ANCILLERY THERAPY USE AND TRACE MINERAL SUPPLEMENTATION IN BEEF CATTLE: IMPACTS ON CLINICAL HEALTH, IMMUNE RESPONSE VARIABLES, ANIMAL PERFORMANCE, AND CARCASS TRAITS

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Abstract: Newly received calves that received antimicrobial treatment for bovine respiratory disease (BRD) were randomly assigned to 1 of 4 experimental ancillary therapy (ANC) groups: flunixin meglumine (NSAID), viral vaccination (VACC), vitamin C (VITC), or no ANC (NOAC). When contrasted with the average of NSAID, VACC, and VITC calves receiving NOAC tended to have heavier BW on d 56, greater ADG and DMI from first BRD treatment through d 28, greater DMI from d 28 through d 56, and had greater DMI from first BRD treatment through d 56 with mortalities and removals excluded ($P = 0.06$, $P = 0.10$, $P = 0.08$, $P = 0.06$, and $P = 0.05$ respectively). Overall morbidity and mortality attributed to BRD were 66.5% and 13.2% respectively. After the receiving period, calves were grouped by previous ANC treatment and the number of times treated for BRD (BRDX) and allocated to finishing pens. The BRDX experimental groups included: never treated for BRD (0X), treated 1 time (1X), 2 times (2X), or 3 or 4 times (3/4X). Ultrasound estimates, BW, and visual appraisal were used to target a common compositional end point for each pen of cattle. No ANC group differences existed for any of variables analyzed ($P \geq 0.26$). With increasing BRDX, days on feed and lung consolidation scores increased linearly ($P \leq 0.01$), while hot carcass weight, dressing percentage, ribeye area, and the percentage of USDA Prime and Choice carcasses decreased linearly ($P \leq 0.03$). An additional experiment examined the effects of copper (Cu), manganese (Mn), and zinc (Zn) supplementation on the clinical signs, immune response variables, and mineral status of calves following a bovine viral diarrhea virus (BVDV) and *Mannheimia haemolytica* (MH) immune challenge. Steers were randomly pairwise assigned to either a mineral supplemented (MIN) or control (CON) experimental treatments. There was a significant ($P < 0.0001$) time by treatment interaction observed for liver Cu levels. Time significantly impacted the concentrations of Cu, Mn, Fe, and Zn within the liver, Cu, Mn, and Zn within the muscle, and Cu, Fe, and Zn within the serum ($P \leq 0.05$). Calves receiving MIN had greater liver Cu ($P = 0.0001$) and Mn ($P = 0.0075$) concentrations compared to CON calves. In contrast, serum Cu and Fe concentrations were increased ($P \leq 0.05$) in CON calves compared to MIN calves. The use of ANC does not appear to positively impact clinical health and could potentially be detrimental to receiving performance in severely immune challenged calves. Calves treated multiple times for BRD are able to reach similar compositional end points as untreated cohorts; however, it may not be possible for treated calves to achieve similar carcass traits. The supplementation of Cu, Mn, and Zn may impact the antibody response to a BVDV and MH immune challenge in calves. When Cu is supplemented to calves receiving a marginally Cu deficient diet, Cu status within the body can be altered.

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NOMENCLATURE

ADG	average daily gain
ANC	ancillary therapy
BAdV	bovine adenoviruses
BCV	bovine respiratory corona virus
BHV1	bovine herpesvirus 1
BIV	bovine immunodeficiency virus
BRD	bovine respiratory disease
BRSV	bovine respiratory synctial virus
BVDV	bovine viral diarrhea virus
BW	body weight
Ca	calcium
CFU	colony forming unit
CIS	clinical illness score
Co	cobalt
CS	subjective clinical score
Cu	copper
CuSO4	copper sulfate
CV	coefficient of variation
DART	depression, appetite, respiration, temperature
DFM	direct-fed microbial
DM	dry matter
DMI	dry matter intake

DOF	days on feed
ECF	extracellular fluid
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
F1	first filial cross
Fb	fibrinogen
Fe	iron
FPT	failure of passive transfer of immunoglobulins
FT	fat thickness
G:F	average daily gain:dry matter intake
HCW	hot carcass weight
HS	*Histophilus somni*
Hp	haptoglobin
IBK	infectious bovine keratoconjunctivitis
IBR	infectious bovine rhinotracheitis
IFN-α	interferon alpha
IFN-γ	interferon gamma
IGF-I	insulin-like growth factor-I
IgG	immunoglobulin G
IL-1	interleukin-1
IL-2	interleukin-2
IL-10	interleukin-10
Iron	Fe
KPH	estimated internal fat (kidney, pelvic, heart)
LM	longissimus muscle
LKT	leukotoxin

LPS	lipopolysaccharide
MB	*Mycoplasma bovis*
MH	*Mannheimia haemolytica*
Mn	Manganese
Mo	Molybdenum
NAHMS	National Animal Health Monitoring System
NCBA	National Cattlemen's Beef Association
NK	natural killer
NPRC	Nutrition and Physiology Research Center
NSAID	non-steroidal anti-inflammatory drug
NRC	National Research Council
PCR	polymerase chain reaction
PI3	parainfluinza virus type 3
PI	persistently infected (with BVDV)
PM	*Pasteurella multocida*
RBC	red blood cell
RR	respiration rate
SD	standard deviation
Se	selenium
TBA	trenbolone acetate
TEMP	rectal temperature
TM	trace mineral
TNF-α	tumor necrosis factor-α
TOS	total observational score
urea N	urea nitrogen
USDA	United States Department of Agriculture

VSP	variable surface lipoproteins
WBC	white blood cell
WC	whole cell
WSBRC	Willard Sparks Beef Research Center
Zn	zinc

CHAPTER I

INTRODUCTION

Bovine respiratory disease (BRD), also known as “shipping fever” or bronchopneumonia, is the most significant production problem confronting the feedlot industry. The BRD complex accounts for the majority of morbidity, mortality, and production losses occurring in feedlots. In a cross-sectional survey conducted by Woolums et al. (2005), BRD was implicated as the leading cause of morbidity and mortality in 561 feedlots in 21 states. Estimated annual economic losses due to BRD are approaching or exceeding $2 billion annually. Chirase and Greene (2001) estimated the annual economic losses due to morbidity, decreased feed efficiency, and treatment costs associated with BRD to be $800-900 million. Bovine respiratory disease is an extremely complex illness complicated by a multitude of stressors, viruses, and bacterial pathogens can potentially contribute to its onset (Duff and Galyean, 2007).

The pathogenesis of BRD typically involves compromised respiratory immune mechanisms and a primary infection with one or more respiratory viruses. The viral infection and the calf's impaired immune response to the virus further compromise the immune system and allow for the colonization of lung tissues by bacteria (Hodgins et al., 2002). Most of the pathogens associated with increased BRD incidence are well

documented in the literature. However, many of these pathogens are also frequently isolated from the respiratory tract of clinically healthy cattle. For example, the most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD, and thus, the principal bacterial pathogen associated with BRD, is *Mannheimia haemolytica* (MH) (Whitley et al., 1992; Booker et al., 2008). However, as an opportunistic pathogen, MH is also frequently isolated from the respiratory tract of clinically healthy calves (Klima et al., 2014).

The prevention and treatment of BRD continues to be a major concern for all of those within the feedlot industry. To aid in BRD prevention, most feedlots vaccinate cattle for bovine viral diarrhea virus (BVDV) (96.6 percent of feedlots), infectious bovine rhinotracheitis (IBR) virus (93.7 percent of feedlots), parainfluenza 3 (PI3) virus (85.1 percent of feedlots), and bovine respiratory syncytial virus (BRSV) (89.5 percent of feedlots) (NAHMS, 2013). Treatment protocols for BRD vary greatly from feedlot to feedlot. However, the standard practice is to administer some class of injectable antimicrobial as the primary form of treatment when treating for suspected BRD in feedlot cattle. Nearly all feedlots (99.0 percent) used an injectable antimicrobial as the initial or primary treatment for BRD (NAHMS, 2013).

It is also common to provide additional treatment, or ancillary therapy (ANC), along with the antimicrobial when treating for suspected BRD. The primary goal of ANC is to improve the response to a BRD challenge in calves treated with antimicrobials, not to replace antimicrobial treatment. This can be accomplished through a variety of mechanisms including: relieving the harmful effects of inflammation, blocking histamine

activity, or boosting immune system function to aid in the defense against infectious pathogens (Apley, 1994).

Three large-scale surveys conducted since 1999 have provided substantial evidence as to the scope of ANC use in commercial feedlots. In 1999, USDA's NAHMS surveyed feedlots in the top 12 cattle feeding states and noted that only 12.8% of these feedlots used a single antimicrobial for the treatment of BRD (NAHMS, 2001). More recently, a survey conducted by Terrell et al. (2011) reported that 48% of veterinarians recommended some form of ANC for the treatment of BRD. In 2011, USDA's NAHMS surveyed feedlots with a capacity of 1,000 or more head in the top 12 cattle feeding states and reported that 55.9% of feedlots used a non-steroidal anti-inflammatory drug (NSAID) and 39.3% used a respiratory vaccine as a component of the initial BRD treatment program for some cattle (NAHMS, 2013). The most commonly used forms of ANC appearing in all surveys included: vitamin C, NSAID, antihistamines, direct-fed microbials (DFM), B vitamins, viral vaccines, and corticosteroids (NAHMS, 2001; Terrell et al., 2011; NAHMS, 2013). While these surveys provide evidence as to the scope of ANC use, there is limited published research on the efficacy of these ANCs.

In addition to ANCs, the feedlot industry has investigated the use of nutritional additives as an additional method to decrease morbidity and mortality due to BRD, while simultaneously enhancing animal performance. One group of nutritional additives that has received consideration would be trace minerals (TM) due to their proposed positive impacts on immune function. The supplementation of TM has been demonstrated to alter immune function measurements and reduced morbidity associated with BRD in some cases (Galyean et al., 1999). The supplementation with TM including: copper (Cu),

manganese (Mn), and zinc (Zn) has become a normal management practice within the feedlot industry to promote maximum performance and immune function, and these TM are frequently included at levels in excess of published requirements in feedlot diets (Vasconcelos and Galyean, 2007).

Research concerning TM supplementation has been extremely inconsistent. This inconsistency is especially evident when investigating ideal diet concentrations and TM forms or sources. However, in some instances organic mineral complexes have demonstrated more bioavailability than traditional inorganic mineral sources. This increased bioavailability is a result of the decreased likelihood of a chelated mineral complex becoming bound to another substance within the upper gastrointestinal tract and thus allowing the mineral to be more readily absorbed across the brush border of the small intestine. The increase in bioavailability of organic TM could allow for improved TM status within the animal in an immune challenge scenario such as a BRD event. However, this has not been demonstrated to date in the published research.

In the last 40 years much knowledge of the pathogenesis of BRD has been acquired and advancements have been made in the production of vaccines and antimicrobials for the prevention and treatment of BRD. There are exponentially more commercially available respiratory vaccines and long lasting antimicrobials for BRD than in any time in previous history. However, the prevalence of BRD in feedlot cattle has not been significantly reduced. Data would indicate that no significant reduction in BRD incidence has occurred over the last 30 to 40 years (Gifford et al., 2012). These data suggest that current BRD prevention and treatment strategies have been ineffective in

reducing and controlling BRD, despite measurable improvements in commercially available vaccines and antimicrobials (Babcock et al., 2006).

The objectives of the experiments presented in this dissertation were to: 1) Evaluate multiple ancillary therapies used in combination with an antimicrobial in newly received high-risk calves treated for bovine respiratory disease; 2) Evaluate the impact of bovine respiratory disease and ancillary therapy use during the receiving period on steer finishing performance, efficiency, carcass characteristics, and lung scores; 3) Evaluate the effects of chelated copper, manganese, and zinc supplementation on clinical signs, immune response variables, and mineral status in calves following natural exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* challenge. The ultimate goal of these experiments would be to expand upon the current knowledge regarding prevention and treatment of BRD and to improve upon the management of cattle subsequent to a BRD challenge to attempt to reduce the overall economic impact BRD to the feedlot industry.

CHAPTER II

REVIEW OF LITERATURE

BOVINE RESPIRATORY DISEASE IN NEWLY RECEIVED CALVES

Bovine respiratory disease (BRD) continues to be the most significant production problem confronting the feedlot industry. The BRD complex accounts for the majority of morbidity, mortality, and production losses occurring in feedlots. In a cross-sectional survey conducted by Woolums et al. (2005), BRD was implicated as the leading cause of morbidity and mortality in 561 feedlots in 21 states. In a review of feedlot data from 1994 to 1999, an average of 12.6 head of cattle died for every 1,000 that were placed on feed (Loneragan et al., 2001). Loneragan et al. (2001) determined that BRD was responsible for 57.1% of the mortality that occurred. Edwards (1996) reported that approximately 70% of all disease that occurred in Midwestern feedlots was attributed to BRD and with a 10% morbidity rate, treatment costs alone would amount to $2 per head for all cattle marketed.

Fulton et al. (2002a) observed that calves treated for BRD once returned $40.64 less, calves treated for BRD twice returned $58.35 less, calves treated for BRD 3 or more times returned $291.93 less than calves that were not treated for BRD (Fulton et al.,

2002a). Chirase and Greene (2001) estimated the annual economic losses due to morbidity, mortality, decreased feed efficiency, and treatment costs associated with BRD to be $800-900 million. More recent estimates of annual economic losses due to BRD in the U.S. are in excess of $1 billion dollars (Hodgins and Shewen, 2004; Griffin et al., 2010; Powell, 2013).

A major issue concerning the estimated incidence of BRD in feedlot cattle is how BRD is identified within the population. There is no standardized case definition for BRD making BRD difficult to define from clinical standpoint. Survey information and the available data would indicate that the clinical diagnosis of BRD is still done almost exclusively by subjective visual observation (Galyean et al., 1999). Due to the subjective nature of clinical diagnosis in feedlots, this can be a concern when attempting to quantify BRD. Even the most experienced veterinarians and pen riders will be ineffective at identifying all calves suffering from BRD in a commercial feedlot. As such, defining BRD incidence based on treatment records commonly underestimates the total effect of BRD. Gardner et al. (1999) reported that the presence of lung lesions at harvest was more highly correlated to decreased performance than animal treatment records. Steers with lung lesions present at harvest and active bronchial lymph nodes returned $73.78 less than steers without lung lesions (Gardner et al. 1999). Only 21% of this loss was attributed to treatment costs while 79% was due to lower hot carcass weights and poorer quality grades (Gardner et al. 1999).

As a result, it would seem that retrospectively correlating the presence of lesions on the lungs of cattle at harvest may be a more valid indicator of overall respiratory disease incidence in cattle. In theory, the majority of cattle that previously suffered from

BRD would have lung lesions present at harvest. However, there are concerns with using this approach to quantify BRD incidence as well. While cattle were never treated for clinical signs of BRD have commonly demonstrated evidence of lung damage as expected, it has also been reported that cattle that were treated for clinical signs of BRD have lacked the presence of any pulmonary lesions (Wittum et al., 1996; Gardner et al., 1999; Thompson et al., 2006; Schneider et al., 2009). Currently, there is not a perfect diagnostic tool to determine the exact incidence of BRD and thus determining the true production and economic losses due to BRD is extremely difficult.

Bovine Respiratory Disease is an extremely complex illness complicated by a multitude of stressors, viruses, and bacterial pathogens that can potentially contribute to its onset (Duff and Galyean, 2007). The way cattle are marketed in the U.S. is inherently responsible for a variety of stressors. In the Southern Plains and Southeast U.S. calves are commonly removed directly from their dams and shipped to a livestock market for sale. At the livestock market, calves are commingled with many other animals of unknown disease and vaccination status. Calves are typically purchased and again commingled into large lots to be loaded on trucks for transportation. The calves are then transported long distances where they are potentially exposed to additional stressors such as exhaust fumes, extreme temperatures, dehydration, malnourishment, and exhaustion (Griffin et al., 2010).

Other factors occurring prior to marketing cattle including prenatal nutrition, colostrum intake, previous nutrition of calves while still on the dam, cattle genetics, individual animal variation, and the previous health and vaccination history of both the herd and individual animals can interact with the marketing stressors to impact BRD

incidence (Duff and Galyean, 2007). Once calves arrive at the feedlot or are received into a stocker or backgrounding program, additional stressors then occur. These post-marketing stressors include processing, (typically includes the administration of vaccinations and implants, and potentially includes dehorning and castration, additional sorting and commingling to form uniform pens, dusty pen environments, and the introduction to new feed and unfamiliar water sources. All of these serve to negatively affect the immune system at a time when the calf is likely going to be exposed to viral and bacterial respiratory pathogens.

The pathogenesis of BRD typically involves compromised respiratory immune mechanisms and a primary infection with one or more respiratory viruses. The principal viral pathogens associated with BRD include: bovine herpesvirus 1 (BHV1) leading to infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza virus type 3 (PI3), bovine respiratory synctial virus (BRSV), and bovine respiratory corona virus (BCV) (Roth and Perino, 1998). The viral infection and the calf's impaired immune response to the virus further compromise the immune system and allow for the colonization of lung tissues by bacteria (Hodgins et al., 2002). Important bacterial pathogens connected to the increased incidence of BRD include: *Mannheimia haemolytica* (MH), *Pasteurella multocida* (PM), *Histophilus somni* (HS), and *Mycoplasma bovis* (MB) (Griffin et al., 2010).

The pathogenesis of BRD can involve a variety of these viral or bacterial pathogens and identifying a true “cause” of BRD is often difficult if not impossible. To further complicate matters, many of these bacterial pathogens are also frequently isolated from the respiratory tract of clinically healthy cattle. For example, the most common

bacterial pathogen isolated from the respiratory tract of calves treated for BRD is *M. haemolytica* serotype 1 (Whitley et al., 1992; Booker et al., 2008). However, *M. haemolytica* is also frequently isolated from the respiratory tract of clinically healthy calves (Klima et al., 2014).

The prevention and treatment of BRD, continues to be a major concern for the feedlot industry. To aid in BRD prevention, most feedlots vaccinate cattle for bovine viral diarrhea virus (BVDV) (96.6 percent of feedlots), infectious bovine rhinotracheitis (IBR) virus (93.7 percent of feedlots), parainfluenza 3 (PI3) virus (85.1 percent of feedlots), and bovine respiratory syncytial virus (BRSV) (89.5 percent of feedlots) (NAHMS, 2013). Treatment protocols for BRD vary greatly from feedlot to feedlot. However, the standard practice is to administer some class of injectable antimicrobial as the primary form of treatment when treating for suspected BRD in feedlot cattle. Nearly all feedlots (99.0 percent) used an injectable antimicrobial as the initial or primary treatment for BRD (NAHMS, 2013).

Risk factors influencing BRD incidence

The BRD complex is an extremely complicated illness that is elicited by a multitude of different risk factors or stressors, viruses, and bacterial pathogens (Duff and Galyean, 2007; Griffin et al., 2010). At its core, BRD is a viral and bacterial disease of the respiratory tract. However, a multitude of risk factors including various stressors can suppress the calf's immune system, allowing for these viral and bacterial pathogens to rapidly multiply within the animal's respiratory tract. Aich et al. (2009) defined these

stressors as “a psychologically perturbing condition occurring in response to adverse external influences capable of affecting physical health.” These risk factors or stressors can occur anytime after conception during the animal’s life. Some of these risk factors can be effectively managed at various phases of beef production, while others simply may not be avoidable and are inherently associated with the marketing of cattle and the current structure of the feedlot industry.

These risk factors or stressors that can lead to increased BRD incidence can be subdivided into pre-marketing stressors, stressors associated with the cattle marketing process, and post-marketing stressors. While they occur at various degrees and at various production stages, these stressors can all cause a suppression of the calf’s immune system, allowing for subsequent pathogen infection and replication within the respiratory tract. The incidence of BRD is not only a feedlot concern. While BRD manifests itself in the stocker or feedlot segments of the beef industry, this is not where the problem is initiated.

The origins of this problem are easily visible when looking at the population statistics of the cow calf production sector of the beef industry. There are 89.3 million head of beef cattle in the U.S. on 729,000 beef cow operations (NCBA, 2013). However the average cow herd size in the U.S. is only 44 head with 90% of cow herds having less than 100 head of cows (NCBA, 2013). The existence of so many cow calf operations and the small average size of these operations results in a huge variation in management and production practices prior to marketing. This substantial variation in pre-marketing production further complicates the stressors placed on calves through marketing channels and effects calf health post-marketing. Some major pre-marketing stress factors

impacting BRD incidence include: prenatal nutrition, colostrum intake, and previous nutrition of these calves while still on the dam, as well as individual cattle genetics and animal variation, and the previous health and vaccination history of the herd and individual animals (Duff and Galyean, 2007).

Where pre-marketing stress is concerned, research has linked poor maternal nutrition during gestation to subsequent decreased calf performance and health (Funston et al. 2012). Previous research conducted by Corah et al. (1975) demonstrated that calves born to first-calf heifers receiving 65% of their required energy intake during the last 90 days of gestation had increased morbidity and mortality rates compared to calves born to first-calf heifers receiving 100% of their required energy intake. Similarly, both Mulliniks et al. (2008) and Larson et al. (2009) stated that there was a reduction in the number of steers treated for BRD in the feedlot when cows were supplemented with additional protein compared to calves from non-protein supplemented dams. Other research has shown improvements in the number of live weaned calves, with no difference in the number of calves treated for BRD before weaning or in the feedlot (Stalker et al. 2006).

Another pre-marketing stressor of concern is the quality and quantity of colostrum consumed by newborn calves. It is widely accepted that the passive transfer of immunoglobulins in colostrum is essential for optimal calf health with colostrum containing effectively all of the essential compounds of bovine cellular and humoral immune defense, including antibodies and important proteins. (Korhonen et al. 2000; Rauprich et al. 2000; Weaver et al. 2000; Stilwell and Carvalho, 2011). There are a multitude of factors, including the timing of colostrum ingestion in relation to birth, the

volume of colostrum ingested, the immunoglobulin concentration of the colostrum ingested, and the age of the dam, that can impact immunoglobulin absorption (Weaver et al. 2000). Wittum and Perina (1995) stated that calves demonstrating a failure of passive transfer (FPT) of immunoglobulins had an increased risk of neonatal and preweaning mortality in addition to an increased risk of preweaning morbidity compared to calves receiving an adequate passive transfer of immunoglobulins. In addition, calves experiencing FPT had decreased feedlot performance, with increased risks of mortality and respiratory morbidity when compared to those calves receiving adequate passive transfer (Wittum and Perina, 1995).

Stilwell and Carvalho (2011) examined the clinical outcomes of calves with adequate transfer or FPT of immunoglobulins as diagnosed by a commercial Immunoglobulin G (IgG) kit. Out of the 97 calves included in the experiment, 60 calves (62%) had a positive test result (plasma IgG > 10 mg/mL), demonstrating protective IgG levels, while 37 (38%) had a negative test result (plasma IgG < 10 mg/mL), demonstrating FPT. Calves from the negative test group displayed a higher morbidity (56.8% vs. 16.7%, respectively) and mortality percentage (16.2% vs. 1.7%, respectively) compared to the positive test group (Stilwell and Carvalho, 2011). The number of antimicrobial treatments required for calves in each group was significantly different also with the positive test group requiring 19 antimicrobial treatments for 60 total animals and the negative test group requiring 38 antimicrobial treatments for 37 animals (Stilwell and Carvalho, 2011).

In addition to prenatal nutrition and colostrum consumption, the nutrition of the calf prior to marketing can also impact the incidence of BRD. While the effects maternal

nutrition during gestation and colostrum consumption are fairly well researched, the effects of early postnatal nutrition and BRD incidence in beef cattle has not been well defined (Duff and Galyean, 2007). Nutrition affects many aspects of the immune system, including: development of lymph tissues, production of mucus, synthesis of immunologically active substances such as leukocytes, antibodies, and cytokines, cell proliferation, cellular signaling, activation, and movement, intercellular killing, and general immune system modulation (Cole, 1996). A major problem concerning when investigating the nutrition and health interaction in calves is that typically the nutritional background or nutrient status of calves used in BRD experiments is not known and impractical to obtain (Duff and Galyean, 2007). Duff and Galyean (2007) suggest that the large variation in nutritional status of calves could help explain the variation in response to nutritional supplements that exists in the literature.

Montgomery et al. (2009) measured plasma glucose, lactate, and urea nitrogen (N) concentrations of 665 crossbred heifers at time of initial processing to determine the incidence of apparent BRD during receiving, and evaluate the effects of BRD on subsequent cattle performance and carcass characteristics. The authors found that at time of initial processing heifers that were eventually treated for apparent BRD had decreased plasma glucose and lactate, and increased plasma urea N concentrations as determined by linear contrasts (Montgomery et al., 2009). The interpretation of these results can be somewhat problematic as the changes in metabolites could be the result of an immune challenge that has already occurred, or the result of nutrient restriction predisposing the calves to an immune challenge. The authors suggested that both methods could be

responsible for the decrease in plasma glucose concentrations observed in heifers eventually treated for BRD (Montgomery et al., 2009).

When considering the decrease in plasma glucose, Steiger et al. (1999) observed a biphasic response when heifers were infused bacterial lipopolysaccharides to simulate a gram-negative bacterial challenge with glucose concentrations increasing initially and then decreasing to concentrations below that of control saline-infused heifers. In addition, Kushibiki et al. (2000), observed a similar biphasic response for plasma glucose levels when injecting heifers with recombinant tumor necrosis factor-α (TNF-α). These 2 studies would suggest that an immune challenge in cattle results in a period of increased plasma glucose levels followed by a period of decreased plasma glucose levels. In contrast, multiple studies have sighted decreased serum glucose levels when calves are in a fasted state. Galyean et al. (1981), Cole et al. (1988), and Schaefer et al. (1990) all reported decreased serum glucose concentrations in calves following a 24 to 36 hour fast. Montgomery et al. (2009) stated that the decreased plasma lactate concentrations observed in heifers treated for BRD might be a result of depleted glycogen stores due to extended fasting which could lead to decreased lactate production via glycogenolysis and anaerobic glycolysis. The authors proposed that the increased plasma urea N concentrations in heifers treated for BRD would suggests that those heifers were catabolic, and supported their previous statement regarding lactate levels and the possibility of decreased glycogen stores at initial processing. (Montgomery et al., 2009).

Other factors such as genetics, breed type, and individual animal variation have been shown to impact BRD incidence. Snowder et al. (2005) aimed to characterize the genetic and environmental factors influencing BRD in cattle. Retrospective data from 9

purebred (Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Pinzgauer, Red Poll, and Simmental), 3 composite breeds (MARC I, MARC II, and MARC III), and a multiple F1 and 3-way crosses 20 year period were evaluated for the effects breed and heterozygosity on BRD incidence (Snowder et al., 2005). The highest incidence rates for BRD were observed in the Braunvieh (19%) and MARC I composites (17%), which contained ¼ Braunvieh (Snowder et al., 2005). Snowder et al. (2005) suggested that the increased incidence of BRD in Braunvieh and Braunvieh composites may be confounded with the breed's higher incidence of calving difficulty. The incidence of BRD ranged from 8 to 12% for all other breed groupings (Snowder et al., 2005).

When evaluating mortality attributed to BRD, the highest mortality rates were observed in the Simmental (18%), MARC III (17%), and Red Poll (16%) breeds, while the lowest mortality rates were observed for Limousin (7%) and Braunvieh (9%) (Snowder et al., 2005). Interestingly, while Braunvieh cattle exhibited the greatest incidence of BRD, the mortality rate for Braunvieh cattle was much less than the overall average of 13% suggesting that while Braunvieh cattle are more susceptible to BRD infection, they were less sensitive to the disease once infected (Snowder et al., 2005). In contrast, the MARC III composite calves had relatively high mortality rates and the relatively low incidence rates (10%) suggesting that MARC III composites were more sensitive to the BRD, although more resistant to BRD infection (Snowder et al., 2005). Increased heterozygosity of calves resulted in a decreased incidence of BRD when compared to purebred cattle (Snowder et al., 2005). In addition, calves that were Continental × British crosses or tropical × British crosses had a lower incidence of BRD than calves that were strictly British × British crosses (Snowder et al., 2005).

In a second retrospective experiment, Snowder et al. (2006) examined the records from 18,112 calves representing the same 9 breeds and 3 composites over a 15-yr period (1987 to 2001), emphasizing that not all breeds were represented in all years. The authors noted that steers were more likely to experience BRD than heifers and cited the stress associated with castration prior to feedlot entry as a probable predisposing cause (Snowder et al., 2006). When the mean incidence was unadjusted for all breeds, the incidence of BRD was lowest for Angus (10.2%), with Charolais, Gelbvieh, and the MARC I and MARC III composite cattle also having lower unadjusted BRD incidences (Snowder et al., 2006). The unadjusted incidence of BRD was greatest for the Pinzgauer, Braunvieh, Simmental, and Limousin breeds (35, 34, 33, and 32%, respectively) (Snowder et al., 2006).

When BRD incidence was adjusted via mixed model analyses, Snowder et al. (2006) indicated that Herefords were generally more susceptible to BRD infection than MARC I and MARC III composite breeds, with the composite breed types having similar susceptibility to BRD when compared to the other purebred breeds. The BRD attributed mortality was greatest for Red Poll calves (9%) compared to the average of all other breeds (4%) (Snowder et al., 2006). The greater mortality rate experienced by Red Poll calves suggests this breed may indeed be more sensitive to BRD infection than other breed types (Snowder et al., 2006). Interestingly, in this experiment Snowder et al. (2006) stated that there was no observed advantage for BRD resistance with increased heterozygosity when contrasting the composite breeds with the purebreds.

The previous health program and vaccination history of a herd or individual animals can also impact BRD incidence. These practices along with other pre-marketing

management decisions can be grouped in to the broad category of "preconditioning." Preconditioning can be thought of as a comprehensive management system that is designed to immunize calves against BRD related pathogens and to reduce or minimize the stressors that cattle potentially experience at the time of marketing (Cole, 1985). Preconditioning can be more specifically subdivided into 3 distinct categories: vaccination practices, surgical procedures, and feeding strategies (Cole, 1985). The preconditioning of calves is a longstanding practice, with the state of Iowa holding a preconditioned cattle sale in 1965 and Oklahoma State University holding the first national preconditioning seminar in 1967, and it is widely accepted by those in academia and industry that the preconditioning of calves is an effective tool for preventing or minimizing and managing BRD. Most feedlot operators believing that the preconditioning of cattle is beneficial in decreasing morbidity and mortality in calves weighing less than 318 kg (NAHMS, 2000).

A major problem that surfaces when reviewing the literature is the determination of what exactly qualifies as preconditioning. Preconditioning is a broad term, and huge variations exist in the literature in the way the term is defined. In a review of preconditioning studies, Taylor et al. (2010b) found that some precondition experiments did not even offer explanation as to what constituted preconditioning. Lynch et al. (1997) determined calves to be preconditioned if they had simply received both viral and *Pasteurella* vaccination prior to weaning. While multiple experiments define calves that were vaccinated and weaned as preconditioned (Karren et al., 1987; Roeber et al., 2001; Macartney et al., 2003; Taylor et al., 2010b). However, Macartney et al. (2003) did not require that calves be trained to a feed bunk, while Karren et al. (1987) and Roeber et al.

(2001) required calves to be acclimated to feed bunks and water troughs. This variation among "preconditioning" studies makes it nearly impossible to compare results across studies and makes interpreting the effects of preconditioning difficult.

While there is substantial variation in the interpretation of what constitutes preconditioning, there are several formalized preconditioning programs in existence today. Many states have developed programs to certify the preconditioning of calves. Two of the better known of these programs being the Texas A&M University Value Added Calf Program and the Oklahoma Quality Beef Network Vac-45 Program. These preconditioning programs have strict requirements that must be met. The requirements typically state that at a minimum: all bull calves be castrated and healed, all horned calves be dehorned and healed, all calves receive an approved vaccination regimen, all calves be weaned for at least 45 days prior to sale, and all calves be tagged with an official program identification tag (Avent et al., 2007).

The preconditioning of calves has demonstrated a reduction in the incidence of BRD in calves as they move from the cow-calf phase to either the stocker or finishing phases of beef production (Thrift and Thrift, 2011). Roeber et al. (2001) observed the feedlot performance and carcass characteristics of calves purchased from 2 different certified preconditioning programs or directly from livestock markets. Morbidity rates of 34.7%, 36.7%, and 77.3% were documented for cattle on the 2 certified preconditioning programs and livestock market calves, respectively (Roeber et al., 2001). Roeber et al. (2001) also reported mortality to be 1.1%, 1.1%, and 11.4% among the same 3 groups of calves. Step et al. (2008) observed vaccination and preconditioning in calves prior to shipment. The authors found that calves preconditioned for 45 days pre-shipping but not

vaccinated and calves both preconditioned 45 days and vaccinated had lower feedlot morbidity levels (5.9% and 9.5%, respectively), compared to calves marketed at weaning (35.1%) and calves assembled from a livestock market (41.9%) (Step et al., 2008). While feedlot mortality was not extremely high and did not differ significantly, the calves assembled from the livestock market and those marketed at weaning had mortality rates of 3.1% and 0.9% respectively, while the calves in both preconditioning treatments experienced 0.0% (Step et al., 2008). Richeson et al. (2012) observed ranch calves preconditioned for 61 days and auction market calves assembled at the time of purchase with and without exposure to a persistently infected bovine viral diarrhea virus (PI-BVDV) pen mate. The authors stated that auction market calves had a 70.4% overall morbidity compared to 6.7% overall morbidity in preconditioned calves (Richeson et al., 2012).

The cattle marketing system in the U.S. is inherently responsible for a wide range of stressors. In the Southern Plains and Southeast U.S. it cannot be assumed that calves are preconditioned or even weaned prior to being shipped to a livestock market for sale. Upon arrival at the livestock market, calves are then commingled with many other animals of unknown disease and vaccination statuses. Once calves are purchased, they are again commingled to form lots to be loaded onto trucks. Once loaded, calves are frequently transported long distances and potentially exposed to exhaust fumes, extreme weather, and temperature fluctuations, or even resold through additional market channels. Dehydration, malnutrition, and exhaustion often occur as a result (Griffin et al., 2010).

Whether or not a calf is formally weaned for a predetermined period of time or simply removed from its dam immediately prior to marketing, the physical separation of

a calf from its dam is invariably a source of stress. This stress resulting from the physical separation from the dam is demonstrated by increased vocalization and pacing by the calf (Newberry and Swanson, 2008). Physical separation has been shown to effect the physiological status of calves. Lay et al. (1998) subjected calves to restricted or ad libitum nursing after 21 days of age. The authors demonstrated that the restricted nursing calves had elevated heart rates and cortisol levels when compared to the ad libitum nursing calves prior to weaning (Lay et al., 1998). However, post weaning the ad libitum group had more calves vocalizing and tended to travel greater distances than the restricted group causing Lay et al. (1998) to propose that the ad libitum group experienced more stress post weaning. This observation led Lay et al. (1998) to suggest that the while the restricted calves were more stressed prior to weaning, the separation and restricted nursing potentially allowed for them to experience less stress post weaning.

Hickey et al. (2003) randomly assigned calves to either a control or abruptly weaned treatment group and observed various physiological measures of stress and immune function. While the interpretation of results from this experiment are somewhat problematic due to complex variables and multiple interactions, the authors did emphasize some interesting results. There was an increase in the neutrophil to lymphocyte ratio that occurred after weaning (Hickey et al., 2003). The authors also stated that the abrupt weaning resulted in increased plasma cortisol and noradrenaline concentrations (Hickey et al., 2003). These changes were accompanied by decreased in vitro interferon-γ production in response to novel mitogen and antigen complexes after weaning (Hickey et al., 2003).

Arthington et al. (2008) allocated steers to 1 of 4 weaning management strategies: weaned on the day of shipping (control), allowed free-choice access to a concentrate feed prior to weaning and shipping (creep-fed), weaned and provided supplemental concentrate feed on pasture before shipping (preweaned), and weaned at 70 to 90 days of age and kept on pasture (early-weaned). Overall receiving ADG was greater for early-weaned steers when compared to control steers (Arthington et al., 2008). During the first week after shipment, early-weaned steers consumed more concentrate and less hay when compared to control steers and preweaned steers and consumed more concentrate and a similar amount of hay when compared to creep-fed steers (Arthington et al., 2008). Arthington et al. (2008) also observed that plasma ceruloplasmin concentrations were less in control steers compared to early-weaned steers on day 0, but that plasma ceruloplasmin concentrations increased rapidly in the control calves over time causing them to be greater in control on day 15 and day 22. Steers that were creep-fed had greater plasma ceruloplasmin concentrations than preweaned steers on day 29 (Arthington et al., 2008). These results led the authors to suggest that early-weaned steers demonstrated improved performance in the feedlot compared to steers weaned directly before feedlot entry and that differences in preweaning strategies appeared to significantly affect the acute phase protein response in steers (Arthington et al., 2008).

Boyles et al. (2007) researched the effects of weaning management strategies on the performance and health of calves during a 28 day feedlot receiving period. The authors evaluated: calves weaned at time of transport, calves weaned and confined in a dry lot for 30 days prior transport, and calves weaned 30 days before transport and allowed fence line contact with their dams (Boyles et al., 2007). Calves weaned at

transport and fence line weaned calves had increased ADG when compared to dry lot weaned calves (Boyles et al., 2007). Morbidity in the feedlot was decreased in fence line weaned calves (15%) when compared to calves weaned at transport and dry lot weaned calves (28% and 38% respectively) (Boyles et al., 2007).

