytica*. In contrast, for BVD steers, total offal weight was similar for steers challenged with *M. haemolytica*. Blood weight expressed as kg ($P = 0.06$) and as % of EBW ($P = 0.05$) was greater for steers challenged with *M. haemolytica* than for steers not challenged with *M. haemolytica*. Weight of the head tended ($P = 0.06$) to be lower for BVD steers compared with steers not exposed to steers PI with BVDV. In addition, weight of the feet, forelegs, and tail were lower ($P \leq 0.03$) for BVD steers than for steers not exposed to steers PI with BVDV. Weight of the feet, forelegs, and tail expressed as a percent of EBW tended ($P = 0.08$) to be greater for steers challenged with *M. haemolytica* than for steers not challenged with *M. haemolytica*. Kidney weight (kg) tended ($P = 0.10$) to respond with a BVD × MH interaction. For steers not exposed to steers PI with BVDV, kidney weight was lower for control steers than for steers challenged with *M. haemolytica*. In contrast, for BVD steers, kidney weight was lower for steers challenged with *M. haemolytica* than for steers not challenged with *M. haemolytica*. No other differences ($P \geq 0.12$) in organ mass due to exposure to steers PI with BVDV or *M. haemolytica* were observed.

DISCUSSION

Immune response by steers used in the present experiment to exposure to steers PI with BVDV and *M. haemolytica* challenge has been reported (Burciaga-Robles et al., 2009). Briefly, BVDV antibody production was increased across time for steers exposed

for 72 h to steers PI with BVDV compared with non-exposed animals, and whole cell *M. haemolytica* antibody concentrations were greater and increased over time in steers challenged with *M. haemolytica*. Rectal temperature increased over time in BVD and MH steers, but the extent and time of the increase depended on the pathogen involved in the challenge. We observed changes in total and differential white blood cell count (WBC), cytokine concentrations, and blood gases consistent with an immune challenge in feedlot cattle (Burciaga-Robles et al., 2009).

In previous experiments, the number of antimicrobial treatments required for BRD has been associated with decreased performance during the feedlot phase (Gardner et al., 1999; Roeber et al., 2001). Roeber et al. (2001) showed that calves that required two or more antimicrobial treatments for BRD had lower ADG during the first 56 d on feed than calves that did not require an antimicrobial treatment, but there was no difference between animals that required one antimicrobial treatment compared with animals that did not require an antimicrobial treatment. This might suggest that calves receiving a single antimicrobial treatment were misdiagnosed or that severity or number of times a BRD event occurs plays a role in how the disease affects overall performance of beef cattle. In the present experiment, ADG was decreased from d -3 to 4 in steers challenged with *M. haemolytica*, and overall (d -3 to finish) ADG tended to be decreased due to exposure of BVDV. Our data suggests that the long-term negative effects associated with BRD might be related to the type and potentially the number of pathogens involved in the BRD event.

Hesseman (2006) concluded that the incidence of calves PI with BVDV arriving at the feedlot is approximately 0.3% of total cattle, and that PI and acutely infected cattle

are the main sources of transmission of this disease to susceptible animals. However, the negative effects that PI calves have on health and performance has been debated. O'Connor et al. (2005) reported that in a population of 5,041 calves (40 pens), the presence of a PI calf had no negative effects on overall health of cattle in the pens. In contrast, Loneragan et al. (2005) attributed an increased risk of developing BRD to animals PI with BVDV present in pens of feedlot cattle. Stevens et al. (2007) reported that the presence of PI calves in a pen increased morbidity (18.8 vs. 29.6%) compared with non-exposed cattle. In addition, these authors evaluated the epidemiological curve of BRD when a PI calf was present in a pen, and reported a twofold increase (15.3 vs. 31.7%) in the number of animals requiring an antimicrobial treatment during the first 7 d on feed. Booker et al. (2008a) evaluated the effects of the presence of a PI animal and also the type of BVDV involved on health and performance of non-exposed cattle. They concluded that the presence of a PI BVDV type 1 animal in a pen resulted in an increased number of BRD treatments and mortalities compared with non-exposed pens, but a PI BVDV type 2 animal had no negative effects on health of finishing cattle. Elam et al. (2008) reported that short-term (60 d) or long-term (215 d) exposure to calves PI with BVDV did not affect DMI or final BW at the end of the finishing period; however, they did observe a tendency for decreased ADG during the first 28 d in exposed cattle, which they attributed to the cost of developing immunity to the wild type strain of BVD. It is important to mention that in the study conducted by Elam et al. (2008), all animals had been vaccinated at least twice against BVDV before entering the feedlot, and at the time of entry to the feedlot received a metaphylactic antimicrobial treatment reducing the possibility of sickness not only to BVDV, but also to *M. haemolytica* and other

microorganisms associated with BRD. In the present experiment, animals exposed to steers PI with BVDV tended to have lower ADG than non-exposed steers across the entire finishing period. Our results suggest that short-term exposure to steers PI with BVDV type 1b can have long-term effects on animal performance.

Olchowy et al. (2000) and Corrigan et al. (2007) have reported effects of *M. haemolytica* in challenge studies on animal performance, although the length of their experiments was relatively short (16 d and 136 h, respectively). In addition, Gånheim et al. (2003, 2005) reported the effects of an aerosolized challenge with BVDV with or without a subsequent *M. haemolytica* challenge. In these studies authors characterized the production of acute phase proteins (Gånheim et al., 2003) and changes in white blood cell counts (Gånheim et al., 2005) due to the bacterial and/or viral challenges, and the length of the experiments were only 23 d. To our knowledge, no long-term effects of acute *M. haemolytica* challenge on performance and carcass characteristics in cattle have been reported. Olchowy et al. (2000) reported decreased ADG of calves challenged with *M. haemolytica* which did not receive an antimicrobial treatment. These results are in agreement with results from the present experiment, in which steers challenged with *M. haemolytica* had lower ADG compared with control steers. However, in the present study the duration of the decreased ADG due to *M. haemolytica* lasted for only 4 d after the *M. haemolytica* challenge. The discrepancy in results might be explained by the difference in age (3 mo vs. 8 mo), breed (Holstein vs. Angus cross) and/or BW (76 vs. 314 kg) of calves used by Olchowy et al. (2000) compared with the present experiment.

Pro-inflammatory cytokines secreted by activated cells of the immune system have been reported to decrease feed intake (Johnson, 1997). In the present experiment,

there was a tendency for decreased DMI during days -3 to 4 for steers with exposure to steers PI with BVDV. In addition, steers exposed to calves PI with BVDV and challenged with *M. haemolytica* tended to have lower DMI than steers on the remaining treatments from d -3 to 4 and from d -3 to finish. According to Hutchenson and Cole (1986), when expressed as a percentage of BW, DMI may be decreased from 1.55 to 0.90% during the first wk after arrival to the feedlot and that depression in DMI can last up to 28 d post arrival. In our study, exposure to steers PI with BVDV decreased DMI as a % of BW during the first 7 d of the experiment compared with animals not exposed to BVD (1.67 vs. 1.99%).

In the present experiment, steers that were either exposed to animals PI with BVDV or challenged with *M. haemolytica* had a negative G:F during d -3 to 4. This might suggest that energy and protein intake during this period was utilized for metabolic priorities other than growth (e.g., in support of the immune response). Haptoglobin synthesis by the liver and the anorexia cascade are stimulated by activated cells of the immune system. Klasing (1998) demonstrated a switch in amino acid utilization from growth to production of acute phase proteins following an immune challenge in chicks. The synthesis of acute proteins by the liver in response to an immune challenge could change the amino acid requirements as well as energy required for maintenance in support of the acute phase response. In the absence of stress or infections, animals will assimilate nutrients into tissues according to their genetic potential and according to age and stage of production (Klassing et al, 1987; Elsasser, 1993). However, during infection, there is an increase in protein turnover (Jepson et al., 1986) due to anorexia induced by cytokine production in response to the pathogen (Klassing, 1998b; Johnson,

1997), increased amino acid requirements for acute phase protein synthesis by the liver (Reeds and Jahoor, 2001), and increased mobilization of protein stores from muscle either by decreased synthesis or increased catabolism (Ling et al, 1997) providing energy precursors for gluconeogenesis or amino acids for protein synthesis by the liver (Espat et al., 1994). As a result of these changes in protein metabolism and or turnover, animals can experience a negative N balance (Ling et al., 1997) reflected in decreased growth (Le Floc'h et al., 2004). Although the most recent edition of the NRC for Beef Cattle (1996) reports that there are no differences in protein requirements in stressed vs. non-stressed calves, recent evidence suggests that different stressors (and disease) can affect the metabolism of dietary protein that could lead to increased protein requirements and increased requirements for specific amino acids (Gilliam et al., 2008; Wagoner et al., 2008). Cole et al. (1986) conducted a series of experiments to evaluate N metabolism in calves challenged with either bovine adenovirus-3 (PI-3) or infectious bovine rhinotracheitis (IBR). Cole et al. (1986) reported that during the first 7 d after inoculation with IBR, calves that developed a febrile response had lower N digestion and N balance that non-febrile calves that were also challenged. In the present experiment, we measured N balance during the acute phase and long-term effects in steers challenged with two of the most common pathogens isolated in BRD cases. Our results indicate decreased N digestibility during the wk of the challenge for calves exposed to BVDV. In addition, N retained tended to be decreased by exposure to steers PI with BVDV during the wk of exposure and was decreased prior to harvest. Prior to harvest, N retained (g/d) tended to be lower for steers exposed to steers PI with BVDV and challenged with *M.*

haemolytica. Our results suggest that acute exposure to BRD pathogens has the potential to have long-term effects on N retained.

Recently, Gilliam et al. (2008) reported N balance of steers with or without supplementation of branched-chain amino acids following (d 2 to 7) a bacterial lipopolysaccharide (**LPS**) intravenous challenge. In their study, there was a lower N retention by steers when LPS was administered, and these differences were due to lower N intake and increased urinary N excretion. In our experiment, total urinary N excretion tended to be greater for animals in the MH group during the first 4 d post *M. haemolytica* challenge. Our results due to *M. haemolytica* challenge are also in agreement with Waggoner et al. (2008) where animals were challenged with LPS, and Cole et al. (1986) who challenged with IBR virus. Cole et al. (1986) reported that in animals challenged with IBR, only animals that developed a rectal temperature of > 39.7^{o}C had increased urinary N excretion for the first 3 d after IBR challenge. Consistent with Cole et al. (1986), rectal temperatures were greater for steers challenged with *M. haemolytica* from 2 to 24 h, reaching a maximum of 41.5^{o}C 6 h after the *M. haemolytica* challenge (Burciaga-Robles et al., 2009). During the subsequent wk (d 7 to 11) results were reversed suggesting a potential compensatory mechanism for urinary N output.

Gilliam et al. (2008) reported a decrease in DMI and fecal N output during the first 6 d after LPS challenge. Our results are in agreement with Gilliam et al. (2008); *M. haemolytica* challenge decreased fecal N output but had no effect on DM digestibility. However, exposure to steers PI with BVDV decreased apparent DM and N digestibility during the wk of exposure in the present experiment. In addition to being associated with BRD, BVDV can also affect other organs in the body, including the digestive tract

(Radostits et al., 2007). For longer-term effects (>7 d) of either LPS (Gilliam et al., 2008) or MH and BVD effects on N retention, no previous reports were found in the literature.

Although our results suggest long-term effects of BVD type 1b on N retention and performance, exposure to steers PI with BVDV and challenge with *M. haemolytica* did not affect carcass characteristics in the present experiment. Roeber et al. (2001) reported that animals requiring only one antimicrobial treatment (exhibiting clinical signs of BRD only once) during the finishing period had decreased performance during the first 56 d on feed, but compensated after that period of time and no differences in carcass quality were detected compared with animals that required at least two antimicrobial treatments for the disease. In the present experiment, our challenge model was administered only once, and carcass quality was not affected. However, differences in carcass merit are difficult to detect with small numbers of animals.

Although reports in the literature exist showing effects that anabolic implants (Hutcheson et al., 1997), postruminal carbohydrate load (McLeod et al., 2007), different winter grazing strategies (Hersom et al., 2004), protein levels (Baldwin et al., 2000), and mineral content of the diet (Soto-Navarro et al., 2004) have on visceral organ mass, to our knowledge this is the first attempt made to characterize the long-term effects of BVDV and *M. haemolytica* on the mass of splanchnic tissues. Hersom et al. (2004) observed differences in several components of the GIT due to different grazing strategies during the growing phase. In addition, Sainz and Bentley (1997) reported differences in visceral organ mass attributed to the utilization of different levels of concentrate or forage in diets. In the present study, no differences in splanchnic organ mass were detected

suggesting organ mass may be more influenced by diet than disease. Gardner et al. (1999) and Thompson et al. (2007) reported a decreased HCW in animals affected by BRD. In our experiment, although HCW was not affected by the experimental treatments, when HCW was expressed as a percentage of EBW, *M. haemolytica* decreased and *M. haemolytica* plus exposure to steers PI with BVDV tended to decrease the proportion of carcass and increase the proportion of total offal relative to EBW, suggesting BRD pathogens might result in nutrient partitioning towards non-edible tissues.

Conclusion

In the present experiment, we observed decreased performance during the first 7 d of the experiment due to *M. haemolyica* challenge, and a tendency for decreased performance across the entire feeding period in animals exposed for 72 h to steers PI with BVDV. In addition, we observed decreased N retention across the feeding period in steers exposed to BVDV and challenged with *M. haemolyica*. Our results suggest that acute exposure to pathogens associated with BRD can have long-term effects on performance of feedlot cattle.

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[1]

Table 1. Composition of diets (DM basis)

Ingredient, % of diet	Adaptation diets			Finisher
	Diet 1	Diet 2	Diet 3	
Corn dent	46.6	55.1	63.1	69.9
Dried corn distillers grain	9.0	11.5	14.0	15.7
Alfalfa hay	40.0	28.0	16.0	6.00
Synergy 19-14[1]	1.00	2.00	3.50	5.00
Wheat midds	1.12	1.12	1.12	1.12
Limestone, 38%	1.27	1.27	1.27	1.22
Dicalcium phosphate	0.25	0.25	0.25	0.25
Salt	0.33	0.33	0.33	0.33
Manganous oxide	0.003	0.003	0.003	0.003
Zinc sulfate	0.020	0.020	0.020	0.004
Potassium chloride	0.260	0.260	0.260	0.260
Magnesium oxide	0.110	0.110	0.110	0.110
Vitamin A-3,000	0.003	0.003	0.003	0.003
Vitamin E-50%	0.002	0.002	0.002	0.002
Rumensin 80[2]	0.018	0.018	0.018	0.018
Tylan 40[2]	0.010	0.010	0.010	0.010
Nutrient, % DM basis[3]				
DM, %	89.3	88.6	87.7	86.9
NE_m, Mcal/kg	1.79	1.85	2.05	2.16
NE_g, Mcal/kg	1.12	1.22	1.33	1.42
NDF, %	26.5	23.0	19.4	16.3
Crude fat, %	3.89	4.52	5.25	5.88
Crude protein, %	14.8	14.4	14.2	14.0
Ca, %	1.05	1.02	0.85	0.70
P, %	0.38	0.39	0.41	0.43
K, %	1.10	1.07	0.91	0.67

ucts, New Orleans, LA.

[2]Elanco Animal Health, Indianapolis, IN.

[3]All values are from laboratory analyses and are presented on a 100% DM basis (except DM).

Table 2. Effects of short term PI BVDV exposure and/or *M. haemolytica* intractracheal challenge on perf
period in beef steers.

	No BVD		BVD			
Item	No MH	MH	No MH	MH	SEM	BVD
BW, kg						
d -3	303	307	307	305	20.8	0.88
d 4	313	302	307	302	26.8	0.72
d 14	316	312	314	305	28.4	0.63
d 28	349	346	346	344	28.9	0.82
d 56	408	407	410	401	34.8	0.85
d 84	474	470	466	461	39.6	0.52
Final[1]	527	528	527	511	16.8	0.43
ADG, kg						
d -3 to 4	1.41	-0.61	-0.01	-0.34	0.97	0.30
d 5 to 14	0.33	0.96	0.60	0.31	0.33	0.57
d 15 to 28	2.36	2.39	2.29	2.79	0.40	0.68
d 28 to 56	2.10	2.17	2.31	2.00	0.26	0.89
d 56 to 84	2.31	2.25	1.99	2.15	0.23	0.30
d 84 to finish	1.42	1.60	1.67	1.38	0.43	0.94
d -3 to finish[1]	1.84	1.82	1.74	1.64	0.08	0.09
DMI, kg/d						
d -3 to 4	5.78	6.46	5.73	4.77	1.26	0.08
d 5 to 14	6.31	6.84	7.60	6.44	1.02	0.42
d 15 to 28	8.30	8.49	9.20	8.38	0.84	0.50
d 28 to 56	9.27	9.52	9.98	9.45	1.07	0.57
d 56 to 84	10.0	10.4	10.5	9.63	0.82	0.78
d 84 to finish	10.8	10.7	11.2	9.64	0.51	0.50
d -3 to finish[1]	9.25	9.66	9.90	8.85	0.72	0.84
DMI, %BW						
d -3 to 4	1.87	2.12	1.83	1.52	0.28	0.05
d 5 to 14	1.94	2.16	2.23	1.92	0.17	0.82
d 15 to 28	2.57	2.66	2.86	2.66	0.15	0.36
d 28 to 56	2.44	2.52	2.64	2.52	0.12	0.45
d 56 to 84	2.28	2.37	2.40	2.23	0.10	0.90
d 84 to finish	2.18	2.16	2.28	1.99	0.09	0.69
d -3 to finish[1]	2.45	2.58	2.64	2.40	0.10	0.97
Gain:Feed, kg/kg						
d -3 to 4	0.243	-0.009	-0.001	-0.071	0.022	0.08
d 5 to 14	0.052	0.140	0.078	0.048	0.052	0.49
d 15 to 28	0.284	0.281	0.248	0.332	0.044	0.75
d 28 to 56	0.227	0.231	0.235	0.216	0.017	0.82
d 56 to 84	0.229	0.217	0.188	0.223	0.016	0.30
d 84 to finish	0.135	0.151	0.149	0.142	0.044	0.90
d -3 to finish[1]	0.201	0.190	0.176	0.187	0.013	0.04

[1]Average days on feed for light block were 126 and 112 for heavy block.

Table 3. Effects of short term PI BVDV exposure and/or *M. haemolytica* intractracheal challenge on DMI, DM digestibility, nitrogen digestibility, and total nitrogen retained in beef steers

Item	No BVD		BVD		SEM	*P* – value		
	No MH	MH	No MH	MH		BVD	MH	BVD*MH
Wk 1 (d 1 to 4)								
DMI, kg	7.05	6.60	6.70	5.22	1.57	0.12	0.09	0.35
Fecal DM output, kg	1.60	1.41	1.88	1.36	0.22	0.49	0.04	0.35
Apparent DM digestibility, %	76.6	78.2	71.1	70.6	4.27	0.004	0.79	0.60
N intake, g	166	155	157	122	36.9	0.12	0.09	0.35
Fecal N output, g	25.3	23.3	28.9	19.9	2.86	0.93	0.04	0.18
Apparent N digestibility, %	84.3	84.7	80.8	81.6	3.28	0.01	0.64	0.87
Urine N output, g	63.7	75.5	63.1	70.3	8.99	0.59	0.09	0.68
Total N output, g	83.0	98.7	89.1	90.3	9.78	0.44	0.32	0.44
N retained, g	76.9	56.5	57.9	32.6	28.5	0.11	0.09	0.85
N retained, % NI	45.4	34.5	40.9	5.75	16.5	0.07	0.01	0.18
Wk 2 (d 7 to 11)								
DMI, kg	6.49	6.91	8.03	6.67	1.22	0.25	0.41	0.12
Fecal DM output, kg	1.65	1.61	1.99	1.44	0.36	0.66	0.15	0.21
Apparent DM digestibility, %	73.9	76.0	75.4	79.1	2.76	0.41	0.30	0.76
N intake, g	145	154	180	149	27.4	0.25	0.41	0.12
Fecal N output, g	27.2	27.4	30.3	25.1	7.19	0.93	0.53	0.50
Apparent N digestibility, %	80.8	81.8	83.6	84.0	2.40	0.30	0.79	0.89
Urine N output, g	60.2	58.4	75.6	57.9	10.5	0.09	0.03	0.07
Total N output, g	81.7	86.8	101	83.0	19.7	0.27	0.36	0.11

N retained, g	51.7	59.5	78.0	66.5	16.9	0.16	0.87	0.40
N retained, % of NI	38.8	38.8	43.7	42.6	3.63	0.22	0.87	0.87
Wk 4 (d 28 to 32)								
DMI, kg	8.47	8.48	8.98	8.69	0.73	0.61	0.84	0.82
Fecal DM output, kg	1.52	1.34	1.58	1.31	0.36	0.94	0.20	0.80
Apparent DM digestibility, %	81.6	83.8	82.2	85.0	3.48	0.65	0.24	0.88
N intake, g	189	190	201	194	16.3	0.61	0.84	0.82
Fecal N output, g	33.5	33.3	36.2	33	6.58	0.79	0.71	0.74
Apparent N digestibility, %	81.7	81.9	81.3	83.0	2.99	0.88	0.70	0.77
Urine N output, g	90.8	105	94.4	83.4	18.0	0.77	0.16	0.06
Total N output, g	124	127	141	116	23.4	0.70	0.18	0.08
N retained, g	65.3	62.3	59.5	78.1	19.1	0.75	0.63	0.50
N retained, % of NI	33.3	30.4	27.5	39.9	10.0	0.76	0.45	0.23
Pre-harvest[1]								
DMI, kg	9.51[a]	11.0[b]	10.3[b]	9.08[a]	0.64	0.38	0.88	0.02
Fecal DM output, kg	1.31	1.41	1.45	1.24	0.19	0.92	0.77	0.40
Apparent DM digestibility, %	85.8	87.1	86.2	86.2	2.21	0.88	0.65	0.67
N intake, g	213[a]	246[b]	232[b]	203[a]	14.4	0.38	0.88	0.02
Fecal N output, g	35.3	34.8	39.9	37.7	7.9	0.44	0.78	0.85
Apparent N digestibility, %	82.7	83.0	82.9	81.3	4.07	0.19	0.61	0.15
Urine N output, g	102	101	131	110	23.4	0.14	0.39	0.42
Total N output, g	137	136	171	147	29.5	0.11	0.38	0.42
N retained, g	76.6	109	72.6	65.0	32.8	0.05	0.29	0.10
N retained, % of NI	33.5	45.0	30.3	31.3	13.4	0.09	0.21	0.29

[a,b]Within a row means with different superscripts are different ($P < 0.05$).

Table 4. Effects of short-term PI BVDV exposure and/or *M. haemolytica* intractracheal challenge on carcass characteristics of finishing steers

Item	No BVD		BVD		SEM	*P*-value		
	No MH	MH	No MH	MH		BVD	MH	BVD*MH
HCW, kg	330	329	328	317	8.08	0.37	0.40	0.49
Dress, %	62.9	62.5	62.6	62.5	1.08	0.78	0.59	0.78
LM area, cm^2	13.3	12.7	13.0	12.6	0.82	0.57	0.21	0.82
12th-rib fat, cm	0.51	0.55	0.53	0.50	0.03	0.67	0.91	0.29
KPH, %	1.91	2.08	2.25	1.91	0.20	0.68	0.68	0.23
Marbling score	403	440	495	420	55.7	0.36	0.62	0.16
Preliminary yield grade	2.93	3.25	2.96	3.06	0.12	0.56	0.11	0.40
Calculated yield grade	2.63	2.98	2.83	2.73	0.27	0.85	0.41	0.16

Table 5. Effects of short-term PI BVDV exposure and/or *M. haemolytica* intratracheal challenge on organ mass of beef steers

Item	No BVD		BVD		SEM	*P*-value		
	No MH	MH	No MH	MH		BVD	MH	BVD*MH
Empty body weight (EBW), kg	470	478	472	458	10.9	0.38	0.76	0.26
HCW, kg	330	329	328	317	8.08	0.37	0.40	0.49
HCW, % of EBW	70.1	68.4	69.3	69.1	0.37	0.87	0.02	0.09
Total offal, kg	140[a]	151[b]	145[a]	142[a]	3.90	0.45	0.29	0.05
Total offal, % of EBW	29.9	31.6	30.7	30.9	0.37	0.87	0.02	0.09
Blood, kg	14.8	18.9	15.3	16.3	1.27	0.44	0.06	0.26
Blood, % of EBW	3.13	3.95	3.23	3.55	0.29	0.59	0.05	0.64
Head, kg	15.6	16.3	15.4	15.1	0.39	0.06	0.63	0.21
Head, % of EBW	3.33	3.40	3.25	3.29	0.06	0.18	0.40	0.82
Hide, kg	39.9	40.9	39.9	38.5	0.93	0.22	0.84	0.19
Hide, % of EBW	8.47	8.52	8.43	8.41	0.17	0.66	0.93	0.82
Feet, legs, and tail, kg	12.3	13.0	11.7	11.9	0.30	0.009	0.17	0.44
Feet, legs, and tail, % of EBW	2.62	2.72	2.47	2.59	0.09	0.03	0.08	0.85
Esophagus, kg	0.32	0.35	0.33	0.36	0.02	0.72	0.32	0.87
Esophagus, % of EBW	0.06	0.07	0.07	0.07	0.005	0.63	0.30	0.77
Reticulo-rumen, kg	10.3	11.0	11.0	11.7	0.79	0.27	0.29	0.95
Reticulo-rumen, % of EBW	2.19	2.29	2.33	2.57	0.15	0.19	0.27	0.64
Omasum, kg	3.37	3.10	3.30	2.72	0.34	0.52	0.23	0.65
Omasum, % of EBW	0.71	0.64	0.69	0.58	0.06	0.55	0.21	0.80
Abomasum, kg	1.42	1.33	1.34	1.45	0.08	0.82	0.89	0.26
Abomasum, % of EBW	0.30	0.27	0.28	0.31	0.01	0.64	0.82	0.12
Small intestine, kg	5.05	4.97	4.91	4.65	0.30	0.45	0.57	0.76
Small intestine, % of EBW	1.07	1.03	1.03	1.00	0.05	0.56	0.56	0.96
Cecum/large intestine, kg	5.02	5.16	5.10	4.56	0.42	0.54	0.64	0.43
Cecum/large intestine, % of EBW	1.06	1.07	1.07	0.98	0.08	0.67	0.64	0.52
Mesenteric/omental fat, kg	16.7	16.8	18.3	16.2	1.00	0.58	0.32	0.29
Mesenteric/omental fat, % of EBW	3.54	3.51	3.87	3.52	0.19	0.39	0.34	0.43
Pancreas, kg	0.51	0.56	0.52	0.51	0.07	0.74	0.64	0.57
Pancreas, % of EBW	0.10	0.11	0.11	0.11	0.01	0.92	0.56	0.76

Spleen, kg	0.94	1.18	1.13	1.23	0.12	0.36	0.21	0.58
Spleen, % of EBW	0.20	0.24	0.23	0.26	0.02	0.29	0.17	0.68
Liver, kg	6.74	6.65	6.64	6.47	0.27	0.57	0.60	0.89
Liver, % of EBW	1.43	1.38	1.40	1.40	0.04	0.89	0.61	0.54
Lungs/heart, kg	8.23	8.09	8.01	8.30	0.32	0.99	0.80	0.52
Lungs/heart, % of EBW	1.74	1.68	1.69	1.80	0.05	0.56	0.61	0.13
Kidneys, kg	1.62	2.09	1.90	1.42	0.45	0.47	0.97	0.10
Kidneys, % of EBW	0.37	0.43	0.44	0.33	0.09	0.74	0.69	0.18

[a,b]Within a row means with different superscripts are different ($P < 0.05$).

CHAPTER V

EFFECTS OF SHORT-TERM FOOD DEPRIVATION AND INTRATRACHEAL CHALLENGE WITH *MANNHEIMIA HEMOLYTICA* ON BLOOD FLOW AND NET PORTAL AND HEPATIC FLUX OF AMINO ACIDS DURING AN ACUTE PHASE IMMUNE RESPONSE IN THE BOVINE

Abstract

Respiratory disease is the most common disease in bovine. During an immune response, cytokines induce protein catabolism and amino acids (AA) are used for the acute phase response. Our objective was to evaluate blood flow and net splanchnic flux of AA during a bovine respiratory disease (BRD) challenge. Twenty two castrated males with chronic indwelling catheters were used. Treatments included: 1) fed *ad libitum* and not challenged (FED/CON); 2) fed *ad libitum* and intratracheally challenged with 6×10^9 CFU/mL of *Mannheimia hemolytica* (FED/CH); 3) deprived of feed for 14 h and not challenged (FAST/CON); and 4) deprived of feed for 14 h and intratracheally challenged with 6×10^9 CFU/mL of *M. hemolytica* (FAST/CH). Arterial, portal, and hepatic blood samples were simultaneously drawn at 1.5 h intervals on the d of challenge. By design, FAST had lower intake than FED, and FED/CH had lower intake than FED/CON (diet × disease, $P = 0.003$). Tumor necrosis factor-α and interleukin-1β increased across time and were greater for CH than CON (disease × h, $P \leq 0.0002$). Arterial concentrations of BCAA, essential AA (EAA), and total AA (TAA) were decreased ($P \leq 0.03$) in CH

compared with CON. Feed deprivation decreased ($P \leq 0.04$) net flux of EAA, non-EAA, and TAA across the PDV. Net removal of EAA, non-EAA, and TAA by the liver was greater ($P \leq 0.02$) for CH compared with CON. BRD results in greater removal of AA by the liver during an acute phase response.

Introduction

Bovine respiratory disease (BRD)[8] involves the complex interaction between stress, bacteria, viruses, and the environment (1). Economic losses have been associated with direct costs due to BRD treatment, but also due to indirect costs associated with decreased animal growth (2,3). During the acute phase of bacterial infection, decreased growth rates have been observed in chicks (4), pigs (5) and cattle (6). Decreased growth rates can be attributed to activation of the immune system with an associated decrease in intake (6,7) and increase in cytokine concentration (8), which can redirect nutrient use away from growth towards mounting an adequate immune response (9,10). Immune response may result in increased nutrient requirements for animals infected with virulent pathogens, which results in decreased animal growth compared with animals not infected (11).

Differences in plasma amino acid (AA) composition due to lipopolysaccharide (LPS) challenge in the castrated male bovine (i.e., steers) have been reported (12) even though the magnitude of the response to an LPS challenge has been suggested to be of smaller duration and magnitude compared to a true pathogen infection (11). During the acute phase response to a pathogen, animals produce significant amounts of nitrogenous compounds (e.g., acute phase proteins, antibodies, cytokines) and develop specific immune cells that can redirect nutrients in support of the immune response (10,11). In addition to the induced anorexia, hyperthermia, and increased acute phase protein synthesis by the liver, cytokines induce muscle protein catabolism and AA are used for gluoconeogenesis and immune cell proliferation (13). However, little is known about how net flux of AA and other nutrients by the portal-drained viscera (PDV) and liver

might be altered due to inflammation and immune response. Better knowledge of net nutrient flux and requirements during an immune challenge could help in the development of feeding and management strategies to preserve both body growth and defenses in stressed animals. The objective of this experiment was to determine blood flow, oxygen consumption, and net flux of AA across the PDV and liver in the bovine fed or deprived of feed for 14 h prior to an intratracheal challenge with *Mannheimia hemolytica*.

Materials and Methods

Animals. All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee. Twenty two castrated male calves (initial body weight = 320 ± 24 kg) were equipped with chronic indwelling catheters to measure blood flow and net nutrient flux across the PDV and liver. Catheters were surgically placed in the portal vein, a hepatic vein, a mesenteric vein, and an adjacent mesenteric artery (14). Catheter patency was maintained by filling catheters with a 1,000 U/mL heparinized-saline solution prior to the start of the experiment and with a 100 U/mL heparinized-saline solution between sampling periods. Animals were allowed a minimum of 14 d to recover from surgery before beginning the initial collection period. Animals were individually housed at the Oklahoma State University Nutrition Physiology Research Center, Stillwater.

Treatments. Prior to the experiment, all animals were determined to be sero-negative to *M. haemolytica* and were considered clinically healthy. Animals were randomly allocated to 1 of 4 treatments arranged as a 2 × 2 factorial: 1) *ad libitum* feeding and not challenged with *M. haemolytica* (FED/CON); 2) *ad libitum* feeding and intratracheally

challenged with *M. haemolytica* (FED/CH); 3) 14-h feed deprivation and not challenged (FAST/CON); 4) 14-h feed deprivation and intratracheally challenged with *M. haemolytica* (FAST/CH). Six animals/treatment were used for the experiment. To facilitate intensive blood collections, sampling occurred during two sampling periods spaced 18 d apart. Two animals from the FED/CON group in the first sampling period were randomly allocated to treatment in the second period. Feed deprivation for the FAST groups occurred beginning 14 h (feed removed at 1700 on d 0) prior to the challenge with *M. haemolytica* (0700 on d 1) and continued throughout the sampling period. Animals were challenged with *M. haemolytica* using the same protocol as described (8). Briefly, animals challenged with *M. haemolytica* received 10 mL of a solution containing 6×10^9 CFU of *M. haemolytica* serotype 1, which was reconstituted and grown prior to the challenge (15). Animals not challenged with *M. haemolytica* were intratracheally dosed with 10 mL of a sterile phosphate-buffered saline solution. Prior to the start of the experiment (14-d diet adaptation period for FED and FAST) and during the sampling period (FED), animals received a pelleted diet formulated to meet or exceed their nutritional requirements (16; Table 1). During periods of feeding, animals were fed twice daily at 0800 and 1700 throughout the experiment.

Sample Collection. Calves were placed in metabolism stanchions in a climate-controlled room (23 to 27°C) 24 h before the initiation of the blood collection period. Rectal temperatures were recorded using a digital thermometer (GLA M-500; GLA Agricultural Electronics, San Luis Obispo, CA) prior to *M. haemolytica* challenge and at 3, 6, 12, 18, and 24 h after the bacterial challenge. At 0600 on the morning of *M. haemolytica* challenge and blood sampling, a priming dose of 20 mL of 10% (wt/vol) para-

aminohippurate (pH = 7.4) was administered through a 0.45 µm sterile filter (Millipore, Bedford, MA) into the mesenteric vein catheter. Para-aminohippurate was continuously infused (PHD 2000 Syringe pump; Harvard Apparatus Inc., Holliston, MA) at 0.7 mL/min for 10 h following the priming dose. Blood was collected 1 h prior to the *M. haemolytica* challenge (0700), and continued every 1.5 h from 0830 until 1600 (total of 7 samples). At every sampling time, 30 mL of blood were simultaneously drawn from the portal vein, hepatic vein, and mesenteric artery catheters into syringes, and blood was placed into tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) for plasma and serum harvest. Blood samples were immediately capped and placed on ice for transport to the laboratory. Immediately after collection, 90 µL of arterial, portal and hepatic whole blood samples were analyzed for blood gasses using a blood gas analyzer (1304 pH/Blood Gas Analyzer, Instrumentation Laboratory, Lexington, MA). An additional 40 µL of whole blood was used to determine packed cell volume. Remaining blood was centrifuged (3,000 × *g*, 4°C, 20 min) and plasma and serum harvested and frozen (−40°C) for further analysis.

Cytokines. Arterial serum samples collected at -0.5, 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, and 24 h of the *M. haemolytica* challenge were analyzed in duplicate for bovine specific tumor necrosis factor-alpha (TNFα; R&D Minneapolis, MN), interleukin-1 beta (IL-1β; Pierce Protein Research Products, Thermo Fischer Scientific, Rockford, IL) and interleukin-6 (IL-6; Pierce Protein Research Products, Thermo Fischer Scientific) as previously described (8). The inter-assay coefficient of variation was below 10% and duplicates with a coefficient of variation greater than 7.5% were re-analyzed.

Serum Haptoglobin. Haptoglobin concentrations in arterial, portal and hepatic serum

samples collected at -0.5, 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, and 24 h of the *M. haemolytica* challenge were analyzed in duplicate using a Bovine Haptoglobin ELISA kit (Immunology Consultants Lab, Portland OR) as previously described (8). The intra- and inter-assay coefficients of variation were below 7.5%.

Plasma Metabolites and Para-Aminohippurate Determinations. Arterial, portal, and hepatic plasma samples collected at -0.5, 1.0, 2.5, 4.0, 5.5, 7.0, and 8.5 h of the *M. haemolytica* challenge were analyzed for para-aminohippurate and metabolite concentrations. Plasma para-aminohippurate concentrations were determined colorimetrically (17). Commercially available kits were used for the colorimetric determination of plasma urea nitrogen (Urea Nitrogen Reagent, Teco Diagnostic, Anaheim, CA), ammonia (Ammonia Reagent Set, Pointe Scientific) and total protein (arterial blood samples only; Total Protein [Biuret] Reagent Set, Pointe Scientific) concentrations. Microplates (Beckman Coulter, Fullerton, CA) were used for all analyses, and absorbance was measured according to manufacturer recommendations for each metabolite using a plate reader (Multiskan Spectrum; Thermo Scientific, Waltham, MA). Intra- and inter-assay coefficients of variation for each metabolite were kept below 5 and 7.5%, respectively.

Free plasma AA were determined via GLC using a commercially available kit (EZ:FAAST #KGO-7165; Phenomenex, Torrance, CA). The kit supplied all necessary reagents for solid phase extraction and derivatization as described (12). Briefly, 100 µL of plasma were derivatized following the manufacturer's procedures and reagents. After a 2 min incubation period, the organic layer was aspirated from the aqueous layer and an aliquot was placed into a glass scintillation vial and analyzed for AA on a gas

chromatograph (Varian CP-3800, Varian, Walnut Creek, CA) using a split injection protocol (2 µL of sample at 250°C) with helium (1.5 mL/min) as the carrier gas. Intra- and inter-assay coefficients of variation for amino acid determination were less than 15%.

Blood Flow and Net Nutrient Flux Calculations. Calculations for blood oxygen concentrations and plasma flows through the portal and hepatic vein and net flux of nutrients across the PDV, hepatic, and total splanchnic vascular beds were calculated as previously described (18,19). A positive net flux indicates a release or absorption of a nutrient, whereas a negative net flux represents uptake or utilization. All animals were sampled for each treatment during the experiment (n = 6). However, hepatic samples were not collected from 1 animal in the FED/CON and 1 animal in the FAST/CH treatment groups, and individual samples were periodically missed due to loss of catheter patency. Therefore, PDV plus hepatic flux does not always equal total splanchnic flux. The hepatic extraction ratio (HER) was calculated using the formula: HER $=(F_{pv}(C_{pv} - C_{hv}) + F_a (C_a - C_{hv}))/F_{pc}Cpv + F_aC_a$, where F_{pv} is the portal plasma flow (L/h), C_{pv}, C_{hv} and C_a are the portal and hepatic vein, and arterial plasma concentrations of the metabolite, and F_a is the hepatic arterial plasma flow (20).

Aromatic AA were calculated as the sum of tyrosine, tryptophan and phenylalanine; BCAA as the sum of leucine, isoleucine and valine; gluconeogenic AA as the sum of alanine, glycine, glutamine, glutamic acid, serine, and threonine; sulfur containing AA as the sum of cysteine and methionine; essential AA (EAA) as the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; non-essential AA (NEAA) as the sum of alanine, asparagine, aspartic acid,

cysteine, glutamine, glutamic acid, glycine, hydroxyproline, proline, serine, and tyrosine; and total AA as the sum of all amino acids reported.

Statistical Analysis

Initial body weight and dry matter intake were analyzed using the Mixed Procedure (SAS, version 9.1; SAS Institute) with animal as the experimental unit. Fixed effects included diet, disease and diet × disease; period was included as a random effect in the model. Blood flow, rectal temperature, cytokine, haptoglobin and metabolite data were analyzed with a repeated measures analysis using a first order autoregressive covariance structure (SAS, version 9.1; SAS Institute). Animal was the experimental unit and the repeated measure statement was sampling time within animal. Fixed effects were diet, disease, time, and all possible interactions, and period was included as a random effect. Results are discussed as significant if $P \leq 0.05$ and as a tendency if $P > 0.05$ and $P \leq 0.10$.

Results

During the adaptation period, DMI expressed as kg/d ($P \geq 0.21$) or as a % of BW ($P \geq 0.13$) did not differ among treatments (Table 2). By design, FAST animals had lower ($P < 0.0001$) DMI than FED animals, and FED/CH animals had lower DMI than FED/CON animals (diet × disease, $P = 0.003$). Rectal temperature was greater for CH than CON animals at 6, 12, 18, and 24 h post challenge regardless of diet (disease × h, $P < 0.0001$; Figure 1). Serum haptoglobin concentration responded with a disease × h interaction ($P < 0.0001$; Figure 2). Serum haptoglobin was similar among treatments across the sampling d, but was greater for CH animals compared with CON animals at 24 h post *M.*

haemolytica challenge. Pre-challenge concentrations of TNF-α, IL-1β, and IL-6 were not different ($P \geq 0.13$) among treatments (Table 2). However, change of TNF-α (disease × h, $P = 0.0002$; Figure 3a) and IL-1β (disease × h, $P < 0.0001$; Figure 3b) concentrations across time was greater for CH than CON animals. Interleukin-6 responded with a diet × h interaction ($P = 0.04$; Figure 4). Change of IL-6 concentration was greater at 5.5, 7.0, and 8.5 h post challenge in FED compared with FAST animals. Change of IL-6 concentration was also greater ($P = 0.01$) for CH compared with CON animals, and the magnitude of increase was greater for FED compared with FAST animals (diet × disease interaction, $P = 0.04$; Table 2).

There were no diet × disease × h interactions ($P > 0.10$) for arterial concentrations of metabolites (Table 3). Concentration of oxygen in arterial blood was lower ($P = 0.01$) for CH compared with CON animals. Ammonia concentration tended ($P = 0.06$) to respond with a diet × disease interaction. Arterial ammonia was lower in FED/CH and FAST/CON animals than FED/CON and FAST/CH animals. Arterial plasma concentrations of urea N, total protein, alanine, asparagine, cysteine, glycine, hydroxyproline, lysine, ornithine, phenylalanine, tryptophan, and glucogenic AA were not affected ($P \geq 0.11$) by treatment. Arterial concentration of aspartic acid was lower ($P = 0.007$) in CH animals compared with CON animals, and tended ($P = 0.06$) to greater in FAST than in FED animals. Arterial concentrations of glutamine ($P = 0.01$) and glutamic acid ($P = 0.004$) were greater in FAST compared with FED animals. Challenge with *M. haemolytica* tended ($P = 0.07$) to decrease arterial concentration of histidine, and decreased arterial concentrations of isoleucine ($P = 0.01$), leucine ($P = 0.02$), methionine ($P = 0.002$), proline ($P = 0.008$), threonine ($P = 0.05$), and valine ($P = 0.02$). In addition,

arterial concentration of BCAA ($P = 0.01$), sulfur-containing AA ($P = 0.02$), EAA ($P = 0.02$), and total AA ($P = 0.03$) were decreased in CH compared with CON animals. Arterial concentration of serine was increased ($P = 0.03$), and concentrations of tyrosine ($P = 0.10$), aromatic ($P = 0.09$), and NEAA ($P = 0.10$) tended to be increased in FAST compared with FED animals.

Animals challenged with *M. haemolytica* had greater ($P = 0.01$) arterial blood flow and tended ($P = 0.06$) to have greater hepatic blood flow than CON animals (Table 4). In addition, portal ($P = 0.08$) and hepatic ($P = 0.09$) blood flow tended to be lower in FAST than FED animals. Oxygen consumption by the PDV ($P = 0.03$), liver (tendency, $P = 0.06$), and TST ($P = 0.01$) was lower in FAST compared with FED animals, and PDV oxygen consumption was increased ($P = 0.03$) in CH compared with CON animals. There were no effects ($P \geq 0.14$) of diet, disease, or diet × disease for net PDV, liver, or TST flux of ammonia or urea N (Table 4). Net flux of haptoglobin across TST responded with a diet × disease interaction ($P = 0.05$). Net removal of haptoglobin was greater for FED/CH and FAST/CON animals compared with FED/CON and FAST/CH animals. Net release of haptoglobin by liver and TST was observed for FAST/CH animals. Net flux of alanine across the liver responded with a diet × disease interaction ($P = 0.01$). Increased removal of alanine by the liver was greater for FED/CH animals than for FAST/CH relative to FED/CON and FAST/CON, respectively. Removal of alanine by TST ($P < 0.03$) and HER ($P < 0.003$) was greater for CH animals than CON animals. Similar to alanine, increased removal of asparagine by the liver tended to be greater for FED/CH animals than for FAST/CH relative to their respective controls (diet × disease interaction, $P = 0.06$). Net TST flux of asparagine acid tended ($P = 0.10$) to be lower in

FAST compared with FED animals, and tended (P = 0.09) to be lower in CH compared with CON animals. There was a diet × disease interaction (P = 0.006) for hepatic extraction ratio. Hepatic extraction ratio increased when FED animals were challenged with *M. haemolytica* and decreased when FAST animals were challenged with *M. haemolytica*. Net PDV release (P = 0.08) and liver removal (P = 0.04) of aspartic acid were lower for FAST compared with FED animals. This resulted in a lower (P = 0.01) HER for aspartic acid in FAST compared with FED animals. Net flux of cysteine across the PDV was lower (P = 0.01) in FAST compared with FED animals, and was lower (P = 0.02) in CH animals compared with CON animals. In addition, there was a net removal of cysteine by TST for CH animals, and a net release for CON animals (disease effect, P = 0.01). Net PDV flux of glutamine responded with a diet × disease interaction (P = 0.04). Net PDV flux of glutamine was lower for FED/CH and FAST/CON animals compared with FED/CON and FAST/CH animals, respectively. In addition, TST flux of glutamine was lower in FAST vs. fed animals (P = 0.005), and CH vs. CON animals (P = 0.02). Net PDV (P = 0.03) and TST (P = 0.01) flux of glutamate were lower and HER was greater (P = 0.004) for FAST compared with FED animals. Net PDV release (P = 0.02) and liver removal (P = 0.002) of glycine were lower in FAST compared with FED animals. In addition, liver (P = 0.003) and TST (P < 0.0001) removal and HER (P = 0.05) of glycine were greater for CH compared with CON animals. Fasting increased removal of histidine by the PDV (P = 0.01) and TST (P = 0.05). In addition, PDV flux of hydroxyproline was lower (P = 0.01) in FAST than in FED animals. Challenge with *M. haemolytica* increased removal of hydroxylproline by the PDV (P = 0.01), liver (P = 0.0001), and TST (P = 0.001), and increased the HER (P = 0.004). Net PDV, liver or

TST flux of isoleucine was not affected ($P \geq 0.10$) by diet or disease. However, HER of isoleucine was greater ($P = 0.05$) in CH compared with CON animals. Net flux of leucine across the liver responded with a diet × disease interaction ($P = 0.05$). Net removal of leucine was greater for FED/CH and FAST/CON animals compared with FED/CON and FAST/CH animals. Fasting increased removal of lysine by the PDV ($P = 0.01$) and TST ($P = 0.03$). In addition, net removal of lysine by the liver ($P = 0.02$) and TST ($P = 0.004$) was greater for CH compared with CON animals. A similar response was observed for ornithine, except ornithine flux across the liver was not affected ($P = 0.13$) by disease. Net PDV flux of phenylalanine was lower ($P = 0.03$) in FAST than in FED animals. The magnitude of difference in removal of phenylalanine by the liver was greater for FED than FAST when animals were challenged with *M. haemolytica* (diet × disease interaction, $P = 0.03$). Similarly, there was a greater removal of proline by the liver for FED than FAST animals when animals were challenged with *M. haemolytica* (diet × disease interaction, $P = 0.007$). Removal of proline by TST ($P = 0.006$) and HER ($P = 0.007$) were greater in CH compared with CON animals. Serine and threonine removal by the liver and removal of threonine by TST was greater ($P \leq 0.05$) in CH than in CON animals. The PDV removed tryptophan ($P = 0.01$) and tyrosine ($P = 0.01$) in FAST compared with a net release in FED animals. In addition, net removal of tryptophan ($P = 0.003$) and tyrosine ($P = 0.03$) by TST was observed in FAST vs. FED animals, and greater ($P \leq 0.03$) removal occurred in CH than in CON animals. The increased HER was greater for FED compared with FAST when animals were challenged with *M. haemolytica* (diet × disease interaction, $P = 0.007$). Removal of valine by the liver was increased ($P = 0.04$) in CH compared with CON animals.

There was a net removal of aromatic AA across the PDV in FAST animals compared with a net release in FED animals ($P = 0.006$; Table 4). In addition, removal of aromatic AA by the liver and TST was greater ($P = 0.02$) for CH than for CON animals. Net liver removal of aromatic AA was greater at 7.0 and 8.5 post *M. haemolytica* challenge for CH vs. CON animals (disease × h interaction, $P = 0.02$; Figure 5a). The magnitude of the increase in HER of aromatic AA was greater for FED than for FAST animals when animals were challenged with *M. haemolytica* (diet × disease interaction, $P = 0.04$; Table 4). There was a tendency ($P = 0.06$) for the liver to remove a greater amount of BCAA in CH compared with CON animals. Net liver removal of BCAA was greater at 2.5 post *M. haemolytica* challenge for CH vs. CON animals (disease × h interaction, $P = 0.05$; Figure 5b). Net PDV flux of glucogenic AA was lower ($P = 0.03$) in FAST than FED animals (Table 4). In addition, the magnitude of the increase in removal of glucogenic AA by the liver was greater for FED than for FAST animals when animals were challenged with *M. haemolytica* (diet × disease interaction, $P = 0.01$). Net liver removal of glucogenic AA was greater at 2.5, 7.0, and 8.5 post *M. haemolytica* challenge for CH vs. CON animals (disease × h interaction, $P = 0.01$; Figure 5c). Total splanchnic tissues removed glucogenic AA in CH animals and released glucogenic AA in CON animals ($P = 0.003$; Table 4). Net PDV flux of sulfur containing AA was decreased ($P = 0.02$) in FAST compared with FED animals, and was lower ($P = 0.04$) in CH compared with CON animals. Feed deprivation decreased ($P \leq 0.04$) net flux of EAA, NEAA, and total AA across the PDV and TST. In addition, net removal of EAA, NEAA, and total AA by the liver and TST was greater ($P \leq 0.02$) for CH compared with CON animals. Disease × h interactions for net liver flux of NEAA, EAA, and total AA are shown in Figure 6a, b,

and c, respectively.

Discussion

Similar to other animal models, decreased intake can be anticipated for morbid/stressed calves (16,21). Decreased intake can occur for 2 or more wk, and therefore nutrient intake during this period might be limiting to achieve adequate immune function and/or maximum growth potential (22). In the present experiment, we deprived animals of feed for 14 h prior to and during the *M. haemolytica* challenge in order to compare potential effects of feed deprivation and bacterial challenge on net nutrient flux. The *M. haemolytica* challenge in FED animals decreased intake compared with CON animals, in agreement with previous data (16,21).

During the acute phase response to a pathogen, cytokines are produced by cells of the immune system (23). The increase in cytokines has been associated with decreased intake (9), consistent with the present experiment. In addition, IL-1β increased 30-fold over pre-challenge concentrations in animals challenged with *M. haemolytica* in the present study, which was associated with increased rectal temperature for at least 24 h post *M. haemolytica* challenge. Rectal temperature response in animals challenged with LPS has responded similarly, but for a shorter duration (12,24). Increased body temperature during a febrile process in humans and rats has been reported to increase metabolic rate by 13% for every 1^{o}C increase in body temperature (25). Therefore, the magnitude and duration of the increase in rectal temperature might impact maintenance requirements in morbid animals due to the increased energy cost for this metabolic process.

Cytokines induce a shift in the partitioning of dietary nutrients away from tissue accretion

towards support of the immune system (26). This change in metabolism of nutrients, including increased lipolysis and protein catabolism (23), results in decreased growth and feed utilization as a consequence of disease. This systemic response has been associated with hypermetabolism and increased energy expenditure in humans (27) and activation of peripheral protein catabolism and increased metabolic activity in splanchnic tissues (28). The increased protein catabolism in peripheral tissues results in increased availability of free AA for acute phase protein synthesis and gluconeogenesis in the liver (29).