Other studies have demonstrated an impact of abrupt weaning on subsequent calf health and performance. Hodgson et al. (2005) found that weaning combined with immediate transport resulted in a failure to upregulate expression of interleukin-10 (IL-10) and caused mortality attributed to BRD to be twice as high when compared to calves that were only subjected to transport and a bacterial challenge. Lynch et al. (2010) determined that abrupt weaning resulted in increased neutrophil counts and diminished phagocytic function in calves. Price et al. (2003) found that allowing fence line contact between beef calves and their dams at weaning reduced the negative effects of immediate separation on calf behavior and growth rate.

The process of transporting calves to the livestock market and then to a subsequent destination results in calves being exposed to an additional social stress while simultaneously being exposed new or different isolates viral and bacterial pathogens. It is not uncommon for BRD to be referred to as “shipping fever” in the classical literature and by those outside of the scientific community today, and for many years transportation was considered the most common predisposing factor for BRD (Stanger et al., 2005; Taylor et al., 2010a). Transportation has been demonstrated to cause an increase in glucocorticoids within the blood and increased glucocorticoid levels have been linked to suppression of the immune system and increased susceptibility to disease (Stanger et al., 2005; Aich et al., 2009). Cernicchiaro et al. (2012) reviewed retrospective data from 13

U.S. commercial feedlots on 16,590 head of cattle to determine the association between BW lost during transport (shrink) and subsequent health and performance in the feedlot. The median shrink experienced by all calves was 3.0% with a mean shrink of 2.4 %, while the mean cumulative BRD morbidity was 10.0% and the mean cumulative mortality was 1.3% (Cernicchiaro et al., 2012). Shrink was significantly associated with BRD morbidity, overall mortality, animal HCW and ADG (Cernicchiaro et al., 2012). These effects were also significantly impacted by gender, season, and arrival BW of the calf (Cernicchiaro et al., 2012).

However, while almost every calf in the U.S. is transported at least 3 times prior to harvest, transportation does not appear to affect all cattle the same and the distance transported may have mixed effects. Ribble et al. (1995) conducted an experiment evaluating the effect of transportation on pneumonia and shrinkage in calves arriving at the feedlot. The authors reported that calves transported for 12 hours had higher morbidity rates than those calves transported for 24 hours (Ribble et al., 1995). Ribble et al. (1995) concluded that other stress factors such as sorting, loading, and time of transit are more strongly associated with the development of BRD than duration of transportation.

Changes in the weather and temperature fluctuations have also been demonstrated to impact BRD incidence in calves. Classically, the highest incidence of BRD is observed during the fall (Kelly and Janzen, 1986; Loneragan et al., 2001 Taylor et al., 2010a). Traditionally calves born in the spring and summer are marketed through livestock markets and ultimately shipped to feedlots or stocker operations during the fall. This seasonality of marketing increases the number of newly received calves through livestock

markets which could potentially result in an increase in the time that calves spend in livestock markets, and could potentially cause delays in the loading, transport, and unloading processes. These changes can impact the length of time calves are exposed to inclement weather and temperature fluctuations at a time when they are experiencing stress, and more susceptible to disease. Wilson et al. (2012) examined the factors influencing subsequent receiving health and performance of 1361 head of "high-risk" steer and bull calves purchased at regional livestock markets received at a single facility in 2010 and 2011. Calves were received in either the spring or fall of the year and purchased by a single order buyer (Wilson et al., 2012). Calves received in the fall had lower ADG and increased morbidity and mortality attributed to BRD than calves received in the spring (Wilson et al., 2012).

Cernicchiaro et al. (2012) reported that shrink had a greater effect on calves arriving during the fall months and that calves arriving during the fall also demonstrated a greater BRD morbidity risk. The authors suggested that the temperature fluctuations commonly observed during the fall and early spring could affect the health and performance of newly received cattle experiencing BW loss after transport (Cernicchiaro et al., 2012). In another experiment, Cernicchiaro et al. (2011) observed several weather factors during the first 45 days subsequent to arrival of calves at the feedlot. For this retrospective experiment, the population included 288,388 total head of cattle that arrived at 9 U. S. commercial feedlots between September and November in 2005 to 2007 (Cernicchiaro et al., 2011). There were 24,947 cases of initial BRD that occurred within this population of cattle during these time periods (Cernicchiaro et al., 2011). The authors found that several weather factors including: maximum wind speed, mean wind chill

temperature, and temperature change in different lag periods were significantly associated with an increased BRD incidence (Cernicchiaro et al., 2011). However, Cernicchiaro et al. (2011) stated that the effects of these variables also depended on several cattle demographic factors including: month of arrival, BRD risk code, BW class, and cohort size. Though it has been accepted that sudden and extreme changes in weather and temperature predispose to calves to BRD, more research is needed to determine the true effect of these changes on BRD.

After calves arrive at a feedlot or a stocker or backgrounding program, they are potentially exposed to even more stressors. These post-marketing stressors include processing, which typically consists of multiple vaccinations, treatment for internal and external parasites, and administration of growth promoting implants and may potentially include dehorning and castration. Additional sorting and commingling of calves occurs to fill pens in an attempt to make those pens more uniform. Calves are frequently exposed to dusty pen environments, and introduced to new feed and water sources. All of these stressors serve to further challenge the immune system at a time when the calf has been or is likely going to be exposed to multiple viral and bacterial pathogens.

Common processing protocols subsequent to arrival at a feedlot or a stocker or backgrounding program include the administration of viral and clostridial vaccines. In the 2011 Feedlot Survey, NAHMS (2013) stated that most feedlots vaccinated some cattle and that the most common vaccinations administered were those for the prevention of BRD. As a percentage of all feedlots, 96.6% vaccinated for BVDV, and 93.7% vaccinated for IBR, while 89.1% vaccinated for BRSV, and 85.1% vaccinated for PI3 (NAHMS, 2013). NAHMS (2013) also stated that 84.4% of feedlots administered a

clostridial vaccine and roughly two thirds of feedlots vaccinated for the most common bacterial agents associated with respiratory disease: HS, MH, and PM.

While vaccination is typically considered a preventative measure for reducing the incidence of BRD, it has hypothesized that vaccination at the time of arrival may actually be detrimental as calves are already stressed, have been exposed to pathogens, and perhaps are experiencing some level of immunosuppression (Perino and Hunsaker, 1997; Richeson et al., 2008). Most recent vaccine research has been focused on comparing different multivalent modified live vaccines composed of different antigen strains. In addition, most vaccine studies published in the literature do not include negative control (non-vaccinated) groups. As a result, it is extremely difficult to determine if vaccination at arrival aids in the prevention of BRD or if it actually may be detrimental. More research must be conducted in order to determine effective respiratory antigen immunization protocols for feedlot cattle.

Richeson et al. (2008) examined the effects of arrival versus delayed viral vaccination on the subsequent health, performance, and serum IBR antibody titers of newly received calves. The authors found that ADG was greater ($P \leq 0.05$) for delayed vaccinated calves throughout the experiment, while the time until first BRD treatment, total treatment cost, and mortality rates did not differ (Richeson et al., 2008). Richeson et al. (2008) stated that the morbidity rates for BRD were high for both calves vaccinated at arrival (71.5%) and delayed vaccinated calves (63.5%) and not statistically different. Positive IBR titer seroconversion was greater for delayed vaccinated calves (Richeson et al., 2008). The increase ADG and IBR antibody titers led the authors to conclude that

delaying vaccination by 14 days may result in a possible improvement in immune response (Richeson et al., 2008).

In a second experiment, Richeson et al. (2009) examined the effects of arrival versus delayed clostridial or viral vaccination on the health, performance, BVDV titers, and immune response measures of newly received calves. The authors found that ADG and morbidity did not differ among the arrival or delayed vaccination treatments (Richeson et al., 2009). Richeson et al. (2009) noted that several differential white blood cell (WBC) measurements were greater when both the clostridial and viral vaccinations were delayed and that this could have been the result of an infection or due to differences in immune status. Richeson et al. (2009) concluded that timing of vaccination did not affect performance or health in high-risk newly received calves.

Estrogenic, androgenic, and combination implants have been used extensively in beef production for many years. Steroidal implants have proven to be one of the best non-nutritional management tools that beef producers can use to improve the performance and efficiency of calves. Implants work by releasing stimulants that act to increase the circulating levels of growth factors thus increasing the secretion of growth hormone and increasing lean tissue. Ultimately, steroid implants alter protein metabolism by increasing the rate protein synthesis and decreasing the rate of protein degradation. Because implants influence metabolism and nutrient partitioning within the body, immune function could be altered as a result (Nichols et al., 2002). If extra nutrients are partitioned for growth, at arrival when the immune system is compromised, implants could have an impact on the incidence of BRD.

Munson et al. (2012) examined the effects of delayed implanting on the health and performance of 1601 high-risk feedlot steers. Steers were either implanted with Revalor-XS (40 mg estradiol and 200 mg trenbolone acetate) at initial processing or 45 days post processing (Munson et al., 2012). The authors found that delaying the time until initial implant tended to reduce morbidity (3.8% decrease) and reduced the percentage of animals that were required to be salvaged or realized (1.8% vs. 3.3%) (Munson et al., 2012). There were no effects of delayed implant administration on retreatment rates, mortality percentage, or lung lesions and adhesion at harvest (Munson et al., 2012). The authors concluded that delaying the timing of implanting did not greatly influence health or performance in calves at a high risk of developing BRD (Munson et al., 2012).

Poe et al. (2103) conducted an experiment to determine the effects of viral vaccination timing with or without a growth implant on the health, performance, complete blood cell counts, and viral antibody titers in high-risk, newly received stocker calves. The percentage of calves treated for BRD and the days until first BRD antimicrobial treatment did not differ among implant treatments (Poe et al., 2103). Calf ADG was also unaffected by the administration of implants (Poe et al., 2103). The authors concluded that growth promoting implants administered on arrival to high-risk stocker calves did not impact health or performance, but noted that the morbidity rate was high with over 80% of calves requiring antimicrobial treatment (Poe et al., 2103).

Dehorning or the “tipping” of horns is a common processing practice in beef production, and nearly all horned cattle entering a feedlot will be dehorned or have their horns tipped. Cattle that have been dehorned are safer to handle and present a lower

injury risk to other cattle, horses, and feedlot personnel (AVMA, 2012b). In addition to the safety concern, cattle with horns also take up additional bunk space in feedlot pens compared to polled cattle, causing intake and behavior concerns. While the dehorning of cattle is necessary from a safety and management perspective, the stress associated with dehorning is well documented. Both physiological and behavioral measurements have been used to evaluate the stress responses to dehorning. While individual responses vary, plasma cortisol concentrations increase rapidly after dehorning, and adrenaline (epinephrine) and noradrenaline (norepinephrine) concentrations have been demonstrated to increase after scoop dehorning young calves (AVMA, 2012b). While the dehorning of horned cattle is desirable, the stress and pain associated with dehorning could have an impact on the incidence of BRD.

While the impacts of dehorning on stress and pain in calves is an area of increasing research, the impact of dehorning on BRD incidence has not been thoroughly examined. Martin et al. (1982) reviewed 3 years of data on 52,889 head of cattle to evaluate the factors associated with death loss and health costs in Ontario feedlot calves. The authors found that when dehorning greater than 30% of the animals within a group, dehorning had a negative impact on health and that dehorning was also associated with increased mortality rates (Martin et al., 1982). It should be noted however that dehorning was only recorded in this data set when greater than 30% of the animals within a group were dehorned (Martin et al., 1982).

In the retrospective experiment by Wilson et al. (2012) examining the factors influencing subsequent receiving health and performance of high-risk calves, calves that were dehorned at initial processing had significantly lower ADG throughout the receiving

period. Calves that were dehorned also tended to receive a second antimicrobial treated more often than calves that were polled (Wilson et al., 2012). The authors found that first treatment morbidity and overall morbidity, while numerically greater for calves that were dehorned, was not significantly different (Wilson et al., 2012). These results led Wilson et al. (2012) to surmise that dehorning negatively impacted calf performance, but may or may not impact calf health.

The castration of bull calves is a common processing practice in beef production, and nearly all bull calves entering a feedlot will be castrated shortly after arrival. The castration of bulls causes them to be safer to handle and presents a lower risk of injury to other cattle, horses, and feedlot personnel (AVMA, 2012a). Castration also improves carcass characteristics. Steers are less likely to be deemed "dark cutters" and produce higher quality grade, more marbled, and more tender carcasses when compared to bulls (AVMA, 2012a). There are several methods by which castration is achieved, but they can be broadly classified into 3 groups: physical, chemical, or hormonal (AVMA, 2012a). Physical castration methods can be subdivided into procedures involving surgical removal of the testes, or methods that irreparably damage the testicles and cause atrophy by interruption of the blood supply.

In typical production settings, physical castration is the most common method of castration used. Coetzee et al. (2010) conducted a survey of 189 U.S. veterinarians to evaluate the prevalence of castration methods. The authors found that surgical castration with a scalpel (57%), followed by removal of the testicles by either manually twisting (44%), or through the use of an emasculator (36%) were the most frequently used surgical castration methods (Coetzee et al., 2010). The most common methods used to

incise the scrotum to conduct surgical castration included using a Newberry Knife (32%) or a conventional knife (14%) (Coetzee et al., 2010). For non-surgical castration methods, the use of elastrator rubber rings (44%), followed by generic banders (22%) were the most commonly used methods (Coetzee et al., 2010).

While the castration of bulls is necessary from both a safety and meat quality perspective, the stress associated with castration has been well documented. All methods of physical castration cause physiological, neuroendocrine, and behavioral changes that are considered as indicators of pain and stress. Animals exhibit changes in behavior indicative of pain during the physical act of castration as well as after the castration procedure. Some of these behavioral responses include: struggling when restrained, kicking of the legs, tail swishing, foot stamping, head turning, restlessness, stiffened gait, reduction of normal social activity, increased lateral recumbency, abnormal standing posture, and reduced feed intake (AVMA, 2012a). While individual responses may vary dependent on castration method, research has consistently demonstrated increases in plasma cortisol concentrations after castration (AVMA, 2012a).

Research has repeatedly demonstrated that castration upon arrival to the feedlot impacts subsequent BRD incidence. When examining the effects of metaphylactic antimicrobial administration on the behavior of feedlot calves, Daniels et al. (2000) reported that calves castrated on arrival had a 92% greater incidence of morbidity compared with those calves that had been castrated before entering the feedlot. In addition, calves castrated on arrival had increased mortality compared to previously castrated calves (3.5% vs. 0% respectively) (Daniels et al., 2000). Daniels et al. (2000)

also reported that previously castrated calves had ADG more than twice that of calves castrated on arrival during a 21 day evaluation period.

Massey et al. (2011) reviewed records from 11 receiving experiments consisting of 3,380 head of calves that took place between 2006 and 2008. Sixty-five percent of all calves arrived as bulls and all bulls were surgically castrated (Massey et al., 2011). Massey et al. (2011) stated that only 28% of calves required antimicrobial treatment for BRD, but it should be noted that all calves received metaphylactic antimicrobial treatment. Calves arriving as steers had a greater probability of never being treated for BRD compared to calves arriving as bulls (90.8% vs. 87.9% respectively) (Massey et al., 2011). There was a greater probability that calves arriving as bulls would require multiple BRD treatments (2.4% vs. 1.8%). (Massey et al., 2011). Massy et al. (2011) also noted a difference in ADG, with calves castrated before arrival gaining 1.55 kg/day and calves arriving as bulls gaining 1.32 kg/day.

Wilson et al. (2011) reviewed the effect of castration methods on the subsequent health and performance of 390 head (266 bulls and 124 steers) of high-risk commingled calves purchased from livestock markets in a 3 state area. Bulls were randomly assigned to 1 of 2 experimental treatments: surgical castration using a Newberry Knife to incise the scrotum, followed by castration by emasculation or non-surgical castration using a Callicrate Bander® (Wilson et al., 2011). The authors found that calves that arrived at the feedlot as bulls had greater first treatment rates, greater retreatment rates, and greater total morbidity than calves that arrived as steers independent of castration method (Wilson et al., 2011). Calves that arrived as bulls also had higher mortality than calves that arrived as steers (Wilson et al., 2011). When considering castration method, Wilson

et al. (2011) noted that surgically castrated bulls had greater first treatment rates and greater total morbidity than bulls that were non-surgically castrated. Steers had improved performance compared to calves that arrived as bulls independent of castration method, and there was no difference in performance between the two castration methods (Wilson et al., 2011).

Coetzee et al. (2012) examined the effects of oral meloxicam administration on the subsequent health and performance of steers relative to bulls that were castrated upon arrival to the feedlot. The calves were divided by castration status at arrival (145 bulls and 113 steers) and randomly assigned to receive either a lactose placebo or oral meloxicam 24 hours prior to processing (Coetzee et al., 2012). All calves received metaphylactic antimicrobial treatment at processing (Coetzee et al., 2012). The percentage of calves "pulled" was significantly greater for calves that were castrated at arrival compared to calves arriving as steers (Coetzee et al., 2012). In addition calves that were castrated at arrival tended to have higher overall morbidity and a greater incidence of BRD (Coetzee et al., 2012). Coetzee et al. (2012) reported that castration reduced ADG and G:F from days 1 to 14, but that by day 28 no effects in performance were apparent. The oral administration of meloxicam did impact the health of castrated calves. Bull calves that received meloxicam prior to castration had reduced pen-level first pull rates and reduced BRD morbidity rates (Coetzee et al., 2012).

While it is understood that BRD is ultimately a viral and bacterial disease of the respiratory tract, the BRD complex is a complicated illness that is prompted by multiple risk factors or stressors. These stressors can occur at any time during a calf's life to impact BRD incidence and can be divided into pre-marketing stressors, stressors

associated with the cattle marketing process, and post-marketing stressors for ease of examination. These stressors act to reduce the calf's immune system, allowing for viral and bacterial pathogens to rapidly multiply within the calf's respiratory tract. Some of these risk factors can be effectively managed, while others simply are unavoidable and are inherently associated with the cattle marketing and structure of the feedlot industry.

Pathogens associated with BRD incidence

While a multitude of risk factors can suppress the calf's immune system, the development of clinical BRD commonly consists of a primary infection with one or more respiratory viruses. The principal viral pathogens associated with BRD include: bovine herpesvirus 1 (BHV1) leading to infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza virus type 3 (PI3), bovine respiratory synctial virus (BRSV), bovine adenoviruses (BAdV), and bovine corona virus (BCV) (Roth and Perino, 1998; Fulton et al., 2011). The initial viral infection combined with the calf's previously compromised immune system allows for the rapid colonization of lung tissues by bacteria (Hodgins et al., 2002). Important bacterial pathogens associated with increased incidence of BRD include: *Mannheimia haemolytica* (MH) (formerly *Pasteurella haemolytica*), *Pasteurella multocida* (PM), *Histophilus somni* (HS), and *Mycoplasma* species (primarily *Mycoplasma bovis*) (MB) (Griffin et al., 2010; Fulton et al., 2011). Many of these bacterial pathogens are frequently isolated from the respiratory tract of clinically healthy cattle. For example, the most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD is MH serotype A1 (Whitley et al., 1992; Booker et al.,

2008; Griffin et al., 2010). However, MH is also frequently isolated from the respiratory tract of clinically healthy calves (Klima et al., 2014; Griffin et al., 2010).

Bovine herpesvirus 1 (BHV1) is the etiological agent responsible for IBR and is in the alpha herpesvirus subfamily that can cause diseases of the respiratory tract, fetal infections and abortions, reproductive tract diseases in both males and females, and severe neonatal disease (Fulton et al., 2009). There are 3 BHV-1 subtypes (BHV1.1, BHV-1.2a, and BHV-1.2b) classified by antigenic and genomic differences (Fulton et al., 2009). In addition, another BHV has been identified in cattle that impacts the central nervous system (Fulton et al., 2009). Viral infection resulting from BHV1 has been associated with a variety of clinical conditions that result from infection of the genitals, respiratory tract, or digestive tract (Hodgins et al., 2002). A dormant infection with BHV1 can exist in the trigeminal ganglia and stress may cause a reactivation of the virus complete with clinical signs and viral shedding (Cusack et al., 2003). Clinical signs range from serous, hyperemic, and edematous membranes, to mucopurulent exudate with focal necrosis, to pseudomembranous inflammation in severe cases (Cusack et al., 2003). The replication of BHV1 occurs within mucosal cells, the submucosa, and connective tissue bordering the tracheal rings (Cusack et al., 2003).

It has been suggested that BHV1 may set in motion the destruction of the respiratory epithelium via the cessation of ciliary activity resulting in loss of function of the mucociliary escalator (Cusack et al., 2003). A secondary respiratory infection then may occur due to the inhalation of the exudates combined with the failure to remove particulates and bacteria from the lungs (Cusack et al., 2003). Necropsies and field observations would support this as a high percentage of cattle diagnosed with BRD and

identifiable IBR have a cranioventral lobar distribution of lesions resembling inhalation pneumonia (Cusack et al., 2003). In addition, BHV1 can cause extreme bronchoconstriction which results in trapping of secretions in the lower airways, impairing lung defense mechanisms and favoring increased bacterial growth (Cusack et al., 2003). Ultimately, infection by BHV1 has been implicated as a cause of immunosuppression that increases the susceptibility to secondary bacterial infections resulting in severe pneumonia (Cusack et al., 2003). This immunosuppression may occur through reductions in neutrophil migration, decreased cell-mediated cytotoxicity, diminished mitogen responses of peripheral blood lymphocytes, and impaired functional activities of alveolar macrophages (Cusack et al., 2003).

Bovine viral diarrhea virus (BVDV) is probably the most researched of all of the viral pathogens associated with BRD with decades of research concerning BVDV prevalence and pathogenesis having been previously conducted (Hodgins et al., 2002; Ridpath, 2010). However, the role of BVDV in the pathogenesis of BRD has been is still under intense debate primarily due to a lack of evidence implicating BVDV as a primary BRD pathogen (Cusack et al., 2003). More specifically the uncertainty of BVDV's role in BRD pathogenesis is a result of the difficulty associated with the determination of total impact of acute, uncomplicated BVDV infections, the high incidence of BRD in persistently infected BVDV animals (PI-BVDV), and the unknown impact of immunosuppression that accompanies acute BVDV infections and predisposes animals to secondary infections, and the impact of increased virulence of pathogens involved in co-infections (Ridpath, 2010). Holland (2009) stated that recent research concerning viral

pathogens involved in field cases of BRD has centered on BVDV with the primary concern being calves that are PI-BVDV.

Regardless of the lack of understanding and agreement on BVDV's exact role in the pathogenesis of BRD, it has been reported that BVDV is the virus most commonly isolated from pneumonic lungs of cattle (Hodgins et al., 2002). The term BVDV encompasses a heterogeneous group of viruses that belong to 2 different species or genotypes, BVDV1 and BVDV2, within the pestivirus genus of the Flavivirus family (Ridpath, 2010). In addition, BVDV is classified by biotype as cytopathic or non-cytopathic based on the presence or absence of visible cytopathic effects in infected cell cultures (Fulton et al. 2002b). Within genotype, BVDV has been further subdivided into subtypes 1a and 1b on the basis of polymerase chain reaction (PCR) and nucleic acid sequencing (Fulton et al. 2002b). In the U.S. the prevalent subtypes include: BVDV1a, BVDV1b, and BVDV2a and in multiple studies, BVDV1b has been the predominant BVDV subtype isolated from calves diagnosed with BRD (Fulton et al. 2002a; Fulton et al. 2002b).

It is suspected that BVDV may facilitate colonization of the lungs by other pathogens (Cusack et al., 2003). Experimental infection of immune-competent, seronegative calves with BVDV has been shown to induce primary BRD in the absence of concurrent infection with other BRD pathogens (Cusack et al., 2003). Fulton et al. (2002a; 2002b) stated that BVDV has been associated with clinical signs and lesions of characteristic of BRD and that the involvement of BVDV in the pathogenesis of BRD has been demonstrated through experimental infections, virus isolation, the identification of BVDV antigen in lesions and other respiratory tract samples from calves, and the

demonstration of active infection through seroconversions in groups of cattle with BRD. This evidence would suggest a primary role for BVDV in the pathogenesis of BRD despite debate within the published research.

The immunosuppressive effect demonstrated through acute BVDV infection may be facilitated by an initial hyperplasia of the germinal centers of lymphoid tissues followed by subsequent lymphoid depletion (Cusack et al., 2003). In addition, BVDV has been shown to impair the production of humoral antibodies, reduce monocyte chemotaxis, and weaken the myeloperoxidase antibacterial system in polymorphonuclear leukocytes (Cusack et al., 2003). Through these mechanisms there is a potential for increased colonization of the lungs by other pathogens causing increased aggravation to pulmonary tissues. The cytopathic effects of BVDV in the respiratory tract cause acute catarrhal inflammation within the nasal cavity and trachea, and have been shown to cause focal intralobular interstitial pneumonia (Cusack et al., 2003). The evidence suggests that BVDV may increase BRD incidence by means of immunosuppression and should be considered a primary respiratory pathogen (Fulton et al. 2002b; Cusack et al., 2003).

Parainfluenza virus type 3 (PI3) has been associated with both acute and chronic pneumonia in cattle and an infection with PI3 is often simultaneous with a BHV1 or BVDV infection. (Hodgins et al., 2002). The replication of PI3 occurs within epithelial cells of both the upper and lower respiratory tract (Cusack et al., 2003). However, despite replication throughout, the damage to respiratory tissues primarily occurs in the lower respiratory tract causing bronchitis, bronchiolitis and alveolitis (Cusack et al., 2003). In the case of acute PI3 viral infections, there is proliferation and necrosis of the bronchiolar epithelium and extensive damage to the cilia and the ciliated cells in small bronchi and

bronchioli (Cusack et al., 2003). The PI3 virus infects alveolar macrophages and thus impairs innate pulmonary defenses (Cusack et al., 2003). The infection of alveolar macrophages by PI3 has been associated with a decreased ability to kill invading bacteria, including inhibition of phagosome-lysosome fusion, and enhanced production of arachidonic acid metabolites which may inhibit other phagocyte functions (Hodgins et al., 2002). Ultimately, cattle can be subjected to a secondary bacterial pneumonia as a result of impaired mucociliary escalator function and depressed cellular immune responses (Cusack et al., 2003).

Bovine respiratory syncytial virus (BRSV) has been recognized as an important viral pathogen in the incidence of BRD and has been demonstrated to act together with various bacterial pathogens to cause pneumonia in cattle (Hodgins et al., 2002). In the U.S., BRSV has commonly been identified in outbreaks of clinical BRD (Cusack et al., 2003). Similar to the other major respiratory viruses, BRSV infection results in destruction of the ciliated respiratory epithelium and the infection of macrophages within the alveoli to reduce local cellular immunity (Cusack et al., 2003). It is presumed that BRSV also negatively impacts the function of neutrophils and lymphocytes (Hodgins et al., 2002). Both ciliated and non-ciliated epithelial cells in the respiratory tract can be effected by BRSV infection, causing necrotizing bronchiolitis and interstitial pneumonia (Hodgins et al., 2002). The prevention of pulmonary clearance through the destruction of the ciliated epithelium causes cattle to succumb to secondary bacterial infections (Cusack et al., 2003).

Bovine adenovirus (BAdV) is encountered throughout the world in cattle populations and 10 serotypes of BAdV have been identified (Hodgins et al., 2002). Viral

infections of BAdV in cattle are well documented, but as with BVDV, the role of BAdV in the pathogenesis of clinical BRD remains controversial (Hodgins et al., 2002). The controversy is primarily due to the fact that while adenoviruses have been occasionally isolated from the respiratory tracts of calves with pneumonia, it is much more common to isolate BAdV from clinically healthy cattle (Hodgins et al., 2002). In contrast to what is observed in cattle, adenovirus infection is very common in sheep, and the isolation of adenovirus from diseased animals occurs frequently (Hodgins et al., 2002). Some serologic experiments provide support for a role of BAdV in the incidence of BRD, while other experiments do not (Hodgins et al., 2002). In an experiment examining the effects of BAdV type 1 on alveolar macrophages a decrease in the expression of Fc receptors was observed along with a decrease in the ability to phagocytize and kill *Candida krusei* were noted in vitro (Hodgins et al., 2002). While it has been suggested that BAdV has a role in BRD pathogenesis, there is simply not enough information available in the published literature to support this hypothesis.

Bovine corona virus (BCV) has received increased attention from researchers in recent years as BRD continues to be a persistent problem despite the widespread use of vaccines for BHV-1 (IBR), BVDV, PI3, and BRSV (Fulton et al., 2011). Active BCV infections are routinely identified through viral isolations from nasal swabs and seroconversions in cattle with BRD and in healthy cattle in numerous studies (Fulton et al., 2011). The virus has also been positively identified in pneumonic lungs, often found in combination with other pathogens (Fulton et al., 2011). Previous research has demonstrated the presence or absence BCV antibodies can be used as a predictor of

antimicrobial treatment in the feedlot, and that BCV can be found in the nasal secretions of calves upon arrival to the feedlot (Fulton et al., 2011).

Necropsy findings of cattle infected with BCV include the presence of epithelial lesions in the turbinates, trachea, and lungs, along with occasional interstitial pneumonia (Fulton et al., 2011). Laboratory experiments have suggested multiple mechanisms as to how BCV may cause a decrease resistance against bacterial pathogens (Hodgins et al., 2002). Isolates of BCV from respiratory tracts with acetylesterase activity capable of releasing acetate from the normal glycocalyx lining have been discovered and it is thought that this modification of the glycocalyx could increase the adhesion of MH and PM to host cells in the lower respiratory tract (Hodgins et al., 2002). The M and E structural proteins of BCV have been demonstrated to induce interferon alpha (IFN-α) synthesis by peripheral blood mononuclear cells (Hodgins et al., 2002). Cytokines such as IFN-α have proinflammatory properties and could induce the infiltration of neutrophils into the lung (Hodgins et al., 2002). The role of BCV in the pathogenesis of BRD is not fully understood, and is an area of emerging research. While BCV should not currently be considered to have a significant impact on BRD incidence due to a lack of evidence implicating BCV as a primary BRD pathogen, more research needs to be conducted to increase knowledge of BCV pathogenesis.

Mannheimia haemolytica (MH) (formerly *Pasteurella haemolytica*), is a small gram-negative, nonmotile, facultative anaerobe that exists as a non-spore-forming rod or coccobacillus belonging to the *Pasteurellaceae* family. There are 12 capsular serotypes of MH based on the original *P. haemolytica* serotype assignments of A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16, and A17.5 (Griffin et al., 2010). Commonly, MH exists as

part of the normal bacterial flora within the upper respiratory tract of healthy calves, and can be found in the nasopharynx and tonsillar crypts (Griffin et al., 2010). While MH serotypes A1 and A2 are known to colonize the upper respiratory tract of healthy calves, serotype A2 is the predominate serotype isolated from clinically normal appearing cattle (Griffin et al., 2010). It has been suggested that MH exists in a commensal relationship with the host animal until conditions within the animal change resulting from a stressor or coinfection (Hodgins and Shewen, 2004; Griffin et al., 2010). However, once this initial commensal relationship is disrupted, MH serotype A1 quickly becomes the predominate organism isolated (Hodgins and Shewen, 2004; Griffin et al., 2010). With great repeatability, the most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD is MH serotype A1, and this serotype has been shown to be responsible for characteristic BRD infection in calves (Whitley et al., 1992; Hodgins and Shewen, 2004; Booker et al., 2008; Griffin et al., 2010).

Once MH has been established within the lung, the resulting tissue damage is a result of multiple virulence factors associated with MH pathogenesis. These virulence factors include: a capsule used for adherence and invasion, outer membrane proteins that induce immune responses, adhesions used for further colonization, neuraminidase that decreases the viscosity of mucus, a lipopolysaccharide (LPS) complex that causes hemorrhaging, edema, hypoxemia, and inflammation, a leukotoxin (LKT) responsible for lysis of leukocytes and platelets, and a quorum-sensing system that is believed to regulate expression of these virulence factors (Hodgins et al., 2002; Griffin et al., 2010). The extracellular fractions of MH can kill bovine neutrophils (Cusack et al., 2003). The presence of lipopolysaccharide endotoxin in the outer membranes is involved in lung

damage. The endotoxin demonstrated a multitude of toxic effects including the initiation of complement and coagulation cascades (Cusack et al., 2003). These cascades result in increased vascular permeability and coagulation which results in the accumulation of inflammatory cells, edema and both fibrin deposition within the lung (Cusack et al., 2003). The endotoxin serves to activate granulocytes and macrophages that help protect against the invading bacteria, but that also lead to increased tissue damage and inflammation (Cusack et al., 2003).

M. haemolytica also produces a specific LKT that is active against phagocytes and impairs phagocytosis and kills macrophages (Cusack et al., 2003). The LKT of MH is a member of the RTX toxins family and is a pore-forming, calcium-dependent cytolysin with specificity for leukocytes and platelets (Hodgins et al., 2002). Low concentrations of this LKT serve to activate neutrophils and induce the apoptosis of leukocytes (Hodgins et al., 2002). Apoptosis of leukocytes would effectively limit the initial inflammatory response, allowing for MH to further colonize and replicate within the lung (Hodgins et al., 2002). This LKT has demonstrated the ability to suppress the respiratory burst of leukocytes, encourage the degranulation of lysosomes, and inhibit phagocytosis and the killing of invading bacteria (Hodgins et al., 2002). All of these LKT activities would improve the ability of MH to rapidly colonize and establish within the lung.

Similar to MH, *Pasteurella multocida* (PM) is also a small gram-negative, nonmotile, facultative anaerobe that exists as a non-spore-forming coccobacillus belonging to the *Pasteurellaceae* family. There are 5 capsular serotypes (A-F) and 16 somatic serotypes (1-16) for PM (Griffin et al., 2010). Also similar to MH, PM can be

isolated from healthy calves and is presumed to exist in a commensal relationship with the host animal in some form until additional predisposing factors resulting from a stressor or coinfection occur (Hodgins and Shewen, 2004; Griffin et al., 2010). Once this commensal relationship is disrupted then PM can quickly colonize and establish within the lung (Hodgins and Shewen, 2004; Griffin et al., 2010). Serotype A3 is the most commonly isolated PM serotype in confirmed BRD cases (Griffin et al., 2010).

Much less is known about the virulence factors of PM compared to those of MH. However, virulence factors are assumed to be similar between the 2 organisms. As demonstrated with MH, PM can also impact neutrophil defenses within the lung. The capsular fraction of PM has been demonstrated to inhibit neutrophil function (Cusack et al., 2003). In addition, PM attracts phagocytes to affected regions of the lungs and destroys them via the production of toxins (Cusack et al., 2003). As such, most of the damage associated with PM is also due to pulmonary inflammation (Cusack et al., 2003). While PM is similar to MH in both structure and function, the 2 bacteria do not necessarily impact calves in the same manner. It has been demonstrated that PM impacts younger calves, and PM is most commonly isolated from younger animals (Hodgins et al., 2002; Step et al., 2005). In addition, PM is considered to be less virulent than MH (Hodgins et al., 2002; Step et al., 2005). In experimental challenge studies, it has taken higher levels of PM to produce primary pneumonia (Hodgins et al., 2002). Interestingly, microbiologists are reporting PM more frequently in fatal BRD cases from feedlots (Griffin et al., 2010). This is somewhat contradictory to decades of previous research implicating MH as the principle bacterial pathogen associated with fatal BRD (Griffin et al., 2010). One potential reasons for this change in the frequency of isolation of PM

compared to MH could be a result of younger cattle being placed on feed in feedlots (Griffin et al., 2010).

As with the other bacteria associated with BRD previously discussed, *Histophilus somni* (HS) (formerly *Haemophilus somnus*) is another small, commensal, gram-negative, nonmotile, facultative anaerobe that exists as a non-spore-forming coccobacillus belonging to the *Pasteurellaceae* family that resides in the respiratory tract of calves. Similar to MH and PM, HS has a lipooligosaccharide endotoxin in the outer membrane of its cell wall (Cusack et al., 2003). This endotoxin causes lesions like those produced by the lipopolysaccharide of MH and PM, but also results in vasculitis and necrosis (Cusack et al., 2003). The endotoxin produced by HS can also cause damage to endothelial cells, alveolar macrophages, and neutrophils (Cusack et al., 2003). In addition to similiarities with MH and PM, HS also produces a histamine and an exopolysaccharide that play an important role in the pathogenesis (Griffin et al., 2010). In most cases, BRD attributed to HS is considered to be more subacute or chronic than that caused by infection by either MH or PM (Hodgins et al., 2002). Clinically, BRD caused by HS is not distinguishable from that caused by the other BRD bacterial pathogens discussed (Griffin et al., 2010).

Mycoplasma species comprise some of the simplest self-replicating life forms and include over 100 different species. Most *Mycoplasma* species are facultative anaerobes and have unique membranes rather than traditional cell walls found in other bacterial BRD pathogens. Multiple *Mycoplasma* species including *Mycoplasma bovis* (MB) have been associated with BRD in feedlot cattle, and the isolation of multiple species of mycoplasmas from pneumonic lungs has been noted in some experiments (Hodgins et al., 2002). *Mycoplasma* species can alter phagocytic function by preventing the activation of

macrophages by other stimuli (Hodgins et al., 2002). In in vitro studies, the killing of *Escherichia coli* by bovine neutrophils has been inhibited by the products of *Mycoplasma* species (Hodgins et al., 2002). Certain species of mycoplasma demonstrate mitogenic activity for lymphocytes, and peptides of some mycoplasma species have been shown to impact T cells (Hodgins et al., 2002). *Mycoplasma* species are not greatly understood and limited information exists about them in the scientific literature.