Haptoglobin is an acute phase protein synthesized in the liver that has been reported to have antibacterial, anti-inflammatory functions during immunological stress (30). In cattle, haptoglobin has been evaluated as a predictor of BRD (31) and correlated with the number of antimicrobial treatments for BRD (32,33,34). In the present experiment, an increase in arterial serum haptoglobin concentrations due to challenge with *M. haemolytica* was observed, although the increase was not measured until 24 h post *M. haemolytica* challenge. These data are in agreement with previous studies utilizing *M. haemolytica* challenge to study the immune response in cattle (8). During the first 6 h after *M. haemolytica* challenge there was no increase in liver flux of haptoglobin in the present experiment. However, other acute phase proteins can be increased prior to haptoglobin (35) which were not measured in the present study.

Although several studies have addressed changes in blood flow and net PDV flux of AA in cattle due to different dietary sources and management strategies (36,37,38,39), to our knowledge, this is the first experiment to measure changes in PDV and liver flux of AA in healthy animals challenged with a BRD pathogen in fed and feed deprived animals. In growing and lactating animals, the splanchnic bed provides the majority of nutrients

required for growth and milk synthesis (36). Therefore, any change in the metabolic status of the animal that affects intake or alters metabolism by splanchnic tissues can have a detrimental effect on growth and lactation of food producing animals. In Holstein calves, feed deprivation decreased portal blood flow when animals were fasted for 36 h (37). In addition, in lactating and non-lactating cows, portal blood flow was lower in feed deprived animals 2 d after feed deprivation occurred, and continued for 6 d, which was the end of the fasting period (36). Hepatic blood flow decreased 2 d after feed deprivation occurred in lactating animals, but remained unchanged in non-lactating cows (36). In the present experiment, feed deprivation tended to decrease portal blood flow compared with fed animals, in agreement with previous results (36,37).

No previous experiments have evaluated the effect of *M. haemolytica* challenge on blood flow across the PDV and liver in cattle. In the present experiment, animals challenged with *M. haemolytica* had increased arterial blood flow and tended to have increased portal and hepatic blood flow, with no interactions with intake. This indicates that when homeostasis is lost due to an infection, the regulation of blood flow across total splanchnic tissues might be regulated by substances released in response to the immune challenge (e.g., cytokines). Specific non-nutritional effects of an immune response are difficult to elucidate due to the complex interactions that occur in infected animals. The increase in blood flow might result in an increased availability of energy and protein substrates to the liver for synthesis of proteins to successfully mount an immune response to the *M. haemolytica* challenge. An increase in splanchnic blood flow in humans 30 min after an intravenous infusion of recombinant IL-6 has been reported (40). However, the authors were not able to conclude if the increased blood flow was due to a direct effect of

IL-6 or another substance as a consequence of the activation of the immune system by IL-6. Lyngsø and others (40) hypothesized that the increased blood flow across the splanchnic tissues was due to an increase in portal blood flow, and unlikely due to an increased arterial blood flow to the liver. In contrast, arterial blood flow was increased and portal blood flow tended to be increased in CH animals compared with CON animals in the present experiment. The discrepancies in results might be explained by the fact that the model utilized attempted to simulate a very small increase in circulating levels of IL-6 as observed 3 h post exercise (40). In the present study, a more drastic increase in cytokines due to the cellular and humoral response to *M. haemolytica* was observed. IL-1, IL-6 and TNFα are the link that exists between the immune system and changes in amino acid metabolism during infection (41). Total plasma AA concentrations have been demonstrated to be decreased during sepsis in humans (42). In cattle (12) and pigs (13), individual AA have been reported to be decreased or increased after LPS (cattle) or complete Freund's adjuvant (pigs) infusion when blood samples were collected from the jugular vein. Plasma concentrations of free AA can decrease due to protein synthesis or amino acid degradation, whereas free AA can be increased by protein degradation or increased absorption from the gastrointestinal tract (13). In homeostasis, AA are added to plasma as blood traverses the gastrointestinal tract (37). In the present experiment, PDV flux of cysteine, glutamine, glutamic acid, glycine, histidine, hydroxyproline, lysine, ornithine, phenylalanine, tryptophan, tyrosine, gluconeogenic, sulfur-containing, essential, non-essential and total AA were decreased when animals were feed deprived. Several studies in many species have reported a net removal of glutamine and glutamic acid by the PDV (43,44,45). Glutamine is the preferred fuel for enterocytes (46). When

dietary sources of glutamine become limiting, or by the enhancing action of corticosteroids on glutamine observed in animal models (47,48,49), an increased PDV uptake of glutamine has been reported. In our experiment, feed deprivation increased net removal of glutamine and glutamic acid by the PDV.

In addition to an increased uptake of glutamine by the PDV, there was a net removal of glutamine across the TST for animals challenged with *M. haemolytica*. The removal of glutamine across the TST in FAST/CH animals can be explained by a decrease in the dietary source of this AA or an increased uptake by the PDV to maintain integrity of the enterocytes or both. The latter is supported by previous data which suggests that most of the glutamine uptake and metabolism occurred in the small intestinal mucosal cells (46). In addition, the increased removal of glutamine across the splanchnic tissues might be explained due to an increased utilization of glutamine by macrophages and lymphocytes in gut associated lymphoid tissue due to the activation and release of these cells into the blood stream (49). Increased glutamine removal might also increase the availability of arginine via citrulline synthesis (50) for the production of nitric oxide, another molecule that has been implicated in the immune response of animals.

In humans, acute phase proteins have a much higher content of aromatic AA when compared to muscle or other dietary sources (51). Therefore, in order for the liver to synthesize acute phase proteins, muscle catabolism may need to occur in order to achieve the requirements for acute phase protein synthesis. It has been proposed that the ratio of endogenous protein that needs to be mobilized relative to exogenous sources for the synthesis of acute phase proteins is 2:1 (52). In the present experiment there was an increased net removal of aromatic AA beginning at 7.5 h post bacterial challenge. The

lag time before the increased removal of aromatic AA by the liver might be explained by the length of time required for the immune cells to release cytokines which impact muscle catabolism and the subsequent release of AA required to support the immune response. This increased requirement for aromatic AA has been proposed to be first limiting for the host animal to mount an immune response while maintaining "normal" growth (51). Because the predominant source of aromatic AA is potentially muscle degradation, changes in the concentration or metabolism of aromatic AA has been described as a potential tool to measure skeletal muscle degradation (53). Increased muscle degradation has been speculated by Reeds and Jahoor (54) to account for the increased N excretion in humans during sepsis due to imbalances between the supply of AA derived from tissue protein and the AA composition of acute phase proteins. Duration of feed deprivation alters AA metabolism, especially the BCAA (45). Increased concentrations of BCAA have been reported (55) with short-term fasting, probably due to muscle protein mobilization, whereas prolonged fasting could induce a protein deficiency and decrease concentrations due to liver uptake being greater than release from muscle or dietary sources (56). In the present experiment, arterial concentrations of BCAA were not affected by feed deprivation, which might be explained by the short period of time (14 h) animals were deprived of feed. In addition to fasting, decreased arterial concentrations of BCAA have been observed in humans with septicemia (57). Animals challenged with *M. haemolytica* had decreased arterial concentrations of BCAA in the present experiment, and BCAA decreased in cattle following an LPS challenge (12). In the present experiment, the decrease in arterial concentration was associated with an increased liver uptake of BCAA during the acute phase of the immune response.

Although BCAA generally escape hepatic extraction to be taken up by periphereal tissues, after intravenous BCAA infusion, hepatic clearance of BCAA was doubled compared with healthy patients (57). The increase in liver removal of BCAA might suggest that BCAA serve as building blocks for proteins involved in the immune response.

In healthy cattle, glucogenic AA can supply between 15 to 36% of the glucogenic substrates removed by the liver (58). In the present study, no differences in arterial concentrations of glucogenic AA were detected due to feed deprivation or bacterial challenge, which does not agree with the data reported in pigs (13) and humans (57). Pigs suffering chronic lung inflammation or humans with septicemia had decreased plasma concentrations of glucogenic AA. However, in the present experiment, a net removal of glucogenic AA by the liver was detected in CH animals, even though glucose concentration and net glucose flux across splanchnic tissues were not affected by feed deprivation or bacterial challenge (data not shown). Increased energy demand due to exercise in dogs results in increased net removal of glutamine and glucogenic AA by the liver (59). In addition, increased gluconeogenic activity in the liver during an immune challenge has been reported *in vitro* in hepatic slices from lactating dairy cows following LPS challenge (60). The increased net removal of glucogenic AA by the liver could limit the supply needed by the host animal for other anabolic purposes. The increased liver uptake of glucogenic AA without changes in glucose concentration could reflect increased energy demand by the liver of the host animal, although hepatic O_2 consumption was not affected by the immune challenge in the present experiment.

Sulfur-containing AA (methionine and cysteine) are important substrates during disease

(41). Methionine has been classified as a nutritionally EAA in animal species and humans. In addition, cysteine is considered a semi-EAA due to the variable capacity of the body to synthesize this AA from methionine (41). In the present experiment, sulfur-containing AA flux across the PDV was lower in FAST and in CH animals, attributed to the decreased DMI. However, the decrease in arterial concentrations of sulfur-containing AA in CH animals might also be attributed to an increased utilization of these AA by peripheral tissues. The decrease in PDV flux of sulfur-containing AA was driven by decreased cysteine, whereas PDV flux of methionine was not affected. Methionine reduces weight loss and improves N balance when added to a protein free diet (61). However, the nitrogen sparing effect was due to cysteine and not methionine per se (62). In our study, the removal of cysteine by the liver in CH animals suggests that cysteine may be required for protein synthesis in response to the immune challenge. In addition, decreased arterial concentration of methionine can result from transsulfuration to cysteine (63) to increase the availability of this AA for adequate protein synthesis in response to an immune challenge. Interestingly, the only other AA that switched from a net liver release to a net liver removal in the present experiment was lysine. In swine reared under stressful conditions, the addition of either 0.1 or 0.2% of crystalline lysine improved performance and increased antibody production to vaccination (64). Our results suggest that growing cattle infected with BRD might have different requirements for AA compared with healthy cattle.

The role of EAA in the immune response has been described in other species (65). Chicks fed diets low in EAA had decreased antibody production in response to an immune challenge. In humans, decreased plasma concentrations and increased clearance

rate of EAA have been reported in patients suffering from septicemia (57). In the present experiment, diet had no effect in plasma concentrations of EAA; however, arterial concentrations of EAA were lower in animals challenged with *M. haemolytica*. Tryptophan was the only EAA whose plasma concentration was decreased due to chronic lung inflammation in fed and fasted pigs (13). Although no difference in arterial concentration of tryptophan was detected in the present experiment, net liver removal was greater in FAST/CH animals than in FED/CON animals. Increased removal of tryptophan during an immune response could lead to decreased availability for growth. An acute decrease in the availability (or increased requirement) of EAA to the host animal has been reported to cause an almost immediate cessation of growth and subsequent weight loss (66). Increased removal of EAA by TST might partially explain decreased growth in cattle suffering from BRD (2).

Hepatic extraction of NEAA provides substrates for synthesis of other metabolites (67). However, substantial or complete removal of NEAA could result in insufficient quantities entering the systemic circulation to support protein synthesis needs by the rest of the body (68). In septicemic patients, NEAA plasma concentrations are lower and total hepatic clearance of these AA are increased (57). These data are in agreement with results of the present experiment, where CH animals had greater liver removal of NEAA and tended to have decreased arterial concentrations. A decrease of NEAA during disease might imply a change in NEAA metabolism in morbid compared to healthy animals. The increased liver removal of NEAA started at 7 h post bacterial challenge and remained increased until the end of the experiment. The time of increased removal of NEAA coincided with the increased removal of aromatic AA. Increased removal of

NEAA by the liver during disease challenge most likely supports synthesis of EAA and proteins required for the immune response.

The overall decreased arterial concentrations of total AA observed in this experiment has also been reported in cattle (12), pigs (13), and humans (57) although to our knowledge, this is the first evidence in growing cattle to report increased net removal of total AA by the liver and total splanchnic tissues in support of an acute phase response. Although many factors remain to be determined, increased demand of AA by total splanchnic tissues could ultimately lead to decreased growth rates in bovine.

Similar to non-ruminant species, inflammation and immune system activation appear to be associated with alterations in nutrient utilization by splanchnic tissues in bovine. In addition, AA may be released from peripheral tissues and shifted to the liver for promotion of hepatic protein synthesis, gluconeogenesis, and oxidation. Decreased plasma concentrations and increased liver uptake of AA may suggest enhanced metabolic clearance and turnover of endogenously released AA. These changes in AA metabolism induced by BRD suggest that AA requirements are different for ruminants undergoing an immune challenge.

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Table 1. Diet composition and nutrient analysis on feed utilized during the experiment.

Ingredient	% DM basis
Wheat midds	37.6
Alfalfa pellets	25.0
Cotton seed hulls	15.0
Soybean hulls	15.0
Soybean meal, 47.7%	3.00
Cane molasses	3.00
Salt	0.25
Calcium carbonate	0.55
Ammonium sulfate	0.50
Selenium 600	0.04
Vitamin A-30,000	0.02
Vitamin E-50	0.003
Zinc sulfate	0.002
Zinc oxide	0.001
Nutrient composition	
NEm, Mcal/kg	1.50
NEg, Mcal/kg	0.81
CP, %	16.1
Fat, %	3.19
NDF, %	52.5
eNDF, %	22.3

ADF, %	30.5

1

Table 2. Effect of fasting with or without a *M. haemolytica* challenge on DMI, rectal temperature and arterial concentrations of cytokines and haptoglobin.

	Fed		Fasted			*P* – value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet ×Disease
BW, kg	322	324	322	308	10.2	0.45	0.56	0.43
DMI								
Adaptation, kg	6.47	5.92	7.15	7.15	0.74	0.21	0.71	0.70
Adaptation, % BW	2.01	1.82	2.19	2.29	0.20	0.13	0.83	0.14
Day of challenge, kg	6.24	1.62	0	0	0.85	< 0.0001	0.003	0.003
Day of challenge, % BW	1.92	0.49	0	0	0.26	< 0.0001	0.003	0.003
Rectal temperature, ^{o}C[1]	38.7	40.0	38.6	39.9	0.15	0.62	< 0.0001	0.80
Haptoglobin, mg/L[1]	0.61	2.06	0.31	1.99	0.28	0.49	< 0.0001	0.67
TNF-α								
Pre challenge, pg/mL	144	77	162	69	70.0	0.85	0.13	0.66
Fold change[1]	3.9	11.3	2.3	8.5	2.4	0.34	0.006	0.80
IL-1								
Pre challenge, pg/mL	247	230	247	237	53.3	0.74	0.80	0.80
Fold change[1]	3.7	30.1	2.9	29.4	9.2	0.92	0.005	0.99

IL-6								
Pre challenge, pg/mL	120	128	122	62	90.3	0.69	0.75	0.69
Fold change[2]	1.14[a]	5.87[b]	1.37[a]	3.02[c]	1.20	0.07	0.01	0.04

[1]Disease × h interaction ($P < 0.001$).

[2]Diet × h interaction ($P < 0.05$).

Table 3. Effects of intratracheal *M. haemolytica* challenge on arterial concentrations of metabolites in fed or fasted beef steers.

Item	Fed		Fasted		SEM	*P* – value		
	No MH	MH	No MH	MH		Diet	Disease	Diet × Disease
Oxygen, m*M*	2.74	2.58	2.80	2.50	0.09	0.87	0.01	0.42
Ammonia, m*M*	0.08	0.07	0.07	0.08	0.01	0.59	0.73	0.06
Urea N, m*M*	4.88	4.15	4.61	4.52	0.50	0.91	0.38	0.49
Total protein, g/L	70.6	73.4	78.5	76.8	4.22	0.19	0.89	0.59
Alanine, µ*M*	157	130	137	132	9.7	0.36	0.11	0.26
Asparagine, µ*M*	11.8	8.7	15.7	11.2	2.65	0.22	0.16	0.81
Aspartic acid, µM^2	3.80	2.76	6.67	2.99	0.82	0.06	0.007	0.11
Cysteine, µ*M*	2.85	1.30	2.36	2.94	0.68	0.39	0.47	0.12
Glutamine, µ*M*	67	77	129	111	17.9	0.01	0.82	0.45
Glutamic acid, µ*M*	156	135	220	196	20.5	0.004	0.28	0.94
Glycine, µ*M*	249	205	203	193	21.9	0.19	0.22	0.42
Histidine, µ*M*	26.2	14.3	28.3	23.1	4.68	0.24	0.07	0.47
Hydroxyproline, µ*M*	13.9	11.6	13.6	14.1	1.35	0.44	0.50	0.30

Isoleucine, µ*M*	123	95	139	101	12.3	0.38	0.01	0.67
Leucine, µ*M*	157	120	177	134	16.9	0.32	0.02	0.84
Lysine, µ*M*	101	63	97	71	23.1	0.94	0.17	0.77
Methionine, µ*M*	5.44	4.38	6.96	3.84	0.61	0.43	0.002	0.10
Ornithine, µ*M*	98.3	69.0	120.0	77.3	28.4	0.60	0.21	0.80
Phenylalanine, µ*M*	44.5	45.1	62.5	46.6	7.02	0.17	0.28	0.24
Proline, µ*M*	65.8	45.1	62.1	50.0	5.18	0.85	0.008	0.59
Serine, µ*M*	71.9	63.5	85.9	81.6	7.17	0.03	0.37	0.77
Threonine, µ*M*	51.7	41.6	61.8	44.7	6.70	0.33	0.05	0.60
Tryptophan, µ*M*	24.8	17.2	33.8	29.9	6.74	0.12	0.39	0.77
Tyrosine, µ*M*	23.0	18.1	37.0	25.6	6.43	0.10	0.21	0.61
Valine, µ*M*	295	220	317	248	29.1	0.30	0.02	0.91
Aromatic amino acids, µ*M*	92	81	133	102	17.8	0.09	0.23	0.59
BCAA, µ*M*	576	436	635	483	57.7	0.36	0.01	0.92
Gluconeogenic amino acids, µ*M*	765	654	819	766	54.2	0.13	0.13	0.59
Sulfur containing amino acids, µ*M*[3]	8.32	5.69	12.40	8.50	2.23	0.54	0.02	0.69

Essential amino acids, µ*M*	830	622	924	704	86.3	0.31	0.02	0.94
Non-essential amino acids, µ*M*	764	641	897	768	78	0.10	0.11	0.96
Total amino acids, µ*M*	1,594	1,263	1,810	1,472	151	0.15	0.03	0.95

[1]Diet × Disease × h interaction ($P = 0.05$).

[2]Diet × h interaction ($P = 0.02$).

[3]Disease × h interaction ($P = 0.05$).

Table 4. Effect an intratracheal *M. haemolytica* challenge on blood flow and net flux of metabolites in fed or fasted beef steers

Item	Fed		Fasted		SEM	*P* –value		
	No MH	MH	No MH	MH		Diet	Disease	Diet × Disease
Arterial blood flow, L/h	136	190	105	179	48.3	0.30	0.01	0.58
Portal blood flow, L/h	536	664	449	519	65.1	0.08	0.11	0.68
Hepatic blood flow, L/h	626	800	526	621	76.3	0.07	0.06	0.66
Oxygen consumption, mmol/h								
PDV	-201	-298	-202	-189	27.0	0.03	0.03	0.14
Liver	-343	-447	-135	-162	95.5	0.06	0.41	0.63
TST	-534	-725	-306	-361	127	0.01	0.25	0.52
HER	1.00	-1.01	-1.02	-1.00	0.005	0.12	0.99	0.92
Ammonia, mmol/h								
PDV	15.9	12.4	11.1	9.9	3.85	0.38	0.64	0.90
Liver	-16.8	-17.0	-14.2	-8.3	6.23	0.36	0.86	0.51
TST[2]	-1.93	-4.32	-1.34	-2.86	3.44	0.14	0.50	0.41
HER[3]	0.904	0.377	0.571	0.375	0.293	0.79	0.23	0.83

Urea N, mmol/h								
PDV	-203	-406	-131	-468	209	0.69	0.42	0.64
Liver	497	542	443	590	227	0.81	0.50	0.35
TST	189	226	386	338	271	0.90	0.72	0.52
HER	0.101	0.059	-0.097	-0.016	0.094	0.15	0.60	0.65
Haptoglobin								
PDV	-0.001	-0.023	-0.006	-0.009	0.008	0.61	0.11	0.22
Liver	-0.57	-15.72	-11.38	2.01	9.06	0.67	0.91	0.09
TST	-0.42	-15.71	-11.02	1.49	7.10	0.69	0.80	0.05
HER	-0.011	0.165	0.156	0.115	0.211	0.68	0.41	0.20
Alanine, mmol/h								
PDV	22.3	12.5	13.7	3.2	7.06	0.19	0.14	0.94
Liver	-10.6	-31.7	-10.6	-16.1	8.71	0.25	<0.001	0.01
TST	7.9	20.9	-3.3	-22.0	7.78	0.40	0.03	0.45
HER[4]	0.085	0.312	0.140	0.334	0.089	0.97	0.003	0.15
Asparagine, mmol/h								
PDV	2.94	1.33	1.41	0.70	0.90	0.18	0.20	0.78
Liver	0.01	-2.55	-1.54	-1.63	1.13	0.43	0.11	0.06

TST	2.40	-0.92	-0.53	-1.82	1.18	0.10	0.09	0.38
HER[4]	0.008	0.824	0.713	0.531	0.226	0.40	0.83	0.006
Aspartic acid, mmol/h								
PDV	1.79	0.95	0.83	0.42	0.48	0.08	0.16	0.56
Liver	-1.07	-0.73	-0.08	-0.09	0.45	0.04	0.71	0.57
TST	0.49	0.27	0.58	0.39	0.51	0.64	0.68	0.78
HER	0.811	0.692	0.479	0.317	0.314	0.01	0.51	0.93
Cysteine, mmol/h								
PDV[3]	0.89	-0.11	-0.14	-0.83	0.37	0.01	0.02	0.69
Liver	0.46	-0.20	0.19	-0.07	0.35	0.54	0.11	0.24
TST[3,4,5]	1.48	-0.30	0.38	-0.86	0.74	0.14	0.01	0.41
HER	1.35	0.91	0.22	0.89	0.80	0.63	0.84	0.67
Glutamine, mmol/h								
PDV	41.84	0.75	-13.97	-3.57	11.90	0.01	0.24	0.04
Liver[5]	-0.56	-8.84	-1.38	-12.3	11.7	0.59	0.15	0.79
TST	39.7	-8.1	-12.4	-20.8	11.5	0.005	0.02	0.09
HER	-0.158	0.389	-0.021	-0.772	0.443	0.45	0.13	0.95
Glutamic acid, mmol/h								

PDV	5.1	-1.1	-23.5	-18.1	10.7	0.03	0.96	0.56
Liver	20.3	17.2	15.2	13.9	11.5	0.23	0.35	0.94
TST[2]	27.7	16.9	7.6	-9.7	11.0	0.01	0.91	0.19
HER	-0.289	-0.120	0.076	0.106	0.100	0.004	0.09	0.54
Glycine, mmol/h								
PDV[6]	28.3	26.2	19.2	5.3	6.95	0.02	0.20	0.34
Liver[6]	-21.8	-55.6	-10.1	-16.8	9.88	0.002	0.003	0.06
TST	5.3	-29.0	6.3	-16.3	6.30	0.09	<0.0001	0.23
HER	0.179	0.380	0.158	0.258	0.096	0.09	0.05	0.34
Histidine, mmol/h								
PDV	8.28	1.74	-1.56	-1.94	2.91	0.01	0.21	0.26
Liver[6]	-0.21	0.33	-1.39	-3.26	2.85	0.11	0.40	0.60
TST[2,4]	5.68	2.60	-3.81	-5.47	3.31	0.05	0.56	0.88
HER	0.21	0.05	0.36	1.92	0.99	0.23	0.45	0.53
Hydroxyproline, mmol/h								
PDV[6]	0.93	0.25	0.13	-1.50	0.41	0.01	0.01	0.27
Liver	1.73	-1.76	-0.14	-1.21	0.61	0.12	0.0001	0.009

TST	0.70	-1.45	0.19	-2.62	0.65	0.45	0.001	0.23
HER	-0.131	0.223	0.085	0.292	0.092	0.14	0.004	0.14
Isoleucine, mmol/h								
PDV	5.28	5.71	3.50	0.06	3.17	0.19	0.59	0.49
Liver	1.21	-4.05	-2.11	-3.97	3.98	0.45	0.10	0.16
TST	6.61	2.47	-0.86	-5.24	4.79	0.25	0.22	0.76
HER	-0.003	0.178	0.102	0.282	0.162	0.83	0.05	0.32
Leucine, mmol/h								
PDV	9.44	8.98	6.03	1.04	3.40	0.07	0.37	0.45
Liver	1.29	-9.88	-2.74	-0.94	5.95	0.82	0.10	0.05
TST[6]	11.5	1.29	-4.37	-2.06	6.14	0.24	0.18	0.19
HER	0.001	0.262	0.114	0.156	0.184	0.68	0.39	0.32
Lysine, mmol/h								
PDV	35.24	7.33	-5.68	-5.33	10.5	0.01	0.16	0.16
Liver[6]	10.21	-16.11	-2.21	-8.48	11.4	0.81	0.02	0.25
TST[5]	41.87	-7.95	-2.43	-15.97	10.2	0.03	0.004	0.10
HER	-0.163	0.671	0.235	0.363	0.358	0.95	0.24	0.28
Methionine, mmol/h								

PDV	1.75	1.35	1.28	1.06	0.50	0.42	0.51	0.84
Liver	-1.02	1.69	-1.16	-0.98	0.46	0.76	0.54	0.27
TST[5]	0.45	-0.39	-0.43	-0.04	0.45	0.56	0.71	0.26
HER[6]	0.449	0.736	0.902	0.689	0.368	0.69	0.43	0.22
Ornithine, mmol/h								
PDV	39.11	3.04	-12.33	-6.52	13.5	0.02	0.24	0.10
Liver[3,4,5]	9.36	-8.04	-0.63	8.37	15.7	0.39	0.13	0.30
TST[3,5]	47.44	-5.33	-7.65	-16.70	15.4	0.03	0.03	0.12
HER[4]	-0.253	0.340	0.347	0.254	0.277	0.41	0.88	0.11
Phenylalanine, mmol/h								
PDV	5.04	4.62	1.59	0.10	2.04	0.03	0.60	0.77
Liver	-3.52	-10.38	-3.45	-3.80	2.84	0.50	0.05	0.03
TST	0.72	-4.86	-3.23	-4.47	3.07	0.87	0.12	0.33
HER	0.158	0.442	0.232	0.302	0.206	0.74	0.27	0.25
Proline, mmol/h								
PDV	4.75	4.80	3.78	1.44	1.31	0.07	0.33	0.31
Liver	-1.52	-9.26	-1.92	-4.11	1.45	0.14	0.0003	0.007
TST	1.99	-3.71	-0.26	-3.42	1.69	0.88	0.006	0.41

HER	0.078	0.232	0.117	0.266	0.09	0.48	0.007	0.23
Serine, mmol/h								
PDV	10.49	9.08	3.98	5.53	3.82	0.16	0.98	0.67
Liver	-3.99	-15.01	-3.79	-8.27	4.10	0.43	0.04	0.37
TST	5.11	-5.74	-2.67	-4.34	3.72	0.47	0.12	0.29
HER	0.082	0.335	0.300	0.209	0.11	0.81	0.95	0.18
Threonine, mmol/h								
PDV	4.56	4.30	4.32	-0.43	2.10	0.19	0.19	0.24
Liver	-0.70	-4.98	-2.06	-3.52	2.08	0.88	0.03	0.15
TST	4.61	-0.32	-1.35	-4.73	2.54	0.07	0.05	0.85
HER	0.020	-.204	0.242	0.315	0.120	0.33	0.34	0.30
Tryptophan, mmol/h								
PDV	6.51	0.83	-2.54	-2.01	2.51	0.01	0.28	0.19
Liver[3,4,5]	2.39	-0.98	-0.004	-2.00	3.05	0.23	0.08	0.28
TST[5]	9.07	-0.08	-2.70	-4.95	2.44	0.003	0.03	0.15
HER[2,4]	-0.207	0.141	0.262	0.330	0.204	0.08	0.77	0.17
Tyrosine, mmol/h								
PDV	10.04	2.69	-0.75	-1.09	3.01	0.01	0.18	0.22

Liver[4]	0.07	-5.26	-1.25	-4.12	4.15	0.78	0.07	0.27
TST[5]	9.62	-3.57	-2.26	-6.04	3.33	0.03	0.01	0.15
HER[2,4]	-0.107	0.697	0.462	0.529	0.292	0.95	0.13	0.007
Valine, mmol/h								
PDV	6.46	4.79	7.40	-4.12	7.82	0.47	0.38	0.54
Liver[6]	4.80	-7.04	-0.31	-2.24	7.91	0.92	0.04	0.17
TST	9.95	0.68	-6.67	-9.08	8.31	0.21	0.23	0.65
HER	-0.024	0.096	0.070	0.160	0.113	0.85	0.18	0.53
Aromatic amino acids, mmol/h								
PDV	22.07	7.60	-0.09	-4.00	6.84	0.006	0.23	0.30
Liver[4]	-1.25	-15.85	-4.41	-9.82	7.84	0.78	0.02	0.07
TST[5]	19.62	-6.99	8.21	-15.88	7.56	0.03	0.02	0.17
HER	-0.084	0.385	0.272	0.357	0.186	0.57	0.09	0.04
Branched amino acids, mmol/h								
PDV	21.0	18.7	18.3	3.6	13.9	0.22	0.38	0.49
Liver[6]	7.31	-20.10	-5.18	-7.24	17.3	0.93	0.06	0.11
TST	28.1	4.7	-12.0	16.6	118.6	0.21	0.19	0.47

HER	-0.013	0.149	0.089	0.175	0.134	0.79	0.23	0.38
Gluconeogenic								
PDV	111.2	63.9	31.6	-7.1	33.9	0.03	0.20	0.88
Liver[4]	8.4	-104.1	-33.7	-42.1	32.0	0.42	0.001	0.01
TST[5]	54.6	-50.6	3.5	-70.7	30.0	0.22	0.003	0.74
HER	-0.030	0.197	0.160	0.199	0.097	0.46	0.09	0.16
Sulfur containing amino acids, mmol/h								
PDV	2.55	1.25	1.08	0.20	0.57	0.02	0.04	0.68
Liver	-0.56	-1.90	-1.00	-1.05	0.52	0.92	0.12	0.06
TST[2,6]	0.85	-0.80	-0.003	-0.69	0.70	0.93	0.52	0.87
HER	0.333	0.539	0.527	0.466	0.214	0.91	0.79	0.29
Essential amino acids, mmol/h								
PDV	84.1	33.3	19.0	-13.1	29.5	0.02	0.17	0.77
Liver[4]	15.4	-54.6	-12.7	-27.8	30.8	0.75	0.01	0.06
TST	92.2	-6.2	-26.3	-52.0	32.1	0.03	0.02	0.25
HER	-0.05	0.19	0.13	0.22	0.14	0.89	0.21	0.26
Non-essential amino acids, mmol/h								

PDV	145.8	49.0	-21.6	-17.7	45.5	0.01	0.28	0.24
Liver[4]	5.13	-87.77	-6.97	41.48	43.4	0.88	0.02	0.19
TST	153	-38.6	-12.9	85.9	43.0	0.04	0.01	0.34
HER	-0.113	0.170	0.184	0.188	0.99	0.37	0.18	0.08
Total amino acids, mmol/h								
PDV	228.5	87.7	-4.8	-31.2	71.6	0.01	0.21	0.39
Liver[4]	22.3	-141.2	-21.8	-69.0	67.3	0.95	0.01	0.08
TST[5]	202.5	-59.5	-28.3	-139.6	68.5	0.02	0.009	0.26
HER	-0.083	0.170	0.150	0.196	0.109	0.63	0.14	0.13

[1]PDV = portal-drained viscera; TST = total splanchnic tissues; HER = hepatic extraction ratio.

[2]Diet × h interaction ($P < 0.01$).

[3]Diet × h interaction ($P \leq 0.05$).

[4]Disease × h interaction ($P < 0.01$).

[5]Diet × Disease × h interaction ($P < 0.05$).

[6]Disease × h interaction ($P \leq 0.05$).

Figure legends

Figure 1. Effect of *M. haemolytica* challenge on rectal temperature in beef steers during the first 24 h post challenge in fed or fasted steers ($P < 0.0001$; SEM = 0.13). [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

Figure 2. Effect of *M. haemolytica* challenge on arterial haptoglobin concentration during the first 24 h post challenge in fed or fasted beef steers ($P < 0.0001$; SEM = 0.62). [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

Figure 3. Fold change in arterial concentration of TNFα (A; diet*h, $P = 0.96$; disease*h, $P < 0.0001$, SEM = 8.37) and IL-1β (B; diet*h, $P = 0.95$; disease*h, $P = 0.0002$, SEM = 2.43) following a *M. haemolytica* challenge in fed or feed deprived beef steers during the first 24 h post challenge. [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

Figure 4. Fold change in arterial concentration of IL-6 following a *M. haemolytica* challenge in fed or fasted beef steers during the first 24 h post-challenge (Diet*hour, $P = 0.04$; Disease*hour, $P = 0.58$, SEM = 1.36). [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

Figure 5. Changes in aromatic (A; diet*h, $P = 0.87$; disease*h, $P = 0.02$, SEM = 12.1), branched chain (B; diet*h, $P = 0.60$; disease*h, $P = 0.05$, SEM = 25.0), and glucogenic (C; diet*h, P = 0.85; disease*h, $P = 0.01$, SEM = 50.6) AA flux across the liver of fed or fasted beef steers during the first 7.5 h following a *M. haemolytica* challenge. [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

Figure 6. Changes in non-essential (A; NEAA; diet*h, $P = 0.40$; disease*h, $P = 0.01$, SEM = 55.2), essential (B; EAA; diet*h, $P = 0.80$; disease*h, $P = 0.001$, SEM = 40.2), and total (C; TAA; diet*h, $P = 0.49$; disease*h, $P = 0.01$, SEM = 101) AA net flux across the liver of fed or fasted beef steers during the first 7.5 h following a *M. haemolytica* challenge. [a,b]Within h, means with different superscript letters are different ($P < 0.05$).

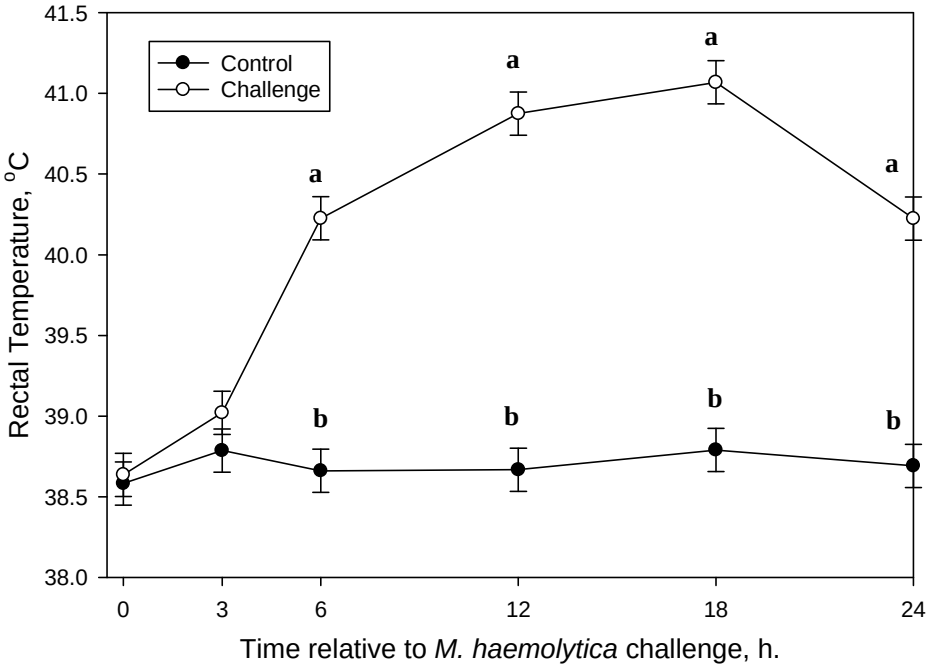
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
Rectal Temperature, °C
Control
Challenge
a
a
a
a
b
b
b
b
0
3
6
12
18
24
Time relative to *M. haemolytica* challenge, h.

Figure 5.1

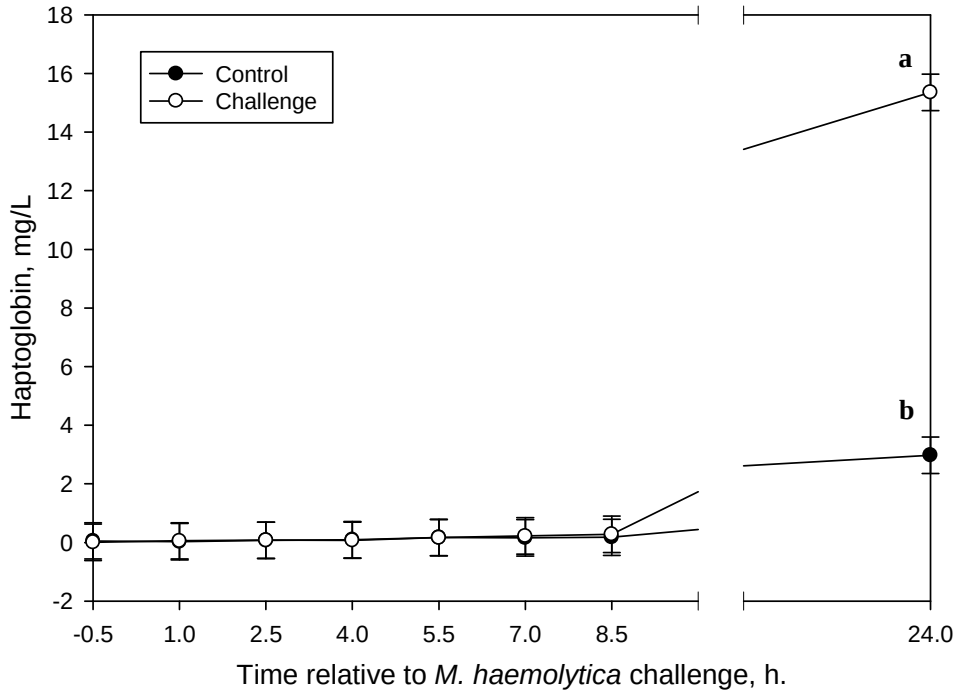
Haptoglobin, mg/L
18
16
14
12
10
8
6
4
2
0
-2
Control
Challenge
a
b
-0.5
1.0
2.5
4.0
5.5
7.0
8.5
24.0
Time relative to *M. haemolytica* challenge, h.

Figure 5.2

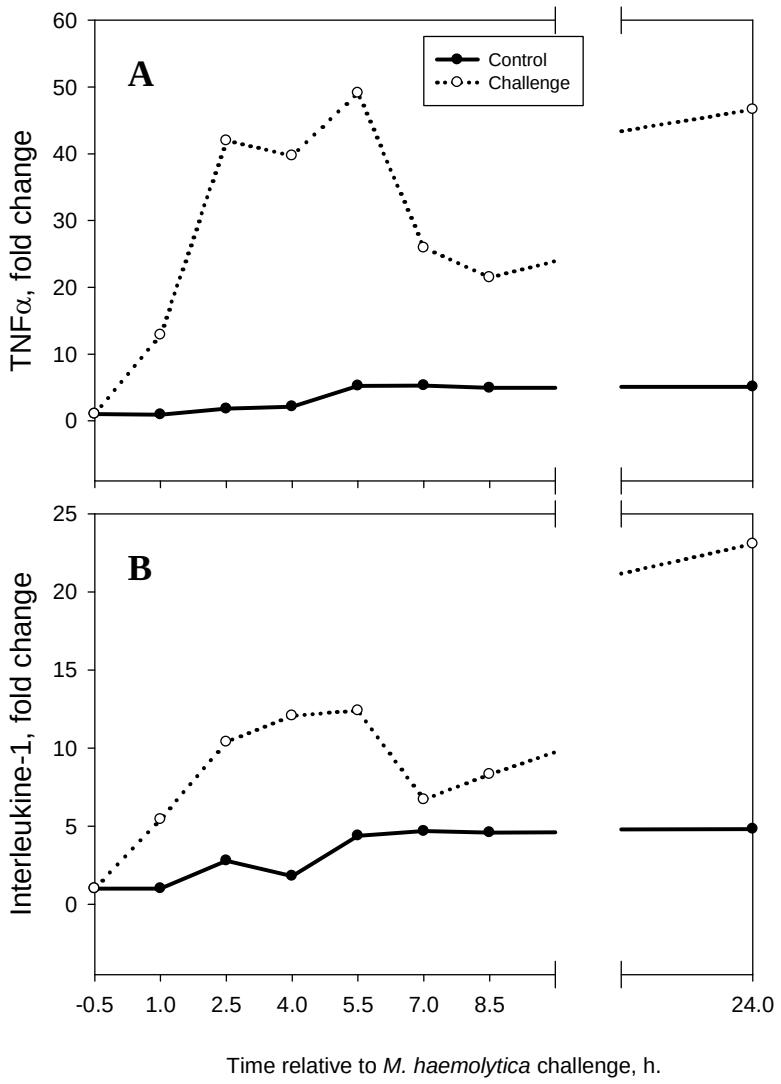
A
B
Control
Challenge
TNFα, fold change
Interleukine-1, fold change
60
50
40
30
20
10
0
25
20
15
10
5
0
-0.5
1.0
2.5
4.0
5.5
7.0
8.5
24.0
Time relative to *M. haemolytica* challenge, h.

Figure 5.3

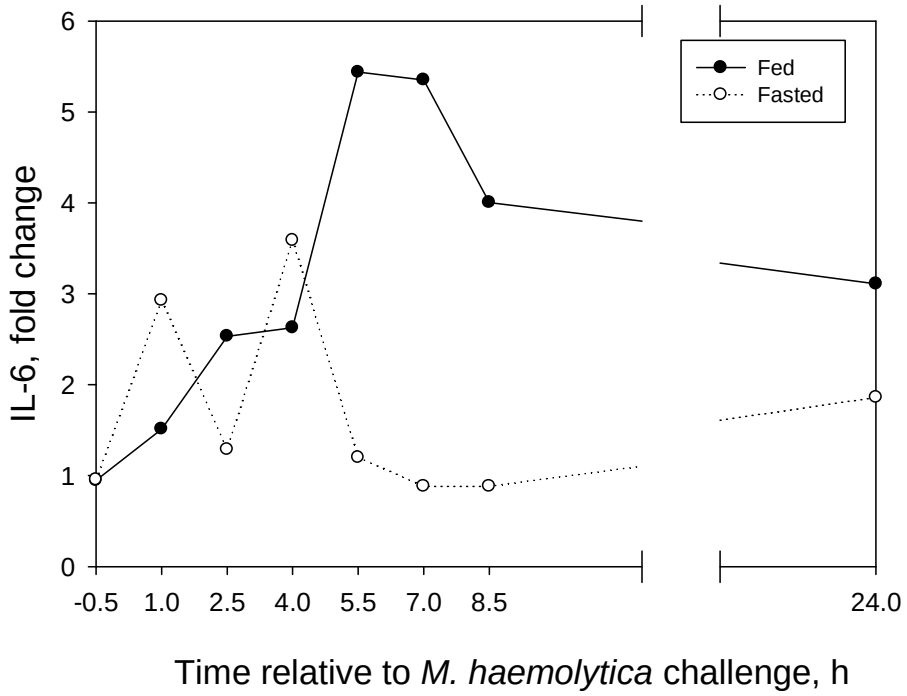
6
5
4
3
2
1
0
IL-6, fold change
Fed
Fasted
-0.5
1.0
2.5
4.0
5.5
7.0
8.5
24.0
Time relative to *M. haemolytica* challenge, h

Figure 5.4

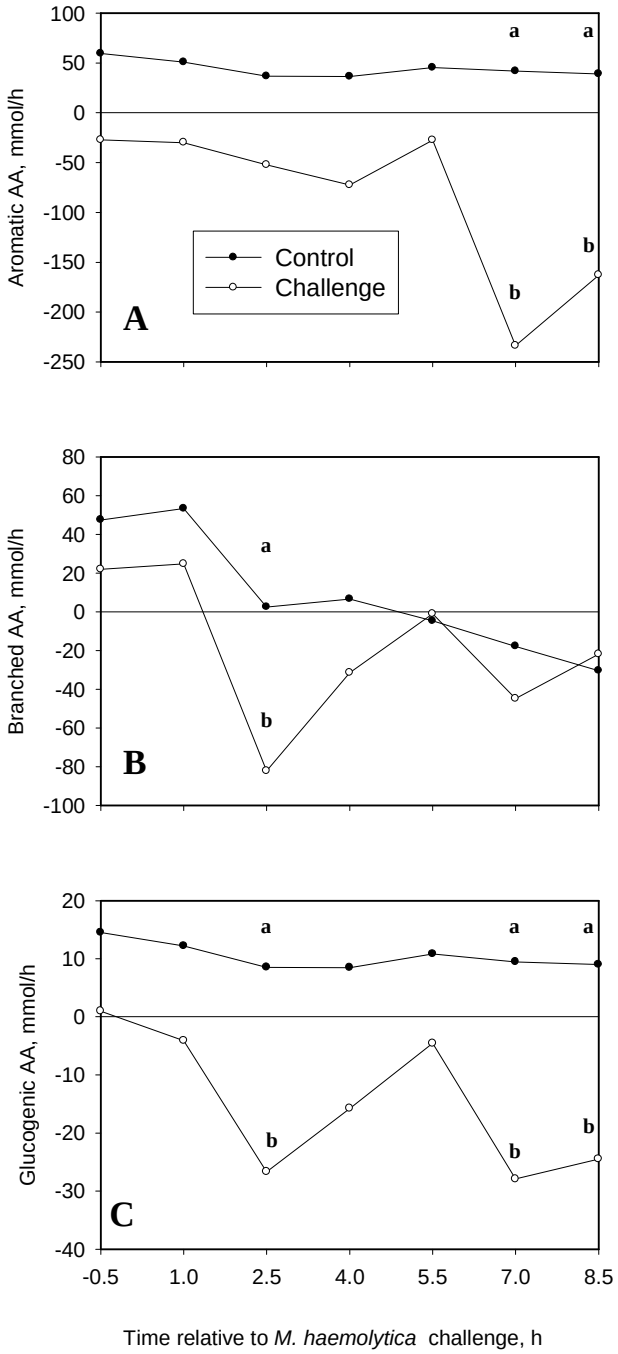
Aromatic AA, mmol/h
100
50
0
-50
-100
-150
-200
-250
a
a
b
b
Control
Challenge
A
Branched AA, mmol/h
80
60
40
20
0
-20
-40
-60
-80
-100
a
b
B
Glucogenic AA, mmol/h
20
10
0
-10
-20
-30
-40
a
a
a
b
b
b
C
-0.5
1.0
2.5
4.0
5.5
7.0
8.5
Time relative to M. haemolytica challenge, h

Figure 5.5

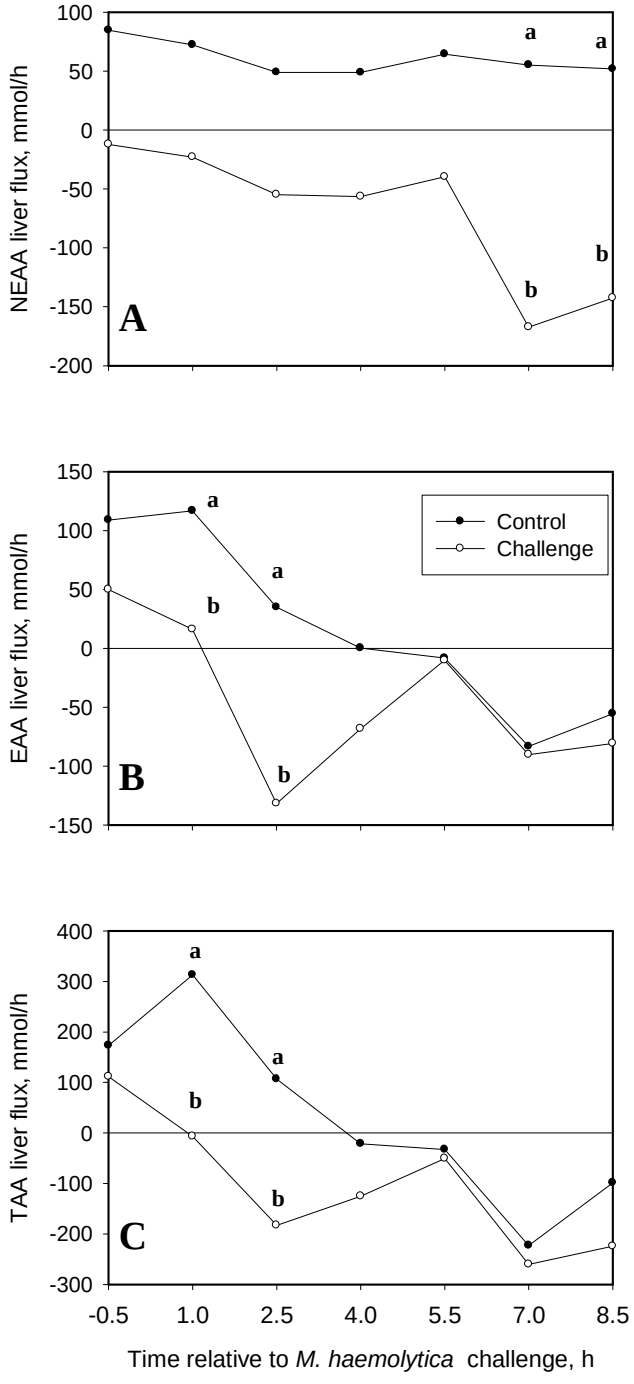
NEAA liver flux, mmol/h
100
50
0
-50
-100
-150
-200
a
a
b
b
A
EAA liver flux, mmol/h
150
100
50
0
-50
-100
-150
a
a
b
b
Control
Challenge
B
TAA liver flux, mmol/h
400
300
200
100
0
-100
-200
-300
a
a
b
b
C
-0.5
1.0
2.5
4.0
5.5
7.0
8.5
Time relative to M. haemolytica challenge, h

Figure 5.6

CHAPTER VI

EFFECTS OF FEED DEPRIVATION AND INTRATRACHEAL CHALLENGE WITH *MANNHEIMIA HAEMOLYTICA* ON BLOOD FLOW AND NET PORTAL AND HEPATIC FLUX OF NUTRIENTS IN BEEF STEERS

ABSTRACT: Bovine Respiratory Disease (BRD) is the most common disease in stocker and feedlot cattle. Although emphasis has been placed on strategies to prevent, diagnose and/or treat BRD, rates of morbidity and mortality have increased. The objective of this experiment was to evaluate blood flow and net splanchnic flux of amino acids during a BRD challenge. Twenty two steers (initial BW = 320 ± 24 kg) with chronic indwelling catheters to measure blood flow and net nutrient flux across the portal drained viscera (PDV) and liver were used for this experiment. The experiment consisted of two, 3 wk sampling periods with 12 animals sampled in each period. During two periods, steers were assigned to one of four treatments: 1) fed ad libitum and not challenged (FED/CON); 2) fed ad libitum and challenged (d 0) with 6×10^9 CFU/mL of *Mannheimia hemolytica* via a tracheal tube (FED/CH); 3) fasted for 72 h and not challenged (FAST/CON); 4) fasted for 72 h and challenged (d 0) with 6×10^9 CFU/mL of *M. hemolytica* via a tracheal tube (FAST/CH). Arterial, portal, and hepatic blood samples were simultaneously drawn 6 times at 1.5 h intervals on d 1, 2, 3, 4, 7, and 14. Statistical analysis was performed for repeated measures using a first-order autoregressive correlation structure for all variables. Diet affected ($P < 0.05$) portal, hepatic, and arterial blood flow, which were greater in FED (460.9, 708.4, and 170.3 L/h, respectively) compared with FAST (426.7, 522.1, and 97.2 L/h, respectively) steers.