Of all the *Mycoplasma* species, MB is perhaps the most researched and understood. However, the role of MB in disease pathogenesis in young calves is much better understood than it is in stocker or feedlot cattle (Griffin et al., 2010). The exact role of MB in BRD pathogenesis is heavily debated as the data defining the relationship of MB to BRD incidence is not as conclusive as it is for other bacterial BRD pathogens (Griffin et al., 2010). However, MB is more widely accepted as a culprit of chronic bronchopneumonia with caseous and possibly coagulative necrosis and is often characterized by a persistent infection that seems unresponsive to many antibiotics (Caswell and Archambault, 2007). Through serology, the movement of MB through herds has been traced, and when naïve cattle are exposed to cattle infected with MB, The pathogen can be found in the naïve cattle within a day (Griffin et al., 2010). Once MB has become established within the respiratory tract, it may persist for the life of the animal in a commensal relationship.

The virulence factors related to MB are associated with 5 variable surface lipoproteins (VSP): VSP-A, VSP-B, VSP-C, VSP-F, and VSP-O (Caswell and Archambault, 2007; Griffin et al., 2010). These VSP have various roles in the development of disease including: evasion of host animal's antibody response,

colonization of mucosal surfaces, adhesion to cells, and suppression of lymphocyte blastogenesis and cytokine secretion (Caswell and Archambault, 2007). In addition, other surface proteins aside from VSP have been shown to facilitate the adhesion of MB to host cells (Caswell and Archambault, 2007). Other virulence factors exhibited by MB include: the production of a polysaccharide toxin and other polysaccharides, the formation of hydrogen peroxide, heat shock protein responses, and the formation of biofilm (Caswell and Archambault, 2007).

A synergistic effect has been observed involving MB and MH with a more severe disease response occurring when MB infected calves are challenged with MH compared to when calves not infected with MB are challenged with MH (Griffin et al., 2010). The presence of lung lesions associated with MB infection range from mild lesions consolidated to cranioventral areas to chronic caseonecrotic bronchopneumonia and 4 main patterns of lung lesions have been observed: caseonecrotic bronchopneumonia, bronchopneumonia with foci of coagulation necrosis, suppurative bronchopneumonia without necrosis, and chronic bronchopneumonia with abscessation (Caswell and Archambault, 2007; Griffin et al., 2010). Some clinical signs that may suggest the presence of a MB infection are: lameness, chronic sickness, and the failure to respond to antimicrobial treatment, but these signs are not specific to only MB cases (Caswell and Archambault, 2007). The clinical signs of MB infected calves with BRD are usually not distinguishable from other BRD causes (Caswell and Archambault, 2007).

While a multitude of risk factors can potentially increase the risk of BRD, the development of clinical BRD is ultimately a result of infection by viral and bacterial pathogens. Frequently pathogenesis occurs through a primary infection by one or

multiple respiratory viruses. The principal infection combined with the calf's previously compromised immune system allows for the rapid colonization and replication within lung tissues by commensal bacteria. This bacterial infection causes inflammation and severe immune responses to occur within the lung tissue leading to the clinical signs associated with BRD.

Management of newly received calves with a high risk of BRD incidence

Given the complex nature of BRD and the multitude of risk factors, viruses, and bacteria involved in BRD pathogenesis, special management considerations should be provided to calves at high risk of developing BRD. The risk factors or stressors that cause calves to be considered at a high risk of developing BRD can occur any time after conception in the calf's life, and while some of these risk factors can be effectively managed at early stages of production, others simply are unavoidable and inherently associated with the cattle marketing system in the U.S. In the Southern Plains and Southeast U.S. it cannot be assumed that calves marketed through typical auction markets have even been weaned prior to being marketed. Through marketing channels calves are commingled with many other animals of unknown disease and vaccination statuses. After being sold, calves are loaded on trucks and frequently transported long distances where they are potentially exposed to exhaust fumes, extreme weather, and temperature fluctuations. While variation in classification of risk levels exists within the industry, upon arrival to the feedlot, calves are generally placed into 1 of 2 risk categories: high-risk or low-risk (Edwards, 1996).

High-risk calves characteristically are younger lighter-weight calves, have not been weaned prior to being marketed, have been assembled from multiple lots of cattle at multiple livestock markets, have potentially been transported long distances, have unknown disease and vaccination histories, may not have been dehorned or castrated, and are highly stressed upon arrival to the feedlot. High-risk calves are typically suffering from dehydration, malnourishment, and exhaustion at the time of arrival (Griffin et al., 2010). Low-risk calves customarily are older or heavier-weight calves, have been weaned prior to marketing and possibly been enrolled into a recognized preconditioning program, come from one or very few sources, arrive with some vaccination or health history, and appear to be less stressed upon arrival to the feedlot. Sanderson et al. (2008) reviewed risk factors associated with BRD in U.S. feedlots and found that cattle arriving in mixed-gender groups, cattle arriving from multiple sources, and cattle transported longer distances were associated with an increased risk for developing initial BRD, while cattle entering the feedlot at heavier body weights were associated with decreased risk of initial BRD. Wilson et al. (2012) observed that calves that were castrated after arrival to the feedlot had lower ADG and higher first treatment rates for BRD than calves that arrived as steers and that calves that were dehorned after arrival to the feedlot had lower ADG than calves that were polled.

Given the array of factors that can dictate the risk level of incoming calves arriving at a feedlot, calves are classified into high-risk or low-risk categories simply based on if they were preconditioned or not. This allows for a simpler classification of risk levels and eliminates unnecessary confusion as to what constitutes high-risk or low-risk. Regardless of how preconditioning is defined, the preconditioning of calves has

proven to reduce the incidence of BRD in calves as they enter the feedlot (Thrift and Thrift, 2011). Multiple studies have demonstrated various benefits of preconditioning (NAHMS, 2000; Roeber et al., 2001; Step et al., 2008; Thrift and Thrift, 2011; Richeson et al., 2012).

The vaccination of clinically healthy, unstressed cattle has shown to be effective in reducing BRD and achieving an optimal vaccine response dependent on providing an efficacious vaccine to an immunocompetent calf (Edwards et al., 2010). As a result of the proven efficacy of vaccines in healthy cattle, the majority of cattle are vaccinated shortly after arrival to the feedlot to help manage BRD pathogens (NAHMS, 2013). Occasionally low-risk calves that arrive at the feedlot with a known vaccination history are not re-vaccinated. As a percentage of all feedlots, 96.6% vaccinated for BVDV, and 93.7% vaccinated for IBR, while 89.1% vaccinated for BRSV, and 85.1% vaccinated for PI3 (NAHMS, 2013). NAHMS (2013) also stated approximately 2 out of 3 feedlots vaccinated for the most common bacterial agents associated with respiratory disease: HS, MH, and PM.

While the vaccination for respiratory viruses, and to some extent bacterial pathogens upon arrival to the feedlot is widespread, there is little scientific evidence to support this management practice (Edwards et al., 2010; Taylor et al., 2010b). Some epidemiologic studies have suggested that the vaccination for respiratory viruses actually leads to increased BRD incidence (Taylor et al., 2010b). It could be interpreted that this data is somewhat confounded, as high-risk calves are more likely to be vaccinated for respiratory viruses. Regardless, there is little justification in the literature for the vaccination of high-risk calves at arrival.

A probable reason for the lack of efficacy of respiratory vaccines in high-risk calves is due to the timing of vaccine administration. Calves under a great deal of stress are unlikely to mount an optimal immune response to the vaccine. Few studies have evaluated the effects of delayed vaccination of high-risk calves, and the results of those studies have been inconsistent. Initial research by Richeson et al. (2008) indicated that delaying vaccination by 14 days may result in a possible improvement in immune response. Conversely in a second experiment, Richeson et al. (2009) determined that timing of vaccination did not affect performance or health in high-risk newly received calves. In addition to vaccine timing, the complicated nature of BRD pathogens and the increased susceptibility of the high-risk calves to these pathogens may also negatively influence vaccine efficacy (Taylor et al., 2010b).

The involvement of multiple bacterial pathogens in combination with the increased risk of BRD incidence observed in high-risk calves may justify the mass antimicrobial treatment of high-risk populations. This prophylactic mass antimicrobial treatment, known as metaphylaxis, has consistently been found to economically and efficiently reduce BRD related morbidity in high-risk calves (Duff and Galyean, 2007; Edwards et al., 2010; Taylor et al., 2010b). Metaphylaxis can be administered via 3 methods: parenterally by subcutaneous, intramuscular, or intravenous injection, through the feed, or in the water (Taylor et al., 2010b). Most commonly, metaphylaxis occurs in the form of an injectable antimicrobial that is administered subcutaneously. While including an antimicrobial in the feed or water in the face of a BRD outbreak could be advantageous, achieving adequate feed or water intake is often a major concern. Calves must have adequate intakes to ensure proper dosage of antimicrobials and the feed and

water intakes of high-risk calves are often depressed for up to a week or more. Currently, the physical injection of antimicrobials is the only way to ensure that calves receive the appropriate dose of antimicrobial.

Metaphylaxis is the instrument most commonly used by feedlot managers and veterinarians to reduce the occurrence of BRD in newly received high-risk feedlot cattle (NAHMS, 2013). As a percentage of all feedlots, 59.3% treated at least some cattle metaphylactically upon arrival. This percentage resulted in approximately 1 in 5 cattle placed on feed (21.3%) being metaphylactically treated (NAHMS, 2013). For calves placed on feed weighing less than 317.5 kg, 92.6% of feedlots with a capacity of 8,000 or more head used metaphylaxis (NAHMS, 2013). This resulted in approximately 2 in 5 calves weighing less than 317.5 kg at arrival (39.2%) receiving metaphylactic treatment for BRD (NAHMS, 2013). The 3 most commonly used antimicrobials used for metaphylaxis were tilmicosin (57.6 percent of feedlots), tulathromycin (45.3 percent), and ceftiofur (39.7 percent) (NAHMS, 2013). Tulathromycin was more commonly used for metaphylactic treatment in cattle weighing less than 317.5 kg at placement compared to cattle weighing more than 317.5 kg at placement (NAHMS, 2013).

Classical metaphylactic work by Lofgreen (1983a) demonstrated that the combination of long-acting oxytetracycline and a sustained-release sulfadimethoxine, decreased BRD-related morbidity from 63.3% in non-medicated control calves to 7.1% in metaphylactically treated calves. Numerous additional studies have demonstrated the effectiveness of tilmicosin, tulathromycin, ceftiofur crystalline free acid, and florfenicol in decreasing BRD related morbidity in newly received, high-risk calves (Duff and Galyean, 2007; Edwards et al., 2010; Taylor et al., 2010b). Interestingly, the

administration of antimicrobials prior to shipping does not appear to be any more effective than administering antimicrobials upon or subsequent to arrival (Edwards et al., 2010; Taylor et al., 2010b). The exact preventative mode of action of metaphylaxis is still unknown, but MH has been shown to be greatly affected by metaphylactic administration programs (Duff and Galyean, 2007). Studies by Frank and Duff (2000) and Frank et al. (2002) reported that both tilmicosin and florfenicol inhibited the colonization of MH within the nasopharynx of cattle (Duff and Galyean, 2007).

The nutritional management of high-risk calves subsequent to feedlot arrival has far-reaching implications on the function of the immune system including: development of lymph tissues, production of mucus, synthesis of immunologically active cells such as leukocytes, antibodies, and cytokines, cell proliferation, cellular signaling, activation, and movement, intercellular killing, and general immune system modulation (Cole, 1996). A major concern with the nutritional management of high-risk calves is that the nutritional background or nutrient status of calves arriving at the feedlot is often unknown and impractical to obtain (Duff and Galyean, 2007). However, it is known that high-risk calves typically suffer from dehydration, malnourishment, and exhaustion upon arrival to the feedlot (Griffin et al., 2010). When reviewing the literature, a great deal of variation in responses to nutritional supplementation is observed in experiments involving high-risk calves. Duff and Galyean (2007) suggest that this variation is partially due to variations in the nutritional status of calves at arrival.

Another major problem in the nutritional management of high-risk calves is simply achieving adequate dry matter intake (DMI) to meet calves' nutrient requirements. High-risk calves have altered eating patterns when compared with unstressed cohorts

(Galyean et al., 1999). The DMI of high-risk calves is extremely variable, and many calves do not achieve adequate DMI for the first couple of weeks on feed. Hutcheson and Cole (1986) stated that DMI for newly received calves ranged from 0.5% to 1.5% of body weight and that the majority of morbid calves do not consume any feed for the first 2 days in the feedlot. Approximately 4 out 5 (83.4%) morbid calves were consuming feed by the end of the first week in the feedlot (Hutcheson and Cole, 1986). The irregular intake of high-risk calves makes the formulation of receiving diets incredibly challenging.

The first inclination when formulating receiving diets would likely be to increase the nutrient density of the diet to compensate for the reduced DMI of high-risk calves. Multiple researchers have demonstrated that the voluntary DMI of lower energy diets containing higher levels of roughage is less than that for higher energy diets in newly received high-risk calves (Galyean et al., 1999). When newly received stressed calves were given a choice among feed mixes varying in concentrate levels, the calves selected diets that contained 72% concentrate during the first week in the feedlot (Lofgreen, 1983b). As a result of altered DMI patterns and the increased energy density of higher concentrate diets, the performance by newly received high-risk calves is typically greater with higher-concentrate (60% concentrate or greater) receiving rations (Galyean et al., 1999).

While increased energy density receiving diets have been shown to improve the performance of high-risk calves, these diets are not without negative aspects. One such negative aspect of feeding high concentrate receiving rations is an increase in BRD morbidity and an increase in the severity of BRD events. Lofgreen (1983b) reported

morbidity rates of 47%, 49%, and 57%, respectively when steam-flaked milo-based receiving diets with concentrate levels of 25%, 50%, and 75% were fed to lightweight, high-risk cattle. Rivera et al. (2005) reviewed dietary roughage and concentrate levels in multiple studies conducted at the Clayton Livestock Research Center. The diets in this review ranged from all hay diets to 75% concentrate rations (Rivera et al., 2005). Morbidity attributed to BRD decreased slightly as roughage concentration in the diet increased (Rivera et al., 2005).

The economic analysis conducted by Rivera et al. (2005) indicated that the decrease in morbidity observed in calves consuming diets with higher roughage concentrations would not compensate for the production losses due to decreased ADG on these same diets. It is also a possibility that the increased morbidity rates observed in calves consuming higher concentrate receiving diets include calves that are misdiagnosed due to the similarity of clinical signs of BRD and subclinical ruminal acidosis. The adaptation of calves to receiving and finishing diets has been associated with inflammatory and acute-phase responses and clinical behavior resulting from digestive upsets associated with dietary adaptation can mimic BRD morbidity behavior (Ametaj et al., 2009; Tizard, 2008).

Dietary protein concentration has also been shown to impact BRD incidence in newly received calves. Similar to what is observed with increasing energy or concentrate level, increasing crude protein concentrations in receiving diets seems to increase BRD incidence, but simultaneously increase ADG and DMI (Galyean et al., 1999). In a review of 15 studies, the relationship between BRD morbidity and crude protein level in the receiving diet was evaluated (Galyean et al., 1999). The regression analysis revealed that

BRD morbidity rates tended to increase with increasing CP level, but that the performance by calves fed higher crude protein diets was equal to or greater than the performance of calves fed lower crude protein diets (Galyean et al., 1999). The authors suggested that this contradictory response could be attributed to 3 possible scenarios: the inaccurate diagnosis of BRD, the sick calves fed higher crude protein diets had increased performance compared to sick calves fed lower crude protein diets, or the healthy calves consuming higher crude protein diets had increased performance compared to healthy calves fed lower crude protein diets (Galyean et al., 1999). Multiple researchers have suggested that additional research is needed to determine the effects of dietary crude protein level on the health and performance of high-risk calves.

The inclusion of minerals and vitamins and the supplementation of minerals and vitamins in excess of published requirements in receiving diets for high-risk calves to aid in immune function has received much consideration. Multiple TM including cobalt (Co), copper (Cu), manganese (Mn), selenium (SE), and zinc (Zn) can affect immune function, but the effects of TM supplementation on performance and immune function in challenge models and field studies are inconsistent (Duff and Galyean, 2007). Much of the recent research involving TM supplementation has focused on the comparison of organic or inorganic TM supplementation. Antioxidant vitamins including vitamin C and vitamin E have also been shown to influence immune function, but again the results from challenge studies and field experiments in calves are inconsistent at best. Duff and Galyean (2007) suggested that adding vitamin E to receiving diets at pharmacological levels (greater than 1000 IU per day) appeared to decrease BRD morbidity. The supplementation of vitamin

A and B vitamins in receiving diets above requirements has not been justified (Duff and Galyean, 2007).

Another concern with managing high-risk calves would be the increased time and labor required to properly care for them. In feedlots, cattle are "pulled" by trained pen riders when they are deemed to be sick almost entirely based on subjective criteria (Galyean et al., 1999). This subjective evaluation will use some criteria based system such as the DART™ system (Pharmacia Upjohn Animal Health, Kalamazoo, MI). In the DART™ system, the pen riders will assign a calf a numerical severity score based on the clinical signs and the severity of those signs. This system is commonly used with modifications as described by Step et al. (2008). The subjective criteria for pulling calves used in the DART™ system consist of depression, abnormal appetite, and respiratory signs.

Signs of depression can include but are not limited to: depressed attitude, lowered head, glazed or sunken eyes, slow or restricted movement, arched back, difficulty standing or walking, knuckling of joints or dragging toes when walking, and stumbling. Signs of abnormal appetite can include: an animal that is completely off feed, an animal eating less than expected or eating extremely slow, a lack of gut fill or gaunt appearance, and obvious body weight loss. Respiratory signs include: labored breathing, extended head and neck (in an attempt to breathe), and audible noise when breathing. A major problem exists in that all of these clinical signs can be very subtle and are entirely subjective to the observation by the pen rider. A substantial amount of art and skill is required to determine which cattle should be pulled and evaluated for BRD treatment. Skilled and trained pen riders are increasingly difficult to find and it is difficult to train

personnel in these skills. Multiple studies have demonstrated that more animals had lung lesions present at slaughter than were treated for BRD, and commonly calves that were never treated had lung lesions present at slaughter (Wittum et al., 1996; Bryant et al., 1999; Gardner et al., 1999; Thompson et al., 2006). These results would indicate that current methods feedlots employ to diagnose and treat calves for BRD are inadequate at detecting calves potentially requiring treatment for BRD.

In a recent survey of feedlot consulting veterinarians, Terrell et al. (2011) investigated labor recommendations for pen riders and doctors for high-risk calves. When asked the number of times per day they recommend that pen riders check high-risk calves, 17.4% recommended checking them once daily, 71.4% recommend checking them twice daily, and 4.4% recommended checking 3 times a day (Terrell et al., 2011). Terrell et al. (2011) also asked the consultants questions about the recommended number of employees required to adequately handle the pulling and treatment of both high-risk and low-risk calves. (Figure 2-2). Veterinarians recommended that a trained pen rider could tend to 2,739 head of high-risk calves and responses ranged from a minimum of 1,000 head to a maximum of 5,000 head per pen rider (Terrell et al., 2011). The same veterinarians recommended that this trained pen rider tend to 5,591 head of low-risk calves (Terrell et al., 2011). The consultants also recommended that a feedlot doctor could attend to 7,083 head of high-risk calves or 15,972 head of low-risk calves (Terrell et al., 2011). Based on these results, it can be determined that consulting feedlot veterinarians feel that high-risk calves require twice the labor and personnel of low-risk calves for adequate BRD detection and treatment.

High-risk calves typically are believed to be younger, have not been weaned, have been assembled from multiple lots at multiple livestock markets, and appear to be highly stressed upon arrival to the feedlot. As a result, high-risk calves require substantially more labor and management than low-risk calves. The successful management of these calves requires a team effort from everyone involved the feedlot operations, especially the nutritionist, veterinarian, and pen riders. With proper management, high-risk calves can be successfully fed, but without proper management, the loss due to increased morbidity and mortality can be exponentially increased.

Impact of BRD incidence on cattle performance and feedlot economics

The incidence of BRD is the most significant production problem facing the feedlot industry and accounts for the majority of morbidity, mortality, and economic losses that occur in feedlots. In a cross-sectional survey conducted by Woolums et al. (2005), BRD was implicated as the leading cause of morbidity and mortality in 561 feedlots in 21 states. Morbidity attributed to BRD accounts for approximately 75% of total morbidity in feedlot cattle (Edwards, 1996). In addition, mortality attributed to BRD accounts for 36 to 80% of total mortality in feedlot cattle (Vogel and Parrott, 1994; Edwards, 1996; Smith, 1998; Chirase and Greene, 2001).

In the 2011 feedlot survey, NAHMS (2013) stated that nearly all feedlots (96.9%) had at least some cattle that were affected by BRD. Across all cattle, BRD was the most common illness affecting 16.2 percent of cattle placed on feed (NAHMS, 2013). Feedlots in the central region (Colorado, Kansas, Nebraska, Oklahoma, and Texas) had twice as

many cattle affected with BRD compared to feedlots in the rest of the U.S. (NAHMS, 2013). NAHMS (2013) reported that the average cost of treatment for BRD in all feedlots was $23.60. In addition to treatment costs, there are other costs and increased labor involved in caring for calves treated for BRD. The majority of feedlots provided bunk hay (95.5%), extra bunk space (88.5%), extra water tank space (86.8%), wind breaks (73.5%), additional bedding (70.7), and shade (65.0%), to calves treated for BRD (NAHMS, 2013). After initial treatment and labor expenses, additional economic losses occur due to retreatments, mortalities, and chronic BRD cases. For cattle treated for BRD that weighed less than 317.5 kg at placement, 14.9% required an additional antimicrobial treatment, 4.0% died, and an additional 2.3% were realized as chronics (NAHMS, 2013).

Additional economic losses result from the decreased performance of calves treated for BRD. Most published research regarding BRD incidence and animal performance have been conducted retrospectively. In most situations, BRD incidence results in decreased ADG and final BW (Smith, 1998; Gardner et al., 1999; Thompson et al., 2006; Schneider et al., 2009). Over the course of a 28 day receiving period, Smith (1996) reported that ADG was decreased by 0.23 kg, and Bateman et al. (1990) reported that ADG was decreased by 0.14 kg less for calves that became sick. Similiarly in a 90 day feeding experiment, calves suffering from a single episode of BRD had 0.18 kg lower ADG than those calves not treated, and calves treated 2 or more times had 0.33 kg lower ADG (Morck et al., 1993).

Erickson et al. (2011) reviewed commercial data on 978 lots (276,116 head of cattle) from 2 feedlots in Canada to evaluate impact of BRD incidence on animal performance. Lots of cattle were categorized by the percentage of the lot treated for BRD

and then analyzed for linear and quadratic effects as BRD incidence increased (Erickson et al., 2011). As more cattle within a pen got sick, DMI decreased linearly and the decrease in DMI was about 0.41 kg less for cattle with 30% or greater incidence of BRD compared to lots with 5% or less incidence of BRD (Erickson et al., 2011). Similarly, as BRD incidence increased, ADG decreased linearly with about a 0.07 kg decrease in ADG for lots where 30% or more of cattle were treated compared to those lots 5% or less incidence of BRD (Erickson et al., 2011). The authors stated that while the impact of BRD incidence on G:F was significant, the effects were sporadic and no clear impact of BRD incidence on G:F was observed (Erickson et al., 2011).

Although the treatment costs, labor costs, and production losses attributable to the treatment of BRD are substantial, the economic impacts of BRD on carcass merit and meat quality are also significant. McNeill et al. (1996) reported that "healthy" steers had greater ADG and 12% more U.S. Choice carcasses than cattle identified as "sick" at some point during the feeding period. Gardner et al. (1999) showed that the net return for steers without lung lesions was $20.03 more than for steers with lung lesions even with non-active lymph nodes. Treatment costs accounted for 25% of this decrease in net return while the remaining 75% decrease in return was due to decreased carcass value (9.4% more USDA Standard carcasses and 3.9% decrease in carcass weight). In addition, when compared to steers without lung lesions, steers with lesions and active lymph nodes had $73.78 less net return of which 21% was due to treatment costs and the remaining 79% was due to lower carcass weight (8.4% less) and decreased quality grade (24.7% more USDA Standard carcasses).

As BRD treatments increase, carcass values and net returns per animal can exponentially decrease. Brooks et al. (2011) examined the economic impact of the number of BRD treatments received by heifers. Heifers treated once for BRD actually returned $10.12 per head more than those heifers never treated (Brooks et al., 2011). However heifers treated twice, 3 times, or deemed chronics returned $11.08, $72.01, and $143.28 less per head than those heifers never treated (Brooks et al., 2011). Schneider et al. (2009) found similar results upon reviewing the records from 5,976 animals fed in Midwestern feedlots to examine the economic impact of increasing BRD treatments. Schneider et al. (2009) observed that decreases in animal performance and carcass merit were associated with a decreases of $23.23, $30.15, and $54.01 in carcass value for cattle treated for BRD once, twice, or 3 or more times respectively when compared to cattle never treated for BRD.

Chirase and Greene (2001) estimated the annual economic losses due to morbidity, decreased feed efficiency, and treatment costs associated with BRD to be $800-900 million. This is the estimate often cited in the introduction of BRD articles, but it was published almost 15 years ago. Other recent estimates indicate that the economic impact to be in excess of $2 billion (Powell, 2013). The problems with these estimates is that they do not include losses of carcass value or the amount of money spent by the feedlot industry on BRD prevention. When considering the total costs to the feedlot industry including losses in carcass value and what is spent on the prevention of BRD, the true cost of BRD may be well in excess of $5 billion.

USE OF ANCILLARY THERAPY IN CALVES TREATED FOR BRD

While the scientific community has gained considerable knowledge about BRD pathogenesis and many advancements have been made in vaccine and antimicrobial production in recent years, the prevalence of BRD in feedlot cattle has not significantly been reduced. In fact, published data would indicate that no significant reduction in the prevalence of BRD has occurred over the last 30 to 40 years (Gifford et al., 2012). It could be argued that these data suggest current BRD prevention and treatment strategies are ineffective in reducing and controlling BRD, despite the wealth of BRD research and measurable improvements in commercially available vaccines and antimicrobials (Babcock et al., 2006). As such, other therapies and treatments have been investigated and employed by the feedlot industry in an attempt to improve the clinical response and general outcomes in calves treated for BRD.

It has become increasingly common to provide an additional form of treatment, or ANC, along with an antimicrobial when treating for suspected BRD. In this case the primary goal of ANC is to improve the calf's response to a BRD challenge, not to replace antimicrobial treatment. Ultimately, the aim of ANC is to typically focus on the calf or the calf's clinical signs rather than the invading pathogens which would be the case with antimicrobials. Vaccines would be one of the few exceptions, in that they are meant to provide pathogen specific antibody responses. The improvement in the calf's response to BRD can be accomplished through a variety of mechanisms. These mechanisms can be divided into 3 broad classes: relieving the harmful effects of inflammation, blocking histamine activity, or boosting immune system function to aid in the defense of infectious pathogens (Apley, 1994). When considering these mechanisms, multiple ANCs can fall

into each class. Anti-inflammatory drugs commonly used for ANC in calves would include corticosteroids and NSAID. Those ANCs with histamine blocking activity would be commonly referred to as antihistamines. Most of the remaining ANCs would fall into the immuno-modulating category, which would include vitamins, minerals, DFM, and vaccines.

Most of the information available concerning ANC use in feedlots comes from extensive surveys of veterinarians or feedlot operators. From the survey data, the same list of the most commonly used ANCs occurs with great repeatability, while the frequency of use for a specific ANC varies some over time and among the different surveys. Across all surveys the most commonly used forms of ANC include: antihistamines, B vitamins, corticosteroids, DFM, NSAID, viral vaccines, and vitamin C (NAHMS, 2001; Terrell et al., 2011; NAHMS, 2013). While these surveys provide evidence as to the scope and frequency of ANC use in feedlots, they provide no information on the modes of action of ANCs or evidence as to the efficacy of ANC use. Very few reviews of ANC use in cattle treated for BRD have been conducted, and there is very limited published research concerning the efficacy of ANC use. The results that do appear in the published literature concerning ANCs and BRD are highly inconsistent.

Justification for ANC use: Proposed modes of action and immune system modulation

While it has become increasingly common to provide ANC along with an antimicrobial when treating calves for suspected BRD, the justification of this practice is not necessarily supported by the current literature. Ultimately the use of ANC is a

function of a real or perceived improvement to a BRD challenge in calves. These mechanisms for this improvement in response can be divided into 3 broad classes: relieving inflammation, blocking histamine activity, or boosting immune system function (Apley, 1994). Multiple ANCs can make up each class, with the majority of ANCs falling into the immunomodulating category.

The majority of anti-inflammatory drugs target arachidonic acid-derived eicosanoids and inhibit their synthesis (Apley, 1994). Eicosanoids include leukotrienes, thromboxane A2, and prostaglandins and can cause a wide variety of physiological changes within the body including: bronchodilation and broncho-constriction, changes in renal blood flow, inhibition of B-cell differentiation, and the proliferation of T-cells (Apley, 1994). Trauma, toxins, or chemical insult can activate phospholipase A2, which causes the release of arachidonic acid from cell membranes. The arachidonic acid is then synthesized into eicosanoids by lipoxygenase or cyclooxygenase (Apley, 1994). Anti-inflammatory drugs frequently used for ANC in calves would include corticosteroids and NSAID.

Corticosteroids prevent the synthesis of eicosanoids through membrane stabilization and by synthesizing lipocortin, which inhibits phospholipase A2 (Apley, 1994). In addition to relieving the effects of inflammation, corticosteroids can also have adverse effects on the immune system. Corticosteroids inhibit interleukin-1 (IL-1) and interleukin-2 (IL-2) production, prevent macrophage migration, interfere with interferon gamma (IFN-γ) function, and inhibit neutrophil activity (Apley, 1994). While corticosteroids can successfully relieve the effects of inflammation, it would seem that

the immunosuppressive effects of corticosteroids could potentially outweigh any potential benefits of the reduced inflammation.

While NSAID also prevent the synthesis of eicosanoids, they accomplish this inhibition through different mechanisms. In the case of NSAID the synthesis is prevented through the inhibition cyclooxygenase (Apley, 1994). It has also been demonstrated that NSAID relieve inflammation through both central and peripheral mechanisms (Apley, 1994). Two of the commonly used NSAID in cattle are acetylsalicylic acid and flunixin meglumine. The NSAID flunixin meglumine has been shown to reduce rectal temperatures and decrease lung consolidation in multiple studies (Apley, 1994; Francoz et al., 2012). While it could be argued that the benefits of NSAID may be important from an animal welfare perspective, it is difficult to determine the economic benefits for the use of NSAID based on inconsistent results in the performance and clinical health of calves given NSAID (Francoz et al., 2012).

Antihistamines work by reducing histamine, which is a chemical released from mast cells in response to a stimulation by antigens of cellular insult (Apley, 1994). Histamine causes bronchoconstriction, vasodilation, and increased capillary permeability through the activation of H1 and potentially H2 receptors (Apley, 1994). Antihistamines reduce histamine production through the blocking of the H1 receptors through competitive antagonism (Apley, 1994). There has been very little research conducted to evaluate the effects of antihistamines as ANC for BRD. Antihistamines have not been demonstrated as effective in treating asthma as it is believed that the bronchoconstriction is caused by leukotrienes rather than histamine (Apley, 1994). If this is the case in calves with BRD as well, antihistamines would likely have no effect in relieving

bronchoconstriction. When critically evaluating the use of antihistamines as an ANC, the short duration of efficacy (6 to 12 hours), rapid elimination from the body, and lack of published research concerning antihistamines should be considered.

The final class of ANCs are those in the immuno-modulating category which attempt to boost immune system function to aid in the defense of infectious pathogens. This class would include DFM, TM, vaccines, and vitamins. The modes of action for the ANCs in this class are extremely varied and mostly unproven. There has been very little research conducted on immuno-modulators and the work that has been published is inconsistent. However, there are multiple ANCs in this class that are used with great frequency in commercial feedlots.

The use of DFM has been well received by feedlot operators as DFM are simply a source of live, naturally occurring microorganisms (Yoon and Stern, 1995). The concept of feeding a DFM to cattle is based on the presumption of potential benefits on gastrointestinal health which would include the establishment of more desirable microflora and prevention of the establishment of pathogenic organisms via competitive exclusion (Krehbiel et al., 2003). Potential beneficial responses observed when feeding DFM to cattle include: increased ADG and improved G:F in feedlot cattle, improved health and increased immunity in young calves, decreased potential for ruminal acidosis, increased propionate concentrations within the rumen, and altered rumen microflora populations (Krehbiel et al., 2003; Guillen, 2009). While there is a considerable amount of evidence demonstrating the effectiveness of DFM on cattle performance, the results have been somewhat inconsistent (Krehbiel et al., 2003). In addition, there is very limited

research on the effects of DFM on cattle health in general and even less on the use of DFM as an ANC.

Another group of the immuno-modulators that has received consideration are TM. This is due the proposed impacts of TM on immune function. The supplementation of TM has been demonstrated to alter immune function measurements and reduced morbidity associated with BRD in some cases (Galyean et al., 1999). The supplementation with TM including: Cu, Mn, and Zn has become a normal management practice within the feedlot industry to promote maximum performance and OPTIMAL immune function, and these TM are frequently included at levels in excess of published requirements in feedlot diets (Vasconcelos and Galyean, 2007). However, the published research concerning TM supplementation at levels greater than published requirements or as an ANC is lacking and has been extremely inconsistent.

A third group of the immuno-modulators would be vaccines. Vaccines attempt to increase immunity to pathogens by increasing antibody titers within the body and initiating a similar immunological response to that of natural exposure to an antigen. Vaccines can be thought of as tools to prime the immune system prior to insult with a viral pathogen by eliciting both specific and non-specific effects on cellular and humoral immunity. Most previous vaccine research in cattle has focused on comparing different multivalent modified live vaccines and many published vaccine studies do not include a negative control. As a result, it is extremely difficult to determine if vaccination at arrival truly aids in prevention of BRD or if it actually may be detrimental to calves as some studies suggest. Data examining the use of vaccines as an ANC for BRD are not available at the current time.

The final group of immuno-modulating ANCs would include vitamins. Water soluble B vitamins and vitamin C have been shown to be critical to immune system function. Specifically the pyridoxine (B6), folic acid (B9), pantothenic acid (B5), and ascorbate (vitamin C) have been shown to have roles in immune function, and the requirements of these water soluble vitamins are increased substantially by stress (Dubeski et al., 1996). Pantothenic acid and ascorbate have been shown to be required for glucocorticoid synthesis in nonruminants and pyridoxine is an important regulator of glucocorticoid function (Dubeski et al., 1996). In addition the supplementation of ascorbate and pyridoxine has demonstrated improvement in cortisol-induced immunosuppression human subjects and laboratory animals (Dubeski et al., 1996).

Ascorbate occurS in higher concentrations in leukocytes than other tissues and these levels are decreased by viral infections or stress (Apley, 1994). In addition, interferon production, lymphocyte proliferation, and antigen clearance are all effected by ascorbate concentrations within the body (Apley, 1994). In a clinical experiment evaluating the effects of ascorbate on neutrophil function in steers, supplemental vitamin C resulted in increased neutrophil oxidative metabolism and tended to increase the ability of neutrophils to phagocytize *S. aureus* (Apley, 1994). As observed with the other immuno-modulating ANCs, the published research supporting the use of vitamins as an ANC for BRD is lacking and has been inconsistent.

Some of the most comprehensive reviews of ANC for BRD have been conducted by Dr. Mike Apley. In his most recent review presented at the CVC in Kansas City in 2010, Dr. Apley chose to focus on the published data concerning the use of steroidal anti-inflammatory drugs and NSAID as an ANC for BRD. In this review, the author cites

multiple studies that demonstrate that NSAID have some beneficial effect as an ANC with the most typical response being a reduction in rectal temperature in calves treated with an NSAID (Apley, 2010). Other clinical responses to NSAID were inconsistent (Apley, 2010). Apley (2010) concluded that there was no published data at the current time that supported the use of antihistamines, B vitamins, DFM, vaccines, or vitamin C as ANCs for BRD.

Prevalence of ANC use in feedlots

There has not been justification in the published research for the use of many if not all ANCs in the treatment of BRD, and the published research that supports the use of ANC is lacking and produced inconsistent results. However, it is still very common to provide ANC, along with an antimicrobial when treating calves for suspected BRD in feedlots. The best evidence of the prevalence of ANC use in feedlots is found in large-scale surveys of consulting veterinarians and feedlot operators. There are 3 large-scale surveys that have been conducted since 1999 that provided substantial evidence as to the scope of ANC use in commercial feedlots.

In 1999, USDA's NAHMS surveyed feedlots in the top 12 cattle feeding states. The feedlots represented 84.9% of all U.S. feedlots at the time and controlled 96.1% of U.S. cattle on feed on January 1, 2000, in feedlots with a 1,000-head-or-more capacity (NAHMS, 2001). The survey noted that only 12.8% of these feedlots used a single antimicrobial for the treatment of BRD, and that the use of 2, 3, or 5 products in combination for BRD treatment was more common than using a single antimicrobial

(NAHMS, 2001). The survey found that the most frequent forms of ANC used in 1,000 head or greater capacity feedlots were: respiratory vaccines, NSAID, antihistamines, B vitamins, a single dose DFM, electrolytes and fluids, corticosteroids, and vitamin C, respectively (NAHMS, 2001).

More recently, Terrell et al. (2011) conducted a survey of 23 feedlot consulting veterinarians that represented approximately 34% of all cattle on feed in the U.S. The authors reported that 48% of veterinarians recommended some form of ANC for the treatment of BRD (Terrell et al., 2011). The most common ANCs recommended by the consulting veterinarians were: vitamin C, NSAID, antihistamines, a single dose DFM, B vitamins, viral vaccines, and corticosteroids, respectively (Terrell et al., 2011). When considering the prevalence of individual ANCs, vitamin C was recommended twice as often as any of the ANCs (Terrell et al., 2011).

Also in 2011, USDA's NAHMS again surveyed feedlots with a capacity of 1,000 or more head in the top 12 cattle feeding states and found that 55.9% of feedlots used NSAID and 39.3% used a respiratory vaccine as a component of the initial BRD treatment program for some cattle (NAHMS, 2013). The survey found that the most frequent forms of ANC used in 1,000 head or greater capacity feedlots were: NSAID, respiratory vaccines, corticosteroids, a single dose DFM, B vitamins, antihistamines, electrolytes and fluids, and vitamin C, respectively (NAHMS, 2013). When looking at the percentage of cattle receiving specific ANCs as a component of initial BRD treatment, 48.5% of cattle received a respiratory vaccine and 34.1% received vitamin C (NAHMS, 2013). The most frequent forms of ANC given on a percentage of cattle basis were:

respiratory vaccines, vitamin C, NSAID, corticosteroids, electrolytes and fluids, a single dose DFM, B vitamins, and antihistamines, respectively (NAHMS, 2013).

Regardless of the survey, the most commonly used forms of ANC have not changed greatly in recent years. However, the prevalence of use of individual ANCs does vary from survey to survey over time. In addition, differences in ANC use are observed if the use of ANC is estimated by evaluating the percent of feedlots or consultants using or recommending a specific ANC or by evaluating the total percent of cattle receiving a respective ANC. Irrespective of the survey, the most commonly used forms of ANC included: antihistamines, B vitamins, corticosteroids, DFM, electrolytes and fluids, NSAID, viral vaccines, and vitamin C, in no particular order (NAHMS, 2001; Terrell et al., 2011; NAHMS, 2013). These surveys provide a great deal of evidence as to the scope of ANC use feedlots, but no evidence as to the efficacy of these ANCs.

Effects of ANC use on the immune status and clinical health of calves

While the use of ANC may not be justified by results within the literature, providing ANC along with an antimicrobial when treating calves for BRD is still incredibly popular with veterinarians and feedlot managers. NAHMS (2013) cited respiratory vaccines, vitamin C, NSAID as the 3 most frequent forms of ANC given to cattle treated for BRD on a percentage of cattle basis. When reviewing the published research concerning the use of these 3 ANCs in calves treated for BRD, it becomes obvious that this area of research is deficient and produced very inconsistent results at present. There is limited published research concerning the effectiveness of various forms

of ANC and no published research comparing multiple commonly administered ANCs within a single experiment or across similar groups of calves.

The vaccination of healthy calves for respiratory viral pathogens is important for preventing BRD and maintaining optimal calf health. However, there is little justification for the vaccination of high-risk calves at arrival to the feedlot even though it is a widespread and accepted management practice (Edwards et al., 2010; Taylor et al., 2010b). Some epidemiologic studies have actually suggested that the vaccinating of calves upon arrival to the feedlot for respiratory viruses actually leads to increased BRD incidence (Taylor et al., 2010b). In addition, most published vaccine research has focused on comparing different multivalent vaccines and the majority of vaccine studies do not include a non-vaccinated negative control. As a result, it is extremely difficult to determine if vaccination at arrival aids in the prevention of BRD or if it actually may be a detrimental to calves. Currently, to the author's knowledge, there are no published studies examining the use of vaccines as an ANC for BRD. This is extremely surprising given that NAHMS (2013) reported that a respiratory vaccine was used as a component of the initial BRD treatment program in 39.3% of feedlots, and that 48.5% of cattle received a respiratory vaccine as part of initial BRD treatment.

Cusack et al. (2005) examined the physiological effects and performance of feeding additional vitamin E and ruminally-protected vitamin C in cattle challenged with BHV1. Angus and Angus crossbred heifer calves were randomly allocated 4 experimental diets in a 2x2 factorial arrangement (Cusack et al., 2005). The diets fed by Cusack et al. (2005) provided either 15 or 185 IU of vitamin E per kg of DM, with or without 3.7 g of ruminally-protected vitamin C per kg of DM. The feeding of ruminally-

protected vitamin C did not result in greater mean plasma ascorbate concentrations, but did result in lower mean total superoxide dismutase concentration in the blood. The feeding ruminally-protected vitamin C did not significantly impact DMI, ADG, or G:F (Cusack et al., 2005). There were interactions between vitamin E and vitamin C leading the authors to conclude that vitamin C may exert physiological effects without increasing plasma concentrations of ascorbate (Cusack et al., 2005). Cusack et al. (2005) also stated that the feeding of ruminally-protected vitamin C might have caused more significant effects on the performance and physiological effects of challenged calves had virus challenge been more severe.

In another experiment, Cusack et al. (2005) examined the effects of injectable vitamin C given at the time of BRD treatment on subsequent cattle health. At the time of BRD treatment, 176 head cattle were alternately administered injectable vitamin C (5 g per head) or not injected (Cusack et al., 2005). Fewer of the cattle injected with vitamin C at the time of BRD treatment died later in the experiment compared to those cattle that were not injected (11% vs. 23%, respectively) (Cusack et al., 2005). This finding led the authors to conclude that mortality rate in cattle with BRD may be reduced by administering injectable vitamin C at the time of antimicrobial administration.

Urban-Chmiel et al. (2011) evaluated the effects of vitamin E and vitamin C on the development of inflammation processes and selected defense mechanisms against MH-induced infections. Simmental calves were assigned to 3 treatments and received subcutaneous injections of vitamin E (750 IU), vitamin C (2.5 g per head), or no vitamin injection (Urban-Chmiel et al., 2011). Calves receiving either of the vitamin injections demonstrated a difference in the sensitivity of leukocytes to the cytotoxic effect of LKT

when compared to the control group (Urban-Chmiel et al., 2011). There were no differences observed in the percentage of cells sensitive to LKT between the calves receiving vitamin E and those receiving vitamin C (Urban-Chmiel et al., 2011). The authors concluded that both vitamin E and vitamin C exerted a protective effect on leukocytes aiding in the defense against MH virulence factors when administered by injection (Urban-Chmiel et al., 2011). Urban-Chmiel et al. (2011) also suggested that these vitamins can be used to support the treatment of BRD in cattle following transport.

Hellwig et al. (2000) examined the use of the NSAID flunixin meglumine as an ANC for BRD in stocker calves purchased from local livestock auctions. Calves with clinical signs of BRD were randomly assigned either a flunixin meglumine and tilmicosin phosphate treated group or tilmicosin phosphate only treatment group (Hellwig et al., 2000). The calves receiving tilmicosin phosphate and flunixin meglumine had a higher percentage of treatment successes (88% vs. 61%) and a lower combined percentage of treatment failures and BRD relapses (5% vs. 38%) than those calves receiving only tilmicosin phosphate (Hellwig et al., 2000). In addition, Hellwig et al. (2000) found that the treatment cost for calves receiving the combination of the NSAID and antimicrobial tended to be less than the treatment cost for calves receiving the antimicrobial alone. Calf ADG was not different among experimental treatments (Hellwig et al., 2000). Based on the clinical results, Hellwig et al. (2000) concluded that the flunixin meglumine tilmicosin phosphate combination was more successful for treating BRD than tilmicosin phosphate alone.

Abundant research and some of the most comprehensive reviews of the use of ANC for BRD have been conducted by Dr. Mike Apley. In a recent review of the

research concerning ANC use presented in 2010, Dr. Apley also chose to focus on the published data concerning the use of anti-inflammatory drugs as an ANC for BRD. In this review, multiple studies that demonstrate that NSAID have some beneficial effect as an ANC are cited with the most typical response observed being a reduction in rectal temperature in calves treated with an NSAID (Apley, 2010). Apley (2010) determined that other clinical responses to NSAID as an ANC were inconsistent. In regard to the use of other ANCs, Apley (2010) concluded that no data published at the current time supported the use of vaccines, vitamin C, or other ANCs for BRD.

Francoz et al. (2012) performed an exhaustive search of the data and then conducted a comprehensive review of the literature concerning the use of ANC for BRD. In order to be included in the review, experiments must have involved the treatment of naturally occurring BRD with antimicrobials and with and without at least 1 ANC. As a result, experimental models, BRD prevention studies, studies evaluating an ANC without a control group, or studies including different antimicrobials in the treatment groups were not included. These stipulations resulted in only 15 articles meeting the criteria (Francoz et al., 2012). Of those experiments, 14 dealt with anti-inflammatory drugs (12 NSAID experiments, 1 steroidal anti-inflammatory drug experiment, and 1 experiment containing both a steroidal anti-inflammatory drug and NSAID) and 1 dealt with immune-modulators (Francoz et al., 2012).

In reviewing the data related to the use of NSAID as an ANC to BRD, the authors concluded that NSAID caused a more rapid decrease in rectal temperature of calves, but did not have any benefit clinical signs, mortality, or calf performance (Francoz et al., 2012). The authors also mentioned that published data were somewhat lacking and too

inconsistent to conclude NSAID used for ANC have any effect on animal performance or mortality (Francoz et al., 2012). The reviewers also suggested that NSAID have the potential to decrease lung lesions at slaughter, but lung consolidation was only evaluated in 2 of the studies (Francoz et al., 2012). It can be argued that reduction in rectal temperature and the potential to reduced lung lesions resulting from NSAID administration may be important from an animal welfare perspective (Francoz et al., 2012). However, it is extremely difficult to justify the economics of NSAID use based on inconsistent improvements in clinical signs and the lack of performance benefit seen in calves (Francoz et al., 2012). As a result of only 1 additional ANC experiment being evaluated outside of the anti-inflammatory drug experiments, the authors concluded that there was no published data at the current time that supported the use of vaccines, vitamin C, or other ANCs for BRD (Francoz et al., 2012).

The use of NSAID as an ANC for BRD has been researched more than the use of any other ANC. In addition, NSAID seem to provide the most consistent response of all of the ANCs that have been examined. The use of vaccines as an ANC for BRD has been not been researched to date, even though the practice is widespread. While there are some experiments that suggest vitamin C has other immuno-modulating capabilities as an ANC, the published research supporting the use of vitamins as an ANC for BRD is somewhat deficient and has proven inconsistent to date. While there is potentially some justification for the use of NSAID as an ANC from an animal welfare perspective, no clinical experiments justifying the use of NSAID as an ANC from an economical or animal performance perspective exist currently.

TRACE MINERAL SUPPLEMENTATION FOR FEEDLOT CATTLE

In addition to ANCs, the feedlot industry has investigated the use of nutritional additives in an attempt to improve the response of calves treated for BRD. One such group of nutritional additives that has received much consideration would be TM. Although TM make up less than 0.01% of the total mass of an organism and are required in extremely small amounts within the diet, many TM are essential for proper growth, development, and immune function. Trace mineral requirements are not well defined, and deficiencies are difficult to isolate due to the inconspicuousness of deficiency signs and the complex interactions that exist within mineral metabolism.

The supplementation of TM has been demonstrated to alter immune function measurements and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have shown no improvements in performance or health variables from the supplementation of TM. Overall, TM research has been very inconsistent when investigating the ideal concentrations and sources of TM supplementation needed for optimum results. Organic mineral complexes have been shown to be more bioavailable than traditional inorganic mineral sources in some cases. This increased bioavailability is a result of chelated mineral complexes being less likely bound to other substances within the upper gastrointestinal tract and thus allowing the mineral to be more readily absorbed across the brush border of the small intestine. The increased bioavailability of organic TM could potentially allow for improved TM status within the animal in an immune challenge scenario such as a BRD event.

The supplementation of TM including Cu, Mn, and Zn has been a common management practice within the feedlot industry for many years and often times these TM are frequently included at levels in excess of published requirements in feedlot diets (Vasconcelos and Galyean, 2007). The primary resource cited for TM supplementation within feedlot diets would be the NRC (2000) Nutrient Requirements of Beef Cattle. Some of the most relevant information available concerning TM in feedlots comes from the published surveys of consulting feedlot nutritionists. From the survey data, the average and most common TM concentrations in feedlot diets are gleaned. While the dietary concentration of specific TM varies some over time as a result of the use of different feed ingredients and increased research, the overall supplement of specific TM has been relatively consistent in recent years.

With respect to actual TM inclusion practices in feedlot diets, 29 consulting feedlot nutritionists indicated recommended concentrations of TM typically 1 to 2 times the NRC (2000) recommendations for beef cattle (Vasconcelos and Galyean, 2007). Interestingly, the average concentrations of TM recommended by the nutritionists in 2007 survey were greater than the concentration of the same TM reported in the previous Galyean and Gleghorn (2001) survey. The supplementation of TM at levels exceeding published nutrient requirements perhaps reflects a goal to err on the side of caution in ration formulation, thus providing a safety factor for both the consulting nutritionist and the feedlot operator (Vasconcelos and Galyean, 2007). Another possible explanation would be that consulting nutritionists supplement TM exceeding published nutrient requirements in order to compensate for a potential depressed TM status of calves entering the feedlot.

Justification for Cu, Mn, and Zn supplementation: Proposed immune system modulation

While the supplementation of TM is not a new management practice, it is becoming increasingly common to include selective TM such as Cu, Mn, and Zn in feedlot diets at levels that exceed published nutrient requirements (Vasconcelos and Galyean, 2007). While these TM make up very small percentage of a calf's body mass and are required in extremely small amounts within the diet, it has been well established that certain TM are essential for overall performance and immune function. In addition to general health and immune function mechanisms, the supplementation of TM has been demonstrated to alter specific immune function measurements and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have demonstrated no improvements in performance or health variables from the supplementation of TM. The role of TM in immune function, combined with the unknown TM status of newly received calves may serve as an explanation for increased TM inclusion in feedlot diets even though justification of the practice is not necessarily supported by the literature.

Copper is important for hemoglobin formation and iron (Fe) absorption, plays and role in bone and connective tissue metabolism, and is necessary for adequate immune function. Copper serves as an essential component of enzymes including lysyl oxidase, cytochrome oxidase, superoxide dismutase, ceruloplasmin, and tyrosinase. In human medicine, Cu has demonstrated a key role in the development and maintenance of a

healthy immune system. Multiple experiments have established that Cu status within the body alters several aspects of neutrophil, monocyte, and T-cell function (Wintergerst et al., 2007). The Cu-containing enzyme superoxide dismutase, which functions in the antioxidant defense against reactive oxygen species, is essential in the disproportionation reaction that converts the superoxide anion to oxygen and hydrogen peroxide (Wintergerst et al., 2007). This disproportionation reaction diminishes damage to lipids, proteins, and DNA (Wintergerst et al., 2007). Published data demonstrating the effects of Cu on the immune response are somewhat limited. This is partially a result of a reduced ability to accurately measure marginal to moderate Cu deficiency in humans due to a lack of specific biomarkers and the fact that Cu status is maintained over a wide range of dietary Cu intakes (Wintergerst et al., 2007).

Manganese plays an important role in growth, bone development, muscular coordination, and carbohydrate and lipid metabolism. Manganese serves as an essential component of enzymes including pyruvate carboxylase, arginase, superoxide dismutase, and others. When laser ablation inductively coupled plasma mass spectrometry was used to categorize metal distribution within tissue sections, it was revealed that tissue abscesses caused by *Staphylococcus aureus* were virtually devoid of detectable Mn (Kehl-Fie and Skaar, 2010). However Kehl-Fie and Skaar (2010) reported that there were extremely high levels of Mn in the surrounding healthy tissue. While all of the factors responsible for the sequestering Mn are unknown, the lack of Mn within the abscess seems to represent an employed strategy to help control infection (Kehl-Fie and Skaar, 2010). These results along with additional data involved in the protein mediated transport of Mn suggest a role for extracellular manganese binding and intracellular manganese

transport in the defense against bacterial infection (Kehl-Fie and Skaar, 2010). In addition, the antioxidant enzyme manganese superoxide dismutase functions in the antioxidant defense against oxidative stress similar to Cu-containing superoxide dismutase. Published data demonstrating the effects of Mn on the immune response are more limited that either Zn or Cu. Limited data would suggest that toxic levels of Mn may actually impair immune function.

Zinc is important for bone and skin development, is an essential component of a number of important metabolic enzymes for protein and carbohydrate metabolism, and is necessary for adequate immune function. In addition, enzymes that require Zn are involved in protein, nucleic acid, and carbohydrate metabolism as well as enzymes associated with immune function. The many immune-related functions of Zn are well documented within human medicine. In vitro and in vivo studies have demonstrated the antioxidant activity of Zn as a cofactor of superoxide dismutase, through the binding and stabilization of protein thiols (Wintergerst et al., 2007). In addition, Zn is involved in the defense against oxidative stress caused by reactive oxygen species produced by activated macrophages (Wintergerst et al., 2007). The thymic hormone thymulin, which requires Zn as cofactor, induces multiple T-cell markers, and encourages T-cell function (Wintergerst et al., 2007). Cytokine release by peripheral blood mononuclear cells is also controlled by Zn (Wintergerst et al., 2007).

Ho et al. (2003) stated that a Zn deficiency in human lung fibroblasts has resulted in induced oxidative stress and increased DNA damage and p53 protein expression. This caused subsequent reduced antioxidant defense and diminished DNA repair mechanisms, which resulted in the lung fibroblasts becoming susceptible to oxidative DNA damage

(Ho et al., 2003). A Zn deficiency also impairs macrophage functions (phagocytosis, intracellular killing activity), neutrophil functions (chemotaxis, generation of the oxidative burst), and natural killer cell activity (Ibs and Rink, 2003). Deficiency of Zn can also cause an imbalance in helper T cell functions. In a Zn deficiency experiment the Th2 cytokine production response was not affected while the Th1 cytokine response was decreased (Wintergerst et al., 2007). Wintergerst et al. (2007) also stated that a deficiency of Zn has resulted in decreased serum thymulin activity, decreased recruitment of T-naïve cells, and a reduction in precursors of cytolytic T cells.

The TM Cu, Mn, and Zn have been demonstrated to play a role in immune function in both human and animal experiments. There is infinitely more research concerning the supplementation of TM and their relation to immune function in human medicine. In human medicine and lab animal models the published data demonstrating the effects of Cu on the immune response are more limited than those of Zn, and data demonstrating the effects of Mn on the immune response are more limited still. However, there is sufficient evidence in both the classical and recent literature to substantiate the role of Cu and Zn in immune function. The role of Mn in immune function is still not well defined. In addition, the research concerning the additional supplementation of TM to feedlot cattle has been very inconsistent. As such, the use of TM as an ANC or the feeding of TM in excess of published requirements when calves are not in a state of deficiency has not demonstrated consistent positive results.

Typical Cu, Mn, and Zn supplementation strategies in feedlots

There is some justification in the published research for role of TM in immune function. However, the published research concerning the supplementation of Cu, Mn, or Zn at levels greater than published requirements or as an ANC is lacking and has produced inconsistent results. As such, there is likely no justification for overfeeding of these TM or for the use of these TM as an ANC at the current time. Regardless, the supplementation of TM including: Cu, Mn, and Zn has become a normal management practice within the feedlot industry for many years primarily to avoid unwanted deficiencies and promote maximum animal performance.

Both consulting nutritionists and academic researchers cite the NRC (2000) Nutrient Requirements of Beef Cattle as the primary resource when formulating TM supplementation levels in feedlot diets. For growing and finishing cattle, TM requirements reported in the NRC (2000) are 10 mg/kg for Cu, 20 mg/kg for Mn, and 30 mg/kg for Zn. Interestingly, these TM are frequently included in commercial feedlot diets at levels in excess of published requirements (Vasconcelos and Galyean, 2007). The best evidence available for the prevalence of TM supplementation and typical dietary TM levels in feedlots is found in large-scale surveys of consulting feedlot nutritionists. Two large-scale surveys conducted since 2000 provide substantial evidence of TM inclusion levels in commercial feedlots.

In 2000, Galyean and Gleghorn (2001) surveyed consulting feedlot nutritionists representing practices in all of the major cattle feeding areas of the U.S. including the Midwest, High Plains, and Southwest. The survey reported the mean and mode TM inclusion levels in both receiving and finishing feedlot diets (Galyean and Gleghorn, 2001). The mean inclusion level for Cu in receiving diets was 20.42 mg/kg with 20

mg/kg being the most recommended inclusion level for Cu (Galyean and Gleghorn, 2001). For Mn, the mean inclusion level in receiving rations was 49.33 mg/kg with 50 mg/kg of Mn being the concentration recommended by the most consulting nutritionists (Galyean and Gleghorn, 2001). In regards to Zn, consulting nutritionists included a mean concentration of 94.06 mg/kg in receiving diets with 150 mg/kg of Zn being the most recommended concentration (Galyean and Gleghorn, 2001).

Interestingly, all 3 TM were included in receiving diets at average concentrations of 2 to 3 times greater than published TM nutrient requirements. This increased supplementation of TM relative to published nutrient requirements perhaps reflects a goal to err on the side of caution in ration formulation, thus providing a safety factor for both the consulting nutritionist and the feedlot operator (Vasconcelos and Galyean, 2007). Another possible explanation could be that consulting nutritionists choose to supplement TM at 2 to 3 times the published nutrient requirements in order to compensate for low DMI intakes of calves during the beginning of the receiving period or to compensate for a potential depressed TM status of calves entering the feedlot. Finally, the supplementation of these TM in excess of published requirements could result from the ability of Cu, Zn, and Mn to alter immune function measurements and reduced morbidity associated with BRD in some cases (Galyean et al., 1999).

The mean Cu inclusion level in finishing rations was 14.75 mg/kg with 20 mg/kg being the most recommended Cu inclusion level (Galyean and Gleghorn, 2001). In the case of Mn, the mean inclusion level in finishing diets was 38.28 mg/kg with 40 mg/kg of Mn being the concentration recommended by the most consulting nutritionists (Galyean and Gleghorn, 2001). Regarding Zn, consulting nutritionists included a mean

concentration of 74.03 mg/kg in finishing rations with 50 mg/kg being the most recommended Zn concentration (Galyean and Gleghorn, 2001). The same trend observed with the receiving diets was also observed in the finishing diets. All 3 TM were included in finishing rations at average concentrations that were greater than published TM nutrient requirements. However, the average concentrations of the 3 TM included in finishing diets were approximately 75% of those included in the receiving diets. This decrease in over formulation as cattle are transitioned from receiving diets to finishing diets suggests that the increased TM supplementation observed in receiving diets is not simply a reflection of the goal to err on the side of caution in ration formulation. Rather it is also a likely a function of consulting nutritionists attempting to compensate for low DMI intakes of calves during the beginning of the receiving period, a potential depressed TM status of calves entering the feedlot, or a perceived benefit to immune function measurements and reduced BRD associated morbidity.

Vasconcelos and Galyean (2007) surveyed consulting feedlot nutritionists representing practices in Texas, Kansas, and Oklahoma (46.43% of respondents); Iowa, Nebraska, Colorado, and South Dakota (31.25% of respondents); Washington and Idaho (8.93% of respondents); Arizona and California (6.25% of respondents); and other states (7.14% of respondents). The 29 respondents represented more than 18 million head of cattle on feed per year or slightly more than 69% of the cattle on feed in the U.S. annually based on USDA estimates (Vasconcelos and Galyean, 2007). In this survey the mean and mode TM inclusion levels were reported for finishing feedlot diets only (Vasconcelos and Galyean, 2007). In 2007, the mean Cu inclusion level in finishing rations was 17.61 mg/kg with 20 mg/kg being the most recommended Cu inclusion level (Galyean and

Gleghorn, 2001). For Mn, the mean inclusion level in finishing diets was 47.86 mg/kg with 50 mg/kg of Mn being the concentration most commonly recommended by consulting nutritionists (Galyean and Gleghorn, 2001). For Zn, consulting nutritionists included a mean concentration of 92.95 mg/kg in finishing rations with 100 mg/kg being the most recommended Zn concentration (Galyean and Gleghorn, 2001).

The same trend in the 2000 survey was observed in the 2007 survey with all 3 TM being included finishing rations at average concentrations greater than current published TM nutrient requirements. Interestingly, the recommended concentrations of Cu, Mn, and Zn in the 2007 survey were higher than the recommended concentration of Cu, Mn, and Zn reported by consulting nutritionists in the 2000 survey. The TM concentrations recommended for finishing diets in the 2007 survey nearly exactly match recommended TM concentrations of receiving diets in the 2000 survey. This resulted in an approximately 25% increase in the concentration of Cu, Mn, and Zn in finishing rations from 2000 to 2007.

From the surveys, it is apparent the supplementation of TM has increased in recent years. It is also clear that Cu, Mn, and Zn are frequently included in commercial feedlot diets at levels 2 to 3 times greater than the published requirements for these TM (Vasconcelos and Galyean, 2007). It would be interesting to know the TM inclusion levels in receiving diets in 2007. Because Vasconcelos and Galyean (2007) did not report receiving diet information, it is unknown if TM inclusion levels increased in receiving rations at the same rate from 2000 to 2007. It is possible the concentration of Cu, Mn, and Zn in finishing rations in 2007 was increased to mimic the concentration of TM seen in 2000 receiving diets while the concentration of TM in receiving diets in 2007 remained

relatively constant. It would also be helpful to have a more recent survey to see if the trend for increased TM supplementation in feedlot diets has continued or if it has plateaued since 2007.

Effects of Cu, Mn, and Zn supplementation on the immune status and clinical health of calves

The supplementation of TM including Cu, Mn, and Zn has been a longstanding management practice within the feedlot industry primarily to avoid unwanted deficiencies and promote maximum animal performance. In addition, there is some evidence suggesting these TM play a role in the improving the clinical health the immune function calves. However, the published research concerning the supplementation of Cu, Mn, or Zn at levels greater than published requirements or as an ANC is somewhat lacking and has produced inconsistent results to date. Regardless, the inclusion of these 3 TM in feedlot diets at concentrations 2 to 3 times greater than published requirements is still incredibly popular with feedlot nutritionists. The role of TM in immune function, combined with the unknown TM status of newly received calves and expected depressed DMI early in the receiving period may serve as a possible explanation for increased TM inclusion even though justification of the practice is not necessarily supported by the published literature.

Much of the available literature concerning Cu supplementation comes from classical Cu deficiency studies. Stabel et al. (1993) fed Holstein steers a semi-purified diet containing only 1.5 mg of Cu/kg and supplemented with no Cu or 10 mg of Cu/kg

for 5 months and then challenged with IBR followed by MH. Serum ceruloplasmin and plasma Cu were both significantly higher in Cu supplemented calves and increased after the MH challenge (Stabel et al., 1993). In addition, Cu concentrations were higher in all tissues for Cu supplemented calves (Stabel et al., 1993). Serum immunoglobulin M tended to be higher in Cu supplemented calves and increased for all calves after the IBR challenge (Stabel et al., 1993). Stabel et al. (1993) observed significantly higher serum IBR antibody titers in the calves that received no Cu supplementation. In contrast, antigen-specific antibodies to MH tended to be higher in Cu supplemented calves on day 21 post challenge (Stabel et al., 1993). The supplementation of Cu did not affect the blastogenic response of lymphocytes and phytohemagglutinin-stimulated blastogenesis was higher in both treatments after the IBR challenge.

Arthington et al. (1996) examined the effects of dietary Cu depletion and subsequent repletion on acute-phase protein concentrations, superoxide dismutase activity, leukocyte numbers, and lymphocyte proliferation in Hereford x Angus crossbred heifers. Heifers were assigned to either a control experimental treatment or a Cu deficient experimental treatment (Arthington et al., 1996). The Cu deficiency was induced in the Cu deficient heifers through the addition of sulfur at 0.3% of the diet and Molybdenum (Mo) to achieve a Mo:Cu ratio of 2.5:1 (Arthington et al., 1996). Arthington et al. (1996) fed the control heifers the basal diet (6.2 mg/kg Cu) with additional $CuSO_4$ to bring the total Cu level to at least 8 mg/kg of Cu. All dietary treatments were delivered for 129 days followed by inoculation with BHV1 (Arthington et al., 1996).

To ensure adequate Cu stores before viral challenge the control heifers were given an injection of cupric glycinate on day 100 and on the day of BHV1 challenge the Mo

supplemented heifers had liver Cu levels of 23.2 mg/kg and the control heifers had liver Cu levels of 90.1 mg/kg (Arthington et al., 1996). Arthington et al. (1996) noted that ceruloplasmin was increased by 48 hours post challenge in the control heifers, but was not increased in the Mo supplemented heifers. Neutrophils were increased on day 129 for the Mo supplemented Cu deficient heifers (Arthington et al., 1996). Plasma fibrinogen was increased by 48 hours after challenge in Mo-supplemented heifers (Arthington et al., 1996). In addition, erythrocyte superoxide dismutase activity was less for Mo supplemented heifers on d 129. The lymphocyte proliferative response to phytohemagglutinin was greater for Mo supplemented heifers post BHV1 challenge.

Sharma et al. (2005) examined Cu status and immune function in Cu deficient heifers supplemented with mineral mixture containing Cu sulfate (CuSO4) or the same mineral mixture with salt replacing the CuSO4. Sharma et al. (2005) observed a significant improvement in serum ceruloplasmin after 30 days and a significant improvement in monoamine oxidase and liver cytochrome oxidase after 60 days on the experimental treatments for the Cu supplemented animals. In addition, Cu supplemented heifers demonstrated significant improvements in hemoglobin levels, total leukocyte counts, and total erythrocyte counts compared to heifers not receiving Cu supplementation after 30 days on their respected treatments (Sharma et al., 2005). When evaluating the effects of Cu supplementation on immune function, Sharma et al. (2005) detected that the phagocytic activity of neutrophils against *Candida albicans* was significantly improved for those heifers supplemented with CuSO4 after 60 days of treatment. Significant improvements in superoxide dismutase activity in RBC, WBC, and whole blood were observed for Cu supplemented heifers.

The supplementation of Cu or adequate dietary Cu inclusion has demonstrated positive effects on immune function in cases of natural or induced Cu deficiencies in many other experiments. Xin et al. (1991) demonstrated that Cu status affected the distribution of Cu within tissues and affected Cu related enzyme activities as well as bactericidal function of neutrophils. Gengelbach et al. (1997) observed superoxide dismutase activity was higher for control or Cu supplemented calves compared to Mo supplemented calves, and that bactericidal activity of neutrophils from Cu supplemented calves tended to be higher than that of Mo supplemented calves. After an IBR and MH challenge, the Cu supplemented calves had higher levels of plasma tumor necrosis factor than MO supplemented calves at weaning and tended to have higher plasma tumor necrosis factor than MO supplemented calves 5 days after IBR inoculation (Gengelbach et al., 1997).

When additional Cu is added to diets that are not Cu deficient, there seems to be little to no effect on immune function. Stress events such as shipping or disease challenges have been shown to impact Cu status within the body (Orr et al., 1990; Nockels et al., 1993). However, this change in Cu status is generally fairly short lived, and does not translate to noticeable changes is immune function or performance. Many experiments have shown no improvements in performance or health variables from the supplementation of additional Cu in excess of animal requirements. Multiple studies have looked at the effect of Cu source on clinical health and immune response of calves, and found no relevant differences between organic or inorganic Cu sources (Stanton et al., 2001; Salyer et al., 2004). In one experiment the source of both Cu and Zn affected the humoral immune response to an ovalbumin vaccine although the effects of TM source

were not consistent for the 2 minerals (Salyer et al., 2004). Overall, the research involving Cu supplementation has been very inconsistent except when a true dietary Cu deficiency exists.

The available data concerning the impact of Mn supplementation on health and immune function is scarce, except in experiments were Mn was given in combination with 2 or more other TM. Only 1 experiment was found evaluating clinical health or immune function involving Mn and 1 other TM. In this experiment, Chirase and Greene (2001) observed the effects of organic and inorganic Mn and Zn supplementation. Chirase and Greene (2001) examined different dietary sources of Mn and Zn that were administered beginning with the fetal stage on the subsequent immune response of transit stressed and virus challenged calves. The treatments supplied 40 mg/kg of Mn and 50 mg/kg of Zn as either through an organic mineral source (Mn and Zn methionine) or through an inorganic mineral source (Mn and Zn oxide) (Chirase and Greene, 2001). A negative control treatment was not included in the experiment.

After weaning, calves were shipped to the feedlot and remained on their respective treatments (Chirase and Greene, 2001). During a 28 day receiving period, steers fed organic Mn and Zn had higher DMI and improved gain compared to steers fed inorganic Mn and Zn (Chirase and Greene, 2001). After this 28 day period, Chirase and Greene (2001) challenged the calves with IBR. During the challenge phase, steers fed Mn and Zn methionine had 20.4% higher DMI than the steers fed Mn and Zn oxide (Chirase and Greene, 2001). The steers fed organic Mn and Zn also had lower rectal temperatures and retained more weight than those fed inorganic Mn and Zn (Chirase and Greene, 2001). Chirase and Greene (2001) stated that these data indicated organic Mn and Zn

lessened the effects of transit stress and IBR infection of calves better than inorganic Mn and Zn. While this experiment does provide some evidence that the feeding of organic mineral sources may be beneficial, it demonstrates little about a specific role of Mn in the clinical health or immune function of calves. It does not determine if the positive effects seen from organic TM supplementation are a result of feeding Mn, Zn, or the combination. There is not enough published research to confirm a role for Mn in the health and immune function of calves at present.

Published data regarding the impact of Zn supplementation on the health and immune function of calves is less prevalent than that for Cu supplementation, but more prevalent than that for Mn supplementation. However, similar to what was observed for Mn, it is uncommon to find this research were Zn was the only TM examined, and in most experiments Zn was given in combination with other TM. It has been documented that stressful events such as transport or a disease challenge have been shown to impact Zn status within the body (Orr et al., 1990; Nockels et al., 1993). It has also been shown that organic or inorganic sources of Zn source do not relevantly affect the clinical health or immune response of calves in multiple studies (Nockels et al., 1993; Stanton et al., 2001; Salyer et al., 2004). In contrast, Chirase and Greene (2001) suggested that organic Zn and Mn lessened the effects of transit stress and IBR infection of calves better than inorganic Zn and Mn. In addition, Salyer et al. (2004) did note that the source of Zn or Cu affected the humoral immune response to an ovalbumin vaccine although the effects of TM source were not consistent for the 2 minerals (Salyer et al., 2004).

Similar to what is observed for Cu, when supplemental Zn is added to diets that are not Zn deficient, there seem to negligible effects on immune function. While stressful

events have been shown to impact Zn status, this change in is generally short lived. In addition, multiple experiments have shown no improvements in performance or health variables resulting from Zn supplementation in excess of animal requirements and found no relevant differences between organic or inorganic Zn sources. In summary the research concerning Zn supplementation in calves has been very inconsistent to date except when a true dietary Zn deficiency exists.

Varied immune and clinical health responses have been observed in studies where a combination of Cu, Mn, and Zn or a combination of 4 or more TM have been administered to calves. George et al. (1997) examined the effects of Co, Cu, Mn, and Zn sources and amounts on the performance and immune function of stressed heifers. Calves were assigned to 3 experimental treatments consisting of inorganic TM at concentrations of 7 mg/kg Co, 37 mg/kg Cu, 58 mg/kg Mn, and 106 mg/kg Zn, organic TM at isoelemental concentrations, or organic TM supplemented at 3 times this concentration for the first 14 days after transport then supplemented at the isoelemental concentrations for the remainder of the experiment (George et al., 1997). There were no differences observed among the treatments for performance variables over the 42 d experiment (George et al., 1997). Calf secondary PI3 antibody titer responses at days 14 and 28 post vaccination, skin swelling responses at 21 days post transport and at 12, 24, and 48 hours post intradermal phytohemagglutinin antigen injection were better for calves receiving organic TM at 3 times the initial concentration than the other organic or inorganic isoelemental groups (George et al., 1997). In addition, when calves were fed increased organic TM for the first 14 days they experienced a 17.2% reduction in BRD incidence when compared to the other organic or inorganic isoelemental groups (George et al.,

1997). Calves receiving organic TM demonstrated improved antibody titer responses to IBR vaccination at days 14 and 28 compared to calves receiving inorganic TM at the same concentration (George et al., 1997). The authors concluded that feeding elevated organic TM early in the receiving period resulted in significant improvements in primary and secondary humoral, and cell-mediated immunity in stressed heifer calves (George et al., 1997).

Rhoads et al. (2003) examined the effects of concentration and source of TM on the performance, immunity, mineral and lipid metabolism, and carcass characteristics of steers. The TM treatments administered consisted of Co, Cu, Mn, and Zn administered at different levels from different sources, and the treatments changed from the receiving period to the finishing period (Rhoads et al., 2003). Due to the treatment structure, there is little value in direct comparisons between the treatments. However, some information can be gleaned from the lack of differences observed to such varied treatments. For example, the antibody titer response to IBR was not different among any of the treatments suggesting that neither TM source nor concentration impacted the response to a viral challenge. The authors concluded that overall results indicated that TM concentration and source had minimal effects on performance and immunity (Rhoads et al., 2003).