Arterial haptoglobin concentration responded with a diet*disease interaction (P = 0.004). Fasting alone caused an increase in arterial haptoglobin concentration, although the increase was greater in Fasted/Challenged steers than Fasted/Control steers. Arterial concentration of total amino acids (TAA) was greater (P = 0.01; 1,966 vs. 1,645 μM) in Control than in Challenged steers. In addition, there was a net removal of TAA (-117.8 mmol/h) by the liver for Challenged steers and a net release for Control steers (21.48 mmol/h; P = 0.03). Although there was no difference (P = 0.22) in arterial concentration of essential AA (EAA), there was a tendency (P = 0.11) for a greater net removal (-65.2 vs. -22.3 mmol/h) of EAA by the liver for the Challenge vs. Control steers. Arterial concentration of nonessential amino acids (NEAA) was greater (P = 0.001) for the Control group (1,173 vs. 924 μM). Similar to TAA, there was a net removal of NEAA (-52.8 mmol/h) by the liver for Challenged steers and a net release for Control steers (42.5 mmol/L; P = 0.02). Based on these results and negative N balance in steers challenged with *M. haemolytica*, it appears that BRD results in greater removal of amino acids by the liver in support of an acute phase response.

Key words: bovine, bovine respiratory disease, feed deprivation, immune response, net nutrient flux

INTRODUCTION

Bovine respiratory disease (**BRD**) involves the complex interaction between stress, bacteria, viruses, and the environment (Duff and Galyean, 2007). Economic losses have been associated with direct costs due to BRD treatment, but also due to indirect costs associated with decreased final BW and carcass quality (Gardner et al., 1999; Thompson et al., 2006). During the acute phase of bacterial infection, decreased growth has been observed in chicks (Klasing et al., 1987), pigs (Kelley et al., 1994) and cattle (Burciaga-Robles et al., 2009a). Decreased growth rates can be attributed to the activation of the immune system with an associated decrease in intake (Klasing and Barnes, 1998; Burciaga-Robles at el., 2009a) and increase in cytokine concentration (Burciaga-Robles et al., 2009b), which can redirect nutrient use away from growth towards mounting an adequate immune response (Klasing, 1988; Spurlock et al., 1997). In addition to activation of the immune system, release of cytokines has been associated with changes in behavior and decreased DMI (Johnson, 1997). Hutchenson and Cole (1986) reported decreased DMI in sick/stressed calves (0.90 ± 0.70% of BW) compared to healthy calves (1.55 ± 0.51% of BW) during the first wk after arrival to a feedlot. In addition, Chirase et al. (1991) reported that the decreased DMI in high stressed/sick calves could remain for two wk, and therefore nutrient intake during this period might be limiting to achieve adequate immune function and/or maximum growth potential (Chirase et al., 1991).

Gilliam et al. (2008) and Waggoner et al. (2008) have reported differences in plasma AA composition due to lipopolysaccharide (**LPS**) challenge in steers, even

though the magnitude of the response to an LPS challenge has been suggested to be of smaller duration and magnitude compared to a true pathogen infection (Sandberg et al., 2007). Although the Beef Cattle National Research Council (NRC, 1996) suggested no difference in crude protein requirements between healthy and stressed calves, during the acute phase response to a pathogen, animals produce significant amounts of nitrogenous compounds (e.g., acute phase proteins, antibodies, cytokines) and develop specific immune cells that can redirect nutrients in support of the immune response (Spurlock, 1997; Sanberg et al., 2007). Therefore, a shift in protein metabolism can be speculated in animals suffering from BRD. In addition to the induced anorexia, hyperthermia, and increased acute phase protein synthesis by the liver, cytokines induce muscle protein catabolism and AA are used for gluoconeogenesis and immune cell proliferation (Melchior et al., 2004). However, little is known about how net flux of AA by the portal-drained viscera (**PDV**) and liver might be altered due to inflammation and immune response in cattle. Better knowledge of net nutrient flux and requirements during an immune challenge would help in the development of new feeding and management strategies to preserve both growth and body defenses in high-stressed calves entering the feedlot. Therefore, the objective of this experiment was to determine the effects of an intratracheal challenge with *Mannheimia hemolytica* on net nutrient flux across the PDV and liver fed or fasted beef steers.

MATERIALS AND METHODS

Animals

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee. Twenty two steers (BW = 320 ± 24 kg) were equipped with chronic catheters to measure blood flow and net flux of nutrients across PDV and liver. Catheters were surgically placed in the portal vein, a hepatic vein, a mesenteric vein, and an adjacent mesenteric artery as described by Ferrell et al. (1991). Catheter patency was maintained by filling catheters with a 1,000 U/mL heparinized-saline solution between sampling days and with a 20 U/mL heparinized-saline solution between sampling times. Steers were allowed a minimum of 14 d to recover from surgery before beginning the initial collection period.

Steers were individually housed at the Oklahoma State University Nutrition Physiology Research Center, Stillwater. The experiment consisted of 18 d during which the animals were kept in individual pens (3.7 × 3.7 m) with the exception of d 0 to 4, 7 to 11 and 14 to 18. During those days, animals were placed in metabolic stanchions to facilitate intensive blood sampling and allow for the collection of total urine and feces (Burciaga-Robles et al., 2009c).

Treatments

Prior to enrollment in the experiment, all steers were determined to be sero-negative to *M. haemolytica* and were considered clinically healthy. Steers were randomly allocated to 1 of 4 treatments arranged as a 2 × 2 factorial as follows: 1) *ad libitum* feeding and not challenged with *M. haemolytica* (**FED/CON**); 2) *ad libitum* feeding and intratracheally challenged (d 0) with *M. haemolytica* (**FED/CH**); 3) 74-h fast and not challenged (**FAST/CON**); 4) 74-h fast and intratracheally challenged (d 0) with *M. haemolytica* (**FAST/CH**). Feed deprivation for the FAST groups occurred 14 h prior to

the challenge with *M. haemolytica* and continued for 60 h during the sampling period. Six steers/treatment were used for the experiment. To facilitate intensive blood collections, sampling occurred during two sampling periods spaced 18 d apart. Two steers from the FED/CON group in the first sampling period were randomly allocated to treatment in the second period. Steers were challenged with *M. haemolytica* using the same protocol as described by Burciaga-Robles et al., 2009b. Briefly, animals challenged with *M. haemolytica* received 10 mL of a solution containing 6×10^9 CFU of *M. haemolytica* serotype 1, which was reconstituted and grown prior to the challenge as described by Mosier et al. (1998). Steers not challenged with *M. haemolytica* were intratracheally dosed with 10 mL of a sterile phosphate-buffered saline solution. With the exception of d where feed was withheld, steers were offered feed for ad libitum intake with feed delivered twice daily at 0800 and 1700. The diet was formulated to meet or exceed nutrient requirements (NRC, 1996). Ingredients and nutrient composition were reported by Burciaga-Robles et al. (2009d).

Sample Collection

Steers were placed in metabolism stanchions in a climate-controlled room (23 to 27°C) 24 h before the initiation of the blood collection period. Rectal temperatures were recorded using a digital veterinary thermometer (GLA M-500; GLA Agricultural Electronics, San Luis Obispo, CA). On d 1, rectal temperatures collected at 3, 6, 12, and 18 h after the bacterial challenge were averaged, and for the remaining d, a daily measurement was performed at 0800. Blood samples were collected on d 1, 2, 3, 4, 7, and 14 of the challenge. At 0600 on the morning of challenge (d 1) and 0700 on all subsequent blood sampling d, a priming dose of 20 mL of 10% (wt/vol) para-

aminohippurate (**PAH**, pH = 7.4) was administered through a 0.45 μm sterile filter (Millipore, Bedford, MA) into the mesenteric vein catheter. Para-aminohippurate was continuously infused (PHD 2000 Syringe pump; Harvard Apparatus Inc., Holliston, MA) at 0.7 mL/min for 9 h following the priming dose. On d 1, blood was collected 1 h prior to the *M. haemolytica* challenge (0700), and continued every 1.5 h from 0830 until 1600 (total of 7 samples). On d 2, 3, 4, 7, and 14, blood was collected beginning at 0800 and continued every 1.5 h until 1530 (total of 6 samples). At every sampling time, 10 mL of blood were simultaneously drawn three times from the portal vein, hepatic vein, and mesenteric artery catheters into syringes, and blood was placed into three 10 mL tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). The first 10 mL were placed into tubes treated with sodium heparin for plasma harvest, the second 10 mL into tubes containing potassium oxalate and sodium fluoride for plasma harvest for glucose analysis, and the third 10 mL were placed into tubes without anticoagulant for serum harvest. After collection, blood samples were immediately capped and placed on ice for transport to the laboratory. Immediately after collection, 90 μL of arterial, portal and hepatic whole blood samples were analyzed using a blood gas analyzer (1304 pH/Blood Gas Analyzer, Instrumentation Laboratory, Lexington, MA) for determination of blood gas concentrations. Calibration of the blood gas analyzer was performed prior to each sampling day using a commercially available calibrator (ContrIL Blood gas control #93630, Instrumentation Laboratory) and automated two-point calibration. An additional 40 μL of whole blood was used to determine packed cell volume. Remaining blood was centrifuged (3,000 × *g*, 4°C, 20 min) and plasma and serum harvested and frozen (−40°C) for further analysis. Two milliliters of plasma and serum from each sampling time were

composited within site (arterial, portal, hepatic) to provide a daily sample, and frozen (−40°C) for further analysis. The pre-challenge samples collected at 0700 on d 1 were excluded from the composite.

Analyses

Mannheimia haemolytica Antibodies. Serum samples from d 0, 7, and 14 were used to determine antibodies to whole bacterial cell and leukotoxin for a formalin killed *M. haemolytica* serotype 1 by an ELISA test as described by Confer et al. (1995; 1996). Antibody responses were expressed as nanograms of immunoglobulin binding based on a set of IgG standards on each plate. The intra- and inter-assay coefficients of variation were below 5%.

Serum Haptoglobin. Haptoglobin concentrations in arterial, portal and hepatic serum samples were analyzed in duplicate using a Bovine Haptoglobin ELISA kit (Immunology Consultants Lab, Portland OR) as described previously (Burciaga-Robles et al., 2009b). The intra and inter assay coefficient of variation were below 7.5%.

Metabolites and Para-Aminohippurate. Daily composites from d 1, 2, 3, 4, 7 and 14 were analyzed for PAH and metabolite concentrations. Plasma PAH concentrations were determined colorimetrically as previously described (Harvey and Brothers, 1962). Commercially available kits were used for the colorimetric determination of plasma glucose (Liquid Glucose [hexoquinase] Reagent Set, Pointe Scientific, Canton, MI), lactate (Lactate Reagent Set, Pointe Scientific), urea nitrogen (Urea Nitrogen Reagent, Teco Diagnostic, Anaheim, CA), ammonia (Ammonia Reagent Set, Pointe Scientific) and total protein (arterial blood samples only; Total Protein [Biuret] Reagent Set, Pointe Scientific) concentrations. Microplates (Beckman Coulter, Fullerton, CA) were used for

all analyses, and absorbance was measured according to manufacturer recommendations for each metabolite using a plate reader (Multiskan Spectrum; Thermo Scientific, Waltham, MA). Intra- and inter-assay coefficients of variation for each metabolite were kept below 5 and 7.5%, respectively.

Free plasma AA were determined via GLC using a commercially available kit (EZ:FAAST #KGO-7165; Phenomenex, Torrance, CA). The kit supplied all necessary reagents for solid phase extraction and derivatization as described by Waggoner et al. (2008). Briefly, AA were extracted from 100 µL of plasma and eluted, the AA were derivatized, and the eluant was allowed to separate into 2 layers. An aliquot of the organic layer was collected into glass scintillation vials and analyzed for AA on a gas chromatograph (Varian CP-3800, Varian, Walnut Creek, CA) using a split injection protocol (2 µL of sample at 250°C) with helium (1.5 mL/min) as the carrier gas. Intra- and inter-assay coefficients of variation for AA determination were less than 15%.

Plasma volatile fatty acids were measured as described by Kristensen (2000) using a HP 5890 Series II GLC (Agilent, Atlanta, GA) with a flame ionization detector and HP 7673 automatic injector (Agilent). The GLC was equipped with a 60 m × 0.25 i.d. fused-silica capillary column chemically bonded with 0.25-um DB5-MS stationary phase (J&W Scientific, Folsom, CA). The injector temperature was set at 250^{o}C. Helium was used as a carrier at a flow rate of 1 mL/min through the column. The column was maintained at a temperature of 75°C for 0.5 min, then temperature was increased to 135°C at a rate of 3°C min^{-1} and maintained at 270°C for 10 min to the end of the run. Peak areas were calculated by the Agilent ChemStation (Revision B.04.01; Agilent).

Blood and Net Nutrient Flux Calculations

Calculations for blood oxygen concentrations and plasma flows through the portal and hepatic vein were calculated as described by Hersom et al. (2003). Briefly, blood flow (L/h) = IRPAH/(CVPAH − CAPAH), where IRPAH is the infusion rate (mg/min) of PAH, and CVPAH and CAPAH are the concentration (mg/L) of PAH in venous and arterial plasma, respectively. Portal and hepatic blood flow were calculated directly, whereas hepatic arterial blood flow was calculated as hepatic blood flow minus portal blood flow.

Net flux of nutrients across the portal-drained viscera (**PDV**), hepatic, and total splanchnic vascular beds were calculated as described by Krehbiel et al. (1992) using the following equations: PDV flux = PBF × (Cp - Ca), Hepatic flux = PBF × (Ch – Cp) + ABF × (Ch – Ca), and total splanchnic flux = PDV flux + hepatic flux, where ABF and PBF are the blood flow rates (L/h) in the artery and portal vein, and Ca, Cp, and Ch are the metabolite concentrations in the arterial, portal, and hepatic plasma samples, respectively. A positive net flux indicates a release or absorption of a nutrient, whereas a negative net flux represents uptake or utilization. The number of steers sampled during each period and number of catheters patent at each site are listed in Table 1. The hepatic extraction ratio (**HER**) has been described as an empirical method to calculate the amount of a nutrient removed (uptake) by the liver as a fraction of the total metabolite present in whole blood or plasma (Delgado-Elorduy et al., 2002), and was calculated according to Brockman and Bergman (1975) using the formula: HER $=(F_{pv}(C_{pv} – C_{hv}) + F_a (C_a – C_{hv}))/F_{pc}Cpv + F_aC_a$, where F_{pv} is the portal plasma flow (L/h), C_{pv}, C_{hv} and C_a are the portal and hepatic vein, and arterial plasma concentrations of the metabolite, and F_a is the hepatic arterial plasma flow.

Aromatic AA were calculated as the sum of tyrosine, tryptophan and phenylalanine; branched chain AA (**BCAA**) as the sum of leucine, isoleucine and valine; gluconeogenic AA as the sum of alanine, glycine, glutamine, glutamic acid, serine, and threonine; sulfur containing AA as the sum of cysteine and methionine; essential AA (**EAA**) as the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; non-essential AA (**NEAA**) as the sum of alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, hydroxyproline, proline, serine, and tyrosine; and total AA as the sum of all amino acids reported.

Statistical Analysis

Performance data were analyzed using the Mixed Procedure of SAS with animal as the experimental unit. Fixed effects included diet, disease and diet × disease; period was included as a random effect in the model. Dry matter intake and blood metabolite concentration and net flux data were analyzed using the Mixed Procedure of SAS with a repeated measures analysis using a first order autoregressive covariance structure with heterogenous variance. A heterogenous variance between subjects was selected due to an increase in variance across treatments, although the pattern of variance remained homogenous within treatments (SAS Institute, 2003). Animal was the experimental unit and the repeated measure statement was sampling time within animal. Fixed effects were diet, disease, time, and all possible interactions, and period was included as a random effect. Results are discussed as significant if $P \leq 0.05$ and as a tendency if $P > 0.05$ and $P \leq 0.10$.

RESULTS

The number of steers sampled during each period and number of catheters patent at each site are listed in Table 1. Luis, we need the statement on catheter patency and the table.

There were disease × d interactions ($P < 0.0001$) for *M. haemolytica* whole cell antibodies (Figure 1a) and *M. haemolytica* leukotoxin (Figure 1b). Antibody titers for *M. haemolytica* whole cells and leukotoxin were greater on d 14 following *M. haemolytica* challenge compared with CON steers. In addition, rectal temperatures were greater for CH than CON steers on d 1 and 2 following the challenge (disease × d interaction, $P < 0.0001$; Figure 2). Dry matter intake averaged across d 0 to 4, 7 to 11 and 14 to 18 responded with a diet × disease × time interaction ($P < 0.01$). Dry matter intake was greater ($P < 0.05$) for FED/CON than FED/CH, FAST/CON, and FAST/CH from d 0 to 4, greater for FED/CON, FED/CH, and FAST/CON than FAST/CH from d 7 to 11, and did not differ among treatments ($P > 0.05$) from d 14 to 18 (Figure 3). Performance data is reported in Table 2. Across the 14-d experiment, FAST steers had lower ($P \leq 0.03$) DMI than FED steers. Interestingly, diet did not affect ($P = 0.75$) ADG; however, ADG was lower ($P = 0.03$) for CH compared with CON steers across the 14-d period. In addition, G:F tended ($P = 0.11$) to be lower CH than CON steers. Feed deprivation did not affect ($P \geq 0.41$) blood gas variables, whereas CH steers had greater blood pH ($P = 0.04$) and lower O_2 saturation ($P = 0.02$), hematocrit ($P = 0.0003$), and O_2 concentration ($P = 0.01$) than CON steers (Table 2). Concentration of acetate in arterial blood was lower ($P < 0.0001$) in FAST compared with FED steers. In addition, arterial concentrations of acetate responded with a diet × d interaction ($P < 0.0001$; Figure 4A). Arterial acetate concentration decreased from d 1 to 3 of feed deprivation,

and remained lower than FED steers through d 14. Arterial blood concentration of propionate was greater in FED/CON and FED/CH steers than FAST/CH steers; FED/CH steers were intermediate (diet × disease, $P = 0.0008$; Table 2). In addition, arterial blood concentrations of butyrate ($P = 0.04$) and β-hydroxybutyrate ($P = 0.02$) were greater in CON steers than CH steers. β-hydroxybutyrate also responded with a diet × d interaction ($P = 0.001$; Figure 4B). On d 2, 3, and 4, β-hydroxybutyrate concentrations increased in FED and decreased in FAST. By d 4 of re-feeding, arterial concentrations returned to pre-feed deprivation levels and remained without changes during the remainder of the experiment. Lactate concentration in arterial blood was greatest for FED/CH steers, intermediate for FAST/CON and FAST/CH steers, and lowest for FED/CON steers (diet × disease, $P = 0.03$; Table 2). In addition, arterial lactate concentration was greater ($P < 0.05$) for CH than CON steers on d 2 and 7 post *M. haemolytica* challenge (diet × d, $P = 0.001$; Figure 5). Across the 14-d experiment, feed deprivation had no effect on arterial glucose concentrations ($P = 0.30$; Table 2); however, it responded with a diet × d interaction ($P = 0.03$; Figure 4C). On d 2 of feed deprivation, glucose concentration was greater for FED steers than FAST steers; however, on d 3, 7 and 14, FAST steers had greater glucose concentrations than FED steers.

There were no diet × disease × d interactions ($P > 0.10$) for arterial concentrations of nitrogenous compounds (data not shown). Arterial concentration of haptoglobin was greater ($P = 0.05$) in CH compared with CON steers (Table 3). In contrast, arterial concentrations of alanine ($P = 0.02$), glutamine ($P = 0.006$), glycine ($P = 0.006$), hydroxyproline ($P = 0.0001$), proline ($P = 0.01$), glucogenic amino acids ($P = 0.002$), non-essential amino acids ($P = 0.01$), and total amino acids ($P = 0.03$) were lower in CH

than CON steers. Similarly, concentrations of asparagine (P = 0.07) and glutamic acid (P = 0.06) tended to be lower in CH compared with CON steers. Proline concentration in arterial blood responded with a diet × disease interaction (P = 0.03). The decrease in proline when steers were challenged with *M. haemolytica* was greater in FAST compared with FED steers. Concentrations of aromatic (P = 0.06) and sulfur containing amino acids (P = 0.07) tended to be lower in FAST compared with FED steers.

Diet × d interactions for arterial concentrations of AA are presented in Figure 5. There were similar diet × d interactions for alanine (P = 0.01; Figure 5A) and histidine (P = 0.02; Figure 5C). During the first 7 d of the experiment concentrations were similar for FED and FAST steers, but on d 14 concentrations in FED steers were grater for both AA. Arterial concentrations of glutamic acid (diet × d, P = 0.0004; Figure 5B) and methionine (diet × d, P = 0.02; Figure 5D) were similar on d 1 of the experiment; however, on d 3 of the feed deprivation period glutamic acid and methionine were greater in FED compared with FAST. Tyrosine concentrations were greater in FED compared with FAST steers on d 3 and 14 (diet × d, P = 0.03; Figure 5E), whereas arterial concentration of serine was greater for FAST compared with FED on d 4 (diet × d, P = 0.05; Figure 5D). Gluconeogenic AA (diet × d, P = 0.02; Figure 5F) and NEAA (diet × d, P = 0.02; Figure 5G) responded in the same fashion where arterial concentrations were increased in FED animals compared with FAST 11 d after re-feeding. (Luis, please check the superscript for glucogenic AA. I believe it's in the wrong spot.)

SFor AA, disease × d interactions for are presented in Figure 7. Glycine (disease × d, P = 0.0002; Figure 7C), glucogenic AA (disease × d, P = 0.001; Figure 7G), and NEAA (disease × d, P = 0.003; Figure 7H) were lower on d 2, 3, and 4 relative to *M.*

haemolytica challenge in CH. Glutamine (disease × d, $P = 0.01$; Figure 7A), and hydroxyproline (disease × d, $P < 0.0001$; Figure 7D) responded in the same fashion than glycine, glucogenic and NEAA, but the decreased concentrations in CH lasted until d 7 relative to *M. haemolytica* challenge. Arterial concentrations of glutamic acid (disease × d, $P = 0.0004$; Figure 7B) and threonine (disease × d, $P = 0.04$; Figure 7F) were lower in CH animals on d2 and 3 following *M. haemolytica* challenge. Phenylalanine was increased (disease × d, $P = 0.01$; Figure 7E) in CH on day 2 relative to *M. haemolytica* but returned to baseline concentrations on day 3 and no further differences were detected.

Arterial blood flow was lower ($P = 0.01$) in FAST compared with FED steers (Table 3). Portal and hepatic blood flow were not affected ($P \geq 0.26$) by treatment. Oxygen consumption across the PDV was lower ($P = 0.005$) in FAST compared with FED steers, and was greater ($P = 0.02$) in CH compared with CON steers. In addition, O_2 consumption across TST was lower ($P = 0.02$) in FAST compared with FED steers. Net PDV release ($P = 0.0006$) and hepatic removal ($P = 0.0008$) of propionate were lower in FAST than in FED steers. Net PDV release of butyrate tended ($P = 0.07$) to be greater and net PDV release of β-hydroxybutyrate was lower ($P = 0.05$) in FAST compared with FED steers. Hepatic extraction of butyrate was greater ($P = 0.05$) in CH compared with CON steers. Similar to PDV release, TST release of β-hydroxybutyrate was lower ($P = 0.03$) in FAST compared with FED steers. Net removal of lactate by the liver ($P = 0.06$) tended to be lower and TST ($P = 0.008$) and HER ($P = 0.001$) were lower in FAST compared with FED steers. In addition, TST removal (tendency, $P = 0.09$) and HER ($P = 0.05$) were lower in CH compared with CON steers. Net release of glucose by the liver (tendency, $P = 0.08$) and TST ($P = 0.04$) were lower in FAST compared with FED steers.

There were no effects ($P \geq 0.44$) of diet, disease, or diet × disease for net PDV, liver, or TST flux of haptoglobin, ammonia or urea N (Table 4). Net flux of alanine across the PDV responded with a diet × disease interaction ($P = 0.02$). Net flux of alanine by the PDV was greatest for FED/CON steers, lowest for FAST/CON steers, and intermediate for FED/CH and FAST/CH steers. Removal of alanine by TST was greater ($P = 0.05$) for FAST steers than FED steers. Removal of asparagine by the liver ($P = 0.01$) was greater for FAST than for FED steers. Net flux of asparagine across TST was greatest for FED/CON steers, lowest for FAST/CON steers, and intermediate for FED/CH and FAST/CH steers (diet × disease interaction, $P = 0.03$). Net liver removal (tendency, $P = 0.06$) and TST flux ($P = 0.01$) of aspartic acid were lower for FAST compared with FED steers. Similarly, net flux of glutamine across the liver ($P = 0.01$) and HER ($P = 0.008$) were lower in FAST compared with FED steers. Net liver ($P = 0.01$) and TST ($P = 0.02$) removal of glutamate were greater for FAST compared with FED steers. In addition, removal of glutamate by TST was greater ($P = 0.01$) for CH compared with CON steers. Net flux of glycine across TST tended to be lower for FED/CH steers compared with steers on the remaining treatments (diet × disease interaction, $P = 0.08$). Fasting tended ($P = 0.10$) to increase removal of histidine by the liver. Challenge with *M. haemolytica* increased ($P = 0.03$) removal of hydroxylproline by the liver. Net flux of isoleucine across the PDV tended to respond with a diet × disease interaction ($P = 0.07$). Net flux of isoleucine was greater for FED/CON and FAST/CH steers compared with FED/CH and FAST/CON steers. Net PDV, liver or TST flux of leucine, lysine, and methionine were not affected ($P \geq 0.16$) by diet or disease. Net removal of ornithine by the liver responded with a diet × disease interaction ($P = 0.05$). Net flux of ornithine was greatest

for FED/CON steers, lowest for FED/CCH steers, and intermediate for FAST/CON and FAST/CH steers. In addition, HER of ornithine was greater ($P = 0.04$) in CH compared with CON steers. Net TST flux of phenylalanine tended ($P = 0.10$) to be lower in FAST than in FED steers, and HER of phenylalanine was greater ($P = 0.04$) in FAST compared with FED steers. Net removal of serine ($P = 0.01$), threonine ($P = 0.03$), and tryptophan ($P = 0.03$) by the liver was greater in FAST than in FED steers. In addition, HER of serine (tendency, $P = 0.09$), threonine ($P = 0.003$), tryptophan ($P = 0.01$), and tyrosine ($P = 0.002$) was greater in FAST than in FED steers. Hepatic extraction of threonine ($P = 0.03$), tryptophan (tendency, $P = 0.09$), and tyrosine ($P = 0.03$) were greater in CH than in CON steers. The liver removed more tyrosine ($P = 0.04$) in CH compared with CON steers.