Kegley et al. (2012) also compared organic Co, Cu, Mn, and Zn to inorganic Co, Cu, Mn, and Zn supplementation in livestock auction purchased stressed beef calves. The calves that were supplemented with organic TM had heavier final BW and ADG compared to the calves that were supplemented with inorganic TM (Kegley et al., 2012). In addition, calves that were supplemented with organic Co, Cu, Mn, and Zn tended to

receive a second treatment for BRD less often (Kegley et al., 2012). The source of TM also impacted antibody response. When allowing for calves that arrived with antibody titers to IBR, the naïve calves receiving organic TM had lower antibody responses to IBR vaccination (Kegley et al., 2012). Although IBR antibody titers were increased for the calves supplemented with inorganic minerals, the fewer calves requiring a second antimicrobial for BRD and improved performance for the calves supplemented with organic TM would suggest an advantage for organic TM supplementation.

Arthington and Havenga (2011) and Richeson and Kegley (2011) both examined the effects of a single injection of trace minerals on the health and performance of calves. Arthington and Havenga (2011) used only 2 treatments, either a subcutaneous injection of a TM solution containing 15 mg/mL of Cu, 10 mg/mL of Mn, 5 mg/mL of Se, and 40 mg/mL of Zn or a saline-injected negative control. Richeson and Kegley (2011) used 3 treatments: a subcutaneous injection of a TM solution containing 10 mg/mL of Cu, 20 mg/mL of Mn, 5 mg/mL of Se, and 20 mg/mL of Zn, a subcutaneous injection of a TM solution containing 16 mg/mL of Cu, 10 mg/mL of Mn, 5 mg/mL of Se, and 48 mg/mL of Zn, or negative control treatment. Arthington and Havenga (2011) discovered that calves receiving injectable TM at the time of vaccination experienced greater neutralizing antibody titers to BHV1 on days 14, 30, and 60 when compared with receiving a saline injection. The results demonstrated that injectable TM do not negatively impact the humoral immune response and may enhance the production of neutralizing antibodies to BHV1 in naïve beef calves (Arthington and Havenga, 2011).

Richeson and Kegley (2011) noted that ADG was greater for calves that received either of the injectable TM treatments compared to the control calves, but the ADG was

not different between the 2 injectable TM treatments. In addition, G:F was improved for both of the injectable TM treatments (Richeson and Kegley, 2011). The calves administered the TM solution containing 10 mg/mL of Cu, 20 mg/mL of Mn, 5 mg/mL of Se, and 20 mg/mL of Zn had a reduced incidence of BRD when compared to the control calves, with the other TM solution being intermediate (Richeson and Kegley, 2011). The average antimicrobial treatment cost was greater for the control calves than for either of the injectable TM treatments (Richeson and Kegley, 2011). These results lead Richeson and Kegley (2011) to conclude that the administration of injectable TM to highly stressed calves at initial processing can result in improved performance, reduced BRD morbidity, and antimicrobial treatment cost.

The supplementation of TM to beef cattle has been researched for many decades. Traditional TM research in beef cattle focused on supplementation in situations of existing or induced TM deficiencies. This research has demonstrated a role for individual TM in health and immune function. However, the supplementation of individual TM in diets that are not deficient in TM seems to have little to no effect on the clinical health and immune function of calves. Recent research has focused on the supplementation of multiple TM at once and the comparison of organic and inorganic TM sources. The results from this research are somewhat inconsistent and difficult to interpret. When 4 TM are fed or injected in combination, it is impossible to determine the impact of individual TM on reported results. In addition, the concentrations of TM making up the treatments in these studies are often not isoelemental making direct comparisons invalid. While there are some experiments that suggest TM do in fact positively impact the health and immune function of calves when supplemented at levels in excess of published

requirements or used as an ANC, the published research supporting this practice is somewhat deficient and has ultimately proven inconsistent to date.

SUMMARY AND CONCLUSIONS FROM THE LITERATURE

Bovine respiratory disease is still the most significant production problem confronting the feedlot industry, accounting for the majority of morbidity, mortality, and production losses that occur in feedlots. Estimated annual economic losses due to BRD are approaching or exceeding of $2 billion annually. However, the true cost to the feedlot industry when losses in carcass value and the amount of money spent on the prevention of BRD are included may be well in excess of $5 billion. Published data would indicate that no significant reduction in the prevalence of BRD has occurred over the last 30 to 40 years (Gifford et al., 2012). This suggests that current BRD prevention and treatment strategies are ineffective in reducing and controlling BRD, despite the wealth of BRD research and measurable improvements in commercially available vaccines and antimicrobials (Babcock et al., 2006).

Bovine Respiratory Disease is an extremely complicated illness and a multitude of risk factors, viruses, and bacterial pathogens can potentially contribute to its onset (Duff and Galyean, 2007). The prevention and treatment of BRD continues to be a major concern for all of those within the feedlot industry. The standard practice is to administer some class of injectable antimicrobial as the primary form of treatment when treating for suspected BRD in feedlot cattle. Nearly all feedlots (99.0 percent) used an injectable antimicrobial as the initial or primary treatment for BRD (NAHMS, 2013). However, it is

also common to provide additional treatment, or ANC, along with the antimicrobial when treating calves for BRD.

The primary goal of ANC would be to improve the response to a BRD challenge in calves treated with antimicrobials, not to replace antimicrobial treatment. There are some experiments that suggest vitamin C has immuno-modulating capabilities as an ANC, but the published research supporting the use of vitamins as an ANC for BRD is somewhat lacking and has proven inconsistent. The use of NSAID as an ANC for BRD has been researched more than the use of any other ANC and NSAID seem to provide the most consistent response of all of the ANCs that have been examined. There is potentially some justification for the use of NSAID as an ANC from an animal welfare perspective, but no clinical experiments justify the use of NSAID from an economical or animal performance perspective. In reviews of ANC research, multiple authors have concluded that there is no published data to support the use of vaccines, vitamin C, or other ANCs for BRD (Apley, 2010; Francoz et al., 2012).

In addition to ANC, the feedlot industry has investigated the use of other nutritional additives as a method to decrease morbidity and mortality due to BRD, while simultaneously enhancing animal performance. The supplementation of TM has been demonstrated to alter immune function measurements and reduced morbidity associated with BRD in some cases (Galyean et al., 1999). The supplementation with TM including: Cu, Mn, and Zn has become a standard management practice within the feedlot industry to promote maximum performance and immune function, and these trace minerals are frequently included at levels in excess of published requirements in feedlot diets (Vasconcelos and Galyean, 2007).

However, research concerning TM supplementation has been extremely inconsistent. Classical research has demonstrated a role for individual TM in health and immune function in the face of a TM deficiency. However, the supplementation of TM in diets that are not deficient in TM seems to have negligible effects on the clinical health and immune function of calves. More recent research has focused on the supplementation of multiple TM and the comparison of organic and inorganic TM sources. These results have been somewhat inconsistent and difficult to interpret. While some experiments that suggest TM do in fact positively impact the health and immune function of calves when supplemented at levels in excess of published requirements or used as an ANC, the published research supporting this practice is somewhat deficient and has ultimately proven inconsistent.

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CHAPTER III

EVALUATION OF MULTIPLE ANCILLARY THERAPIES USED IN COMBINATION WITH AN ANTIMICROBIAL IN NEWLY RECEIVED HIGH-RISK CALVES TREATED FOR BOVINE RESPIRATORY DISEASE

ABSTRACT

Ancillary therapy (ANC) is commonly provided in conjunction with an antimicrobial when treating calves for suspected bovine respiratory disease (BRD). This experiment evaluated the effects of 3 ANC in combination with an antimicrobial in high-risk calves treated for BRD. Newly received crossbred steers (n = 516; initial BW = 217 ± 20 kg) were monitored by trained personnel for clinical signs of BRD. Calves that met antimicrobial treatment criteria (n = 320) were then randomly assigned to 1 of 4 experimental ANC: intravenous flunixin meglumine injection (NSAID), intranasal viral vaccination (VACC), intramuscular vitamin C injection (VITC), or no ANC (NOAC). Steers receiving VACC tended (P = 0.10) to require a second BRD treatment less frequently than steers receiving NSAID or NOAC. Calves receiving NSAID or VITC tended (P = 0.09) to require a third BRD treatment less often than calves receiving NOAC. Of calves treated 3 times for BRD, those receiving NOAC had lower (P = 0.05) severity scores than those receiving VACC or VITC and heavier (P = 0.02) BW than those receiving NSAID, VACC, or VITC at the time of third treatment. Between the

second and third BRD treatments, calves receiving NOAC also had greater (P = 0.03) ADG than those receiving VACC or VITC and tended (P = 0.06) to have greater ADG than those receiving NSAID. Calves receiving NOAC tended (P = 0.07) to have heavier BW on d 28 than NSAID, VACC, or VITC with mortalities and removals excluded. There was no difference in rectal temperature among the experimental ANC. When contrasted with the average of NSAID, VACC, and VITC with mortalities and removals excluded, calves receiving NOAC tended to have heavier BW on d 56 (P = 0.06), greater ADG (P = 0.10) and DMI (P = 0.08) from first BRD treatment through d 28, greater DMI from d 28 through d 56 (P = 0.06), and had greater DMI from first BRD treatment through d 56 (P = 0.05). After the receiving period, a subset of calves (n = 126) were allocated to finishing pens to evaluate the effects ANC administration on finishing performance, carcass characteristics, and lung scores. No ANC treatment differences were observed for any of the variables analyzed in the finishing experiment ($P \geq 0.26$). Responses observed to the 3 ANC used in these experiments were negligible. The use of NSAID, VACC, and VITC do not appear to positively impact clinical health and could potentially be detrimental to performance during the receiving period in high-risk exposed calves.

Key Words: ancillary therapy, bovine respiratory disease, flunixin meglumine, high-risk calves, viral vaccine, vitamin C

INTRODUCTION

Bovine respiratory disease (BRD) is the most significant production problem for the feedlot industry, accounting for the majority of morbidity, mortality, and decreased production in feedlots with estimated annual economic losses in excess of $2 billion (Powell, 2013). The standard protocol when treating for suspected BRD in feedlot cattle is to administer an injectable antimicrobial. However, it is also common for veterinarians to prescribe an additional treatment, or ancillary therapy (ANC), along with the antimicrobial. The goal of ANC is to improve the response to a BRD challenge in calves treated with antimicrobials, not to replace antimicrobial treatment. This can be accomplished by relieving the harmful effects of inflammation, blocking histamine activity, or boosting immune system function to aid in the defense against infectious pathogens (Apley, 1994).

In 1999, USDA's NAHMS surveyed feedlots in the top 12 cattle feeding states and noted that only 12.8% of these feedlots used a single antimicrobial for the treatment of BRD (NAHMS, 2001). More recently, a survey conducted by Terrell et al. (2011) reported that 48% of veterinarians recommended some form of ANC for the treatment of BRD. After this experiment was conducted, NAHMS (2013) released an updated survey detailing additional ANC use in feedlots. The most common forms of ANC listed in the surveys included: antihistamines, B vitamins, corticosteroids, direct-fed microbials (DFM), non-steroidal anti-inflammatory drugs (NSAID), viral vaccines, and vitamin C (NAHMS, 2001; Terrell et al., 2011; NAHMS, 2013). While these surveys provide evidence as to the scope of ANC use, there is limited published research on the efficacy of these ANC. The objective of this experiment was to evaluate the effects of 3 of the most common ANC used in combination with an antimicrobial on the performance,

health, and immune response variables of newly received high-risk calves treated for clinical bovine respiratory disease.

MATERIALS AND METHODS

All procedures for the present experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Animal Care and Use Protocol AG-12-11).

Cattle description and initial processing

Over the course of 1 week, 516 crossbred steers and bulls (BW at arrival = 217 ± 20 kg) were purchased at livestock auctions throughout Oklahoma and transported (average distance = 135 km) to the Willard Sparks Beef Research Center at Oklahoma State University. Upon arrival at the feed yard, calves were individually weighed and visually inspected for noticeable deformities or abnormalities. Hide color, horn status, and sex was recorded and an uniquely numbered ear tag was placed in the left ear of each calf. Calves were then commingled into holding pens, given ad libitum access to prairie hay and water, and allowed to rest 24 to 48 hours prior to initial processing.

At processing, a blood sample (9 mL) was collected via jugular venipuncture for subsequent serum analysis (Corvac™ Serum Separator Tube, Tyco Healthcare Group LP, Mansfield MA). Initial processing consisted of vaccination for Infectious Bovine Rhinotracheitis (IBR) virus, Bovine Viral Diarrhea Virus (BVDV) Types 1 and 2,

Parainfluenza 3 (PI3) virus, and Bovine Respiratory Syncytial Virus (BRSV; BRD Shield; Novartis, Greensboro, NC), *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii* and *Clostridium perfringens* Types C and D (Caliber 7; Boehringer-Ingelheim, St. Joseph, MO), and treatment for the control of internal and external parasites (Ivomec Plus; Merial, Duluth, GA). Individual BW were obtained, bulls (n = 355) were surgically castrated by incising the scrotum with a Newberry castrating knife followed by emasculation by a single individual. Any calves with horns (n = 57) had their horns tipped with a Keystone dehorner.

Receiving phase pen management and diet

After processing, groups of calves were gate cut and returned to receiving pens where they received ad libitum access to a common receiving ration and water. Receiving pens were 12.2 × 30.5 m soil-surfaced open-air pens with 12.2 m concrete bunk at the front of each pen. A 76 L concrete water tank (Model J 360-F; Johnson Concrete, Hastings, NE) was shared between 2 pens and was cleaned 3 times per week throughout the experiment. The common wet corn gluten feed based receiving ration was formulated to meet or exceed NRC (2000) nutrient requirements (Table 1). The ration was fed to all cattle twice daily at 0700 h and 1300 h in a 274-12B Roto-Mix Forage Express mixer wagon (Roto-Mix, Dodge City, KS) to the nearest 0.45 kg of that day's feed call. Long stem prairie hay was fed at 0.454 kg per head per d for 5 d. Ration samples were collected once per week and dried in a forced air oven for 48 h at 60ºC to determine DM.

Ration samples were composited gravimetrically and analyzed at a commercial laboratory (Servi-Tech Inc., Dodge City, KS) for nutrient composition (Table 1).

Assessment for clinical signs of BRD and antimicrobial administration

Calves remained in the receiving pens and were allocated to an experimental ANC only after they were identified as demonstrating subjective clinical signs of BRD, met treatment criteria, and were administered an antimicrobial. During the receiving period, calves were visually monitored twice daily by trained evaluators throughout the experiment for clinical signs characteristic of BRD. The evaluation employed criteria based on the DART™ system (Pharmacia Upjohn Animal Health, Kalamazoo, MI) with some modifications as described by Step et al. (2008). The subjective criteria used for pulling calves consisted of depression, abnormal appetite, and respiratory signs. Signs of depression included but were not limited to: depressed attitude, lowered head, glazed or sunken eyes, slow or restricted movement, arched back, difficulty standing or walking, knuckling of joints or dragging toes when walking, and stumbling. Signs of abnormal appetite included: an animal that was completely off feed, an animal eating less than expected or eating extremely slow, a lack of gut fill or gaunt appearance, and obvious body weight loss. Respiratory signs included: labored breathing, extended head and neck (in an attempt to breathe), and audible noise when breathing. The evaluators assigned a calf a severity score from 0 to 4 based on the clinical signs and the severity of those signs.

A score of 0 was assigned for a clinically normal appearing calf. A score of 1 was assigned for mild clinical signs, 2 for moderate clinical signs, 3 for severe clinical signs, and 4 for a moribund animal. For a calf to be assigned a score of 4, the calf was unable to rise, or had extreme difficulty standing, walking, or breathing. These animals required immediate assistance. The objective criteria used to determine if antimicrobial treatment was necessary was rectal temperature. All calves assigned a severity score of 1 to 4 were taken to the processing chute for rectal temperature measurement (GL M-500, GLA Agricultural Electronics, San Luis Obispo, CA). Any animal that was pulled with a severity score of 1 or 2, and had a rectal temperature of 40°C or greater received an antimicrobial according to label instructions. If a calf was pulled with a severity score of 1 or 2 and had a rectal temperature of less than 40°C, no antimicrobial treatment was administered, and the calf was returned to its receiving pen after evaluation. Any animal with a severe clinical signs (severity score = 3 or 4), received an antimicrobial according to label instructions regardless of rectal temperature. In extreme cases the antimicrobial may have been administered in the home pen if the calf was deemed unable to make the walk to the working facility.

Prior to antimicrobial administration, an accurate BW was obtained to calculate the appropriate dosage and a blood sample was collected (Corvac™ Serum Separator Tube, Tyco Healthcare Group LP, Mansfield MA). Antimicrobial doses were calculated by rounding the calf's current BW up to the nearest 11.3 kg. All antimicrobials were administered subcutaneously per manufacturer's label directions following Beef Quality Assurance Guidelines (NCBA, 2001). The first antimicrobial treatment was administered on the left side of the animal, and subsequent injections were given on alternating sides of

the animal. The severity score, temperature, BW, and antimicrobial dosage administered (or no treatment administered) was recorded for every calf that was pulled for exhibiting clinical signs of BRD. A maximum of 4 antimicrobial treatments were administered during the experiment.

The first time antimicrobial treatment criteria were met, gamithromycin 150 mg/mL (Zactran; Merial, Duluth, GA) was administered at the rate of 1 mL/24.9 kg of BW. A moratorium was observed after gamithromycin administration before a second antimicrobial treatment could be administered. This moratorium was 240 h for calves with a severity score of 1 or 2, and 96 h for calves with a severity score of 3 or 4. If antimicrobial treatment criteria were met a second time, florfenicol 300 mg/mL (Nuflor; Intervet/Schering-Plough, Desoto, KS) was administered at the rate of 1 ml/7.56 kg of BW. After florfenicol administration, a 96 h moratorium was observed before a third antimicrobial treatment could be administered regardless of severity score. If antimicrobial treatment criteria were met a third time, ceftiofur crystalline free acid 200 mg/mL (Excede; Pfizer, New York City, NY) was administered at the rate of 1 ml/30.2 kg of BW. After ceftiofur crystalline free acid administration, a 168 h moratorium was observed before a fourth antimicrobial treatment could be administered regardless of severity score. If antimicrobial treatment criteria were met a fourth time, a second dose of ceftiofur crystalline free acid was administered as described above.

Allocation to experimental ANC treatments and ANC administration

Once a calf was "pulled" for suspected BRD, met the treatment criteria described, and received an antimicrobial, it was then randomly assigned to 1 of 4 ANC treatments. The 4 ANC treatments consisted of: an intravenous flunixin meglumine injection (NSAID), an intranasal viral vaccination (VACC), an intramuscular vitamin C injection (VITC), or no ANC (NOAC). The VITC experimental ANC treatment consisted of 10 mL per calf of Vita-Jec® C (Aspen; Liberty, MO) containing 250 mg of sodium ascorbate per mL injected intramuscularly. The NSAID experimental ANC treatment consisted of 2 mL per 45.4 kg of BW of Suppressor® (RXVeterinary; Westlake, TX) containing 50 mg of flunixin per mL injected intravenously. The VACC experimental ANC treatment consisted of 2 mL per calf of Inforce 3® (Zoetis; Florham Park, NJ) viral respiratory vaccine containing IBR-PI3-BRSV isolates administered intranasally. The NOAC experimental treatment consisted of no experimental ANC, and only an antimicrobial injection was administered at the time of BRD treatment. Calves received their respective experimental ANC at all subsequent BRD treatments.

After calves were administered their experimental ANC, they were allocated to a new home pen that previously had been randomly assigned to their respective ANC group. The calves remained in these pens for the duration of the experiment with the exception of mortalities and removals. These pens had the same dimensions and pen structure of those receiving pens previously described. A group of 4 pens (1 per ANC group) remained open until 80 head of calves (20 per experimental ANC) were allocated to those pens. The average length of time it took to fill a group of pens was 3 d and half the pens on the experiment were filled in 2 d. The date when a group of pens were closed was determined to be d 0 for that group of 4 pens.

Receiving phase data collection, calculations and statistical analysis

A shrunk BW was obtained for all animals upon arrival. Unshrunk BW were obtained at the time of initial BRD treatment and all subsequent BRD treatments. Interim BW were determined for all animals by weighing all pens and individual animals on d 28 and 56 after pens were closed. Body weights obtained at the time of BRD treatments and on d 28 and 56 are presented with a calculated 2% shrink. Individual BW and DOF were used to calculate individual ADG. Individual BW and ADG values were averaged within a pen to obtain pen means. Actual head days within a pen and total feed consumption were used to calculate DMI. Average DMI and ADG for the pen were used to calculate G:F.

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) with pen serving as the experimental unit. Contrasts were performed for the main effects and for the average of the 3 experimental ANC treatments (NSAID, VACC, and VITC) vs. the control (NOAC). Results were considered significantly different where $P \leq 0.05$. Tendencies are discussed where $0.05 > P \leq 0.10$. Data were analyzed with mortalities and removals included (deads in) and with mortalities and removals excluded (deads out). For the deads out analysis, the feed consumptions for mortalities and off experiment calves were removed from the pen at a calculated maintenance intake level. The NRC (2000) equation NEm = 0.077 Mcal/$EBW^{0.75}$ and ration NEm concentration were used to calculate maintenance DMI.

Finishing phase cattle management

After the receiving period, calves remained in their home pens and received ad libitum access to the common receiving ration (Table 1) and water for 2 to 3 additional weeks. After this additional period, (average total DOF = 86) a subset of 126 calves were allocated to a finishing experiment. Body weight at arrival was not different for the subpopulation of calves used for the finishing experiment ($P = 0.70$). For the finishing experiment, all previous experimental ANC were maintained, but calves were also allocated by the number of antimicrobial treatments administered during the receiving period for a separate experiment. This resulted in 6 pens or replications of each ANC treatment (2 pens of calves that received 1 antimicrobial treatment, 2 pens of calves that received 2 antimicrobial treatments, and 2 pens of calves that received 3 or 4 antimicrobial treatments). After the respective experiments were completed, the data were analyzed for interactions between the experimental ANC treatments and the number of antimicrobials administered. The lack of significant interactions allowed for the integrity of both experiments to be maintained.

Prior to allocation to finishing pens, all steers were administered 200 mg trenbolone acetate (TBA) and 40 mg estradiol (Revalor XS; Merck Animal Health, Summit, NJ) in the caudal aspect of the right ear per manufacturer's directions. The goal was to harvest all calves at a common physiological end point regardless of DOF, while still maintaining the integrity of the pen and shipping truck load lots. This was accomplished through the use of the ultrasound estimates, BW, and visual appraisal. Calves were harvested in 2 groups (DOF = 166 or 197). For the last 28 DOF, all steers

were fed ractopamine hydrochloride (Optaflexx 45; Elanco Animal Health, Indianapolis, IN) at 300 mg•steer^{-1}•d^{-1}.

Finishing phase pen management and diet

Finishing pens were 4.57 × 15.24 m in area with a 4.57 m long concrete bunk at the front of the pen. The pens contained a 4.57 × 4.42 m concrete pad with the remainder of the pen being soil surfaced. The pens were under partial cover, with the bunk and pad being covered by an overhang. A 76 L concrete water tank (Model J 360-F; Johnson Concrete, Hastings, NE) was shared between 2 pens and was cleaned 3 times per week throughout the experiment.

The common finishing ration was formulated to meet or exceed NRC (2000) nutrient requirements (Table 2). Adaptation to the finishing diet was accomplished using a two-ration blend method where each day the percentage of finishing diet delivered was increased by approximately 4.6% DM and the percentage of receiving diet (Table 1) delivered was decreased by approximately 4.6% DM until only the finishing diet was being fed. Following adaptation, the finishing ration was fed to all cattle twice daily at 0700 h and 1300 h in a 274-12B Roto-Mix Forage Express mixer wagon (Roto-Mix, Dodge City, KS) to the nearest 0.45 kg of that day's feed call. Ration samples were collected once per week, and dried in a forced air oven for 48 h at 60º centigrade to determine dry matter. Ration samples were composited gravimetrically and analyzed at a commercial laboratory (Servi-Tech Inc., Dodge City, KS) for nutrient composition (Table 2).

Finishing phase data collection, calculations and statistical analysis

Unshrunk BW were obtained at the time of allocation to finishing pens and at approximately 45 d intervals thereafter. All BW were shrunk a calculated 4%. Individual BW and ADG values were averaged within a pen to obtain pen mean BW and ADG. Ultrasound estimates were taken at 91 and 138 DOF. Carcass data along with lung consolidation and lung adhesion scores were obtained at the harvest facility by trained personnel from West Texas A&M University. Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) with pen serving as the experimental unit. Initially data were analyzed for an interaction between experimental ANC and the number of antimicrobials administered. Only one interaction existed (marbling score) for all variables analyzed. Due to the lack of significant interactions, the integrity of the experiment was able to be maintained, and data were subsequently analyzed on the basis of ANC only. Results were considered significantly different where ($P \leq 0.05$). Tendencies are discussed where ($0.05 > P \leq 0.10$). Contrasts were performed for the main effects and for the average of the 3 experimental ANC (NSAID, VACC, and VITC) vs. the control (NOAC). Data were analyzed with mortalities (4; 3 digestive and 1 BRD) included in the analysis (deads in). One NOAC calf, 1 VACC calf, and 2 VITC calves died during the experiment. Due to calves being harvested at varying DOF, harvest group was included in the model statement for the analysis of marbling number and the percentage of USDA Prime and Choice carcasses.

RESULTS

Receiving phase calf performance within BRD treatment intervals

Calf performance within BRD treatment intervals is presented in Table 3. The BW of calves at the time of the initial BRD treatment was not different between the 4 experimental ANC ($P = 0.75$). There was also no difference ($P = 0.75$) in the BW of calves among ANC groups at the time of second antimicrobial administration for BRD. Between the first and second BRD treatments, calves on all experimental ANC treatments lost an average of 0.71 kg per d, but there was no difference among ANC ($P = 0.92$).

By the time calves received a third antimicrobial treatment for BRD, those calves receiving NOAC had significantly heavier BWs compared to the other 3 ANC groups ($P = 0.02$). This was a result of the calves receiving NOAC gaining 0.06 kg per d between the second and third BRD treatments, while the calves receiving NSAID, VACC, and VITC lost an average of 1.81 kg during the same interval. The ADG of the NOAC calves was significantly greater than the ADG for VACC and VITC calves and tended to be greater than the ADG for NSAID calves during this time period ($P = 0.03$). There were no differences ($P = 0.94$) in BW among ANC by the time a fourth antimicrobial was administered. In addition, ADG between the third and fourth BRD antimicrobial treatments and the overall ADG between the first and fourth BRD antimicrobial treatments was not different between ANC groups ($P = 0.95$ and $P = 0.78$, respectively). When the average of the 3 experimental ANC (NSAID, VACC, and VITC) was contrasted against NOAC, BW at the time of the third BRD treatment, and the ADG

between the second and third BRD treatments were greater for calves receiving NOAC as expected based on the mean separations with the overall model ($P \leq 0.01$).

Retreatment percentages and BRD retreatment intervals

The data for BRD retreatment percentages and the length of time between BRD treatments are also presented in Table 3. Calves that received VACC tended to require a second antimicrobial treatment for BRD less frequently than calves receiving either NOAC or NSAID ($P = 0.10$). However, when comparing ANC means, there was no difference between calves receiving VACC or VITC and VITC was also not different from NOAC or NSAID ($P \geq 0.17$). Calves that received NSAID or VITC tended to require a third antimicrobial treatment for BRD less often than calves receiving NOAC ($P = 0.09$). When comparing individual ANC means, NSAID was not different from VACC or VITC and NOAC was not different from VACC ($P \geq 0.13$). When the average of NSAID, VACC, and VITC was contrasted against NOAC, calves receiving NOAC did receive a third antimicrobial treatment more often ($P = 0.04$). There were no significant differences between ANC for the length of time until the first, second, third, or fourth BRD antimicrobial treatments ($P \geq 0.21$). However, it should be noted that calves receiving NOAC received both their third and fourth BRD treatments at numerically greater days after arrival. This numerical difference resulted in a significant increase ($P = 0.05$) in the length of time until the third BRD treatment when the average of the 3 experimental ANC was contrasted against NOAC.

Subjective clinical severity scores and rectal temperatures

The data for subjective clinical severity scores and rectal temperatures at the time of BRD treatment are contained in Table 4. There was a tendency ($P = 0.10$) for calves receiving VACC to have lower clinical severity scores than calves receiving NOAC or VITC at the time of initial BRD treatment. Clinical severity scores for calves receiving NSAID were not different from any other ANC at the time of initial BRD treatment ($P > 0.10$). At the time of the second antimicrobial treatment, there were no differences ($P = 0.27$) in clinical severity scores among ANC groups. At the time of the third BRD treatment, calves receiving NOAC had lower ($P = 0.05$) clinical severity scores than calves receiving VACC or VITC, while the severity scores of calves receiving NSAID were not different ($P > 0.10$) from any of the other ANC. Upon receiving a fourth antimicrobial treatment, there was again no difference among any of the ANC groups ($P = 0.79$). There were no differences in rectal temperatures among any of the ANC at any BRD treatment event throughout the experiment ($P \geq 0.21$).

Removals and mortality attributed to BRD

Data concerning mortality attributed to BRD and removal of calves from the experiment are reported in Table 4 as well. There were no differences ($P = 0.55$) in the mortality percentages among the ANC groups, while the calves receiving NOAC did exhibit numerically decreased mortality compared to the other ANC groups. There were also no differences in removals among the ANC groups ($P = 0.13$). However, when the average of the 3 ANC was contrasted against the NOAC group, calves receiving NOAC

were removed from the experiment at a greater rate ($P = 0.05$). The numerical decrease in mortality and increase in removals for calves receiving NOAC resulted in no difference ($P = 0.98$) in the combined percentage of calves that were not able to complete the experiment due to BRD-related mortality or removal from the home pen as a result of lameness or the inability to compete.

Receiving phase performance with mortalities and removals included

The performance data including mortalities and removals (deads in data) is included in Table 5. No differences ($P \geq 0.23$) existed between any of the individual ANC for any of the performance or efficiency data when evaluated on a deads in basis. However, when the average of NSAID, VACC, and VITC was contrasted against NOAC, there was a tendency ($P \leq 0.07$) for calves receiving NOAC to have heavier BW on d 28 and d 56 of the experiment. There was also a tendency ($P = 0.07$) for NOAC calves to have greater DMI from d 28 to d 56 when compared to the average of the other 3 ANC.

Receiving phase performance with mortalities and removals excluded

The performance data with mortalities and removals excluded (deads out data) is found in Table 6. When the mortalities and removals were excluded from the analysis, there was a tendency ($P = 0.07$) for calves receiving NOAC to have heavier BW on d 28. When the average of NSAID, VACC, and VITC was contrasted against NOAC, the same tendencies observed in the deads in data for calves receiving NOAC to have heavier BW

(P = 0.06) on d 56 and greater DMI (P = 0.06) from d 28 to d 56 were observed in the deads out data. In addition, calves receiving NOAC tended to have greater ADG (P = 0.10) and DMI (P = 0.08) from the time of the first BRD treatment to d 28 when comparing NOAC to the 3 other ANC. Finally, when contrasting the average of NSAID, VACC, and VITC against NOAC, calves that received NOAC had greater DMI (P = 0.05) over the length of the receiving period.

Finishing performance, efficiency, lung scores, and carcass characteristics

No ANC differences were observed in the overall model for any of the variables analyzed in the finishing experiment ($P \geq 0.26$). When average of the NSAID, VACC, and VITC was contrasted against NOAC, a tendency was observed for improved efficiency over a single interval. During the first 45 d of the finishing period, calves receiving NOAC demonstrated improved G:F. No other differences were observed for performance or efficiency during finishing for the contrast of the 3 ANC versus NOAC. There were also no differences observed in ultrasound measurements, lung scores, or carcass characteristics among any of the ANC ($P \geq 0.26$). This was also true for the contrast of the 3 ANC versus NOAC ($P \geq 0.14$).

DISCUSSION

The goal of ANC administration is to focus on the overall health of the calf or improving the calf's clinical signs rather than treating the invading pathogens responsible

for the illness as would be the case with antimicrobials. The use of vaccines as an ANC would be one of the few exceptions, in that they attempt boost the immune system by providing a pathogen-specific antibody response. The improvement in the calf's response resulting from ANC administration can be theoretically accomplished through a variety of mechanisms and these mechanisms can subsequently be divided into 3 broad classes. The 3 classes of ANC can potentially impact overall calf health or calf's clinical signs by: relieving the harmful effects of inflammation, reducing histamine activity, or improving immune system function to aid in the defense against BRD pathogens (Apley, 1994).

The objective of this experiment was to evaluate the effects of 3 of the most commonly used ANC that also had significantly different intended effects and modes of action in combination with an antimicrobial within a common population of newly received, high-risk calves that were treated for BRD. It has been established that there is widespread use of ANC within the feedlot industry through surveys conducted by NAHMS (2001), Terrell et al. (2011), and NAHMS (2013). However, there is limited published research concerning the effectiveness of the various forms of ANC and there is no published research to the author's knowledge comparing multiple commonly administered ANC within a single experiment or across similar groups of calves. According to the most recent survey detailing ANC use conducted by NAHMS (2013), the 3 ANC used in this experiment were the 3 most frequent forms of ANC given on a percentage of cattle treated basis in commercial feedlots.

In addition to comparing multiple ANC within a single experiment across a similar group of calves, the present experiment aimed to observe the effects of ANC in a group of newly-received, high-risk calves originating from multiple livestock auctions. If

only a small incidence of BRD occurs within the experiment, any observed responses to the ANC could be viewed as less valid. The calves in this experiment experienced a significant natural immune challenge resulting in a first treatment morbidity of 66.5% and mortality attributed to BRD of 13.2% when considering the initial population of 516 head. The final goal of the experiment was to make sure that calves were experiencing a natural BRD challenge prior to receiving an ANC. In the present experiment, only calves that met antimicrobial treatment criteria were enrolled in the experiment to ensure that the effects observed were only evaluated in morbid calves.

It is difficult to compare the results of this experiment to others published in the literature. When reviewing the published research concerning the use of these 3 specific ANC in calves treated for BRD, it becomes obvious that this area of research is deficient and has produced very inconsistent results to date. Much of the research that exists consists of the evaluation of a single ANC. These studies may or may not include a negative control, and they are often conducted on a small number of animals and not well replicated. The use of NSAID as an ANC for BRD have been researched to a greater extent than the use of any of the other ANC and NSAID seem to provide the most consistent response of all the ANC that have been examined. There are a couple recent reviews concerning the use of ANC that bear mentioning. These ANC reviews essentially become reviews of anti-inflammatory drugs and more specifically NSAID due to the lack of published controlled field experiments for other ANC.

The responses observed to the 3 ANC used in this experiment were largely negligible. For all of the variables measured, positive responses to ANC administration were only observed on 2 occasions during the receiving period. The first being that calves

receiving VACC tended to require a second antimicrobial treatment for BRD less frequently than calves receiving NOAC. The second being that calves receiving either NSAID or VITC tended to require a third BRD treatment less often when compared to calves receiving NOAC. The tendencies for improvements in BRD treatment percentages in the case of these 2 variables could potentially be the result of numerical differences in the intervals between BRD treatments, the intervals from arrival to mortality, and the intervals from first BRD treatment to mortality between the experimental treatments.

There were no differences in the time intervals for calves receiving from 1 to 4 antimicrobial treatments for BRD existed among the individual ANC groups. However, when the average of the 3 experimental ANC was contrasted against NOAC, a significant increase in the length of time until the third antimicrobial was administered was observed for calves receiving NOAC. In addition, calves that received the 3 experimental ANC all received their final BRD treatment in numerically fewer days than calves receiving NOAC. Of calves that suffered BRD attributed mortality, the calves receiving NOAC lived numerically longer from the time of arrival and lived at least 4 d longer after receiving their initial BRD treatment than any of the other ANC.

Calves receiving NSAID were treated for BRD the final time 3 d sooner than calves receiving NOAC. In addition, calves that suffered mortality that had received NSAID did on average 19 d after receiving an initial BRD treatment and 25 d after arrival. This was numerically less than the 23 d after receiving a BRD treatment and 26 d after arrival for calves receiving NOAC. Calves receiving VACC received their final BRD treatment an average of 6 d sooner than calves receiving NOAC and VACC calves that died, did so an average of 15 d after receiving their first antimicrobial treatment 21 d

after arrival. Calves that received VITC were treated for BRD the final time an average of 5 d sooner than calves receiving NOAC and VITC calves that died, did so an average of 19 d after receiving their first antimicrobial treatment 25 d after arrival. These numerical differences in intervals between BRD treatments, intervals from arrival to mortality, and intervals from first BRD treatment to mortality may have simply caused for calves on the NOAC treatment to receive numerically more antimicrobials over certain treatment intervals. Total antimicrobials administered were not different among ANC groups and ranged from 137 to 150.

For many of the variables measured, calves receiving NOAC demonstrated statistically or numerically positive responses in relation to the other ANC. This was especially true when average of the NSAID, VACC, and VITC was contrasted against NOAC. Regardless of one's opinion of ANC use, the improvements observed in cattle receiving NOAC were somewhat surprising. Some of these advantages for NOAC are of clinical importance, while others may serve to simply advocate that there is little justification for ANC use in high-risk calves suffering from an extreme natural immune challenge.

Calves receiving NOAC had heavier BW at the time of third BRD treatment and improved ADG between the second and third BRD treatments compared to the other ANC. These differences must be critically evaluated as calves receiving NOAC received their third BRD treatment approximately a week after calves receiving NSAID, VACC, or VITC received their third BRD treatment. As a result, it would be expected that the calves receiving NOAC would weigh more at this time. However, the fact that calves receiving NOAC actually gained BW between the second and third BRD treatments,

while the calves receiving the other 3 ANC all lost BW during the same BRD treatment interval should not be disregarded. In addition, the subjective clinical scores at the time of third BRD treatment were significantly lower for calves receiving NOAC compared to calves receiving VACC or VITC. While this is a subjective measurement, it suggests that calves receiving NOAC exhibited less visible depression and had improved appetites and respiratory signs at this time compared to the other ANC.

The importance of the results previously discussed and the perceived improvement in calves receiving NOAC could certainly be argued. This argument can be made with variables that are not measured over equal intervals for all ANC or with variables that are by nature subjective. In order to circumvent this, performance and efficiency were also measured across fixed intervals that were the same for all pens and ANC. These data were also presented both with mortalities and removals included and mortalities and removals excluded due to the numerical differences in intervals from the time of initial BRD treatment until mortality previously discussed among the ANC. The results of these data provide perhaps the most compelling evidence in favor of NOAC compared to the other 3 ANC. When mortalities and removals were included in the analysis, there are no differences between any of the individual ANC for any of the performance and efficiency data when evaluated. However, when the average of NSAID, VACC, and VITC was contrasted against NOAC, there was a tendency for calves receiving NOAC to have heavier BW on d 28 and d 56 of the experiment greater DMI from d 28 to d 56.

When mortalities and removals were excluded in the analysis the results favoring the use of NOAC compared to the other 3 ANC were magnified. This difference resulted

primarily from removing any of the differences in intervals from the time of initial BRD treatment until mortality previously discussed. Only calves that received an ANC and completed the 56 d receiving experiment were included in this analysis. With the mortalities and removals excluded, the calves receiving NOAC tended to have heavier BW on d 28 in the overall model. When the average of the 3 ANC was contrasted against NOAC, the same tendencies observed in the deads in data for calves receiving NOAC to have heavier BW on d 56 and greater DMI from d 28 to d 56 were still observed. In addition calves receiving NOAC tended to have greater ADG and DMI from the time of the first BRD treatment to d 28 and had greater DMI over the length of the entire experiment when compared to the 3 other ANC.

The expectation would be that any performance differences observed in the 56 d receiving period would likely disappear by the time calves were fed for an additional 166 or 197 d. It would be also expected that any ANC treatment administered within 30 d of arrival would not significantly impact finishing performance or carcass characteristics. This was the case and at the end of the finishing period, no ANC differences were observed in the overall model for any of variables analyzed. When average of the NSAID, VACC, and VITC was contrasted against NOAC for the finishing data, only 1 tendency was observed for a single 45 d interval with calves receiving NOAC demonstrating improved G:F for the first 45 d of the finishing period. There were no other differences observed for performance, efficiency, ultrasound measurements, lung scores, or carcass characteristics for the 3 ANC contrasted against NOAC.

When reviewing the published research concerning the use of these 3 specific ANC in calves treated for BRD, very few controlled well-replicated studies are found

except in the case of NSAID. There are also a few good review papers concerning ANC in calves treated for BRD. A recent review of the research concerning ANC conducted by Apley (2010) chose to focus on the published data concerning the use of anti-inflammatory drugs as an ANC for BRD. In this review, multiple studies that demonstrated some beneficial effect of NSAID as an ANC are cited with the most typical response observed being a reduction in rectal temperature in calves treated with an NSAID (Apley, 2010). Apley (2010) determined that other clinical responses to NSAID as an ANC in the research were inconsistent.

Francoz et al. (2012) also conducted a comprehensive review of the literature concerning ANC use. For an experiment to be included in the review, the experiment must have involved the treatment of naturally occurring BRD with antimicrobials and with and without at least 1 ANC. As a result of this criteria, experimental models, BRD prevention studies, studies evaluating an ANC without a control group, or studies including different antimicrobials within the treatment groups were not included. When studies not meeting these stipulations were removed from consideration, it resulted in only 15 articles meeting the criteria (Francoz et al., 2012). Of those 15 experiments, 14 dealt with anti-inflammatory drugs (12 NSAID experiments, 1 steroidal anti-inflammatory drug experiment, and 1 experiment containing both a steroidal anti-inflammatory drug and NSAID) and 1 dealt with immune-modulators (Francoz et al., 2012).

Upon reviewing the data related to the use of NSAID as an ANC to BRD, the authors concluded that NSAID caused a more rapid decrease in rectal temperature of calves, but did not in any way benefit clinical signs, mortality, or calf performance

(Francoz et al., 2012). The authors did mention that published data were lacking and too inconsistent to conclusively determine the effects on calf performance or mortality when NSAID were used as an ANC for BRD (Francoz et al., 2012). Francoz et al. (2012) suggested that NSAID have the potential to decrease lung lesions at slaughter, but noted that lung consolidation was only evaluated in 2 of the studies (Francoz et al., 2012). Based on the results of this review, it could be argued that the reduction in rectal temperature and the potential to reduced lung lesions resulting from NSAID administration may be important from an animal welfare perspective (Francoz et al., 2012). However, it would be extremely difficult to justify the economics of NSAID use based on inconsistent improvements in clinical signs and the lack of performance benefits seen in calves receiving a NSAID as ANC for BRD (Francoz et al., 2012).

When reviewing individual research experiments concerning ANC use for calves with BRD, several studies using NSAID were found. Within this literature, the most consistent response observed when a NSAID is used as an ANC for BRD calves is a more rapid reduction in rectal temperature after NSAID administration. However this response is often short-lived and typically there is no difference in rectal temperature when measured at the end of the evaluation period. Many of these studies involving the use of NSAID such as the one conducted by Hellwig et al. (2000) examine the use of a single NSAID antimicrobial combination as an ANC for BRD calves. Hellwig et al. (2000) randomly assigned calves with clinical signs of BRD either a flunixin meglumine and tilmicosin phosphate treatment group or tilmicosin phosphate only treatment group. The calves receiving the tilmicosin phosphate and flunixin meglumine combination had a higher percentage of treatment successes (88% vs. 61%) and a lower combined

percentage of treatment failures and BRD relapses (5% vs. 38%) than those calves receiving only tilmicosin phosphate (Hellwig et al., 2000). In addition, Hellwig et al. (2000) found that the treatment cost for calves receiving the combination of the NSAID and antimicrobial tended to be less than the treatment cost for calves receiving the antimicrobial alone. Hellwig et al. (2000) did not mention or report rectal temperate changes for calves in this experiment. However, based on the clinical results, Hellwig et al. (2000) concluded that the flunixin meglumine tilmicosin phosphate combination was more successful for treating BRD than tilmicosin phosphate alone.

When reviewing the published data, the most consistent response to NSAID administration is a more rapid decrease in the rectal temperature of calves compared to a control or other treatments. In the current experiment, the administration of NSAID as an ANC did not decrease the rectal temperature of calves at subsequent BRD. Granted, rectal temperature was only measured in calves that met treatment criteria at the time of BRD treatment. It is quite possible that calves receiving NSAID could have demonstrated improvements in the reduction of rectal temperature if rectal temperature was obtained a few hours after NSAID administration. This experiment attempted to mimic commercial production settings as much as possible and pulling multiple calves up at short intervals to obtain hourly rectal temperatures was not a priority of the current experiment. That is not to say that changes in rectal temperature were unimportant in the current experiment, but rather the concern was with prolonged or sustained changes in rectal temperature over the course of time. It should also be noted, however, that the interval between BRD treatments was relatively short for many calves, and this was especially the case with the first and second BRD treatments. As such, if calves receiving NSAID demonstrated any

prolonged improvement in rectal temperatures it could have been detected and this was not the case.

It has also been suggested in the research that NSAID have the potential to decrease lung lesions at harvest in treated calves. Our data did not support these findings. In the present experiment, the administration of NSAID as an ANC did not reduce lung consolidation or adhesion at harvest. Calves receiving NSAID actually had numerically increased lung consolidation compared to calves receiving NOAC. Conversely, calves receiving NSAID had a slight numerical decrease in lung adhesion scores when compared to calves receiving NOAC.

In regard to the use of other ANC, reviewers have not observed reasons to justify their use. In the review conducted by Apley (2010), the author concluded that no data published at the current time supported the use of vaccines, vitamin C, or other ANC for BRD. Similar to Apley (2010), Francoz et al. (2012) concluded that there was no published data that currently supported the use of vaccines, vitamin C, or other ANC for BRD. This was primarily a result of Francoz et al. (2012) only being able to evaluate 1 additional ANC experiment outside of the anti-inflammatory drug experiments included in the review.

When reviewing the research, no experiment utilizing VACC as an ANC for BRD was found. This is extremely surprising given that NAHMS (2013) reported that a respiratory vaccine was used as a component of the initial BRD treatment program in 39.3% of feedlots, and that 48.5% of cattle received a respiratory vaccine as part of initial BRD treatment. It is well established that the vaccination of healthy calves for respiratory

pathogens is important for preventing BRD and maintaining optimal calf health. However, there is little justification for the vaccination of high-risk calves at arrival to the feedlot even though it is a widespread and accepted management practice (Edwards et al., 2010; Taylor et al., 2010). Some epidemiologic studies have actually suggested that the vaccinating of calves upon arrival to the feedlot for respiratory viruses actually leads to increased BRD incidence (Taylor et al., 2010). This data is somewhat confounded as preconditioned low-risk calves would be less likely to be vaccinated on arrival. As a result the increased BRD incidence observed in calves vaccinated upon arrival to the feedlot is not necessarily a causal response. In addition, most published vaccine research has focused on comparing different multivalent vaccines and the majority of vaccine studies do not include a non-vaccinated negative control. As a result, it is extremely difficult to determine if vaccination of high-risk calves at or shortly after arrival aids in the prevention of BRD or if it actually may be a detrimental.

Because no published research was found evaluating the use of VACC as an ANC for BRD, comparisons to the data in the present experiment are not possible. It is interesting to note that while not significant, calves that died after receiving VACC in the current experiment did so numerically sooner than any other ANC treatment. This was true regardless if the interval was measured from the time of arrival to the feedlot, or from the time of the first BRD treatment. This ultimately resulted in the VACC group receiving the fewest total antimicrobial treatments (137 doses) for BRD. However, this was only 8 to 13 total antimicrobial treatments less than the other 3 ANC groups.

When reviewing individual research experiments concerning ANC use for calves with BRD, a few experiments utilizing VITC as an ANC for BRD were found. In an

experiment conducted by Cusack et al. (2005), the authors examined the effects of injectable vitamin C given at the time of BRD treatment on subsequent cattle health. At the time of BRD antimicrobial treatment, 176 cattle were alternately administered injectable vitamin C (5 g per head) or not injected (Cusack et al., 2005). Fewer of the cattle injected with vitamin C at the time of BRD treatment died later in the experiment compared to those cattle that were not injected (11% vs. 23%, respectively; Cusack et al., 2005). The results led the authors to conclude that mortality rate in cattle with BRD may be decreased by administering injectable vitamin C at the time of antimicrobial administration.

Urban-Chmiel et al. (2011) evaluated the effects of vitamin E and vitamin C on the development of inflammation processes and selected defense mechanisms against MH-induced infections. Calves were assigned to 3 treatments and received subcutaneous injections of vitamin E (750 IU), vitamin C (2.5 g per head), or no vitamin injection (Urban-Chmiel et al., 2011). Calves receiving either of the vitamin injections demonstrated a difference in the sensitivity of leukocytes to the cytotoxic effect of LKT when compared to the control group (Urban-Chmiel et al., 2011). There were no differences observed in the percentage of cells sensitive to LKT between the calves receiving vitamin E and those receiving vitamin C (Urban-Chmiel et al., 2011). The authors concluded that both vitamin E and vitamin C exerted a protective effect on leukocytes aiding in the defense against MH virulence factors when administered by injection (Urban-Chmiel et al., 2011). Urban-Chmiel et al. (2011) also suggested that these vitamins can be used to support the treatment of BRD in cattle following transport.

In the current experiment, VITC had no major effects on morbidity or mortality. In fact calves administered VITC had numerically higher mortality and combined mortality and removals when compared to NOAC. While leukocyte sensitivity was not measured in the current experiment as with Urban-Chmiel et al. (2011), there was no evidence to support the use of VITC as an ANC for BRD as those authors suggested. Calves that died after receiving VITC in the current experiment did so at the same time interval as calves receiving NSAID, but numerically sooner than calves receiving NOAC. This was true regardless if the interval was measured from the time of arrival to the feedlot or from the time of the first BRD treatment.

Conclusions

There is widespread use of ANC for BRD in commercial feedlots as evidenced by multiple published surveys. The goal of ANC therapy is to improve the response to a BRD challenge in calves treated with antimicrobials, and there is potential justification for ANC use based on the modes of actions of various ANC. However, published research has yet to prove that ANC other than NSAID are effective in commercial settings in response to a natural BRD challenge. While NSAID have demonstrated the ability to reduce rectal temperatures in treated calves, the response is usually short-lived and not evident at the end of the evaluation period. It has also been suggested that NSAID reduce lung consolidation at harvest, but this has only been demonstrated in a few experiments. For all of the variables measured in the current experiment, positive responses to ANC administration were only observed on 2 occasions during the receiving

period. However, there were numerous positive responses to NOAC administration observed for multiple variables in the receiving period. The lack of positive responses observed to the 3 ANC used in this experiment combined with the improvements observed in calves receiving NOAC lead us to conclude that the use of NSAID, VACC, and VITC as an ANC for BRD does not appear to be warranted because NSAID, VACC, and VITC do not appear to positively impact clinical health of calves treated for BRD. In addition, this experiment suggests that ANC use could potentially be detrimental to calf performance during the receiving period if administered to calves experiencing a severe natural immune challenge.

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Table 3-1. Composition of common receiving diet[1]

Ingredient (%)[1]	
Wet corn gluten feed[2]	48.8
Grain sorghum hay	30.0
Dry-rolled corn	15.0
Dry supplement B-273[3]	5.2
Nutrient Composition[1,4]	
NE_m, Mcal/kg	1.69
NE_g, Mcal/kg	1.07
TDN, %	71.60
Crude protein, %	17.40
Crude fat, %	1.90
NDF, %	39.90
ADF, %	21.40
Calcium, %	0.68
Phosphorus, %	0.67
Magnesium, %	0.36
Potassium, %	1.15
Sulfur, %	0.27

[1]All values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Dry supplement B-273 was formulated to contain (% DM basis): 38.46% ground corn, 30.36% limestone, 21.04% wheat midds, 6.92% urea, 1.03% magnesium oxide, 0.618% zinc sulfate, 0.38% salt, 0.119% copper sulfate, 0.116% manganese oxide, 0.05% selenium premix (contained 0.6% Se), 0.311% vitamin A (30,000 IU/g), 0.085% vitamin E (500 IU/g), 0.317% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.195% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[4]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Table 3-2. Composition of common finishing diet[1]

Ingredient (%)[1]	
Dry-rolled corn	48.14
Wet corn gluten feed[2]	15.00
Dried distillers grains plus solubles	15.00
Prairie hay	9.00
Liquid supplement[3]	6.54
Dry supplement B-273[4]	3.12
Dry supplement B-283[5]	3.20
Nutrient Composition[1,6]	
NE_m, Mcal/kg	2.23
NE_g, Mcal/kg	1.54
TDN, %	89.55
Crude protein, %	18.85
Crude fat, %	5.00
NDF, %	22.35
ADF, %	10.40
Calcium, %	0.96
Phosphorus, %	0.52
Magnesium, %	0.28
Potassium, %	1.03
Sulfur, %	0.31

[1]All values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Synergy 19-14 (Westway Feed Products; New Orleans, LA).
[4]Dry supplement B-273 was formulated to contain (% DM basis): 38.46% ground corn, 30.36% limestone, 21.04% wheat midds, 6.92% urea, 1.03% magnesium oxide, 0.618% zinc sulfate, 0.38% salt, 0.119% copper sulfate, 0.116% manganese oxide, 0.05% selenium premix (contained 0.6% Se), 0.311% vitamin A (30,000 IU/g), 0.085% vitamin E (500 IU/g), 0.317% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.195% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[5]Dry supplement B-283 was formulated to contain (% DM basis): 40.47% limestone, 36.26% ground corn, 19.73% wheat midds, 2.47% salt, 0.312% zinc sulfate, 0.071% copper sulfate, 0.064% manganese oxide, 0.029% selenium premix (contained 0.6% Se), 0.202% vitamin A (30,000 IU/g), 0.056% vitamin E (500 IU/g), 0.207% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.127% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[6]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Table 3-3. The effects of ancillary therapies used in combination with an antimicrobial on performance between bovine respiratory disease treatments, retreatment percentages, and retreatment intervals

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Treatment BW[3], kg							
1st treatment	214	213	211	212	2.60	0.75	0.38
2nd treatment	217	208	212	210	7.14	0.49	0.17
3rd treatment	221^{a}	198^{b}	195^{b}	198^{b}	8.05	0.02	<0.01
4th treatment	188	181	185	190	12.9	0.94	0.84
Average daily gain[4], kg							
1st to 2nd	-0.50	-0.74	-0.68	-0.91	0.60	0.92	0.58
2nd to 3rd	0.06^{ay}	-1.36^{abz}	-2.10^{b}	-1.60^{b}	0.55	0.03	0.01
3rd to 4th	-0.52	-0.34	-0.65	-0.76	0.65	0.95	0.93
1st to 4th	-0.86	-0.85	-1.17	-1.18	0.39	0.78	0.57
Retreatments[5], %							
2nd treatment	48.8^{a}	51.3^{a}	37.5^{b}	43.8^{ab}	10.9	0.10	0.30
3rd treatment	55.4^{y}	39.0^{z}	49.4^{yz}	40.7^{z}	12.3	0.09	0.04
4th treatment	24.4	25.7	36.5	50.7	16.3	0.44	0.37
3rd treatment of 1st	30.0	25.0	21.3	22.5	9.18	0.26	0.08
4th treatment of 1st	8.75	8.75	12.5	15.0	5.42	0.50	0.41
Time to treatment[6], d							
1st treatment	7.60	7.36	7.39	7.36	1.34	0.78	0.33
2nd treatment	11.6	9.35	11.3	8.53	2.38	0.70	0.47
3rd treatment	20.4	13.2	14.3	13.3	2.74	0.21	0.05
4th treatment	25.4	22.5	19.2	20.6	4.04	0.65	0.29

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ANC; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the combined average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Treatment BW was the BW in kg with a calculated 2% shrink at the time of BRD treatment.

[4]Treatment ADG was calculated from the shrunk (2%) BW in kg and individual DOF between the BRD treatments.

[5]Retreatment percentages were calculated by taking the number of calves treated for BRD divided by the number of calves treated for BRD the previous time in the case of 2nd treatment, 3rd treatment, and 4th treatment, or by taking the number of calves treated for BRD divided by the number of calves treated for BRD initially in the case of 3rd treatment of 1st and 4th treatment of 1st.

[6]Average length of time in days from arrival until the BRD treatment.

Table 3-4. The effects of ancillary therapies used in combination with an antimicrobial on clinical severity scores, rectal temperatures, and mortalities and removals

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Severity score[3]							
1st treatment	1.14[y]	1.13[abyz]	1.04[bz]	1.18[a]	0.04	0.10	0.55
2nd treatment	2.43	2.88	2.43	2.68	0.29	0.27	0.28
3rd treatment	2.43[a]	2.92[ab]	3.24[b]	3.14[b]	0.20	0.05	0.01
4th treatment	2.94	3.17	3.20	3.06	0.23	0.79	0.41
Rectal temperature[4], °C							
1st treatment	40.7	40.7	40.8	40.8	0.07	0.21	0.19
2nd treatment	40.4	40.3	40.5	40.3	0.16	0.80	0.94
3rd treatment	40.0	39.7	40.0	39.8	0.36	0.83	0.49
4th treatment	39.4	39.5	39.0	39.0	0.35	0.56	0.47
Off experiment, %							
Mortality	16.3	22.5	20.0	22.5	8.86	0.55	0.20
Removals[5]	6.25	0.00	3.75	1.25	1.80	0.13	0.05
Combined off	22.5	22.5	23.8	23.8	9.67	0.98	0.82

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ANC; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the combined average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Subjective clinical severity score (1 = mild clinical signs, 2 = moderate clinical signs, 3 = severe clinical signs, and 4 = extreme clinical signs or a moribund animal) assigned by trained personnel. For a calf to be assigned a clinical severity score of 4, the calf was not be able to rise, or had extreme difficulty standing, walking, or breathing.

[4]Rectal temperature in degrees Celsius at the time of BRD treatment.

[5]Percentage of calves removed from the experiment due to lameness or the inability to compete in the home pen (includes surviving chronic BRD cases).

[6]Combined percentage of mortalities and removals for the experiment.

Table 3-5. The effects of ancillary therapies used in combination with an antimicrobial on receiving performance with mortalities and removals included

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Body weight[3], kg							
1st treatment	214	213	211	212	2.60	0.75	0.38
d 28	250	243	244	244	3.36	0.26	0.07
d 56	288	279	280	280	4.03	0.25	0.06
Average daily gain[4], kg							
1st treatment – d 28	1.19	1.03	1.15	1.04	0.09	0.40	0.21
d 28 – d 56	1.25	1.20	1.27	1.22	0.07	0.93	0.85
1st treatment – d 56	1.27	1.16	1.21	1.17	0.06	0.47	0.17
Dry matter intake[5], kg							
1st treatment – d 28	5.04	4.57	4.80	4.82	0.54	0.40	0.17
d 28 – d 56	7.95	7.29	7.58	7.48	0.24	0.23	0.07
1st treatment – d 56	6.34	5.77	6.04	6.05	0.41	0.32	0.13
Gain:Feed[6]							
1st treatment – d 28	0.238	0.232	0.246	0.219	0.02	0.72	0.79
d 28 – d 56	0.157	0.165	0.167	0.164	0.01	0.75	0.32
1st treatment – d 56	0.200	0.205	0.202	0.196	0.01	0.93	0.95

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ANC; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the combined average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Treatment BW was the BW in kg with a calculated 2% shrink.

[4]Treatment ADG was calculated from the shrunk (2%) BW in kg and DOF between the time periods.

[5]Treatment DMI was calculated by taking DMI in kg for a pen for the period shown divided by the actual number of head days within each pen including mortalities and removals (deads in).

[6]Treatment G:F was calculated by taking the pen ADG in kg divided by the pen average DMI in kg for the time periods.

Table 3-6. The effects of ancillary therapies used in combination with an antimicrobial on receiving performance with mortalities and removals excluded

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Body weight[3], kg							
1st treatment	216	212	213	213	2.91	0.73	0.31
d 28	253[a]	245[b]	245[b]	246[b]	2.80	0.07	0.01
d 56	288	279	281	280	4.03	0.25	0.06
Average daily gain[4], kg							
1st treatment – d 28	1.28	1.12	1.15	1.12	0.08	0.39	0.10
d 28 – d 56	1.25	1.20	1.27	1.22	0.07	0.93	0.85
1st treatment – d 56	1.27	1.16	1.21	1.17	0.06	0.47	0.17
Dry matter intake[5], kg							
1st treatment – d 28	5.43	4.76	5.04	5.14	0.46	0.19	0.08
d 28 – d 56	8.05	7.37	7.58	7.66	0.22	0.21	0.06
1st treatment – d 56	6.70	6.03	6.28	6.37	0.31	0.15	0.05
Gain:Feed[6]							
1st treatment – d 28	0.238	0.245	0.233	0.220	0.02	0.81	0.81
d 28 – d 56	0.155	0.164	0.167	0.160	0.01	0.69	0.33
1st treatment – d 56	0.189	0.194	0.193	0.185	0.01	0.87	0.84

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ancillary therapy; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the combined average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Treatment BW was the BW in kg with a calculated 2% shrink.

[4]Treatment ADG was calculated from the shrunk (2%) BW in kg and DOF between the time periods.

[5]Treatment DMI was calculated by taking DMI in kg for a pen for the period shown divided by the actual number of head days within each pen excluding mortalities and removals (deads out). Mortalities and removals were backed out of the pen at a calculated maintenance DMI (NEm= 0.077 Mcal/$EBW^{0.75}$).

[6]Treatment G:F was calculated by taking the pen ADG in kg divided by the pen average DMI in kg for the time periods.

Table 3-7. The effects of ancillary therapies used in combination with an antimicrobial on subsequent finishing performance and efficiency of crossbred steers

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Days on feed[3], d	182	182	192	182	6.54	0.61	0.65
Body weight[4], kg							
Initial	292	288	281	287	12.7	0.95	0.67
d 45	372	367	359	363	13.8	0.92	0.59
d 91	440	432	423	424	12.4	0.73	0.34
d 138	503	498	489	494	11.5	0.85	0.51
Final	563	560	562	560	7.09	0.99	0.83
Average daily gain[5], kg							
Initial - d 45	1.81	1.76	1.73	1.69	0.06	0.52	0.22
d 46 - d 91	1.44	1.41	1.39	1.32	0.08	0.75	0.51
d 92 - d 138	1.33	1.41	1.42	1.50	0.06	0.34	0.14
d 139 - final	1.44	1.54	1.38	1.57	0.11	0.60	0.63
Initial - final	1.49	1.51	1.47	1.51	0.04	0.91	0.88
Dry matter intake[6], kg							
Initial - d 45	8.57	8.65	8.73	8.58	0.23	0.96	0.77
d 46 - d 91	9.71	9.75	10.1	10.1	0.33	0.73	0.47
d 92 - d 138	10.1	9.78	10.2	10.5	0.31	0.48	0.86
d 139 - final	10.2	9.87	9.91	10.4	0.30	0.60	0.65
Initial - final	9.63	9.49	9.74	9.82	0.23	0.76	0.84
Gain:feed[7]							
Initial - d 45	0.212	0.203	0.198	0.197	0.01	0.26	0.07
d 46 - d 91	0.149	0.145	0.139	0.130	0.01	0.31	0.22
d 92 - d 138	0.132	0.145	0.140	0.144	0.01	0.49	0.16
d 139 - final	0.140	0.155	0.140	0.151	0.01	0.54	0.40
Initial - final	0.155	0.159	0.151	0.154	<0.01	0.61	0.98

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ancillary therapy; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Average of days on feed (DOF) for all pens within an experimental treatment.

[4]Treatment BW was the BW in kg with a calculated 4% shrink.

[5]Treatment ADG was calculated from the shrunk (4%) BW in kg and DOF between the time periods.

[6]Treatment DMI was calculated by taking DMI in kg for a pen for the period shown divided by the actual number of head days within each pen.

[7]Treatment G:F was calculated by taking the pen ADG in kg divided by the pen average DMI in kg for the time periods.

Table 3-8. The effects of ancillary therapies used in combination with an antimicrobial on ultrasound estimates, lung scores, and carcass characteristics of crossbred steers

	Experimental ancillary treatment[1]					*P*-value[2]	
Variable	NOAC	NSAID	VACC	VITC	Pooled SEM	Overall *P*-value	Ancillary vs. NOAC
Ultrasound estimates[3]							
d 91 REA, cm^2	80.3	79.2	75.1	78.4	2.51	0.50	0.36
d 91 12^{th}-rib fat, cm	0.81	0.76	0.81	0.75	0.05	0.74	0.53
d 91 IMF	4.21	4.15	4.50	4.14	0.18	0.47	0.81
d 138 REA, cm^2	87.6	87.4	82.2	86.7	2.20	0.29	0.40
d 138 12^{th}-rib fat, cm	0.92	0.90	0.93	0.91	0.07	0.99	0.98
d 138 IMF	4.46	4.18	4.55	4.16	0.19	0.41	0.47
Lung scores[4]							
Consolidation[5]	0.61	1.03	1.07	0.72	0.19	0.26	0.14
Adhesion[6]	0.80	0.69	0.89	0.89	0.18	0.84	0.91
HCW, kg	363	360	361	359	5.42	0.95	0.64
Dressing percentage	64.5	64.2	64.3	64.1	0.35	0.82	0.40
REA, cm^2	92.3	90.9	89.3	90.2	2.12	0.79	0.39
12th-rib fat, cm	1.34	1.26	1.46	1.32	0.11	0.59	0.94
KPH fat, %	1.99	2.00	2.05	2.08	0.08	0.85	0.60
Marbling score[7]	407	415	443	418	20.2	0.61	0.42
Prime and choice[8], %	42.8	56.1	65.1	41.7	11.5	0.41	0.37
Yield grade	2.68	2.63	2.95	2.75	0.20	0.68	0.67
Liver Score[9]	0.56	0.33	0.36	0.50	0.31	0.94	0.65

[1]Experimental ancillary therapy (ANC) treatments administered at each bovine respiratory disease (BRD) treatment: NOAC = antimicrobial only, no ancillary therapy; NSAID = intravenous flunixin meglumine injection; VACC = revaccination with an intranasal viral vaccine; VITC = intramuscular vitamin C injection.

[2]*P*-values are included for the overall F-test and the contrast of NOAC vs. the average of the NSAID, VACC, and VITC. Within a row means with different superscripts (a,b,c) differ ($P \leq 0.05$) and means with different superscripts (x,y,z) tend to differ ($P \leq 0.10$).

[3]Ultrasound estimates of rib eye area (REA), 12th-rib fat thickness, and intramuscular fat were taken on d 91 and d 138 by Chad Gordon of Ultrasound Technologies.

[4]Lung scores were obtained by trained personnel from West Texas A&M University.

[5]Lung consolidation: 0 = clinically normal, healthy lung with < 5% consolidation of lung tissue, 1 = ± 5% consolidation of lung tissue or mycoplasma like lesion, 2 = > 5%, but < 50% consolidation of lung tissue, missing lung, or mycoplasma like lesion, 3 = > 50% consolidation of lung tissue, missing lung, or mycoplasma like lesion.

[6]Lung adhesion: 0 = clinically normal, healthy lung, 1 = minor threadlike fibrous adhesion, 2 = extensive fibrous adhesion.

[7]Rib eye area.

[8]Marbling scores: 400 = $Small^{00}$, 500 = $Modest^{00}$.

[9]Pecentage of calves having prime or choice carcasses within each pen.

[10]Liver Score: 0 = no abscesses, 1 = A-, 2 = A, 3 = A+, 4 = telangiectasis, 5 = distoma (fluke damage), and 6 = fecal contamination.

CHAPTER IV

IMPACT OF BOVINE RESPIRATORY DISEASE DURING THE RECEIVING PERIOD ON STEER FINISHING PERFORMANCE, EFFICIENCY, CARCASS CHARACTERISTICS, AND LUNG SCORES

ABSTRACT

Bovine respiratory disease (BRD), also known as "shipping fever" or bronchopneumonia, is the most significant production problem for the feedlot industry, accounting for the majority of morbidity, mortality, decreased production, and economic losses in feedlots. The objective of this experiment was to evaluate the effect of BRD incidence during the receiving period on subsequent finishing performance, efficiency, carcass characteristics, and lung scores of feedlot steers. Prior to the initiation of this experiment, some calves were enrolled in an experiment evaluating ancillary therapy use. During the receiving period, crossbred steers (n = 516; initial BW = 217 ± 20 kg) were monitored daily by trained personnel for clinical signs of BRD. Overall morbidity and mortality attributed to BRD were 66.5% and 13.2% respectively. After the receiving period, a subset of calves (n = 174) were grouped by previous experimental treatment and the number of times treated for BRD (BRDX) and allocated to finishing pens. The BRDX experimental groups included: never treated for BRD (0X), treated 1 time (1X), 2 times (2X), or 3 or 4 times (3/4X). Arrival BW did not differ among calves utilized in this experiment

(P = 0.17). However, BRDX during the receiving period decreased calf performance, resulting in BW of 324, 316, 285, and 260 kg for 0X, 1X, 2X, and 3/4X, respectively, at the start of the finishing phase (P < 0.001). Ultrasound estimates, BW, and visual appraisal were used to target a common compositional end point based on 12th rib fat thickness (average days on feed; DOF = 182) for each pen of cattle. There was no difference ($P \geq 0.83$) in 12th rib fat thickness among BRDX at harvest. Data were analyzed using the MIXED procedure of SAS with pen (n = 32; 8 per BRDX group) serving as the experimental unit. The lack of significant interactions between BRDX and previous experimental ancillary therapy treatments allowed for the integrity of both experiments to be maintained. With increasing BRDX, DOF and lung consolidation scores increased linearly ($P \leq 0.003$), while HCW, dressing percentage, rib eye area (REA), and the percentage of USDA Prime and Choice carcasses decreased linearly ($P \leq 0.03$). These results suggest that with additional DOF, calves treated multiple times for BRD are able to reach similar compositional end points as their untreated cohorts; however, it may not be possible for these calves to ever reach the same quality and yield potential.

Key Words: bovine respiratory disease, carcass characteristics, finishing performance, high-risk calves, lung scores.

INTRODUCTION

Bovine respiratory disease (BRD) is a complex illness and a multitude of stressors, viruses, and bacterial pathogens can potentially contribute to its onset (Duff and Galyean, 2007). Development of clinical BRD frequently occurs via a primary infection with 1 or more respiratory viruses. The initial viral infection combined with a compromised immune system then allows for the rapid colonization of bacteria within the lungs (Hodgins et al., 2002). The BRD complex accounts for the majority of morbidity, mortality, decreased production, and economic losses in feedlots.

Woolums et al. (2005) implicated BRD as the leading cause of morbidity and mortality in 561 feedlots in 21 states. Morbidity attributed to BRD can account for approximately 75% of total morbidity, and mortality attributed to BRD accounts for 36 to 80% of total mortality in feedlot cattle (Vogel and Parrott, 1994; Edwards, 1996; Smith, 1998; Chirase and Greene, 2001). In a 2011 survey, NAHMS (2013) stated nearly all feedlots (96.9%) had cattle that were affected by BRD and that BRD was the most common illness in feedlots affecting 16.2% of cattle placed on feed. Economic losses from BRD result from antimicrobial treatments, increased labor, mortalities, chronic BRD cases, and decreased performance of calves treated for BRD. Powell (2013) estimated annual economic losses resulting from BRD to be in excess of $2 billion.

In an experiment with a slightly different treatment structure, Holland et al. (2010) examined the effects of previous BRD incidence in heifers. The authors suggested that when calves were harvested at a common endpoints, animals requiring multiple antimicrobial treatments for BRD were able to produce carcasses with similar value to untreated animals given additional days on feed (DOF). The objective of this experiment

was to evaluate the impact of BRD incidence during the receiving period on subsequent finishing performance, efficiency, carcass characteristics, and lung scores of steers.

MATERIALS AND METHODS

All procedures for the present experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Animal Care and Use Protocol AG-12-11).

Cattle and receiving period

Over the course of 1 week, 516 crossbred steers and bulls (BW at arrival = 217 ± 20 kg) were purchased at livestock auctions throughout Oklahoma and transported (average distance = 135 km) to the Willard Sparks Beef Research Center (WSBRC) at Oklahoma State University. Upon arrival at the feed yard, calves were individually weighed and visually inspected for noticeable deformities. Hide color, horn status, and sex were recorded and an uniquely numbered ear tag was placed in the left ear of each calf. Calves were then commingled into receiving pens, given ad libitum access to prairie hay and water, and allowed to rest 24 to 48 hours prior to initial processing.

Initial processing consisted of vaccination for protection against Infectious Bovine Rhinotracheitis (IBR) virus, Bovine Viral Diarrhea Virus (BVDV) Types 1 and 2, Parainfluenza 3 (PI3) virus, and Bovine Respiratory Syncytial Virus (BRSV; BRD Shield; Novartis, Greensboro, NC), *Clostridium chauvoei*, *Clostridium septicum*,

Clostridium novyi, *Clostridium sordellii* and *Clostridium perfringens* Types C and D (Caliber 7; Boehringer-Ingelheim, St. Joseph, MO), and treatment for the control of internal and external parasites (Ivomec Plus; Merial, Duluth, GA). Individual BW were obtained, bulls (n = 355) were surgically castrated by incising the scrotum with a Newberry castrating knife, then by emasculation by a single individual, and all horns (n = 57) were tipped with a Keystone dehorner.

Assessment for clinical signs of BRD and antimicrobial administration

During the receiving period, calves were visually monitored twice daily by trained evaluators throughout the experiment for clinical signs characteristic of BRD. The evaluation employed criteria based on the DART™ system (Pharmacia Upjohn Animal Health, Kalamazoo, MI) with some modifications as described by Step et al. (2008). The subjective criteria utilized for pulling calves consisted of depression, abnormal appetite, and respiratory signs. Signs of depression observed included but were not limited to: depressed attitude, lowered head, glazed or sunken eyes, slow or restricted movement, arched back, difficulty standing or walking, knuckling of joints or dragging toes when walking, and stumbling. Signs of abnormal appetite included: an animal that was completely off feed, an animal eating less than expected or eating extremely slow, a lack of gut fill or gaunt appearance, and obvious body weight loss. Respiratory signs included: labored breathing, extended head and neck (in an attempt to breathe), and audible noise when breathing. The evaluators assigned a calf a severity score from 0 to 4 based on the clinical signs and the severity of those signs.