Feed deprivation increased net removal of aromatic (tendency, $P = 0.06$), gluconeogenic ($P = 0.003$), non-essential ($P = 0.003$), and total amino acids ($P = 0.02$) across the liver (Table 4). In addition, challenge with *M. haemolytica* increased liver removal of aromatic ($P = 0.01$), gluconeogenic ($P = 0.04$), and non-essential ($P = 0.04$) amino acids. Similarly, removal of aromatic ($P = 0.05$) and non-essential ($P = 0.02$) amino acids by TST was greater in CH than in CON steers. Feed deprivation increased the HER for aromatic ($P = 0.005$), gluconeogenic ($P = 0.009$), non-essential ($P = 0.008$), and total amino acids ($P = 0.01$), and challenge with *M. haemolytica* increased HER for aromatic ($P = 0.04$), gluconeogenic ($P = 0.05$), non-essential ($P = 0.05$), and total amino acids (tendency, $P = 0.08$). Net PDV flux of sulfur containing amino acids was greater ($P = 0.03$) in FAST compared with FED steers.

DISCUSSION

In the present experiment, peak rectal temperature was reached 24 h post *M. haemolytica* challenge, and remained elevated 48 h post challenge. Similarly, immune challenges with bacterial LPS in cattle (Gilliam et al., 2008, Reuter et al., 2008; Waggoner et al., 2008) and pigs (Webel et al., 1997) has resulted in increased rectal temperature, although the increase in rectal temperature is usually for a shorter period of time than observed in the present experiment. Burciaga-Robles et al. (2009c) reported the acute changes in cytokines, rectal temperature, portal and hepatic blood flow and net nutrient flux across the PDV, liver, and TST during the d of *M. haemolytica* challenge following the initial 14 h of feed deprivation. Based on those results and results from the present experiment, we conclude that our challenge model was successful in developing clinical and physiological signs associated with BRD.

A febrile response has been reported to be a beneficial response by the host rather than being a negative consequence of the pathogen (Blatteis, 2003), because pathogen growth is sensitive to changes in body temperature. In addition, increased body temperature can have a positive effect on the host immune response (e.g., activation of specific proteins; Sanberg et al. 2007). Increased body temperature during a febrile process in humans and rats (Dantzer, 2002) has been reported to increase metabolic rate by 13% for every 1^{o}C increase in body temperature. Therefore, the magnitude and duration of the increase in rectal temperature might impact maintenance requirements in morbid animals due to the increased energy cost for this metabolic process.

Haptoglobin is an acute phase protein produced by the liver, and increased concentrations have been reported by Carter et al. (2002), Berry et al. (2004), and Step et

al. (2008) in calves experiencing clinical signs of BRD. In the present experiment, CH steers had increased haptoglobin compared with CON steers. Melchior et al. (2004), using complete Freund's adjuvant as an immune challenge in fed or fasted pigs, reported increased haptoglobin concentrations 10 d after the challenge. In our study and the study of Melchior et al. (2004), haptoglobin concentration was not affected by feed deprivation.

To account for potential effects that intake has on nutrient digestion and metabolism, pair feeding has been suggested for use in experiments comparing animals challenged with a pathogen vs. non-challenged animals (Sandberg et al., 2007). Because pathogen exposure induces anorexia in host animals, potential effects of decreased intake should also be accounted for when developing feeding strategies for morbid animals in production settings (Kyriazakis et al, 1998). In the present experiment, we observed a compensatory response in intake for FED/CH, and FAST/CON steers by d 7 to 11, although intake in FAST/CH remained below the remaining treatment groups. In addition, ADG was decreased for CH compared with CON steers. Therefore the model used in the present experiment was effective at simulating a BRD event across the 14 d experimental period.

Blood gas

Blood gas components and acid base equilibrium in cattle can be altered during respiratory disease (Carlson, 1996). Normal blood pH in cattle has been reported to range 7.35 to 7.45 (Cingi et al. 2009). In our study, blood pH was increased in CH (7.48) when compared with CON (7.45) steers and could be considered as marginally alkalotic. Parker et al. (2007) reported no differences in blood pH due to 48 h transportation with

feed and water deprivation, which is in agreement with results in our study where a 72 h fasting did not influence blood pH.

Hemoglobin and packed cell volume were decreased in CH steers compared with CON steers. This data is in agreement with data reported by Corrigan et al. (2007), where heifers challenged with *M. haemolytica* had a decrease in hemoglobin and packed cell volume attributed to inflammation of lung tissue. In a previous experiment (Burciaga-Rbles et al. 2009a) using a similar experimental model, we reported a decrease in hemoglobin and a tendency for lower packed cell volume, similar to the present experiment.

Arterial concentrations of energy metabolites

Loerch and Fluharty (1999) reported that periods of feed and water deprivation associated with marketing strategies of beef cattle could have a negative effect on the rumen environment. In addition, Baldwing (1967) reported that total rumen bacteria were decreased after a 48 h period of feed and water deprivation. Galyean et al (1981) reported that rumen bacteria counts were decreased after a 32 h feed deprivation, but the counts returned to pre-feed levels by 72 h after re-feeding occurred. Ruminal fermentative capacity (**RFA**) has been reported to be a measurement of the capacity of rumen microorganisms to ferment substrate. Baldwin et al (1967) reported that RFA is decreased to 0% after 48 h of feed and water deprivation, and Hutchenson and Cole (1981) reported that this negative effect could last as long as 5 d.

The end products of ruminal fermentation include acetate, propionate, and butyrate, which are either metabolized by or absorbed across the rumen wall where they are transported to the liver. These VFA are the major source of energy in the normally-

fed ruminant (Kristensen, 2005). However, little is known about peripheral VFA metabolism under conditions of environmental stress or immune challenge, when energy requirements are suddenly increased. In ruminants, acetate is an important substrate for oxidation (Annison and Armstron, 1970) and fat synthesis (Pethick, 1981; Smith and Crouse, 1984). In the present experiment, arterial concentrations of acetate were not affected due to *M. haemolytica* challenge, but were decreased due to feed deprivation. These results are in agreement with those reported by Lomax and Baird (1983) who observed a decline in acetate after 24 h of feed deprivation in fasted dairy cows. Arterial acetate concentrations continued to decline to d 6 to approximately 95% of pre-fasting levels. The decrease in acetate concentration in fasted animals suggests that the majority of acetate production and absorption is related to DMI, most likely resulting in ruminal microbial and fermentation alterations due to lack of substrate.

In homeostasis, propionate derived from ruminal fermentation is considered the major gluconeogenic precursor, and the conversion rate from propionate to glucose has been reported to range from 32 to 73% (Seal and Reynolds, 1993). However, propionate concentration depends on the availability of substrate for fermentation in the rumen (Bergman, 1990; Owens et al., 2008). In addition, during periods of increased energetic demands, propionate conversion to glucose has been reported to increase (Wiltrout and Satter, 1972; Steel and Leng, 1973; Drackley et al., 2001). In the present experiment, arterial concentrations of propionate were higher in FED/CON, intermediate for FAST/CON and FED/CH, and lowest for FAST/CH. Although propionate is a potent glucogenic compound, it is difficult to assess its precise contribution to glucose production in the whole animal (Bergman , 1990). Arterial

concentrations of propionate can only be increased if availability of substrate is increased or if specific disorders of propionate metabolism exist. The decreased arterial concentrations of propionate in the present study in response to *M. haemolytica* challenge without increased concentrations of glucose may indicate that propionate can be used for metabolic activities in peripheral tissues when energy demands are increased, as suggested by Bell et al. (1974). Increased energy requirements in muscle or other peripheral tissues could lead to a repartitioning of nutrients from non-oxidative pathways (nutrient storage) to oxidative pathways in which circulating VFA might be used as energy substrate (Hocquette et al., 1998).

Arterial concentrations of butyrate were decreased in CH steers in the present experiment. Although butyrate is mainly taken up by epithelial tissues of the gastrointestinal tract and liver, minimum amounts can enter peripheral circulation (Bergman, 1990), and in peripheral tissues be rapidly oxidized or used for lipogenesis or milk fat synthesis (Black et al., 1961; Annison et al., 1963). Similar to butyrate, arterial β-hydroxybutyrate (**βHB**) concentration was lower in CH compared with CON in the present study. Waldron et al. (2003) reported a linear decrease in βHB in lactating cows infused with different doses of LPS. In addition, Steiger et al. (1999) reported a decrease in βHB in dairy heifers shortly after being infused with LPS.

Burciaga-Robles et al. (2009b) reported no difference in lactate concentrations in steers following a *M. haemolytica* challenge, in contrast with the present experiment. However, the concentration of lactate was decreased in steers exposed to steers persistently infected with BVDV type 1b. In contrast, Montgomery et al. (2008) reported decreased lactate concentrations in heifers treated against BRD when compared to non-

treated heifers. Coghe et al. (2000) suggested the use of plasma lactate as a diagnostic/prognosis tool in animals suffering from BRD, suggesting that during respiratory disease, a decrease in oxygen transferred from the lungs to arterial blood due to increased respiration would decrease aerobic metabolism. However, increased plasma lactate was only a good prognosis tool when plasma lactate levels were increased above 4 m*M* when animals died within the following 24 h. In the present experiment, a decrease in arterial blood oxygen concentration and increased plasma lactate might suggest that successful respiratory infection altered respiration rate and oxygen dynamics in CH steers. In addition, increased lactate plasma concentrations have been associated with an increased glucogenolysis and a reduced capacity of peripheral tissues to utilize lactate in response to an immune challenge (Harada el al., 1994; Mizock, 1995).

Glucose concentrations in this study were not affected by *M. haemolytica* challenge, but decreased during the first three d of feed deprivation and then increased above pre-feeding levels after re-feeding. Our results are in agreement with Galyean et al. (1981) and Cole et al. (1988) who reported a decrease in serum glucose when steers were fasted for 32 and 24 h, respectively. Heitman and Bergmann (1980) reported that during short periods of glucose deficit, AA from skeletal muscle can be released and used for glucogenesis. However, during long periods of negative energy balance due to decreased intake or increased nutrient requirements, Thompson and Wu (1991) reported a combination of adaptation mechanisms to overcome the energy sparing from energy and protein, including increased peripheral oxidation of VFA and increased formation of ketone bodies (βHB and acetoacetate). However, Robinson and Williams (1980) also suggested that the concentrations of these metabolites in peripheral circulation could be

decreased because they are utilized as oxidative fuels by the host. Changes in arterial concentrations of energy related compounds are in general agreement with previous results related to periods of metabolic stress due to negative energy balance, associated disease, decreased DMI, and increased energy demands.

Arterial concentrations of nitrogenous compounds

Urea-N concentrations have been used as an indicator of protein balance in cattle (Ndlovu et al., 2007). In the present experiment, feed deprivation for 72 h had no effect on arterial concentration of ammonia and urea. This data is in agreement with results of Lapierre et al. (2000) who reported no effect of increasing DM, energy, and CP intake on arterial concentrations of these metabolites. Yambayamba et al. (1996) reported lower concentrations of urea N in feed restricted heifers compared to heifers deprived of feed for 48 h. In contrast, Hayden et al. (1993) and Cole et al. (1988) have reported increased concentrations of urea N in feed restricted animals compared to an ad libitum-fed group. Discrepancies in results across experiments might be explained from previous protein nutrition status and the capability of N recycling in ruminant animals. Webel et al. (1997) reported increased concentrations of urea N in pigs challenged with LPS compared to a non-challenged control group. The increase in urea N was associated with an acute stage of proteolysis associated with the immune response. In cattle, Orr et al. (1988) reported an increase in urea N in steers challenged with infectious bovine rhinotracheitis virus. These authors restricted DMI to 1% of BW which might have increased the negative effect of the viral infection and resulted in a greater muscle catabolism and increase urea N. We did not observe similar results with *M. haemolytica* challenge. More recently, Montgomery et al. (2008) reported a linear increase in urea N

in heifers not treated up to treated three times against BRD. Therefore, an increase in plasma urea N could be an indicator of severity and duration of BRD other than just morbidity associated with the disease.

One of the main characteristics of the immune response is hypercatabolism, increased oxygen consumption and energy expenditure, activation of periphereal catabolism, and specially augmented metabolism in the hepatosplanchnic region (Dahn et al., 1987; Barton and Cerra, 1989). This increased catabolism of periphereal tissues (muscle) provides substrates to the liver for glucogenesis and synthesis of acute phase proteins (Reid et al., 2004). In general, AA concentrations in patients with sepsis (Druml et al., 2000), cattle following LPS challenge (Gilliam et al. 2008; Waggoner et al. 2008), and pigs after Freud's complete adjuvant injection (Melchior et al., 2004) are decreased compared to healthy individuals, and the extent of this difference has been used as a marker of the severity and prognosis of the disease process (Freund et al., 1979). Burciaga-Robles et al. (2009c) reported the changes in AA concentrations during the first 8.5 h of the present experiment. Interestingly, across 14 d *M. haemolytica* challenge decreased arterial plasma concentrations of alanine, glutamine, glycine, hydroxyproline, proline, gluconeogenic, NEAA and total AA concentrations ($P < 0.05$). Although a decrease in AA concentrations following inflammatory disease could reflect the decrease in feed intake (Melchior et al. 2004), there was no differences in DMI by d 14 of the present experiment. In addition, feed deprivation had no effect on arterial plasma concentrations of individual AA except proline. Therefore, differences in AA concentrations in the present experiment associated with *M. haemolytica* challenge might be attributed to an extended effect of the disease on AA metabolism. A decrease in

peripheral concentrations of AA has been proposed by Melchior et al. (2004) to result from an increased utilization of AA as substrates for energy by peripheral tissues, utilization of the AA by cells of the immune system as precursors of energy or protein molecule synthesis, or from entering specific pathways related to body defense. Gilliam et al. (2008) and Waggoner et al. (2008) reported a decrease in methionine, lysine, leucine, isoleucine, phenylalanine, tryptophan, glycine, serine, aspartate, glutamine and ornithine in response to an LPS challenge; however, the length of their experiment was only 12 h suggesting that changes in this AA were more related to changes occurring in the acute phase of the immune response as described previously for this experiment (Burciaga-Robles et al. 2009c). In pigs (Melchior et al. 2004), plasma concentrations of AA after Freud's adjuvant injection were lower in fed compared to fasted pigs, suggesting that when AA are supplied during the immune response, they are more rapidly metabolized in challenged animals. However, in our experiment and during the acute phase previously reported (Burciaga-Robles et al. 2009c), the interactions between feed deprivation and *M. haemolytica* challenge were minimum.

In the present experiment, decreased arterial concentrations of glucogenic AA were observed in CH animals. As described previously, during an immune challenge there is an increased energy substrate demand due to metabolic changes in energy metabolism, increased glucose utilization, and oxidation of VFA to meet the energy demands, but in addition an increased catabolism of glucogenic AA occurs (Druml et al., 2001). Increased gluconeogenic activity in the liver during an immune challenge has been reported previously *in vitro* in hepatic slices from lactating dairy cows following LPS challenge (Waldron et al., 2003).

Over the 14 d experiment, arterial concentrations of BCAA were not affected by *M. haemolytica* challenge, in contrast to our previous report indicating decreased concentration of this group of amino acids (Burciaga-Robles et al., 2009). In contrast, arterial concentrations of NEAA were still decreased after 14 d in CH animals, which could indicate an increased requirement for this group of AA. Aromatic and sulfur-containing AA tended to be decreased in feed deprived steers. This may be attributed to the long-term effects that feed deprivation has on rumen bacterial populations and altered ruminal fermentation that has been previously described (Galyean et al., 1981; Hutchenson and Cole, 1996). Increasing peripheral availability of these AA may be beneficial during the first 14 d after arrival to the feedlot. The overall decrease in arterial concentrations of total AA observed in this experiment has also been reported in humans (Druml et al., 2001), pigs (Melchoir et al., 2004), and cattle (Waggoner et al., 2006; 2008) and the degree of change has been associated with the severity of the infectious process (Druml et al. 2001).

Blood flow

Blood flow is key to the dispersion and metabolism of absorbed nutrients through the PDV and liver tissues (Hersom et al., 2003). In general, blood flows were similar to those previously reported for growing steers (Reynolds and Huntington, 1998; Hersom et al. 2003). Blood flow during the first 6 h post *M. haemolytica* challenge has been reported by Burciaga-Robles et al. (2009c). In contrast to the acute response, only arterial blood flow was increased in FAST compared with FED steers averaged across the 14 d experiment, whereas blood flow across the PDV and liver were not different. Lomax and Baird (1983) reported decreased arterial, portal and hepatic blood flow in

lactating cows after 2 d of feed deprivation. The decrease in arterial blood flow remained during the 6 d feed deprivation period followed by a numeric twofold increase of arterial blood flow 3 d following re-feeding. Although the increase was not statistically different, the increase in arterial blood flow in lactating dairy cows after re-feeding might be related to physiological mechanisms to increase the flow of nutrients to peripheral tissues in order to meet the demands associated with production, whereas in non- lactating cows with steady-state nutrient requirements those changes were not observed. On the d of *M. haemolytica* challenge, we observed increased hepatic and arterial blood flow due to the bacterial challenge (Burciaga-Robles et al., 2009c). The possible effects of immune related proteins on changes in blood blow were discussed in that paper. Interestingly, the acute effect of *M. haemolytica* challenge is in agreement with the results provided by Lyngso et al. (2002) when changes in TST blood flow were studied in relationship to IL-6 infusion.

Oxygen consumption and net flux of energy related metabolites

Several authors (Huntington et al., 1988; Burrin et al., 1989; Freetly and Ferrell, 1991; Reynolds et al., 1992) have reported increased O_2 consumption by splanchnic tissues with increases in energy (or DMI) intake, which is in agreement with the present experiment when steers were deprived of feed for 74 h. In contrast, CH animals had greater PDV O_2 consumption that CON. These data are difficult to explain, and show the degree of complexity in metabolic changes that can occur after an immune challenge. Rokyta et al. (2004) observed that total hepatosplanchnic blood flow and oxygen delivery increased significantly in critically ill human subjects and returned to baseline after cessation of the illness. Vasoconstrictor-mediated alterations could modulate splanchnic

response to nutrient intake and immune challenge and explain the increased hepatic blood flow we reported during the acute response to this challenge (Burciaga-Robles et al., 2009). Marla et al. (2004) reported an increase in TST oxygen consumption when enteral or parenteral nutrition was provided to septic patients when compared to healthy patients. However, no previous reports were available on the possible effects of an immune challenge on increased PDV O_2 consumption. Reynolds et al. (1992) reported a greater variation in TST O_2 consumption in dairy cattle attributed to higher DMI, but also due to differences in productive state. During an immune challenge the relationship between DMI and O_2 consumption might be altered.

In the present experiment, net flux of propionate across the PDV was decreased in FAST compared with FED steers. Because the availability of propionate in portal blood is related to the amount produced by rumen microorganisms during fermentation of carbohydrates (Bergman 1990; Owens et al. 2008), the decrease in propionate flux is most likely explained by the alteration of RFA described previously (Hutchenson and Cole, 1981). Although rumen production of propionate was not measured in the present experiment, decreased production or increased utilization by ruminal epithelia most likely resulted in decreased net PDV flux. In addition, net propionate removal by the liver was decreased in FAST animals. Propionate is the major glucose precursor in the liver of ruminant animals (Aiello et al., 1989). In lactating dairy cows, Reynolds et al. (1987) estimated that 90% of portal propionate was removed by the liver and accounted for 55% of glucose output. In the present experiment, decreased portal flux of propionate was associated with a tendency for a decreased output of glucose by the liver.

Net flux of nitrogenous compounds

Feed deprivation followed by *M. haemolytica* challenge had no effect on PDV, liver or TST flux of ammonia or urea-N. However, net liver removal of hydroxyproline, tyrosine, aromatic, glucogenic, NEAA, and TAA were increased in response to *M. haemolytica* challenge. Reid et al. (2004) reported that acute phase proteins found in humans have a much higher content of aromatic AA compared to muscle or dietary sources. Therefore, in order for the liver to synthesize acute phase proteins, muscle catabolism may need to occur in order to achieve the requirements for acute phase protein synthesis. Reeds et al. (1994) proposed that the amount of muscle protein that needs to be mobilized for the synthesis of acute phase proteins is 2:1 (Luis, relative to what, i.e., what is the ratio "endogenous:???"). Interestingly, CH steers had lower ADG compared with CON. Although we didn't measure AA turnover in muscle, the combination of increased net removal of aromatic AA, increased haptoglobin concentrations, and lower ADG may support the hypothesis of Le'Floch, et al. (2004), who proposed that the increased requirements of aromatic AA due to an immune challenge might be the limiting step for animals to achieve an adequate immune response and maintain growth. Increased net removal of aromatic AA was also observed during the acute phase of the experiment (Burciaga-Robles et al., 2009), and might suggest that aromatic AA are limiting in calves suffering from BRD.

In humans, glucose and free fatty acids have been reported as the preferred sources of energy substrate in healthy individuals, and AA can act as a functional energy source in cases of increased demand or increased muscle catabolism (Kaesler et al., 2003). In healthy beef cattle, glucogenic AA can supply between 15 to 36% of the glucogenic substrates removed by the liver (Matras and Preston, 1989). In the present

study, no differences in arterial concentrations of glucogenic AA were detected due to feed deprivation or bacterial challenge during the acute response to the immune challenge. However, across 14 d arterial concentrations of glucogenic AA were decreased in CH animals, similar to data reported in pigs (Melchior et al., 2004) and humans (Druml et al., 2001). Pigs suffering chronic lung inflammation (Melchior et al., 2004) or humans with septicemia (Druml et al., 2001) had decreased plasma concentrations of glucogenic AA. In the present experiment, net removal of glucogenic AA by the liver was detected in CH steers, even though glucose concentration and net glucose flux across TST were not affected feed deprivation or bacterial challenge. This increased liver uptake of glucogenic AA without changes in glucose concentration could reflect increased energy demand by the liver of the host animal, although hepatic O_2 consumption was not affected by the immune challenge. Iwashita et al. (2005) reported that during increased energy demand in dogs due to exercise, net removal of glutamine and glucogenic AA by the liver was increased. In addition, increased gluconeogenic activity in the liver during an immune challenge has been reported *in vitro* in hepatic slices from lactating dairy cows following LPS challenge (Waldron et al., 2003). Increased net removal of glucogenic AA could limit the supply needed by the host animal for other anabolic purposes. This increase in liver uptake and decrease in arterial concentrations of glucogenic AA, in addition to decreased portal propionate flux, decreased propionate removal, tendency for decreased liver release of glucose and decreased ADG provides insight into changes in metabolic demands due to BRD. These effects remain across 14 d after an immune challenge and without animals showing further clinical (visual appearance) or physiological (rectal temperature back to normal)

signs of disease.

Hepatic extraction of non-essential AA (**NEAA**) provides substrates for synthesis of other metabolites (Bergman, 1973). Substantial or complete removal of NEAA can result in insufficient quantities entering the systemic circulation to support protein synthesis needs by the rest of the body (Lobley et al., 1996). In septicemic patients, NEAA plasma concentrations were lower and total hepatic clearance of these AA were increased compared with healthy patients (Druml et al., 2001). These data are in agreement with results of the present experiment, where CH steers had greater net liver removal of NEAA and decreased arterial concentrations. A decrease of NEAA during disease might imply a change in NEAA metabolism in morbid compared with healthy cattle. The increased liver removal of NEAA was observed during the acute response of the immune challenge and remained 14 d after the immune challenge occurred. Increased removal of NEAA by the liver during disease challenge most likely supports synthesis of EAA and proteins required for the immune response.

In addition to the *M. haemolytica* challenge, feed deprivation resulted in increased liver removal of aspartic acid, glutamic acid, serine, threonine, tryptophan, tyrosine, gluconeogenic and TAA, and a decreased liver output of glutamine. Glutamine is the most abundant free AA and serves as an energy source for rapidly proliferating cells, especially enterocytes and immune cells (Castell, 2003). Feed deprivation has been described as a stressful condition in cattle (Warriss et al., 1995) and also has been associated with increased corticosteroid levels (Werniki et al., 2006). In dogs (Humbert et al., 2001), rats (De Blaauw et al., 2004) and humans (Darmaun et al., 1988), corticosteroids have been reported to enhance de novo synthesis of glutamine, most likely

in skeletal muscle (Karinch et al.., 2001). Karinch et al. (2001) suggested that an increased period of stress resulting in de novo synthesis of glutamine could lead to glutamine depletion, which might ultimately lead to decreased growth rates and immunosupression. In addition, Ziegler et al. (1992) suggested that the requirement of glutamine might be higher during disease due to a prolonged muscle catabolic state attempting to meet the body demands for glutamine.

Conclusion

Similar to non-ruminant species, feed deprivation, inflammation and immune system activation appear to be associated with alterations in short and long-term (14 d) nutrient utilization by splanchnic tissues. Amino acids may be released from peripheral tissues and shifted to the liver for promotion of hepatic protein synthesis and gluconeogenesis. In addition to decreased plasma concentrations, liver uptake of AA may also be increased suggesting enhanced metabolic clearance and turnover of endogenously released AA. These changes in AA metabolism induced by BRD might suggest that AA requirements are different for ruminants undergoing an acute immune challenge and can remain increased even 14 d after the immune challenge occurs even though clinical or physiological manifestations of disease are no longer detectable.