A score of 0 was assigned for a clinically normal appearing calf. A score of 1 was assigned for mild clinical signs, 2 for moderate clinical signs, 3 for severe clinical signs, and 4 for a moribund animal. For a calf to be assigned a score of 4, the calf was not be able to rise, or had extreme difficulty standing, walking, or breathing. These animals required immediate assistance. The objective criteria used to determine if antimicrobial treatment was necessary was rectal temperature. All calves assigned a severity score of 1 to 4 were taken to the processing chute for rectal temperature measurement (GL M-500, GLA Agricultural Electronics, San Luis Obispo, CA). Any animal that was pulled with a severity score of 1 or 2, and had a rectal temperature of 40°C or greater received an antimicrobial according to label instructions. If a calf was pulled with a severity score of 1 or 2 and had a rectal temperature of less than 40°C, no antimicrobial treatment was administered, and the calf was returned to its receiving pen after evaluation. Any animal with a severe clinical signs (severity score = 3 or 4), received an antimicrobial according to label instructions regardless of rectal temperature. In extreme cases the antimicrobial may have been administered in the home pen if the calf was deemed unable to make the walk to the working facility.

Prior to antimicrobial administration, an accurate BW was obtained to calculate the appropriate dosage and a blood sample was collected (Corvac™ Serum Separator Tube, Tyco Healthcare Group LP, Mansfield MA). Antimicrobial doses were calculated by rounding the calf's current BW up to the nearest 11.3 kg. All antimicrobials were administered subcutaneously per manufacturer's label directions following Beef Quality Assurance Guidelines (NCBA, 2001). The first antimicrobial treatment was administered on the left side of the animal, and subsequent injections were given on alternating sides of

the animal. The severity score, temperature, BW, and antimicrobial dosage administered (or no treatment administered) was recorded for every calf that was pulled for exhibiting clinical signs of BRD. A maximum of 4 antimicrobial treatments were administered during the experiment.

The first time antimicrobial treatment criteria were met, gamithromycin 150 mg/mL (Zactran; Merial, Duluth, GA) was administered at the rate of 1 mL/24.9 kg of BW. A moratorium was observed after gamithromycin administration before a second antimicrobial treatment could be administered. This moratorium was 240 h for calves with a severity score of 1 or 2, and 96 h for calves with a severity score of 3 or 4. If antimicrobial treatment criteria were met a second time, florfenicol 300 mg/mL (Nuflor; Intervet/Schering-Plough, Desoto, KS) was administered at the rate of 1 ml/7.56 kg of BW. After florfenicol administration, a 96 h moratorium was observed before a third antimicrobial treatment could be administered regardless of severity score. If antimicrobial treatment criteria were met a third time, ceftiofur crystalline free acid 200 mg/mL (Excede; Pfizer, New York City, NY) was administered at the rate of 1 ml/30.2 kg of BW. After ceftiofur crystalline free acid administration, a 168 h moratorium was observed before a fourth antimicrobial treatment could be administered regardless of severity score. If antimicrobial treatment criteria were met a fourth time, a second dose of ceftiofur crystalline free acid was administered as described above. Once a calf was "pulled" for suspected BRD, met the treatment criteria, and received an antimicrobial, it was then randomly assigned to 1 of 4 ancillary therapy treatments. The 4 ancillary therapy treatments consisted of: an intravenous flunixin meglumine injection, an intranasal viral vaccination, an intramuscular vitamin C injection, or no ancillary therapy.

Finishing phase cattle management

After the receiving period, calves remained in their home pens and received ad libitum access to a common receiving ration (Table 1) and water for 2 to 3 additional weeks. After this additional period (average total DOF = 86), a subset of 174 calves were allocated to a finishing experiment. This subset included 126 head of calves that were treated for BRD from the receiving ancillary therapy experiment and an additional 48 head of calves that were not treated for BRD during the receiving period. Body weight at arrival was not different for the subpopulation of calves used for the finishing experiment ($P = 0.17$). For the finishing experiment, all previous experimental ANC were maintained, but calves were allocated by the number of times treated for BRD (BRDX). The BRDX experimental groups included: never treated for BRD (0X), treated 1 time (1X), 2 times (2X), or 3 or 4 times (3/4X). Data were analyzed for interactions between ANC and number of BRD treatments administered. Only one interaction existed (marbling score) for all variables analyzed. Due to the lack of significant interactions, the integrity of the experiment was able to be maintained, and data were subsequently analyzed on the basis of BRDX only.

Prior to allocation to finishing pens, all steers were administered 200 mg trenbolone acetate (TBA) and 40 mg estradiol (Revalor XS; Merck Animal Health, Summit, NJ). The ultimate goal was to harvest all calves at a common compositional end point with an emphasis on achieving a 12th rib fat thickness of at 1.27 cm regardless of DOF, while still maintaining the integrity of the pen and shipping truck load lots. This

was accomplished through the use of the ultrasound estimates, BW, and visual appraisal. Calves were harvested in 2 groups (DOF = 166 or 197). For the last 28 DOF, all steers were fed ractopamine hydrochloride (Optaflexx 45; Elanco Animal Health, Indianapolis, IN) at 300.75 mg•steer^{-1}•d^{-1}.

Finishing phase pen management and diet

Finishing pens were 4.57 × 15.24 m in area with a 4.57 m long concrete bunk at the front of the pen. The pens contained a 4.57 × 4.42 m concrete pad with the remainder of the pen being soil surfaced. The pens were under partial cover, with the bunk and pad being covered by an overhang. A 76 L concrete water tank (Model J 360-F; Johnson Concrete, Hastings, NE) was shared between 2 pens and was cleaned 3 times per week throughout the experiment.

The common finishing ration was formulated to meet or exceed NRC (2000) nutrient requirements (Table 2). Adaptation to the finishing diet was accomplished using a two-ration blend method where each day the percentage of finishing diet delivered was increased by approximately 4.6% DM and the percentage of receiving diet (Table 1) delivered was decreased by approximately 4.6% DM until only the finishing diet was being fed. Following adaptation, the finishing ration was fed to all cattle twice daily at 0700 h and 1300 h in a 274-12B Roto-Mix Forage Express mixer wagon (Roto-Mix, Dodge City, KS) to the nearest 0.45 kg of that day's feed call. Ration samples were collected once per week, and dried in a forced air oven for 48 h at 60ºC to determine DM.

Ration samples were composited gravimetrically and analyzed at a commercial laboratory (Servi-Tech Inc., Dodge City, KS) for nutrient composition (Table 2).

Finishing phase data collection, calculations and statistical analysis

Unshrunk BW were obtained at the time of allocation to finishing pens and at approximately 45 d intervals thereafter. All BW were shrunk a calculated 4%. Individual BW and ADG values were averaged within a pen to obtain pen mean BW and ADG. Ultrasound estimates were taken at 91 and 138 DOF. Carcass data, lung consolidation scores, and lung adhesion scores were obtained by trained personnel from West Texas A&M University at harvest. Lung consolidation and lung adhesion scores were converted to a numeric scale and individual values were averaged within a pen to obtain pen mean lung scores. Lung consolidation scores consisted of: 0 = clinically normal, healthy lung with < 5% consolidation of lung tissue, 1 = ± 5% consolidation of lung tissue or mycoplasma-like lesion, 2 = > 5%, but < 50% consolidation of lung tissue, missing lung, or mycoplasma-like lesion, 3 = > 50% consolidation of lung tissue, missing lung, or mycoplasma like-lesion. Lung adhesion scores consisted of: 0 = clinically normal, healthy lung, 1 = minor threadlike fibrous adhesion, 2 = extensive fibrous adhesion.

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) with pen serving as the experimental unit. Contrasts were performed for the linear and quadratic effects of BRDX. Contrasts were not considered unless the overall model was significant ($P \leq 0.05$) or tended to be significant ($0.05 > P \leq 0.10$). Data were analyzed with mortalities (4; 3 digestive and 1 BRD) included in the analysis (deads in).

One calf from the 2X group and 3 calves from the 3/4X group died during the experiment. Due to circumstances beyond our control, the calves harvested at 197 DOF experienced a longer chill time at the plant prior to grading. Due to calves being harvested at varying DOF and there being a difference in chill time prior to grading between the harvest groups, harvest group was included in the model statement for the analysis of carcass quality parameters.

Carcass value and economic analysis

The average base price on the premium grid at the time of harvest was $199.87 per 45.4 kg. This base price was then adjusted for any individual premiums or discounts based on the carcass data and then multiplied by the individual HCW for each animal to calculate a carcass value for each calf. This carcass value represents the actual value of the calf at the time of harvest. After carcass values were calculated for each BRDX group, the actual cost of antimicrobials used in the experiment was subtracted from each respective BRDX group. For 3/4X, the average of 3 or 4 antimicrobials was used. Next additional labor and other expenses were accounted for at an assumed cost of $7.25 per antimicrobial administered. Yardage was then adjusted based on actual DOF for a pen and a yardage cost of $0.40 steer^{-1}•d^{-1}. Finally, feed cost was also adjusted based on actual DMI for a pen and a ration cost of $301.85 per 907.2 kg on a DM basis. These adjustments resulted in what is termed as total calf value. Total calf value would be the carcass value adjusted for actual costs resulting from BRD treatment including: the purchase price of antimicrobials, increased labor costs associated with treatment and care,

adjustments in yardage costs based on variations in DOF, and adjustments in feed costs based on variations in DOF.

RESULTS

Finishing performance and efficiency

The performance and efficiency data is shown in Table 3. Arrival BW prior to the receiving experiment did not differ among calves utilized in this experiment ($P = 0.17$) for BRDX. However, the incidence of BRDX during the receiving period did impact calf ADG during this period. As a result, there was a linear decrease ($P < 0.001$) in initial BW at the start of the finishing phase resulting in initial BW of 324, 316, 285, and 260 kg for 0X, 1X, 2X, and 3/4X, respectively. Interval BW obtained on d 45, d 91, and d 138 all exhibited the same linear decrease ($P < 0.001$) as BRDX increased. The difference in BW among the BRDX groups decreased throughout the finishing period, resulting in final BW difference of only 16 kg between 0X and 3/4X compared to the initial BW difference of 64 kg between the same 2 BRDX groups. However, there was still a linear decrease ($P = 0.01$) in the final BW taken prior to harvest for increasing BRDX.

During the first 45 d of the finishing period, there was no difference ($P = 0.68$) in ADG among the BRDX groups. Conversely, from d 46 to d 91 and from d 92 to 138 there was a linear increase ($P \leq 0.002$) in ADG as BRDX increased. From d 139 to the end of the experiment, this difference in ADG was no longer present ($P = 0.40$). However when ADG was calculated for the entire finishing period, a linear increase ($P = 0.05$) in ADG was observed as BRDX increased.

There was also a linear increase ($P = 0.004$) in DMI observed from the start of the finishing period until d 45 as BRDX increased. There was a tendency for DMI to be different from d 92 through d 138, however no linear or quadratic trend ($P \geq 0.12$) could be determined to separate the DMI of heifers, regardless of BRDX group. No other differences in DMI ($P \geq 0.49$) were detected among the BRDX groups. When DMI was evaluated as a percentage of average BW, a significant linear increase ($P \leq 0.005$) was observed for all intervals as well for the entire finishing period. There was a tendency for a quadratic effect from d 92 through d 138 of the experiment for DMI as a percentage of average BW.

Gain:feed was not different ($P = 0.20$) among BRDX during the first 45 d of the finishing period. However, the improvement in ADG with increasing BRDX from d 46 to d 91 and from d 92 to 138 combined with similar DMI among BRDX groups during these same intervals resulted in linear improvements ($P \leq 0.01$) in G:F over the next 2 intervals. From d 139 to the end of the experiment, this difference in G:F was no longer present ($P = 0.26$). In addition, overall G:F was not significantly different ($P = 0.22$) among BRDX groups.

Common compositional end point projections and days on feed

On d 91, ultrasound estimates (Table 4) indicated that there was a significant linear decrease ($P < 0.001$) in rib eye area (REA) and that intramuscular fat tended ($P = 0.10$) to decrease linearly (linear, $P = 0.06$) as the number of BRD treatments increased. By d 138, there was still a linear decrease ($P = 0.01$) in REA as BRDX increased, but

there was no longer a difference ($P = 0.32$) in intramuscular fat according to ultrasound. The 12th rib fat thickness of calves was not different ($P \geq 0.21$) among BRDX groups at d 91 or d 138 according to ultrasound estimates. Based on the ultrasound estimates, the average BW and ADG of calves within each pen, and visual appraisal, an individual harvest date was projected for each pen. Logistics dictated that calves would need to be shipped in groups large enough to evenly fill trucks and also allow for efficient carcass data collection. Ultimately, the pens were fairly easily separated into early and late harvest groups by their individual predicted harvest dates and all pens were able to be harvested in 2 groups (DOF = 166 or 197). Days on feed (Table 3) did increase linearly ($P = 0.002$) as BRDX increased. At harvest, there were no differences ($P = 0.83$) in compositional maturity based on 12th rib fat thickness among any of the BRDX groups. There was actually a numerical increase in 12th rib fat thickness observed for 2X and 3/4X calves compared to 0X.

Carcass characteristics and lung consolidation and adhesion scores

Hot carcass weight followed the same pattern as final BW with increasing BRDX resulting in a linear decrease ($P \leq 0.001$) in HCW. In addition to the decrease in HCW due to differences in BW, there was also linear decrease ($P \leq 0.001$) in dressing percentage which further impacted HCW. Rib eye area also decreased in a linear fashion ($P = 0.03$) when BRDX increased. However, when REA was evaluated as a percentage of HCW no differences were observed among the BRDX groups ($P = 0.52$). There tended ($P = 0.06$) to be a linear decrease in the percentage of USDA Prime and Choice carcasses

(linear, $P = 0.03$) as the number of BRD treatments increased. No differences ($P \geq 0.26$) for 12th rib fat thickness, kidney pelvic and heart fat, marbling score, USDA Yield Grade, or liver score were observed among the BRDX groups. Lung consolidation increased linearly ($P \leq 0.01$) as BRDX increased. Conversely, there were no differences ($P = 0.47$) in lung adhesion among BRDX groups with calves receiving 1 BRD treatment having the numerically highest adhesion scores.

Carcass value and economic analysis

The average carcass price per 45.4 kg was not different ($P = 0.25$) among BRDX groups and ranged from $197.99 to $200.41. However, decreases in BW and dressing percentage as BRDX increased resulted in larger differences in total carcass value as BRDX increased. Calves never treated for BRD had an average carcass value of $1,643.80, while 1X, 2X, and 3/4X groups returned $1,612.67, $1,589.01, and $1,540.46, respectively resulting in a linear decrease ($P = 0.001$) in total carcass value. When total calf value was calculated by adjusting for the cost of antimicrobials, increased labor, and variation in yardage and feed consumption due to differences in DOF, the differences in value among BRDX groups became more magnified. The total calf value for 0X remained the same, while 1X, 2X, and 3/4X groups had total calf values of $1,605.93, $1,476.91, and $1,413.35, respectively (linear $P < 0.0001$). These differences in total calf value resulted in losses of $37.87, $166.89, and $230.46 for 1X, 2X, and 3/4X, respectively when compared to 0X. The greatest single economic loss experienced by all calves that were treated for BRD was a loss of carcass value at the time of harvest.

DISCUSSION

The overall morbidity (66.5%) and mortality (13.2%) attributed to BRD within this experiment fell within the range of expected morbidity and mortality rates for high-risk steers and bulls of similar backgrounds at our facility. In other recent experiments conducted at the WSBRC utilizing high-risk, livestock auction-sourced steers and bulls purchased during the fall total morbidity has ranged from 31.4% to 68.0%. Mortality attributed to clinical BRD in these same experiments ranged from 1.49% to 13.9%. These morbidity and mortality percentages would be supported by surveys and reviews within the literature. In a survey conducted by Woolums et al. (2005), the authors implicated BRD as the leading cause of morbidity and mortality in 561 feedlots in 21 states. Edwards (1996) estimated that BRD accounts for approximately 75% of total morbidity in feedlot cattle. Mortality attributed to BRD has been estimated to account for between 36 to 80% of total mortality in feedlot cattle depending on the source (Vogel and Parrott, 1994; Edwards, 1996; Smith, 1998; Chirase and Greene, 2001). From a 2011 survey, NAHMS (2013) identified that nearly all feedlots (96.9%) had cattle affected by BRD and that BRD was also the most common illness in feedlots affecting 16.2% of cattle placed on feed. In addition, it was reported that feedlots in the central region (Colorado, Kansas, Nebraska, Oklahoma, and Texas) had twice as many cattle affected with BRD compared to feedlots in the rest of the U.S. (NAHMS, 2013). It should also be noted that this average includes all cattle received into feedlots, not only high-risk calves as in the case of the current experiment.

An advantage to the increased morbidity and numbers of animals requiring multiple treatments was that it afforded the ability for replication of BRD treatment groups within a finishing experiment. This particular set of calves provided an excellent opportunity to examine our objectives. The primary objective of this experiment was to evaluate the effect of BRD incidence during the receiving period on subsequent finishing performance, efficiency, carcass characteristics, and lung scores of feedlot steers. In our opinion, to accurately determine the effects of BRD incidence all calves must be allowed to reach their quality and yield potential regardless of the number of BRD treatments received. Obviously, this cannot be accomplished by feeding all calves to equal days on feed, so our second objective was to attempt to harvest each pen of calves at a common compositional end point. Our target was to harvest all pens when calves within that pen reached a 12th rib fat thickness of 1.27 cm. This goal was successfully achieved through the use of ultrasound estimates, BW projections, and visual appraisal, as all BRDX groups were harvested between 1.28 and 1.40 cm of 12th rib fat. Finally, this experiment aimed to quantify the actual economic losses due to increasing BRDX for a specific group of cattle under recent market conditions.

Calves were enrolled in a 56 day receiving experiment once they received an initial BRD treatment. After the receiving period, calves remained in their receiving pens for an additional 2 to 3 additional weeks (average total DOF = 86). This time frame attempted to ensure that all BRD treatments, including up to 4 antimicrobial treatments when necessary, would occur prior to the finishing period. This approach would also be supported by the literature. Thompson et al. (2006) reported that 87% of all BRD treatments had occurred within 35 d of arrival, and Babcock et al. (2009) stated that 74%

of morbidity occurred during the first 42 d on feed. There was 1 calf from the 3/4X group that did require BRD treatment after the initiation of the finishing experiment. This calf ultimately died and the death was attributed to BRD.

This experiment was very similar to one conducted by Holland et al. (2010). The major differences between that experiment and the present experiment being sex and the treatment structure of BRDX. In the experiment by Holland et al. (2010), heifers were used. In the present experiment, all calves used arrived as steers or bulls. Also, Holland et al. (2010) classified BRD treatment groups (BRDX) as: never treated for BRD (0X), treated for BRD 1 time (1X), treated for BRD 2 times (2X), treated for BRD 3 times (3X), or chronically ill (CI). In the present experiment, up to 4 antimicrobials were administered, and calves receiving 3 or 4 antimicrobials were combined into one BRDX group. There were several calves in the 3/4X group in the present experiment that could have been classified as CI according to the classification provided by Holland et al. (2010). To be classified as CI by Holland et al. (2010), a calf must have been administered 3 antimicrobial treatments and at least 48 h had to have passed after the last antimicrobial was given. In addition, the calf must have been enrolled in the experiment for longer than 21 d and have experienced a net loss of BW over the most recent 21-d period. Finally a calf must have been assigned a clinical severity score of 3 or 4 on the day of classification.

By assembling the BRDX groups the way we did in the present experiment, we were able to provide greater replication of calves experiencing a severe natural BRD challenge and receiving multiple antimicrobial treatments. This treatment structure allowed us to have 8 pens per BRDX, even though we had fewer steers in some of the

pens receiving multiple BRD treatments. With the treatment structure employed by Holland et al. (2010), there were only 2 pens of CI animals, while there were at least 6 pens of heifers for the other BRDX groups. If Holland et al. (2010) had more CI heifers in their experiment and combined them with the 3X group, the treatment structures would be essentially the same between the 2 experiments. Waggoner et al. (2007) reported the mean DOF for cattle that were never treated to be 193 d, for cattle treated 1 time to be 200 d, and for cattle treated 2 or more times to be 212 d. The 19 d difference in DOF between healthy calves and those treated 2 or more reported by Waggoner et al. (2007) is consistent the difference in DOF for the same groups of calves within the present experiment. In this experiment, calves in the 2X and 3/4X groups required an additional 17 DOF on average.

Holland et al. (2010) also used a similar combination of ultrasound estimates, calf performance, and visual appraisal to target a common compositional end point (an average 12th rib fat thickness of 1.27 cm) for all heifers. There were 3 harvest dates (DOF = 152, 174, and 189) used by Holland et al. (2010) compared to 2 harvest dates in the present experiment. In their experiment heifers treated 0X, 1X, and 2X were all harvested at the same average DOF, while heifers in the 3X and CI groups were on feed for an average of 19 and 26 more days, respectively. In the present experiment, all BRDX groups were harvested at different average DOF (Holland et al., 2010).

Arrival BW prior to initiation of the receiving phase did not differ among the BRDX groups (10 kg difference between 0X and 3/4X) in the present experiment. However, increasing BRDX during the receiving period decreased calf ADG, resulting in a linear decrease in BW at the start of the finishing phase (64 kg difference between 0X

and 3/4X). These results are in agreement with Holland et al. (2010) where arrival BW was also not different among BRDX groups. Similarly Gardner et al. (1999) and Waggoner et al. (2007) also demonstrated that arrival BW was not different between cattle that were never treated for BRD and cattle that required antimicrobial treatment for clinical signs of BRD. In addition, Holland et al. (2010) observed a linear decrease in BW as BRDX increased at the start of the finishing phase resulting from a linear decrease in ADG during the receiving period. This linear decrease in BW during the receiving period associated with increasing BRDX is also supported by data from other experiments (Gardner et al., 1999; Thompson et al., 2006; Schneider et al., 2009).

The depression in ADG observed in the present experiment was consistent with other results in the published literature. During the pre-finishing period (average DOF = 86), steers in 1X, 2X, and 3/4X groups in the present experiment had decreases in ADG of 6.7%, 32.9%, and 52.4%, respectively when compared to 0X. This was similar to the decreases in ADG reported by Holland et al. (2010) who reported a 59% decrease in ADG for heifers in the 3X group. It should be noted that this was over a 63 d period, and an improvement in ADG would be expected for those heifers from d 63 to d 86. This initial loss of ADG during the pre-finishing period in the current experiment resulted in a 7.4% decrease in ADG for calves in the 3/4X group compared to the 0X group from the time of arrival to harvest. This result was similar to the overall decrease in ADG from arrival to harvest reported by Holland et al. (2010) who stated that heifers receiving 3 antimicrobial treatments had a decrease in ADG of 8.7% during this time.

After the decrease in ADG during the pre-finishing period observed for morbid steers, a compensatory gain response was observed for those calves during the finishing

period. The interval improvements in ADG for treated calves resulted in improved overall ADG during the finishing phase for calves treated for BRD. These results are in contrast to those reported by Holland et al. (2010) who reported no difference in overall finishing period ADG. Similar to those results reported by Holland et al. (2010), Thompson et al. (2006) observed a decrease in ADG during the first 35 d on feed for morbid animals, but no difference in ADG from d 35 until slaughter. Although a compensatory gain response was observed during the finishing phase in the present experiment, it is important to note that steers treated for BRD were not able to fully compensate in regards to BW even with additional DOF.

Holland et al. (2010) reported no linear differences in the final BW of heifers for the 0X, 1X, 2X, and 3X groups. This was in contrast to the results of the present experiment where there was still a linear decrease in the final BW present just prior to harvest as BRDX increased. However, it is interesting to note that when contrasting 3X heifers with CI heifers, there was a significant decrease in final BW for CI heifers (Holland et al., 2010). The differing results observed in the final BW of these 2 experiments could simply be the result of how the BRDX groups were structured for those calves receiving 3 or more antimicrobial treatments that suffered from an extreme BRD challenge.

Experiments by Roeber et al. (2001) and Waggoner et al. (2007) evaluated animals in 2 separate Ranch to Rail programs where animals were also slaughtered on a market ready basis. Similar to the findings of Holland et al. (2010), final BW was not different among animals administered varying antimicrobial treatments. However Roeber et al. (2001) and Waggoner et al. (2007) did both find a numerical decrease (8 and 18 kg,

respectively) in final BW for calves requiring 2 or more antimicrobial treatments. In a review of individual carcass characteristics of 33,073 steers fed in commercial pens linked to previous BRD treatments, Erickson et al. (2011) reported a significant linear decrease in final BW among steers as BRDX increased.

Much of the data investigating the effects of BRD on feedlot cattle performance results from correlating individual cattle performance to treatment records on a retrospective basis. This retrospective approach has also been conducted using the presence of lung lesions or lung scores at harvest. Finally, other experiments have simply evaluated the performance of a pen or lot of cattle with high incidence of BRD. These experiments provide good evidence of performance of treated cattle on a large commercial scale. However, none of these methods are ideal, as they either cannot account for DMI and efficiency measures, or they simply lump non-treated and cattle receiving differing numbers of BRD treatments within a pen or lot together to determine intake and efficiency of the pen or lot.

The treatment structure of the present experiment allowed for the measurement of DMI of feedlot cattle that had required different numbers of antimicrobial treatments for BRD during the receiving period. Obviously without knowing which calves would eventually require treatment, we were not able to measure intake during the receiving period, but previous data would suggest that the morbid calves in our experiment were likely consuming less feed than healthy animals during this time. It is likely that much of the decrease in pre-finishing ADG observed in the current experiment by calves that required antimicrobial treatment for BRD could be the result of less frequent visits to the feed bunk and decreased DMI by these calves. Because all calves in the present

experiment received the same diets during both the receiving and finishing phases, differences in performance resulting from nutrition would only be related to differences in DMI by morbid animals.

High-risk calves have been shown to have altered eating patterns when compared with unstressed cohorts (Galyean et al., 1999). In addition, the DMI of high-risk calves is extremely variable, and many calves do not achieve adequate DMI for the first couple of weeks on feed. Hutcheson and Cole (1986) stated that DMI for newly received calves ranged from 0.5% to 1.5% of body weight and that the majority of morbid calves do not consume any feed for the first 2 days in the feedlot. It was also reported that approximately only 4 out 5 (83.4%) morbid calves were consuming feed by the end of the first week in the feedlot (Hutcheson and Cole, 1986). These results can cause sick calves to have only 58%, 68%, and 88% of the DMI compared to healthy animals during the first, fourth, and eighth weeks after arrival in the feedlot (Hutcheson and Cole, 1986).

There was a linear decrease in DMI as BRDX increased for the first 45 d of the present experiment. However, no other trends were observed for the remainder of the experiment and overall DMI was not different among BRDX groups. These results would be similar to those reported by Holland et al. (2010) who noted a linear decrease in DMI during the first 65 DOF. However, when DMI was evaluated as a percentage of mean BW in the present experiment, a linear increase was observed for all intervals as well for the entire finishing period. This increase in DMI as a percentage of BW would serve as additional evidence of the attempted compensation for lost performance that occurred during the receiving period among treated calves. Holland et al. (2010) reported a linear improvement or tendency linear for improvement in DMI as a percentage of BW over 2

intervals, but no difference in DMI as a percentage of BW over the length of the experiment.

In the present experiment there was no difference in G:F among BRDX during the first 45 d of the finishing period. However, the improvement in ADG with increasing BRDX from d 46 to d 91 and from d 92 to 138 combined with similar DMI among BRDX groups during these same intervals resulted in linear improvements in G:F over these 2 intervals. From d 139 to finish, calves in the 2X and 3/4X groups became numerically less efficient resulting in overall G:F not being significantly different among the BRDX groups. This result was in contrast to the efficiency data reported by Holland et al. (2010) who noted a linear increase in overall G:F for increasing BRDX.

The ultimate goal of the present experiment was to harvest all calves at a common compositional end point regardless of DOF. Due to the desired collection of carcass data and lung scores at harvest combined with the distance to the harvest facility from WSBRC, logistical concerns compelled us to harvest calves in as few harvest groups as possible while maintaining our goal to harvest all calves at a common compositional end point. By using a combination of animal performance, ultrasound estimates, and visual appraisal we were able to project an ideal individual harvest date for each pen. After this was accomplished, pens were fairly easily separated into early and late harvest groups by their individual predicted harvest dates and all pens were able to be harvested in 2 groups.

Based on the measure of 12th rib fat thickness as well as overall carcass fat evidenced through KPH percentage and USDA Yield Grades, we were successful at harvesting all steers at the same compositional end point. Only a 0.12 cm difference the

12th-rib fat thickness was observed between steers across BRDX treatment groups. Cattle in the 3/4X group were finished to the greatest numerical fat thickness and was actually a numerical increase in 12th rib fat thickness observed for both 2X and 3/4X calves compared to 0X. By ensuring that calves treated multiple times for BRD had slightly numerically higher fat thickness, we were confident that these calves were given ample time to reach their quality and yield potential. These results were similar to those described by Holland et al. (2010) who also reported no differences in 12th-rib fat and overall carcass fatness, except for in the CI heifers.

In the present experiment, HCW followed the same pattern as final BW with increasing BRDX resulting in a linear decrease in HCW. In addition to the decrease in HCW due to final BW, there was also linear decrease in dressing percentage which caused a further decrease in HCW. This was in contrast to the data reported by Holland et al. (2010) who noted no linear differences in HCW or dressing percentage. The results reported by Roeber et al. (2001) support the findings of the present experiment. Roeber et al. (2001) reported that there was a significant decrease in both HCW and dressing percentage for calves receiving 2 or more antimicrobial treatments. In the review of commercial data by Erickson et al. (2011), a significant linear decrease in HCW was observed among steers as BRDX increased.

In the present experiment REA also decreased in a linear fashion when BRDX increased. These results were also in contrast to those described by Holland et al. (2010) who reported no differences in the REA of heifers with increasing BRDX. Roeber et al. (2001) and Waggoner et al. (2007) also reported no significant decrease in REA with increasing BRD treatments however both did find a numerical decreases in the REA of

calves requiring 2 or more antimicrobial treatments. Erickson et al. (2011), reported significant linear decrease in the REA of steers in commercial feedlots as BRDX increased. When REA was examined as a percentage of HCW in the present experiment, no differences existed among BRDX groups. This would suggest the reduction in REA observed in the present experiment is primarily the result of decreased HCW as BRDX increases.

In addition quality grade seemed to be negatively impacted in the current experiment as there also tended to be a linear decrease in the percentage of USDA Prime and Choice carcasses as the number of BRD treatments increased. Again these findings were different than those of Holland et al. (2010), who reported no difference in the percentages USDA Choice or Select carcasses among the BRD treatment groups. No differences for kidney pelvic and heart (KPH) fat, marbling score, USDA Yield Grade, or liver score were observed among the BRDX groups in the present experiment. These results are similar to those described by Holland et al. (2010). Holland et al. (2010) reported a tendency for a linear decrease in marbling score as the number of BRD treatments increased. In the present experiment there was a numerical decrease in marbling score as BRDX increased, but this difference of 45 units from Small^{06} to Small^{51} was not statistically significant.

The results for a decrease in quality grade and a numerical decrease in marbling score in the present experiment are similar to those reported elsewhere in the literature. Schneider et al. (2009) reported that 16% fewer calves that were treated for BRD graded USDA Choice compared to non-treated calves. Roeber et al. (2001) reported that there was a significant decrease in marbling score for calves receiving 2 or more antimicrobial

treatments. In a review of commercial data, Erickson et al. (2011) reported significant linear decreases in both marbling score and the percentage of USDA Choice carcasses of steers as antimicrobial treatments for BRD increased. However the impacts of BRD treatment on quality grade and marbling score have proved to be somewhat inconsistent. Waggoner et al. (2007) and Garcia et al. (2010) reported no decrease in marbling score for cattle requiring BRD treatment. Garcia et al. (2010) observed a tendency for treated cattle derived from sires of 7 *Bos taurus* breeds to have a decreased percentage of USDA Choice carcasses. Interestingly, Garcia et al. (2010) observed no difference in the percentage of USDA Choice carcasses for treated cattle derived from sires of tropically adapted *Bos taurus* and *Bos indicus*-influenced breeds.

In the present experiment, lung consolidation scores increased linearly as BRDX increased. Conversely, there were no differences in lung adhesion scores among BRDX groups. Calves receiving 1 BRD treatment actually had the numerically highest lung adhesion scores in the current experiment. In the experiment by Holland et al. (2010), a slightly different lung scoring system was utilized as described by Bryant et al. (1999). In their experiment, the presence and severity of lung lesions (score = 0, 1, 2, or 3) were evaluated along with a simple presence or absence of interlobular adhesions or missing lobes indicating thoracic adhesions (score = 0 or 1) (Holland et al., 2010). There were no significant differences in any of the lung score data presented by Holland et al. (2010).

In theory, the majority of cattle that previously suffered from BRD would have lung lesions present at harvest. Wittum et al. (1996) and Bryant et al. (1999) previously indicated that the presence of pulmonary lesions was a better predictor than treatment records for losses in ADG resulting from BRD. One reason for this finding would be that

cattle that were never treated for clinical signs of BRD have commonly demonstrated evidence of lung damage at harvest. However using the presence of lung lesions at harvest as a predictor of previous BRD incidence is not without fault, as it has also been reported that cattle that have be treated for clinical signs of BRD have lacked the presence of any detectable pulmonary lesions at harvest (Wittum et al., 1996; Gardner et al., 1999; Thompson et al., 2006; Schneider et al., 2009). Holland et al. (2010) argued that even though the lung lesion data were not different between the BRD treatment groups in their experiment, that the differences in preconditioning phase performance, combined with increased haptoglobin concentrations and rectal temperatures in treated heifers indicated that the BRD treatments administered were related to an active infection. Currently, there is no perfect diagnostic tool to determine the exact incidence of BRD in feedlot cattle and thus determining the true production and economic losses due to BRD is extremely difficult. However, we would agree with the assessment Holland et al. (2010) that measuring finishing performance, carcass characteristics, and ultimately economic losses due to BRD based on antimicrobial treatment for the clinical signs of BRD is appropriate and accurate.

It has been well documented that as BRD treatments increase, carcass values and overall net return per animal can exponentially decrease. Waggoner et al. (2007) reported the mean loss in total value for cattle that were treated 1 time to be $28.52, and the mean loss in total value for cattle for cattle treated 2 or more times to be $172.67. Schneider et al. (2009) reviewed the records from 5,976 animals fed in Midwestern feedlots and reported similar results for the economic impact of 1 BRD treatment. However, the economic loss resulting from multiple antimicrobial treatments was not as severe

according to Schneider et al. (2009). The authors observed that decreases in animal performance and carcass merit were associated with a decrease of $23.23, $30.15, and $54.01 in carcass value for cattle treated for BRD once, twice, or 3 or more times respectively when compared to cattle never treated for BRD (Schneider et al., 2009). Brooks et al. (2011) examined the economic impact of BRD treatments for the heifers in the experiment conducted by Holland et al. (2010). Interestingly, heifers treated once for BRD actually returned $10.12 per head more than those heifers never treated (Brooks et al., 2011). However heifers treated 2 times, 3 times, or deemed chronically ill returned $11.08, $72.01, and $143.28 less per head than those heifers never treated (Brooks et al., 2011).

In the current experiment those calves never treated for BRD had an average carcass value of $1,643.80, while 1X, 2X, and 3/4X groups had average carcass values of $1,612.67, $1,589.01, and $1,540.46, respectively. When a total calf value was calculated by adjusting for the cost of antimicrobials, increased labor, and variation in yardage and feed consumption due to differences in DOF, these differences in value were increased. The total calf value for 0X remained $1,643.80, while 1X, 2X, and 3/4X groups had total calf values of $1,605.93, $1,476.91, and $1,413.35, respectively. These differences in total calf value resulted in economic losses of $37.87, $166.89, and $230.46 for 1X, 2X, and 3/4X, respectively when compared to 0X. The greatest single economic loss for all calves that were treated for BRD was due a loss of carcass value at the time of harvest. The second greatest economic loss for 1X calves was due to antimicrobial cost. In addition, calves in the 1X group received a positive adjustment for yardage and feed cost due to a numerical decrease in DOF resulting from a numerical improvement in

performance compared to calves in the 0X group. The second greatest economic loss for 2X and 3/4X calves was due to increased feed cost resulting from increased DOF.

These economic values obviously change and those presented in this experiment are specific to this lot of cattle, and the prices and market conditions at the time they were marketed. These values are dependent on the price of cattle at the time of harvest, the choice select spread, and grid premiums and discounts. In addition, the cost of inputs greatly impacts the total value of the calf at harvest. The average cost of antimicrobial treatment administered in this experiment was $13.73. However, NAHMS (2013) reported that the average cost of antimicrobial treatment for BRD in all feedlots was $23.60. Regardless of how the economic data presented is viewed, to accurately estimate the total economic losses resulting from BRD our opinion is that calves treated for BRD should be allowed additional DOF in order reach similar compositional end points compared to untreated cohorts. In addition, our opinion is that all costs associated with BRD treatment and the additional DOF including: antimicrobial treatment costs, additional labor, additional yardage, and additional feed consumption should be considered.

Conclusions

The calves in this experiment experienced a significant natural immune challenge resulting in a first treatment morbidity of 66.5% and mortality attributed to BRD of 13.2% for the initial lot of 516 calves. The incidence of clinical BRD prior to the finishing phase resulted in decreased BW and ADG. However, after allocating calves by

previous BRD treatments prior to finishing, a compensatory gain response was observed as those calves previously treated for BRD attempted to compensate for lost performance that occurred during the receiving period. Although a compensatory gain response was observed during the finishing phase, it is important to note that steers treated for BRD were not able to fully compensate in regards to BW even with additional DOF.