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Table 6.1. Effects of feed deprivation and *M. haemolytica* (MH) challenge on performance of fed or fasted beef steers.

	Fed		Fasted			*P* - value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet × Disease
BW, kg								
d 0	317	324	326	310	10.2	0.82	0.66	0.26
d 14	338	339	355	314	13.2	0.74	0.15	0.74
DMI, d 0 – 14								
kg/d	7.12	6.93	6.44	4.86	0.62	0.03	0.08	0.27
% of BW	2.17	2.00	1.83	1.55	0.16	0.02	0.11	0.62
ADG, kg								
d 0 – 14	1.25	0.89	1.67	0.22	0.39	0.75	0.03	0.17
G:F, kg/kg								
d 0 – 14	0.185	0.140	0.252	-0.035	0.99	0.59	0.11	0.23

Table 6.2. Effects of intratracheal *M. haemolytica* (MH) challenge on arterial blood gas components and concentrations of energy metabolites in fed or fasted beef steers.

	Fed		Fasted			*P* – value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet × Disease
pH	7.45	7.47	7.45	7.48	0.007	0.70	0.04	0.36
pCO_2, mm Hg	36.8	38.0	37.0	35.5	1.47	0.41	0.91	0.31
Bicarbonate, m*M*[1]	26.4	27.1	26.8	26.8	0.39	0.94	0.35	0.33
O_2 saturation, %	98.4	98.1	98.4	98.3	0.36	0.89	0.02	0.86
Hemoglobin, g/100 mL	8.84	8.52	9.01	8.45	0.26	0.85	0.10	0.65
Packed cell volume, %	26.4	23.8	26.0	23.1	0.65	0.42	0.0003	0.82
Blood base, m*M*	3.58	4.19	3.67	4.17	0.39	0.92	0.16	0.89
CO_2, m*M*[1]	26.6	28.0	26.9	26.8	0.86	0.56	0.37	0.28
O_2, m*M*	2.61	2.50	2.64	2.44	0.05	0.75	0.01	0.41
Acetate, m*M*[2]	1.31	1.11	0.79	0.80	0.07	<0.0001	0.22	0.19
Propionate, m*M*	0.026[a]	0.016[b,c]	0.017[c]	0.015[b]	0.0001	0.004	0.0008	0.01
Butyrate, m*M*	0.033	0.019	0.020	0.015	0.004	0.09	0.04	0.35
β-hydroxybutyrate, m*M*[2]	0.60	0.51	0.57	0.40	0.05	0.21	0.02	0.41

Lactate, mM^3	0.307	0.682	0.589	0.613	0.073	0.17	0.01	0.03
Glucose, mM^2	4.44	4.40	4.56	4.56	0.19	0.30	0.97	0.92

[1]Disease × d interaction ($P < 0.05$).
[2]Diet × d interaction ($P \leq 0.01$).
[3]Disease × d interaction ($P < 0.01$).

Table 6.3. Effects of intratracheal *M. haemolytica* (MH) challenge on arterial concentrations of nitrogenous metabolites in fed or fasted beef steers.

	Fed		Fasted			*P* – value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet × Disease
Haptoglobin, mg/L	0.021	0.123	0.042	0.425	0.127	0.19	0.05	0.25
Ammonia, m*M*[1]	0.065	0.089	0.095	0.090	0.009	0.12	0.35	0.14
Urea, m*M*	4.33	4.50	3.60	4.79	0.53	0.67	0.19	0.33
Alanine, μ*M*[2]	157	154	161	132	7.23	0.21	0.02	0.08
Asparagine, μ*M*	16.6	14.3	20.8	12.9	2.71	0.60	0.07	0.30
Aspartic acid, μ*M*	5.12	6.56	6.04	6.01	0.94	0.83	0.45	0.43
Cysteine, μ*M*	4.37	3.59	3.66	5.20	0.90	0.61	0.67	0.20
Glutamine, μ*M*[3]	171	139	171	122	13.9	0.55	0.006	0.55
Glutamic acid, μ*M*[2,3]	214	185	207	185	13.2	0.80	0.06	0.80
Glycine, μ*M*[3]	251	203	223	185	14.8	0.11	0.006	0.73
Histidine, μ*M*[4]	40.0	41.4	37.7	39.8	4.25	0.64	0.68	0.93
Hydroxyproline, μ*M*[3]	19.3	15.3	21.2	13.4	1.30	0.98	0.0001	0.14
Isoleucine, μ*M*	108	108	110	90	8.08	0.27	0.21	0.21

Leucine, μ*M*	138	144	135	126	10.5	0.33	0.85	0.47
Lysine, μ*M*	127	118	137	113	11.0	0.82	0.13	0.50
Methionine, μ*M*[4]	7.23	7.17	6.80	5.74	0.69	0.18	0.42	0.46
Ornithine, μ*M*	119	125	110	73.8	18.3	0.11	0.40	0.27
Phenylalanine, μ*M*[3]	43.2	47.9	41.4	43.6	2.84	0.27	0.22	0.65
Proline, μ*M*	64.6[a]	63.3[b]	64.5[a]	51.6[b]	2.70	0.03	0.01	0.03
Serine, μ*M*[4]	96.1	88.4	95.3	94.6	5.69	0.63	0.45	0.52
Threonine, μ*M*[1]	56.6	52.1	61.5	44.7	7.71	0.86	0.17	0.42
Tryptophan, μ*M*	30.5	29.8	32.7	26.8	2.78	0.87	0.22	0.35
Tyrosine, μ*M*[4]	34.5	33.3	36.3	29.8	4.19	0.83	0.35	0.52
Valine, μ*M*	258	253	250	215	18.9	0.21	0.27	0.40
Aromatic amino acids, μ*M*	111	117	104	96.6	7.88	0.06	0.74	0.28
Branched amino acids, μ*M*	502	523	483	424	41.1	0.15	0.64	0.33
Gluconeogenic, μ*M*[3,4]	949	823	920	765	42.8	0.31	0.002	0.72
Sulfur containing amino acids, μ*M*	12.3	11.4	10.2	9.83	0.98	0.07	0.51	0.80
Essential amino acids, μ*M*	812	802	818	878	57.7	0.43	0.28	0.37

Non-essential amino acids, μM[3,4]	1115	968	1091	889	50.3	0.30	0.01	0.57
Total amino acids, μM	1965	1813	1949	1630	110	0.36	0.03	0.44

[1]Disease × d interaction ($P < 0.05$).
[2]Diet × d interaction ($P \leq 0.01$).
[3]Disease × d interaction ($P \leq 0.01$).
[4]Diet × d interaction ($P < 0.05$).

Table 6.4. Effects of intratracheal *M. haemolytica* (MH) challenge on blood flow and energy metabolite flux across the PDV, liver and TST fed or fasted beef steers.

	Fed		Fasted			*P* – value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet × Disease
Arterial blood flow, L/h	164	145	110	111	16.6	0.01	0.59	0.52
Portal blood flow, L/h	503	566	473	493	48.1	0.29	0.39	0.65
Hepatic blood flow, L/h	621	695	583	572	71.0	0.26	0.65	0.54
Oxygen consumption, mmol/h								
PDV	-131	-233	-103	-117	25.3	0.005	0.02	0.07
Liver	-215	-215	-133	-201	53.4	0.36	0.51	0.51
TST	-355	-459	-233	-291	59.7	0.02	0.17	0.69
HER								
Acetate, mmol/h								
PDV	196	135	124	129	38.3	0.13	0.27	0.21
Liver	97.6	46.6	51.4	38.5	87.9	0.57	0.51	0.69
TST[1]	227	175	160	165	110	0.52	0.69	0.63
HER	0.165	0.041	0.043	0.413	0.242	0.57	0.57	0.26

Propionate, mmol/h								
PDV[1]	77.2	64.7	29.5	37.8	12.9	0.0006	0.81	0.25
Liver[2]	-69.7	-58.1	-16.5	-32.3	14.8	0.0008	0.81	0.19
TST[3]	4.67	7.98	10.7	5.49	2.61	0.48	0.70	0.10
HER	-0.546	-0.656	-0.438	-0.666	0.110	0.45	0.14	0.39
Butyrate, mmol/h								
PDV	16.6	21.0	35.1	50.7	14.0	0.07	0.45	0.67
Liver	-14.8	-25.8	-27.9	-55.3	18.3	0.23	0.28	0.64
TST	0.02	-7.29	9.04	-1.81	8.88	0.21	0..31	0.99
HER[3]	0.116	-0.425	0.637	-0.264	0.496	0.35	0.05	0.62
β-hydroxybutyrate, mmol/h								
PDV	0.055	0.077	0.036	0.034	0.015	0.05	0.51	0.44
Liver	0.089	0.069	0.037	0.054	0.016	0.14	0.47	0.60
TST	0.151	0.126	0.095	0.089	0.02	0.03	0.44	0.64
HER	-0.153	-0.108	-0.090	-0.137	0.041	0.67	0.98	0.25
Lactate, mmol/h								

PDV[2]	65.9	94.1	63.4	43.5	22.2	0.22	0.84	0.26
Liver	-138	-157	-122	-91.8	34.9	0.06	0.79	0.49
TST[3]	-85.4	-37.0	-19.8	-13.7	28.8	0.008	0.09	0.33
HER[3]	1.38	0.30	0.16	0.45	0.20	0.001	0.05	0.15
Glucose, mmol/h								
PDV	-60.7	-20.1	-69.4	-43.5	15.9	0.83	0.73	0.89
Liver	270	172	102	33	103	0.08	0.33	0.86
TST	258	223	30.5	-46.5	211	0.04	0.54	0.78
HER	-0.153	-0.108	-0.090	-0.137	0.041	0.67	0.98	0.25

[1]Diet × d interaction ($P < 0.05$).
[2]Diet × d interaction ($P \leq 0.01$).
[3]Disease × d interaction ($P < 0.05$).

Table 6.5. Effects of intratracheal *M. haemolytica* (MH) challenge on nitrogenous metabolite flux across the PDV, liver and TST in fed or fasted beef steers.

	Fed		Fasted			P – value		
Item	No MH	MH	No MH	MH	SEM	Diet	Disease	Diet × Disease
Haptoglobin								
PDV	-0.42	14.6	3.33	2.97	14.7	0.77	0.60	0.58
Liver	-4.91	-3.37	-5.78	-4.94	18.5	0.94	0.94	0.98
TST	-5.15	15.3	9.58	-8.47	31.0	0.87	0.96	0.51
Ammonia, mmol/h								
PDV	7.19	8.17	3.14	7.78	3.24	0.47	0.37	0.55
Liver	-10.4	-9.73	-3.05	-6.42	7.58	0.44	0.85	0.76
TST	-1.91	-2.27	-1.58	0.67	3.22	0.55	0.72	0.63
HER	0.251	0.246	0.246	0.197	0.098	0.78	0.78	0.82
Urea N, mmol/h								
PDV	-168	-118	-114	-265	103	0.77	0.97	0.71
Liver	284	496	368	402	208	0.80	0.61	0.95
TST	318	264	111	269	130	0.46	0.88	0.60

HER[1]	0.053	0.016	0.029	0.004	0.087	0.81	0.69	0.94
Alanine, mmol/h								
PDV	22.1[a]	15.3[b]	10.3[c]	16.7[b]	2.99	0.08	0.95	0.02
Liver	-11.9	-15.6	-12.4	-19.2	5.78	0.71	0.34	0.77
TST	9.84	0.20	-2.05	-2.34	3.81	0.05	0.17	0.20
HER	0.109	0.157	0.162	0.289	0.074	0.20	0.23	0.58
Asparagine, mmol/h								
PDV	3.97	2.84	4.10	4.20	1.12	0.49	0.63	0.57
Liver	-0.65	-1.31	-7.11	-3.03	1.69	0.01	0.29	0.15
TST	3.84	1.34	-3.04	1.44	1.56	0.03	0.51	0.03
HER	0.126	0.262	2.41	0.39	0.82	0.14	0.25	0.19
Aspartic acid, mmol/h								
PDV	2.46	1.92	2.60	1.80	0.53	0.98	0.19	0.79
Liver	-0.82	-0.52	-1.76	-2.31	0.77	0.06	0.85	0.55
TST	1.81	1.30	0.85	-0.13	0.51	0.01	0.12	0.62
HER	0.26	0.45	0.48	1.05	0.27	0.12	0.15	0.47

Cysteine, mmol/h								
PDV	-0.989	0.242	-0.474	-0.395	0.927	0.94	0.47	0.53
Liver	3.27	3.46	2.11	2.11	0.94	0.18	0.92	0.91
TST	2.69	3.89	1.64	1.87	0.98	0.12	0.46	0.61
HER	-0.497	-0.453	-0.540	-0.409	0.111	0.99	0.41	0.68
Glutamine, mmol/h								
PDV	-27.3	-8.88	-6.80	-3.06	13.5	0.33	0.41	0.58
Liver	60.3	30.0	13.3	2.31	14.5	0.01	0.14	0.48
TST	22.4	8.52	8.95	1.51	12.2	0.40	0.38	0.74
HER	-0.305	-0.199	-0.028	0.030	0.093	0.008	0.36	0.79
Glutamic acid, mmol/h								
PDV	12.6	4.66	12.2	-1.58	14.6	0.81	0.45	0.83
Liver	22.7	-5.89	-22.7	-25.8	13.4	0.01	0.21	0.31
TST	30.1	-5.06	-10.5	-25.8	11.8	0.02	0.08	0.66
HER	-0.104	1.67	0.40	0.38	0.57	0.46	0.11	0.10
Glycine, mmol/h								

PDV	13.8	10.1	19.3	13.2	4.99	0.37	0.30	0.79
Liver	-7.60	-23.4	-10.2	-7.85	9.00	0.45	0.43	0.29
TST	7.63	-15.0	7.55	7.44	6.76	0.09	0.08	0.08
HER	0.074	0.308	0.144	0.097	0.101	0.45	0.32	0.14
Histidine, mmol/h								
PDV	-2.23	1.09	3.82	2.79	2.88	0.16	0.67	0.43
Liver	-4.97	-10.1	-9.81	-15.0	3.17	0.12	0.10	0.99
TST	-8.13	-9.53	-6.60	-10.6	3.52	0.95	0.42	0.69
HER	0.485	1.75	1.34	1.90	0.64	0.43	0.16	0.58
Hydroxyproline, mmol/h								
PDV	0.80	1.44	1.45	0.92	1.06	0.95	0.95	0.57
Liver	1.24	-0.22	-1.97	-1.64	-1.09	0.03	0.56	0.40
TST	1.41	0.04	-0.46	-0.50	0.98	0.22	0.43	0.45
HER	0.307	0.104	0.221	0.301	0.249	0.81	0.80	0.56
Isoleucine, mmol/h								

PDV	11.4	4.34	7.19	9.23	2.65	0.90	0.31	0.07
Liver	-5.24	-3.95	-7.45	-9.86	5.22	0.42	0.91	0.71
TST	7.20	1.19	-0.25	1.20	5.64	0.43	0.67	0.49
HER	0.100	0.105	0.172	0.228	0.082	0.25	0.71	0.76
Leucine, mmol/h								
PDV	9.55	7.94	9.08	13.5	4.39	0.55	0.74	0.48
Liver	-1.41	-3.51	-6.12	-10.4	6.99	0.39	0.63	0.86
TST	8.76	1.09	2.88	5.51	9.94	0.93	0.70	0.59
HER	0.072	0.082	0.114	0.154	0.078	0.44	0.73	0.83
Lysine, mmol/h								
PDV	3.12	17.2	14.8	12.8	5.99	0.52	0.29	0.16
Liver	-2.23	-23.8	-19.7	-20.1	9.94	0.48	0.25	0.27
TST	2.25	-3.74	-6.25	-5.97	10.2	0.58	0.77	0.75
HER	0.177	0.994	0.542	0.487	0.325	0.88	0.28	0.21
Methionine, mmol/h								
PDV	1.64	1.47	1.67	1.31	0.31	0.81	0.39	0.75

Liver	-0.385	-0.402	-0.952	-0.870	0.564	0.34	0.95	0.92
TST	1.35	0.91	0.78	0.43	0.44	0.22	0.36	0.91
HER	0.127	0.109	0.270	-0.401	0.156	0.15	0.70	0.62
Ornithine, mmol/h								
PDV[1]	-5.04	-4.81	4.91	8.57	7.59	0.13	0.79	0.82
Liver	10.3[a]	-16.1[b]	-9.90[c]	-9.85[c]	7.10	0.29	0.05	0.05
TST	1.80	-23.0	-5.22	-0.40	9.82	0.41	0.29	0.12
HER	-0.095	0.781	0.295	0.697	0.316	0.62	0.04	0.45
Phenylalanine, mmol/h								
PDV	5.29	5.11	5.12	4.52	1.52	0.79	0.79	0.88
Liver[1]	-2.46	-3.82	-5.13	-8.12	2.30	0.11	0.32	0.70
TST	3.20	0.31	0.06	-3.00	2.02	0.10	0.13	0.96
HER[1]	0.103	0.148	0.271	0.370	0.090	0.04	0.44	0.76
Proline, mmol/h								
PDV	4.89	2.65	4.43	6.00	1.28	0.25	0.78	0.13
Liver	-1.68	-0.86	-3.93	-4.64	2.53	0.21	0.98	0.74

TST	3.71	0.53	0.60	2.18	2.28	0.73	0.71	0.27
HER	0.047	0.045	0.114	0.179	0.065	0.11	0.61	0.59
Serine, mmol/h								
PDV	10.4	10.2	13.2	12.1	4.66	0.60	0.88	0.92
Liver	-1.17	-8.26	-17.5	-16.3	5.08	0.01	0.54	0.40
TST	5.75	3.06	-3.79	-3.27	5.30	0.34	0.41	0.36
HER	0.077	0.249	0.398	0.258	0.099	0.09	0.86	0.11
Threonine, mmol/h								
PDV	-0.18	4.08	5.39	6.10	6.21	0.53	0.68	0.77
Liver	6.62	-6.64	-9.39	-12.0	5.86	0.03	0.29	0.55
TST	4.31	-3.56	-3.09	-4.48	4.48	0.40	0.34	0.53
HER	0.0007	0.331	0.483	0.863	0.167	0.003	0.03	0.87
Tryptophan, mmol/h								
PDV	-0.94	1.70	1.60	1.67	1.18	0.26	0.23	0.25
Liver	0.32	-2.66	-3.57	-4.86	1.47	0.03	0.13	0.54
TST	-0.753	-1.20	-2.15	-2.59	1.78	0.41	0.79	0.99

HER	0.021	0.196	0.286	0.451	0.104	0.01	0.09	0.95
Tyrosine, mmol/h								
PDV	2.50	4.01	2.94	4.08	1.86	0.88	0.46	0.91
Liver	-1.20	-7.41	-6.10	-8.32	2.16	0.16	0.04	0.33
TST	0.95	-2.92	-2.82	3.77	2.07	0.25	0.23	0.46
HER	0.068	0.413	0.536	0.717	0.122	0.002	0.03	0.48
Valine, mmol/h								
PDV	12.2	4.64	6.17	18.6	7.24	0.57	0.72	0.15
Liver	-16.2	-15.0	-12.0	-11.2	15.6	0.77	0.93	0.97
TST	-6.00	-12.3	-6.8	10.7	17.1	0.75	0.98	0.27
HER	0.140	0.133	0.185	0.121	0.102	0.85	0.70	0.76
Aromatic amino acids, mmol/h								
PDV	1.32	3.74	5.59	7.95	3.73	0.35	0.51	0.99
Liver	2.57	-9.65	-6.86	-14.7	3.98	0.06	0.01	0.57
TST	3.23	-8.02	-1.37	-6.74	4.16	0.76	0.05	0.53
HER[1]	-0.023	0.150	0.258	0.555	0.120	0.005	0.04	0.59

Branched amino acids, mmol/h								
PDV	28.0	6.97	22.0	39.2	19.5	0.49	0.91	0.32
Liver	-21.0	-25.7	-25.4	-41.6	24.3	0.66	0.65	0.80
TST	11.5	-27.3	-3.01	-4.96	36.9	0.80	0.66	0.51
HER	0.109	0.122	0.143	0.167	0.08	0.62	0.81	0.94
Gluconeogenic amino acids, mmol/h								
PDV	27.1	35.6	54.8	44.5	33.5	0.56	0.97	0.77
Liver	83.1	-25.7	-61.5	-83.5	33.6	0.003	0.04	0.18
TST	105	10.7	-20.7	-34.2	-27.6	27.5	0.11	0.02
HER	-0.071	0.102	0.147	0.212	0.062	0.009	0.05	0.36
Sulfur containing amino acids, mmol/h								
PDV	-0.14	1.38	1.05	5.88	2.89	0.03	0.25	0.54
Liver	2.43	3.02	1.08	-4.96	3.26	0.14	0.38	0.29
TST	3.50	4.28	2.26	2.43	1.46	0.29	0.74	0.83
HER	-0.138	-0.189	-0.100	0.687	0.345	0.17	0.26	0.20
Essential amino acids, mmol/h								

PDV	38.9	50.7	58.4	71.2	19.1	0.28	0.50	0.97
Liver	-26.9	-66.4	-75.6	-96.3	38.0	0.28	0.41	0.79
TST	26.5	-23.3	-21.9	-7.82	43.0	0.69	0.65	0.44
HER	0.070	0.179	0.242	0.262	0.080	0.11	0.41	0.57
Non-essential amino acids, mmol/h								
PDV	31.5	50.5	67.8	54.1	31.3	0.50	0.92	0.58
Liver	88.7	-36.8	-72.2	-90.8	36.5	0.003	0.04	0.12
TST	91.1	14.7	-3.55	-24.9	29.1	0.06	0.02	0.14
HER	-0.068	0.110	0.155	0.204	0.058	0.008	0.05	0.26
Total amino acids, mmol/h								
PDV	64.4	101	131	127	47.2	0.31	0.70	0.65
Liver	59.8	-107	-156	-195	69.4	0.02	0.12	0.33
TST	105	-38.7	-28.6	-35.1	108	0.32	0.26	0.30
HER	-0.024	0.138	0.186	0.231	0.060	0.01	0.08	0.31

[1]Diet × d interaction ($P \leq 0.05$).

Figure legends

Figure 6.1. Changes in *M. haemolytica* whole cell (A; disease, $P < 0.0001$, SEM = 0.10; disease*d, $P < 0.0001$, SEM 0.30) and *M. haemolytica* leukotoxin (B; disease, P = 0.0003, SEM = 0.031; disease*d, $P < 0.0001$; SEM 0.074) serum antibody concentrations in fed or fasted beef steers following a *M. haemolytica* challenge. [a]Within d, means with a superscript letters are different ($P < 0.05$).

Figure 6.2. Effect of *M. haemolytica* challenge on rectal temperature during the first 7 d post challenge in fed or fasted beef steers ($P = 0.0006$; SEM = 0.10; disease*d, $P < 0.0001$, SEM = 0.031). [a]Within d, means with different superscript letters are different ($P < 0.05$). [b]Within , means with different superscript letters are different ($P = 0.07$)

Figure 6.3. Effect of *M. haemolytica* challenge on DMI in fed or fasted beef steers during a 14 d period (diet*w, $P = 0.21$, SEM = 0.61; disease*w, $P = 0.27$ SEM = 0.61 ; diet*disease*w $P = 0.01$; SEM = 0.86). [a]Within w, means with a superscript letters are different ($P < 0.05$).

Figure 6.4. Changes in arterial plasma concentrations of acetate (A; diet*d, $P = <0.0001$; disease*d, $P = 0.67$, SEM = 0.09), β-hydroxybutyrate (B; diet*d, $P = 0.01$; disease*d, $P = 0.24$, SEM = 0.07), and glucose (C; diet*d, P = 00.03; disease*d, $P = 0.89$, SEM = 0.40) in fed or fasted beef steers following a *M. haemolytica* challenge. [a]Within d, means with superscript letters are different ($P < 0.05$).

Figure 6.5. Changes in arterial plasma concentrations of alanine (A; diet*d, $P = 0.01$; disease*d, $P = 0.60$, SEM = 9.11), glutamic acid (b; diet*d, $P = 0.0004$; disease*d, $P = 0.0001$, SEM = 16.3), histidine (C; diet*d, $P = 0.02$; disease*d, $P = 0.55$, SEM = 5.68), methionine (D; diet*d, $P = 0.02$; disease*d, $P = 0.31$, SEM = 1.01), Serine, (E; diet*d, $P = 0.05$; disease*d, $P = 0.16$, SEM = 7.00), tyrosine (F; diet*d, $P = 0.03$; disease*d, $P = 0.24$, SEM = 5.43), glucogenic AA (G; diet*d, $P = 0.02$; disease*d, $P = 0.03$, SEM = 51.1), and NEAA (H; diet*d, $P = 0.02$; disease*2, $P = 0.002$, SEM = 64.1) in fed or fasted beef steers following a *M. haemolytica* challenge. [a]Within d, means with superscript letters are different ($P < 0.05$).

Figure 6.6. Changes in arterial plasma concentrations of lactate (A; disease*d, $P = <0.001$; day*d, $P = 0.18$, SEM = 0.08) in response to a *M. haemolytica* challenge in fed or fasted beef steers. [a]Within d, means with superscript letters are different ($P < 0.05$).

Figure 6.7. Changes in arterial plasma concentrations of glutamine (A; disease*d, $P = <0.001$; diet*d, $P = 0.14$, SEM = 16.4), glutamic acid (b; disease*d, $P = 0.0004$; diet*d, $P = 0.007$, SEM = 16.3), glycine (C; disease*d, $P = 0.0002$; diet*d, $P = 0.16$, SEM = 21.3), hydroxyproline (D; disease*d, $P = <0.0001$; diet*d, $P = 0.25$, SEM = 1.25), phenylalanine, (E; disease*d, $P = 0.01$; diet*d, $P = 0.12$, SEM = 3.45), threonine (F; disease*d, $P = 0.04$; diet*d, $P = 0.56$, SEM = 9.57), glucogenic AA (G; disease*d, $P = 0.001$; diet*d, $P = 0.02$, SEM = 51.1), and NEAA (H; disease*d, $P = 0.003$; diet*d, $P = 0.0.02$, SEM = 64.1) in response to a *M. haemolytica* challenge in fed or fasted beef steers. [a]Within d, means with superscript letters are different ($P < 0.05$).

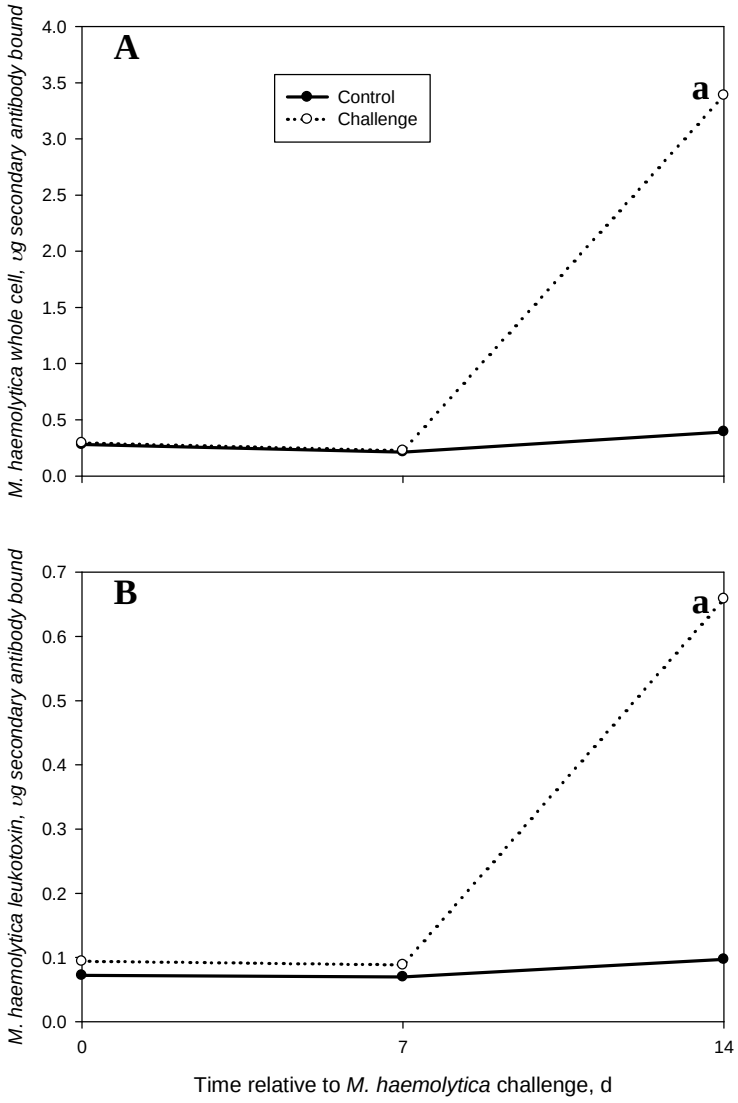
A
B
a
a
Control
Challenge
M. haemolytica whole cell, ug secondary antibody bound
M. haemolytica leukotoxin, ug secondary antibody bound
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
7
14
Time relative to M. haemolytica challenge, d

Figure 6.1

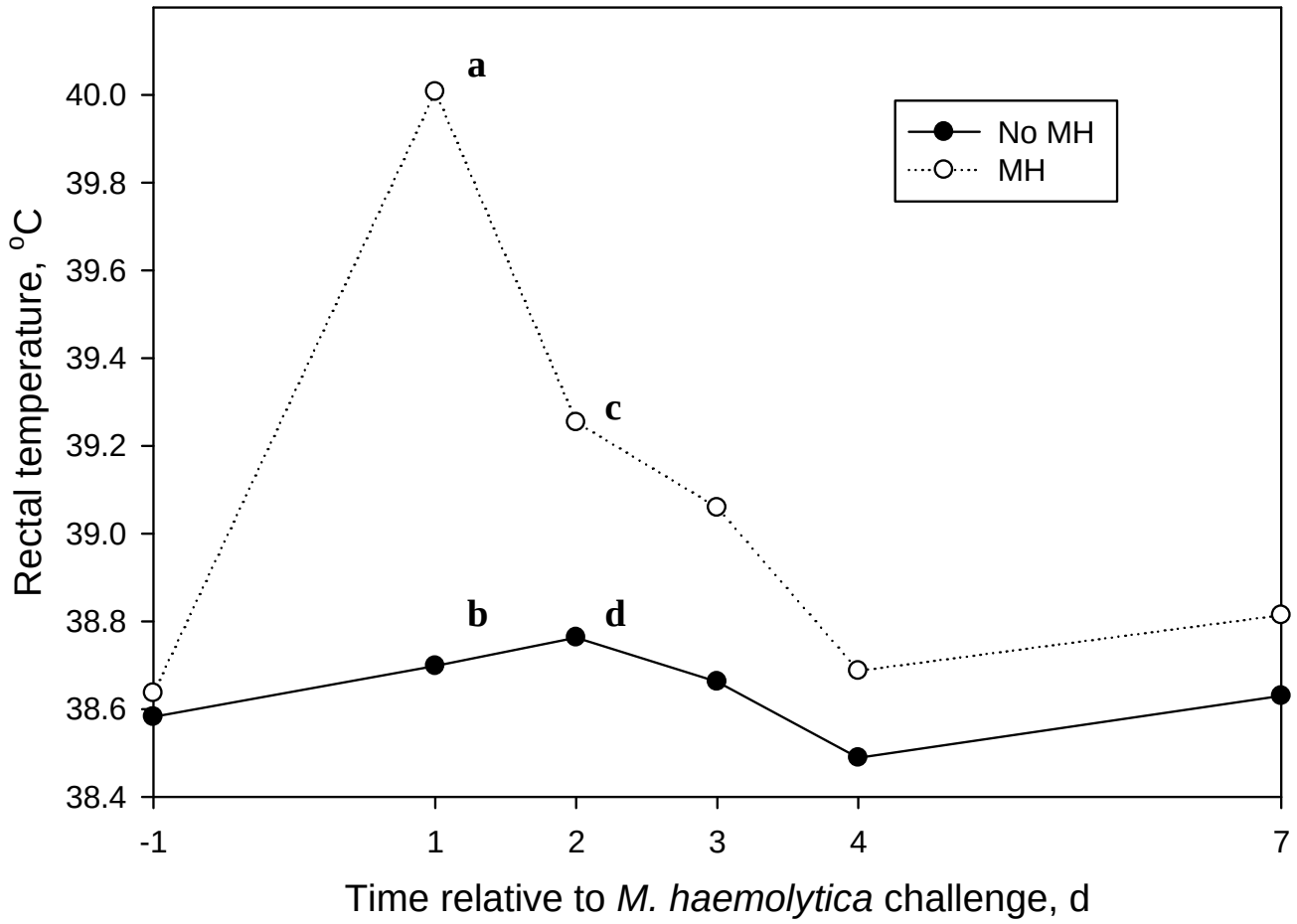
a
b
c
d
No MH
MH
Rectal temperature, °C
40.0
39.8
39.6
39.4
39.2
39.0
38.8
38.6
38.4
-1
1
2
3
4
7
Time relative to M. haemolytica challenge, d

Figure 6.2

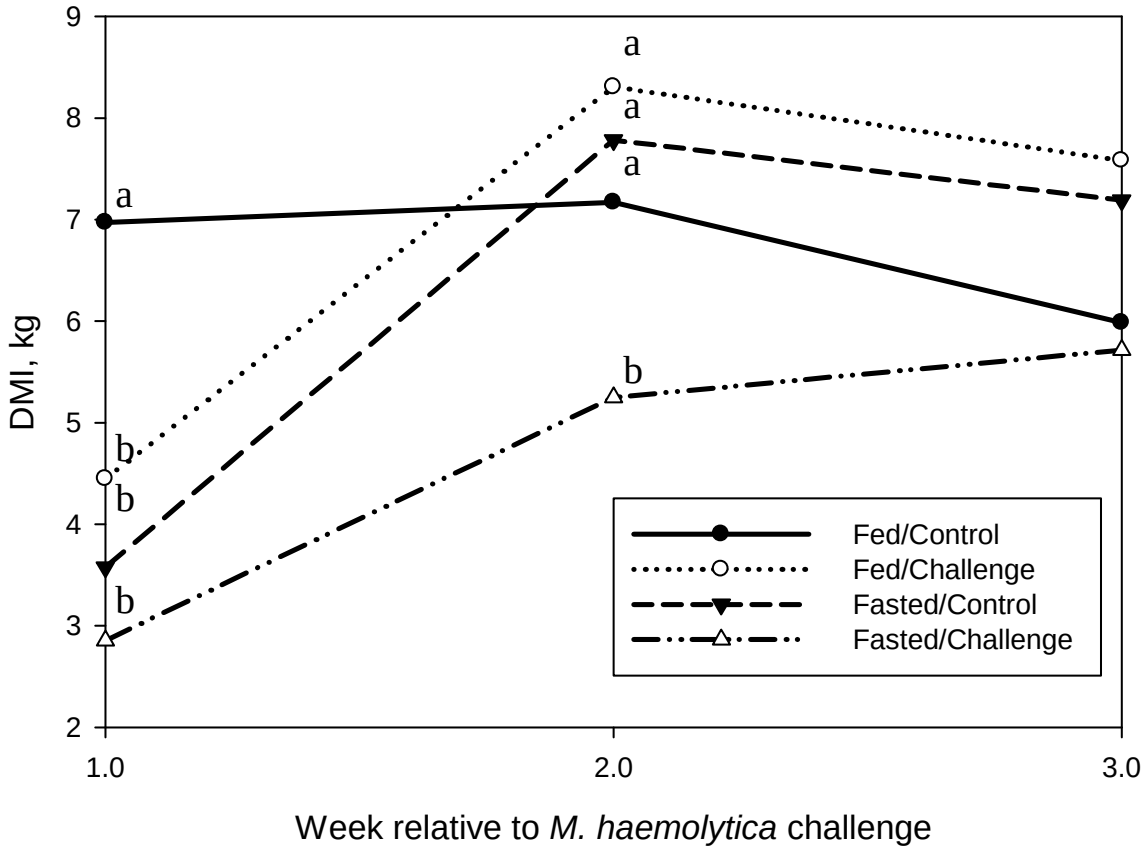
9
8
7
6
5
4
3
2
DMI, kg
1.0
2.0
3.0
Week relative to *M. haemolytica* challenge
a
a
a
b
b
b
b
Fed/Control
Fed/Challenge
Fasted/Control
Fasted/Challenge

Figure 6.3

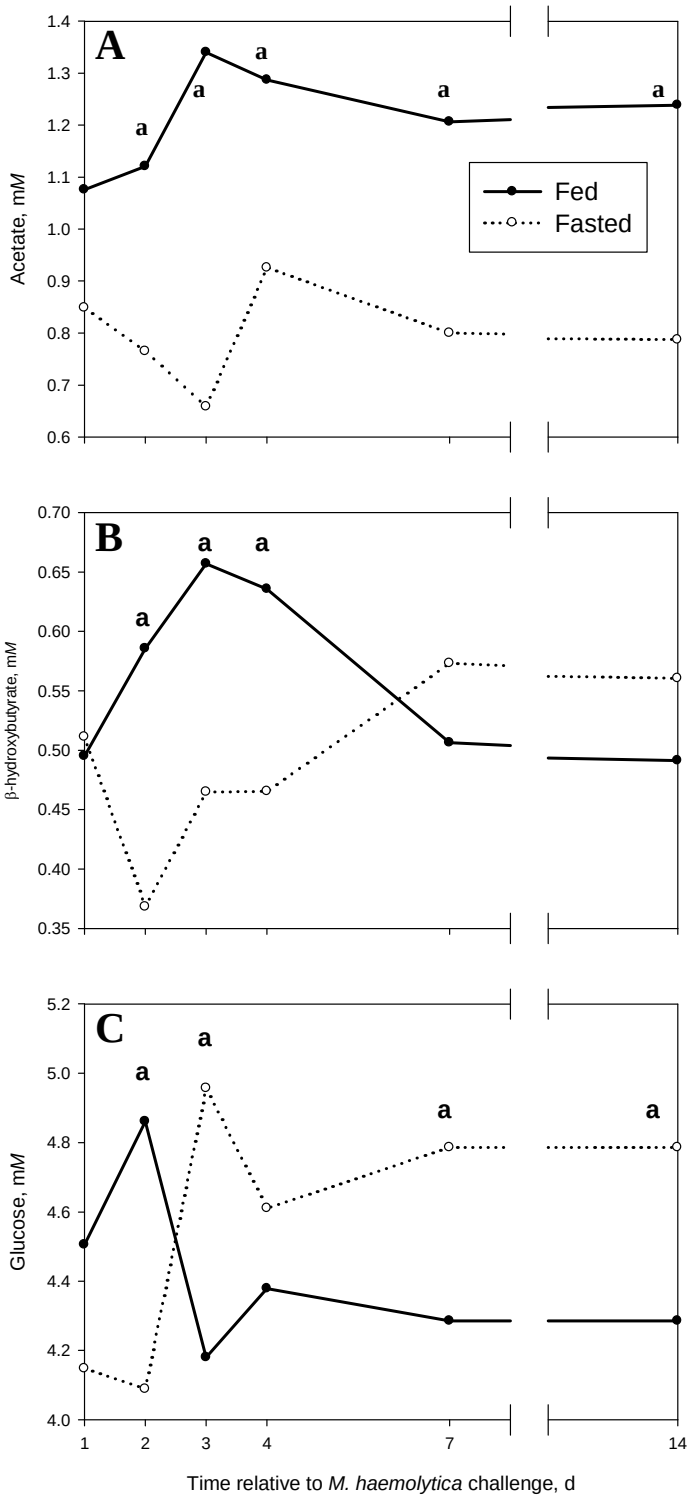
A
B
C
Acetate, m*M*
β-hydroxybutyrate, m*M*
Glucose, m*M*
Fed
Fasted
a
Time relative to *M. haemolytica* challenge, d

Figure 6.4

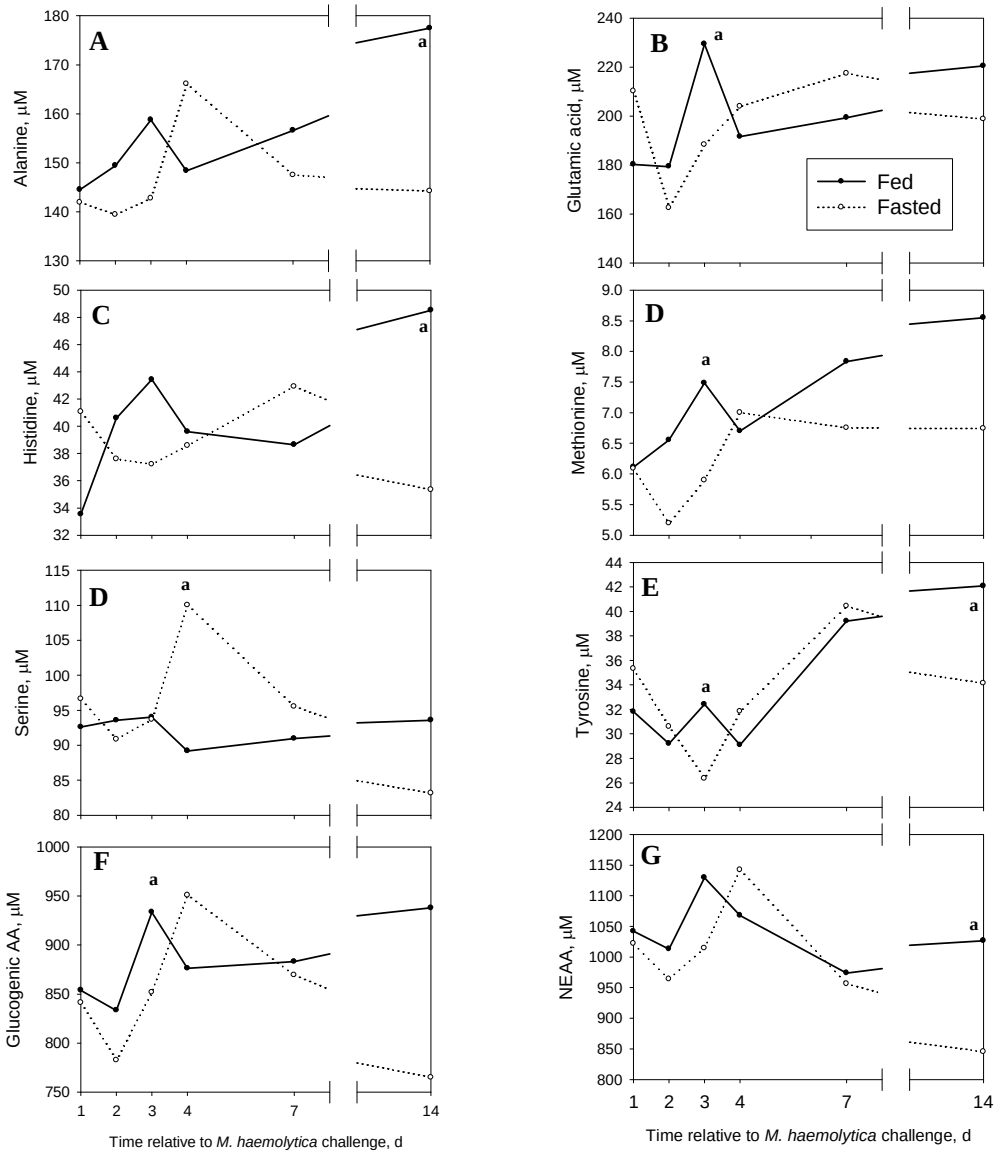
A
Alanine, µM
B
Glutamic acid, µM
Fed
Fasted
C
Histidine, µM
D
Methionine, µM
D
Serine, µM
E
Tyrosine, µM
F
Glucogenic AA, µM
G
NEAA, µM
a
Time relative to M. haemolytica challenge, d
Time relative to M. haemolytica challenge, d

Figure 6.5

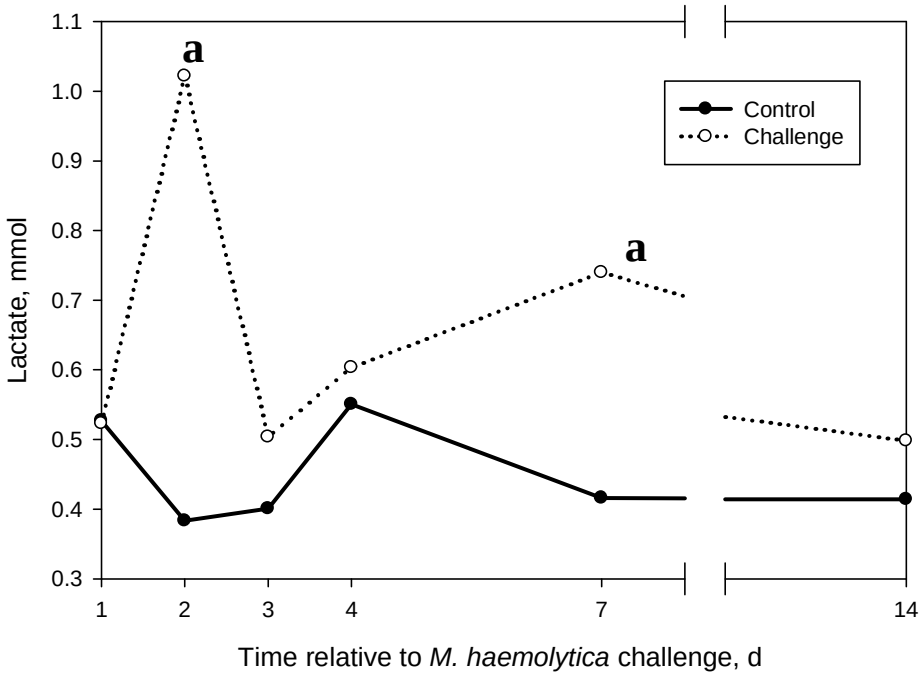
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Lactate, mmol
a
a
Control
Challenge
1
2
3
4
7
14
Time relative to *M. haemolytica* challenge, d

Figure 6.6

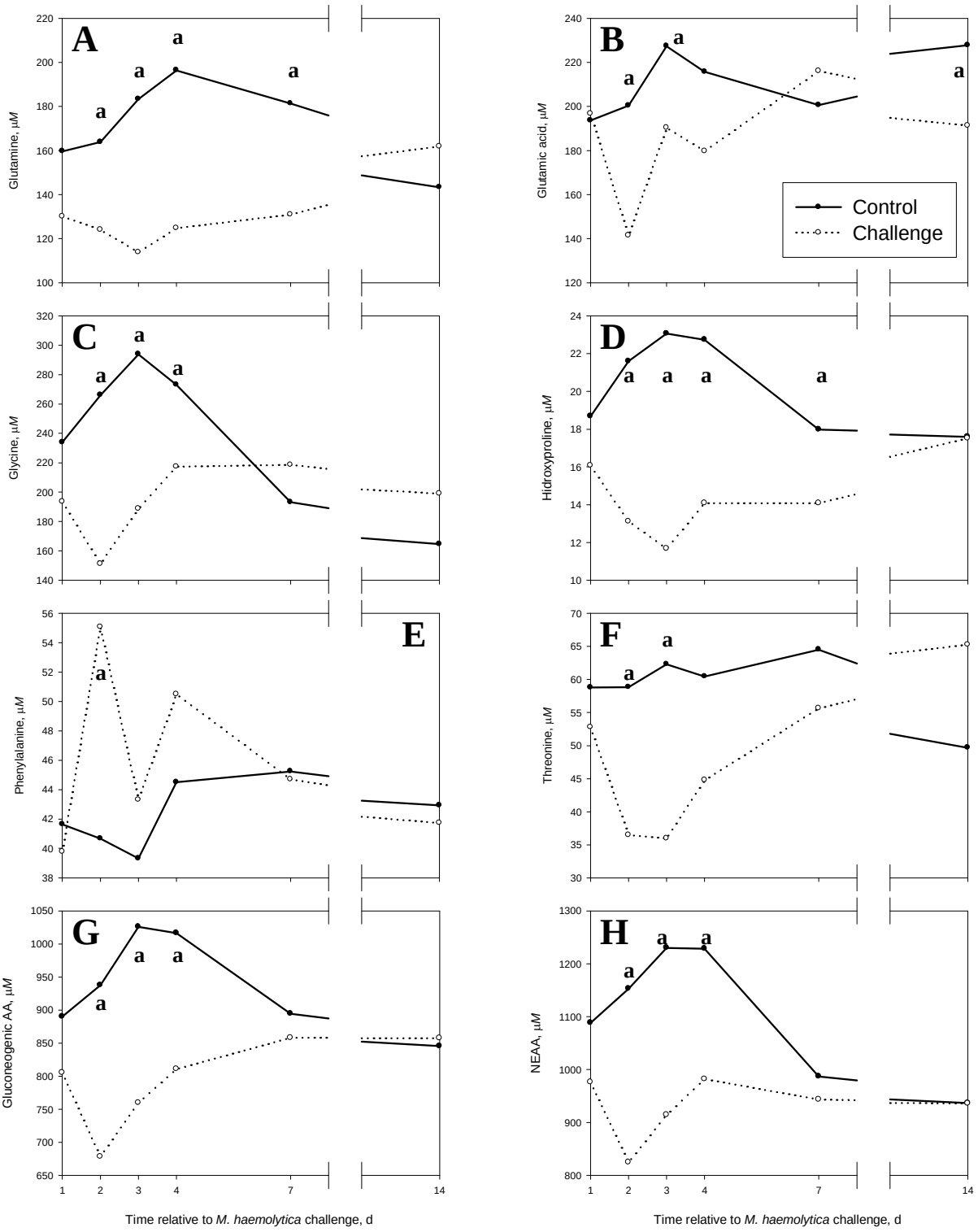
A
B
C
D
E
F
G
H
Glutamine, µM
Glutamic acid, µM
Glycine, µM
Hidroxyproline, µM
Phenylalanine, µM
Threonine, µM
Gluconeogenic AA, µM
NEAA, µM
Control
Challenge
Time relative to M. haemolytica challenge, d
Time relative to M. haemolytica challenge, d

Figure 6.7

VITA

Luis Octavio Burciaga-Robles

Candidate for the Degree of

Philosophy Doctor

Dissertation: TYPE FULL TITLE HERE IN ALL CAPS

Major Field: Animal Nutrition

Biographical:

Born in Chihuahua, Chihuahua mexico on April 7, 1977. Son of Jesus Rogelio Burciaga Barrios and Maria Graciela Robles Villa; Brother of Jesus Humberto

Personal Data:

Education:

Master in Science Major in Animal Production. Universidad Autonoma de Chihuahua, Mexico, December 2002.

Medico Veterinario Zootecnista. Universidad Autonoma de Ciudad Juarez, Mexico, December 2000.

Name: Luis Octavio Burciaga-Robles Date of Degree: May, 2009
Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECTS OF BOVINE RESPIRATORY DISEASE ON IMMUNE RESPONSE, ANIMAL PERFORMANCE, NITROGEN BALANCE, AND BLOOD AND NUTRIENT FLUX ACROSS TOTAL SPLANCHNIC TISSUES IN BEEF STEERS

Pages in Study: 244 Candidate for the Degree of Philosophy Doctor

Major Field: Animal Nutrition

Scope and Method of Study:

Scope and Method of Study: Objectives were to evaluate the effects of bovine respiratory disease (**BRD**) on immune response, cattle performance, carcass characteristics, visceral organ mass, apparent nutrient digestibility, nutrient retention, and blood flow and net flux of nutrients across the portal-drained viscera (**PDV**) and liver. Steers were challenged with bovine viral diarrhea virus (**BVDV**) and/or *Mannheimia haemolytica* (**MH**). Findings and Conclusions: Steers challenged with BVDV and/or MH showed alterations in the immune system that were ultimately reflected in decreased performance and nitrogen balance. In addition, plasma amino acid concentrations were decreased and liver removal increased suggesting enhanced turnover of endogenously released amino acids. These changes in amino acid metabolism induced by BRD suggest that amino acid requirements are different for cattle undergoing an acute immune challenge and can remain increased even 14 days after the immune challenge occurs even though clinical or physiological manifestations of disease are no longer detectable.

ADVISER'S APPROVAL: Dr. Clint R. Krehbiel