The steers in this experiment were able to be successfully harvested at a common compositional end point through the use of ultrasound estimates, BW projections, and visual appraisal. At harvest, all BRDX groups exhibited 1.28 and 1.40 cm of 12th rib fat. To achieve this, DOF had to increase linearly as BRDX increased. Increasing BRDX resulted in a linear increase in the consolidation of lung tissue, while lung adhesion scores were not adversely affected BRDX. When slaughtered at a common endpoint, REA and the percentage of USDA Prime and Choice carcasses were negatively impacted by increasing BRDX; however, the differences in base carcass price per 45.4 kg were minimal among the experimental BRDX treatments. The most significant economic losses were an effect of losses in carcass value primarily due to HCW losses resulting from decreased final BW and dressing percentages as BRDX increased. After the initial loss in carcass value due to BRD, the next most significant economic losses were a result of the additional feed cost and the antimicrobial treatment cost.

With additional DOF calves treated multiple times for BRD can reach the same compositional end point as untreated cohorts; however, these calves will likely never reach the same quality and yield potential and consequently carcass value. The overall net return of calves treated for BRD is further decreased due the costs associated with BRD treatment and the additional DOF including: antimicrobial treatment costs, labor,

yardage, and feed consumption. Therefore, the economic losses of calves requiring multiple antimicrobial treatments for BRD can be minimalized with additional DOF, but not eliminated. In this experiment, the incidence of clinical BRD resulted in a 2.3%, 10.2%, and 14.0% decrease in the total value of calves at harvest for calves treated once, twice, and 3 or 4 times, respectively.

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Table 4-1. Composition of common receiving diet[1]

Ingredient (%)[1]	
Wet corn gluten feed[2]	48.8
Grain sorghum hay	30.0
Dry-rolled corn	15.0
Dry supplement B-273[3]	5.2
Nutrient Composition[1,4]	
NE_m, Mcal/kg	1.69
NE_g, Mcal/kg	1.07
TDN, %	71.60
Crude protein, %	17.40
Crude fat, %	1.90
NDF, %	39.90
ADF, %	21.40
Calcium, %	0.68
Phosphorus, %	0.67
Magnesium, %	0.36
Potassium, %	1.15
Sulfur, %	0.27

[1]All values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Dry supplement B-273 was formulated to contain (% DM basis): 38.46% ground corn, 30.36% limestone, 21.04% wheat midds, 6.92% urea, 1.03% magnesium oxide, 0.618% zinc sulfate, 0.38% salt, 0.119% copper sulfate, 0.116% manganese oxide, 0.05% selenium premix (contained 0.6% Se), 0.311% vitamin A (30,000 IU/g), 0.085% vitamin E (500 IU/g), 0.317% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.195% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[4]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Table 4-2. Composition of common finishing diet[1]

Ingredient (%)[1]	
Dry-rolled corn	48.14
Wet corn gluten feed[2]	15.00
Dried distillers grains plus solubles	15.00
Prairie hay	9.00
Liquid supplement[3]	6.54
Dry supplement B-273[4]	3.12
Dry supplement B-283[5]	3.20
Nutrient Composition[1,6]	
NE_m, Mcal/kg	2.23
NE_g, Mcal/kg	1.54
TDN, %	89.55
Crude protein, %	18.85
Crude fat, %	5.00
NDF, %	22.35
ADF, %	10.40
Calcium, %	0.96
Phosphorus, %	0.52
Magnesium, %	0.28
Potassium, %	1.03
Sulfur, %	0.31

[1]All values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Synergy 19-14 (Westway Feed Products; New Orleans, LA).
[4]Dry supplement B-273 was formulated to contain (% DM basis): 38.46% ground corn, 30.36% limestone, 21.04% wheat midds, 6.92% urea, 1.03% magnesium oxide, 0.618% zinc sulfate, 0.38% salt, 0.119% copper sulfate, 0.116% manganese oxide, 0.05% selenium premix (contained 0.6% Se), 0.311% vitamin A (30,000 IU/g), 0.085% vitamin E (500 IU/g), 0.317% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.195% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[5]Dry supplement B-283 was formulated to contain (% DM basis): 40.47% limestone, 36.26% ground corn, 19.73% wheat midds, 2.47% salt, 0.312% zinc sulfate, 0.071% copper sulfate, 0.064% manganese oxide, 0.029% selenium premix (contained 0.6% Se), 0.202% vitamin A (30,000 IU/g), 0.056% vitamin E (500 IU/g), 0.207% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.127% Tylan 40 (Elanco Animal Health; Indianapolis, IN).
[6]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Table 4-3. Effect of 0, 1, 2, or 3 or 4 antimicrobial treatments for bovine respiratory disease (BRD) during the receiving period on subsequent finishing performance and efficiency of steers

	Antimicrobials administered[1]					*P*-value[2]		
Variable	0X	1X	2X	3/4X	Pooled SEM	Overall *P*-value	Linear Contrast	Quadratic Contrast
Days on feed[3], d	174	170	193	189	4.51	0.002	0.002	1.00
Animal BW[4], kg								
Initial	324	316	285	260	5.98	<0.001	<0.001	0.18
d 45	402	395	364	336	6.71	<0.001	<0.001	0.12
d 91	459	456	425	408	7.20	<0.001	<0.001	0.34
d 138	517	522	486	480	6.65	<0.001	<0.001	0.41
Final	568	572	560	552	4.89	0.04	0.01	0.25
Average daily gain[5], kg								
Initial - d 45	1.74	1.76	1.78	1.70	0.05	0.68	0.66	0.29
d 46 - d 91	1.23	1.33	1.31	1.53	0.05	0.005	0.002	0.28
d 92 - d 138	1.23	1.41	1.30	1.53	0.04	<0.001	<0.001	0.53
d 139 - final	1.50	1.60	1.39	1.46	0.09	0.40	0.40	0.85
Initial - final	1.41	1.51	1.43	1.54	0.03	0.02	0.05	0.83
Dry matter intake[6], kg								
Initial - d 45	8.94	8.95	8.60	8.35	0.15	0.02	0.004	0.38
d 46 - d 91	9.83	10.0	9.70	10.0	0.26	0.78	0.86	0.85
d 92 - d 138	9.81	10.1	9.68	10.5	0.24	0.07	0.12	0.27
d 139 - final	9.74	10.1	9.89	10.3	0.26	0.49	0.26	0.97
Initial - final	9.56	9.78	9.47	9.75	0.18	0.57	0.75	0.86
Dry matter intake[7], % of BW								
Initial - d 45	2.46	2.52	2.65	2.80	0.03	<0.001	<0.001	0.16
d 46 - d 91	2.29	2.36	2.46	2.69	0.06	<0.001	<0.001	0.18
d 92 - d 138	2.01	2.07	2.13	2.37	0.05	<0.001	<0.001	0.06
d 139 - final	1.80	1.85	1.89	1.99	0.04	0.04	0.005	0.66
Initial - final	2.14	2.21	2.24	2.40	0.04	<0.001	<0.001	0.17
Gain:feed[8]								
Initial - d 45	0.194	0.196	0.207	0.204	<0.01	0.20	0.06	0.64
d 46 - d 91	0.126	0.133	0.136	0.154	0.01	0.01	0.002	0.36
d 92 - d 138	0.126	0.139	0.135	0.146	<0.01	0.05	0.01	0.83
d 139 - final	0.155	0.158	0.140	0.142	0.01	0.26	0.11	0.94
Initial - final	0.148	0.154	0.151	0.158	<0.01	0.22	0.09	0.93

[1]Number of times treated for BRD (BRDX): never treated for BRD (0X), treated once for BRD (1X), treated twice for BRD (2X), or treated 3 or 4 times for BRD (3/4X).
[2]*P*-values are included for the overall F-test and the linear and quadratic contrast for the number of times treated for BRD.
[3]Average of days on feed (DOF) for all pens within an experimental treatment.
[4]Treatment BW was the BW in kg with a calculated 4% shrink.
[5]Treatment ADG was calculated from the shrunk (4%) BW in kg and DOF between the time periods.
[6]Treatment DMI was calculated by taking DMI in kg for a pen for the period shown divided by the actual number of head days within each pen.
[7](DMI/Average BW for the time period) × 100.
[8]Treatment G:F was calculated by taking the pen ADG in kg divided by the pen average DMI in kg for the time periods.

Table 4-4. Effect of 0, 1, 2, or 3 or 4 antimicrobial treatments for bovine respiratory disease (BRD) during the receiving period on lung consolidation and adhesion and carcass characteristics of steers

	Antimicrobials administered[1]					*P*-value[2]		
Variable	0X	1X	2X	3/4X	Pooled SEM	Overall *P*-value	Linear Contrast	Quadratic Contrast
Ultrasound estimates[3]								
d 91 REA, cm^2	81.3	84.1	77.0	73.7	1.49	<0.001	<0.001	0.05
d 91 12th-rib fat, cm	0.82	0.82	0.81	0.72	0.04	0.21	0.08	0.25
d 91 IMF	4.55	4.29	4.42	4.04	0.15	0.10	0.04	0.70
d 138 REA, cm^2	88.9	89.6	84.6	83.8	1.71	0.05	0.01	0.65
d 138 12th-rib fat, cm	0.94	0.93	0.91	0.91	0.05	0.95	0.60	0.99
d 138 IMF	4.60	4.28	4.49	4.24	0.15	0.32	0.22	0.83
Lung scores[4]								
Consolidation[5]	0.38	0.54	1.06	0.97	0.16	0.01	0.003	0.42
Adhesion[6]	0.73	0.96	0.67	0.83	0.14	0.47	0.99	0.81
HCW, kg	372	369	360	353	3.66	0.004	<0.001	0.63
Dressing percentage	65.5	64.6	64.2	64.0	0.27	0.003	<0.001	0.23
REA[7], cm^2	91.8	93.9	90.8	87.3	1.56	0.05	0.03	0.09
REA[8], % of BW	24.7	25.4	25.3	24.7	0.44	0.52	0.97	0.15
12th-rib fat, cm	1.33	1.28	1.36	1.40	0.09	0.83	0.49	0.63
KPH fat, %	2.17	2.01	2.08	2.00	0.06	0.26	0.16	0.55
Marbling score[9]	451	428	426	406	16.7	0.29	0.10	0.91
Prime and choice[10], %	70.3	56.5	60.2	36.2	9.15	0.06	0.03	0.54
Yield grade	2.81	2.60	2.75	2.91	0.16	0.59	0.53	0.26
Liver score[11]	0.67	0.23	0.63	0.46	0.27	0.65	0.86	0.61

[1]Number of times treated for BRD (BRDX): never treated for BRD (0X), treated once for BRD (1X), treated twice for BRD (2X), or treated 3 or 4 times for BRD (3/4X).

[2]*P*-values are included for the overall F-test and the linear and quadratic contrast for the number of times treated for BRD.

[3]Ultrasound estimates of rib eye area (REA), 12th-rib fat thickness, and intramuscular fat were taken on d 91 and d 138 by Chad Gordon of Ultrasound Technologies.

[4]Lung scores were obtained by trained personnel from West Texas A&M University.

[5]Lung consolidation: 0 = clinically normal, healthy lung with < 5% consolidation of lung tissue, 1 = ± 5% consolidation of lung tissue or mycoplasma like lesion, 2 = > 5%, but < 50% consolidation of lung tissue, missing lung, or mycoplasma like lesion, 3 = > 50% consolidation of lung tissue, missing lung, or mycoplasma like lesion.

[6]Lung adhesion: 0 = clinically normal, healthy lung, 1 = minor threadlike fibrous adhesion, 2 = extensive fibrous adhesion.

[7]Rib eye area.

[8](REA/HCW) × 100.

[9]Marbling scores: 400 = $Small^{00}$, 500 = $Modest^{00}$.

[10]Pecentage of calves having prime or choice carcasses within each pen.

[11]Liver Score: 0 = no abscesses, 1 = A-, 2 = A, 3 = A+, 4 = telangiectasis, 5 = distoma (fluke damage), and 6 = fecal contamination.

Table 4-5. Effect of 0, 1, 2, or 3 or 4 antimicrobial treatments for bovine respiratory disease (BRD) during the receiving period on carcass value at harvest and feedlot economics

	Antimicrobials administered[1]					*P*-value[2]		
Variable	0X	1X	2X	3/4X	Pooled SEM	Overall *P*-value	Linear Contrast	Quadratic Contrast
Final shrunk body weight, kg	568	572	560	552	4.89	0.04	0.01	0.25
Dressing percentage	65.5	64.6	64.2	64.0	0.27	0.003	<0.001	0.23
Hot carcass weight, kg	372	369	360	353	3.66	0.004	<0.001	0.63
Actual carcass price[2], $/45.4 kg	200.41	198.24	201.01	197.99	1.27	0.25	0.43	0.74
Total carcass value[3], $	1,643.80	1,612.67	1,589.01	1,540.46	20.6	0.01	0.001	0.68
Carcass value difference from previous BRDX, $	0.00	-31.13	-23.66	-48.55	18.0	0.31	0.10	0.86
Carcass value difference from 0X, $	0.00	-31.13	-54.79	-103.34	18.0	0.003	<0.001	0.63
Antimicrobial cost[4], $	0.00	-14.40	-29.60	-46.97	0.00	<0.001	<0.001	<0.001
Additional labor cost[5], $	0.00	-7.25	-14.50	-25.38	0.00	<0.001	<0.001	<0.001
Additional yardage cost[6], $	0.00	1.55	-7.75	-6.20	1.49	<0.001	<0.001	1.00
Additional feed cost[7], $	0.00	13.36	-60.25	-48.56	12.0	<0.001	<0.001	0.94
Total calf value[8], $	1,643.80	1,605.93	1,476.91	1,413.35	23.2	<0.001	<0.001	0.58
Total calf value difference from previous BRDX, $	0.00	-37.87	-129.02	-63.57	20.9	0.002	0.006	0.02
Total calf value difference from 0X, $	0.00	-37.87	-166.89	-230.46	20.9	<0.001	<0.001	0.54

[1]Number of times treated for BRD (BRDX): never treated for BRD (0X), treated once for BRD (1X), treated twice for BRD (2X), or treated 3 or 4 times for BRD (3/4X).

[2]Pen averages of actual carcass base prices. The base carcass price on the grid was $199.87 per 45.4 kg.

[3]Base carcass price multiplied by HCW.

[4]Actual cost of antimicrobial treatment. For 3/4X, the antimicrobial cost was averaged for those calves that received 3 or 4 antimicrobials.

[5]An estimate of $7.25 per antimicrobial treatment was assumed to cover the cost of labor, and any additional costs related to BRD treatment.

[6]Actual yardage based on pen DOF relative to 0X. Yardage was $0.40 $steer^{-1} \cdot d^{-1}$.

[7]Actual feed cost based on pen DMI and pen DOF relative to 0X. The ration cost $301.85 per 907.2 kg on a DM basis.

[8]Total carcass value adjusted for additional costs associated with antimicrobial treatment, labor, yardage, and feed consumption.

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CHAPTER V

EFFECT OF COPPER, MANGANESE, AND ZINC SUPPLEMENTATION ON THE PERFORMANCE, CLINICAL SIGNS, AND MINERAL STATUS OF CALVES FOLLOWING EXPOSURE TO BOVINE VIRAL DIARRHEA VIRUS TYPE 1B AND SUBSEQUENT *MANNHEIMIA HAEMOLYTICA* INFECTION

ABSTRACT

Trace mineral (TM) supplementation has been demonstrated to alter immune function and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). The objective of this experiment was to determine the influence of dietary copper (Cu), manganese (Mn), and zinc (Zn) supplementation on the performance, clinical signs, and mineral balance of calves following a bovine viral diarrhea virus (BVDV) and *Mannheimia haemolytica* (MH) immune challenge. Steers (n = 16; BW = 225 ± 20 kg) from a single ranch were processed, weaned, and randomly pairwise assigned to either the mineral supplemented (MIN) or control (CON) experimental treatments. The MIN calves received 150 mg of Cu, 130 mg of Mn, and 320 mg of Zn daily while the CON calves received the basal diet with no additional Cu, Mn, or Zn supplementation. The basal diet contained sufficient Mn and Zn, but inadequate Cu based on NRC (2000) published nutrient requirements. After 46 d on the experimental treatments, all calves were naturally exposed to heifer persistently infected (PI) with BVDV type 1b for 4 d and

then subsequently intratracheally challenged with MH. Data were analyzed using the GLIMMIX procedure of SAS with sampling time serving as a repeated measure and calf serving as the experimental unit. The immune challenge was validated via increased BVDV antibody titers, MH whole cell (WC) and leukotoxin (LKT) antibody titers, rectal temperatures (TEMP), and subjective clinical scores (CS). Calf performance ($P \geq 0.48$) was not affected by mineral supplementation. Mineral supplementation also did not impact the CS or TEMP of calves ($P \geq 0.53$). There was a significant ($P < 0.001$) time by treatment interaction observed for liver Cu levels. Time significantly impacted the concentrations of Cu, Mn, Zn, and Fe within the liver, Cu, Mn, and Zn within the muscle and Cu, Zn, and Fe within the serum ($P \leq 0.03$). Calves receiving MIN had greater ($P < 0.01$) liver Cu and Mn concentrations compared to CON calves. In contrast, serum Cu and Fe concentrations were increased ($P \leq 0.05$) in CON calves compared to MIN calves. Mineral supplementation did not impact mineral levels within the muscle ($P \geq 0.38$). The supplementation of Cu, Mn, and Zn can potentially impact TM status in response to an immune challenge in calves. When Cu is supplemented to calves receiving a marginally Cu deficient diet, Cu status within the body can be altered.

Key Words: bovine respiratory disease, bovine viral diarrhea virus, immune challenge, *Mannheimia haemolytica*, mineral supplementation

INTRODUCTION

Bovine respiratory disease (BRD) is the most significant production problem for the feedlot industry, accounting for the majority of morbidity, mortality, and decreased production in feedlots with estimated annual economic losses in excess of $2 billion (Powell, 2013). The supplementation of trace minerals (TM) has been demonstrated to alter immune function and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have shown no improvements in performance or health variables from the supplementation of TM.

Overall, TM research has been very inconsistent when investigating the ideal concentrations and sources of TM supplementation needed for optimum results. The supplementation of TM including Cu, Mn, and Zn has been a common management practice within the feedlot industry for many years and often times these TM are included at levels in excess of published requirements (Vasconcelos and Galyean, 2007). The role of TM in immune function, combined with the unknown TM status of newly received calves may explain the increased TM inclusion in feedlot diets despite the inconsistent responses in the literature.

Organic mineral complexes have been shown to be more bioavailable than traditional inorganic mineral sources. This increase in bioavailability is a result of chelated mineral complexes being less likely bound to other substances within the upper gastrointestinal tract and thus allowing the mineral to be more readily absorbed across the brush border of the small intestine. The increased bioavailability of organic TM could potentially allow for improved TM status within the animal in an immune challenge scenario such as a BRD event. The objective of this experiment was to determine if copper (Cu), manganese (Mn), and zinc (Zn) supplementation effected the performance,

clinical signs, and mineral balance of calves following a BVDV and MH immune challenge.

MATERIALS AND METHODS

All procedures for the present experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Animal Care and Use Protocol AG-12-5).

Cattle description and initial processing

An Angus-based commercial cow herd was identified as a potential source to supply calves for this experiment. An initial pool of 18 bull calves were selected from a single pasture. Eighty days prior to the initiation of the experiment, all bull calves were tagged with an individually numbered tag, given an initial vaccination for clostridial pathogens (Covexin 8; Merck Animal Health, Summit, NJ), and surgically castrated at the ranch of origin. Blood samples were collected and analyzed as described by Burciaga-Robles et al. (2010) to ensure calves were seronegative to the pathogens to be used in the challenge portion of this experiment. On 24 d prior to initiation of the experiment, a second blood sample collected and analyzed to confirm steers remained seronegative to the pathogens to be used in the challenge portion of this experiment. Ear notches were also obtained and tested via immunohistochemistry (IHC) by a commercial diagnostic lab to ensure no calves were persistently infected (PI) with BVDV.

Eleven days prior to the experiment, calves received a second vaccination for clostridial pathogens (Covexin 8; Merck Animal Health, Summit, NJ), were vaccinated for viral pathogens excluding BVDV (Inforce; Zoetis, Florham Park, NJ), received a vaccine for infectious bovine keratoconjunctivitis (IBK) (Autogenous Bacterin; Newport Laboratories, Worthington, MN), and were treated for the control of internal and external parasites (Ivermax Plus; Norbrook Laboratories, Lenexa, KS). Calves also received a prophylactic dose of tilmicosin phosphate (300 mg per mL) administered at the rate of 1.5 mL per 45.4 kg of BW (Micotil; Elanco Animal Health, Indianapolis, IN) and a fly tag (Corathon; Bayer, Shawnee Mission, KS). After processing, all steers were transported 97 km to the Animal Science Equine Center at Oklahoma State University. This facility was chosen to prevent exposure of the recently weaned calves to any other cattle at the other University research facilities. Calves remained at the Equine Center for a 6 d weaning period.

Challenge model and facility management

Five days prior to initiation the experiment, the steers were gathered and transported 6 km to the Nutrition and Physiology Research Center (NPRC) at Oklahoma State University. Calves were weighed, and BW in combination with initial antibody titers were used to allocate 16 steers to experimental treatments. Calves were then placed in metabolic stanchions with headlocks for a 5 d adaptation period. Each stanchion had an individual automatic water bowl and each calf was provided with an individual feed trough. Calves were placed in metabolic stanchions prior to the experiment to allow for

adaption of calves to the stanchions and automatic water bowls. Metabolic stanchions were only used during this experiment during the adaptation period (5 d) and the intense sampling period (d 0 through d 7).

After the adaptation period, the experiment was initiated. All time will be referenced in relation to the MH challenge as d 0. All calves were randomly assigned individual 3.05 × 3.66 m slatted floor pens on d -46. Each pen had access to 2 automatic water bowls and each calf was provided with an individual feed bunk. Calves remained in these individual pens for the first 42 d of the experiment.

The BVDV and MH challenge model described by Burciaga-Robles et al. (2010) was utilized in this experiment with slight modifications. Pre BVDV challenge samples were collected on d -4. After this sampling, all calves were comingled in a common pasture for 4 d with an animal previously confirmed as being PI positive with BVDV1b via IHC and genotyping as described by Fulton et al. (2006). During this time, all animals shared a single common water tank, and were fed together in 3 portable 3 m feed bunks.

On d 0, calves were gathered and placed in the metabolic stanchions. Pre-MH challenge sampling took place at h 0. Immediately after h 0 sampling, all steers received 10 mL of a solution containing 6×10^9 CFU of MH serotype 1 that was reconstituted and grown prior to the challenge as described by Mosier et al. (1998). The MH was delivered via intratracheal bronchoalveolar lavage by a licensed veterinarian as described by Dowling et al. (2002), with slight modifications. Briefly, steers were restrained and a broncheoalveolar lavage tube (Bivona Medical Technology, Gary, IN) was gently inserted into the ventral meatus of a nostril, passed on into the trachea, and positioned

within 2 to 3 cm of the tracheal bifurcation. The MH challenge solution was then delivered in a way such that the challenge solution would be allowed to enter both lungs. No adverse effects of the challenge procedure itself were observed.

For the next 7 d, calves remained in the stanchions and were monitored several times daily. Intensive sampling occurred during this time. After the 7 d intensive sampling period, calves were returned to their respective individual 3.05 × 3.66 m slatted floor pens for the duration of the experiment. On d 14 and d 28, calves were gathered, briefly restrained in a manual squeeze chute, and sampled again. The calves did remain in our possession after the experiment was terminated, and an additional sampling occurred on d 68. All calves were comingled and received the same diet from d 28 to d 68.

Common diet and feeding

While on pasture with their dams prior to weaning, cows and calves received no mineral supplementation. The only TM calves would have had received would have been provided through the milk of their dams or forage consumed on the ranch. Upon arrival to the Horse Unit, calves were given ad libitum access to water, Bermuda grass hay, and the common receiving ration minus the dry supplement (Table 1). After transportation to the NPRC and being placed in stanchions, the Bermuda hay was removed, and the calves continued to receive ad libitum access to water and the common receiving ration minus the dry supplement. On d -46, a common dry supplement (Table 1) was included in the ration. This supplement was formulated to meet or exceed NRC (2000) nutrient

requirements except for Cu, Mn, and Zn. The common supplement contained no additional Cu, Mn, or Zn from organic or inorganic sources.

The calves continued to receive ad libitum access to the common receiving ration, with supplement now included, and water for the duration of the experiment. Feed was delivered twice daily at 0800 h and 1500 h. Bucket feeders were cleaned and orts were weighed back weekly. To ensure that the calves still received their respect experimental mineral treatments during the 4 d BVDV challenge, calves were gathered each morning at 0700 h and sorted into their respective experimental treatments. Each group then received 11.3 kg of the common receiving ration and their respective experimental top dress. After all 11.3 kg was consumed, all calves were returned to the common pasture with the PI animal. Ration samples were collected daily, and dried in a forced air oven for 48 h at 60ºC to determine dry matter. All daily ration samples were composited gravimetrically and analyzed at a commercial laboratory (Servi-Tech Inc., Dodge City, KS) for nutrient composition (Table 1).

Experimental mineral treatments

Starting on d -46 calves on the MIN experimental treatment received a ground corn top dress daily containing 150 mg of Cu, 130 mg of Mn, and 320 mg of Zn in the form of Cu methionine, Mn methionine, and Zn methionine, respectively (Mintrex Cu, Mn, and Zn; Novus International, Inc., St. Charles, MO). Calves on the CON experimental treatment received a top dress daily that only contained ground corn. Top dresses were batched daily and weighed out on a gram scale to the nearest 0.01 g. The

kitchen mixer was cleaned daily before mixing the CON top dress. The CON top dress consisted of 1000 g of ground corn mixed for 5 min and 125 g of the top dress was placed in 8 identical plastic color coded containers.

The mixer was cleaned again prior to making the MIN top dress. The MIN top dress consisted of 968 g of ground corn and 32 g of Mintrex mineral (8 g of Mintrex Cu, 8 g Mintrex Mn, and 16 g Mintrex Zn) on an AF basis. This mixture was mixed for 5 min and 125 g of the top dress was placed in 8 identical plastic color coded containers. This resulted in 121 g of ground corn with 1 g Mintrex Cu, 1 g of Mintrex Mn, and 2 g of Mintrex Zn per MIN container. Top dresses were then delivered to each individual pen or stanchion immediately after delivery of the common ration and gently mixed in. All feed bunks, pens, stanchions, and containers were color coded to match their respective experimental treatment.

Data collection, calculations and statistical analysis

Unshrunk BW were obtained a d -46, d-4, and d 28. All BW reported were shrunk a calculated 2%. Individual BW and experiment DOF was utilized to calculate individual ADG. Experimental DOF and total feed consumption on a DM basis were used to calculate individual average DMI. Average DMI and ADG were then utilized to calculate G:F.

Data were analyzed using repeated measures analysis with the GLIMMIX procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) with steer serving as the experimental unit. Various covariance structures within models were compared. The

covariance structure that best fit the data in the present experiment was a non-structured covariance. In the few instances where model optimization could not be completed, a Toeplitz or banded Toeplitz covariance structure was employed. Sampling time served as the repeated measure and *P*-values are reported for the effect of treatment, time, and the interaction of time and treatment. If a time × treatment interaction was present, a slice output option was used to determine the time points at which treatments were different.

One animal was excluded from the performance analysis due to decreased performance resulting from suffering a mechanical injury prior to the challenge portion of the experiment. The calf was able to recover and was not removed from the experiment, and thus was included in the remainder of the analysis. For the mineral analysis, extreme outliers (samples > 3 SD ± μ) were excluded from the analysis. The removal of any data points from the mineral analysis was completed prior to the linking of individual samples to experimental treatments to ensure unbiased removal of data points. No other data was excluded from the analysis.

Rectal temperatures, respiration rates, and subjective clinical severity scores

Rectal temperatures (TEMP), respiration rates (RR), and clinical severity score (CS) were recorded at h 0, 2, 4, 6, 12, 18, 24, 48, 72, 96, and 168 (d 7). Rectal temperatures were recorded using a digital thermometer (GLA M-500; GLA Agricultural Electronics, San Luis Obispo, CA). Respiration rates were measured by counting flank movements for 30 s while a second person recorded time with a stopwatch. The same person counted flank movements at each sampling time. In addition, all steers were

monitored by trained personnel throughout the experiment for clinical signs characteristic of BRD. The evaluation employed criteria based on the DART™ system (Pharmacia Upjohn Animal Health, Kalamazoo, MI) with some modifications as described by Step et al. (2008). The subjective criteria utilized for evaluating calves consisted of depression, abnormal appetite, and respiratory signs. Signs of depression observed included but were not limited to: depressed attitude, lowered head, glazed or sunken eyes, slow or restricted movement, arched back, difficulty standing or walking, knuckling of joints or dragging toes when walking, and stumbling. Signs of abnormal appetite included: an animal that was completely off feed, an animal eating less than expected or eating extremely slow, a lack of gut fill or gaunt appearance, and obvious body weight loss. Respiratory signs included: labored breathing, extended head and neck (in an attempt to breathe), and audible noise when breathing. The evaluators assigned each calf a CS from 0 to 4 based on the clinical signs and the severity of those signs.

A score of 0 was assigned for a clinically normal appearing calf. A score of 1 was assigned for mild clinical signs, 2 for moderate clinical signs, 3 for severe clinical signs, and 4 for a moribund animal. For a calf to be assigned a score of 4, the calf would not be able to rise, or had extreme difficulty standing, walking, or breathing and would have required immediate assistance. No calves reached a severity score of 3 or 4 in the present experiment.

Tissue sampling for determination of trace mineral status

Whole blood was collected via the jugular vein using an 18 gauge needle and serum separator tube (Corvac Serum Separator Tube; Tyco Healthcare Group LP, Mansfield, MA) at multiple time points throughout the experiment. The whole blood samples were allowed to clot for 12 to 24 h at 4°C. After the clotting time, chilled blood samples were centrifuged at 2,500 × g for 20 min. Serum was aliquoted to 2 mL microcentrifuge tubes and immediately frozen at -20°C until further analyses could be completed. Serum samples that were collected on d -70, -4, 7, 14, 28, and 68 relative to the MH challenge were used for mineral analysis.

Muscle and liver biopsy samples were collected from each calf on d -46, d -4, and d 28. Muscle and liver biopsy samples were also placed into 2 mL microcentrifuge tubes and immediately frozen at -20°C until mineral analyses could be completed. Biopsies were collected from the LM and liver using the procedure described by Sexten et al. (2012) with slight modifications. To obtain the biopsies, steers were briefly restrained in a manual squeeze chute, hair was removed from both biopsy sites using surgical clipper blades, and a local anesthetic (lidocaine HCl, 20 mg/mL, 6 mL/biopsy site) was administered after a preliminary scrub with iodine. The biopsy sites were then surgically scrubbed 3 times with a commercially available iodine scrub followed by rinsing with a 70% isopropyl alcohol solution. After the third scrub and rinse, commercial iodine solution was sprayed on the injection sites.

After ensuring the biopsy sites were thoroughly anesthetized, a scalpel was utilized to make an approximately 1 cm stab incision for the insertion of the biopsy needles. For the muscle biopsy, the incision was made between the 12th and 13th ribs approximately 5 cm lateral to the vertebrae, and a sterile Bergstrom biopsy needle was

used to obtain an approximately 865 mg sample of muscle tissue. For the liver biopsy, the incision was made between the 11th and 12th ribs approximately 25 cm lateral to the vertebrae, and 2.1 mm × 15.2 cm 14-gauge Tru-cut biopsy needle inserted directed cranially and ventrally toward the opposite elbow. The biopsy needle was advanced through the peritoneum, through the diaphragm, and into the liver to obtain an approximately 85 mg sample of liver tissue.

Trace mineral analysis

Serum samples were allowed to thaw at 4°C. After thawing, microcentrifuge tubes containing serum were vortexed and 1 mL of serum was pipetted into large plastic express microwave tubes. One mL of nitric acid was added to each microwave tube, and each tube was plugged and capped. Microwave tubes were vortexed and loaded onto the microwave rack. Tubes were microwaved on a programed cycle at maximum of 1600 watts for 15 min. After completion, the microwave tubes were removed and allowed to cool. After cooling, the contents of the microwave tubes were triple rinsed with milliequivalent water into 5 mL volumetrics. The contents of each volumetric were filtered through a syringe filter into screw cap culture tubes. The filtered samples were then analyzed for mineral concentration by inductively coupled plasma mass spectrometry (ICP-MS) in triplicate (7700 Series ICP-MS; Agilent Technologies, Santa Clara, CA).

Muscle and liver samples were thawed at 4°C. A volume of milliequivalent water was used to flush the samples out of the microcentrifuge tubes and transfer them into

glass capped test tubes. The samples with added water were placed in a drying oven and dried for approximately 24 h at 110°C to obtain an accurate DM for each sample. The samples were then placed in a muffle furnace for 6 h to 15 h at 550°C until all samples were completely ashed. Two mL of nitric acid was added to each glass test tube and the tubes were capped placed in heating racks and heated on hot plates at 260°C until the contents became dissolved in solution. The sample contents were triple rinsed with milliequivalent water into 10 mL volumetrics. The contents of each volumetric were filtered through a syringe filter into screw cap culture tubes. The filtered muscle samples were then analyzed for mineral concentration by inductively coupled plasma mass spectrometry (ICP-MS) in triplicate (7700 Series ICP-MS; Agilent Technologies, Santa Clara, CA). Due to anticipated increased mineral concentrations in the liver, the filtered liver samples were analyzed for mineral concentration by inductively coupled plasma optical emission spectrometry (ICP-OES) in triplicate (Optima 2100 DV; PerkinElmer, Inc., Wellesley, MA).

RESULTS

Effectiveness of BVDV and MH challenge model

The immune challenge model described by Burciaga-Robles et al. (2010) was validated in this experiment. The BVDV and MH challenges were both proved successful via increased antibody titers for BVDV as well as for MH WC and MH LKT. In addition, TEMP and CS both significantly increased during the challenge, then returned to normal

levels. The responses observed to the immune challenge were consistent with previous experiments by Burciaga-Robles et al. (2010) and others using this challenge model.

Calf performance and efficiency

Calf performance and efficiency data are presented in Table 2. There was no time × treatment interaction ($P \geq 0.82$) for any of the performance or efficiency data measured. The BW of calves at the initiation of the experiment was not different between the experimental mineral treatments. In addition, mineral supplementation did not affect BW pre or post challenge ($P = 0.78$). There were also no differences in the ADG ($P = 0.78$) or DMI ($P = 0.48$) of calves between the experimental mineral treatments. With ADG and DMI both being similar between treatments, there was also no difference in G:F between treatments ($P = 0.95$). Time significantly impacted all performance and efficiency variables ($P \leq 0.001$).

Subjective clinical severity scores

The data for CS are presented in Figures 1a and 1b. For the first 24 h following the MH challenge, there was a tendency for a time × treatment interaction ($P = 0.09$). This interaction resulted from calves on the CON treatment having higher ($P < 0.05$) CS at h 12, while calves on the MIN treatment had numerically higher CS at h 18 and h 24. While time did significantly impact CS ($P < 0.0001$) during the first 24 h post MH

challenge, treatment did not (P = 0.87). The greatest CS for MIN calves was observed at h 18. For CON calves, the greatest CS was observed at h 18 and h 24.

When looking at the prolonged (7 d) effect of mineral supplementation on CS following an immune challenge, there was no time × treatment interaction (P = 0.15). Similar to what was observed with the immediate (24 h) response, time was significant (P < 0.0001), but treatment did not have a significant effect on CS (P = 0.53). Most calves returned to a CS of 0 (clinically normal) within 48 h of the MH challenge. All calves had returned to a CS of 0 by d 7.

Rectal temperatures

Calf TEMP is presented in Figures 2a and 2b. For the first 24 h post MH challenge, there was no time × treatment interaction (P = 0.16). Mineral treatment did not affect TEMP (P = 0.78) during the first 24 h post MH challenge. However, time did significantly impact TEMP during this period (P < 0.0001). The greatest TEMP for MIN calves occurred at h 6, while the greatest TEMP for CON calves occurred at h 12. By h 24 TEMP were below 39.5°C for both treatments.

For the week long effect of mineral supplementation on TEMP following an immune challenge, there was no time × treatment interaction (P = 0.12). Similar to the immediate (24 h) response, treatment did not significantly impact TEMP (P = 0. 66). However, time again was significant (P < 0.0001) during the first 7 d post MH challenge. After the initial increase following the MH challenge and falling below 39.5°C at 24 h, calves did not exhibit any increase in TEMP from 48 h to 168 h (d 7).

Respiration rates

Calf RR data are presented in Figures 3a and 3b. For the first 24 h after the MH challenge, there was no time × treatment interaction ($P = 0.65$). Mineral supplementation did not affect RR ($P = 0.35$) during the first 24 h post MH challenge. However, time did significantly impact RR during this period ($P = 0.001$). The greatest RR for all calves occurred at h 12.

For the 7 d effect of mineral supplementation on RR following an immune challenge, there was no time × treatment interaction ($P = 0.31$). Similar to the immediate (24 h) response, treatment did not significantly impact RR ($P = 0. 52$). However, time was significant ($P = 0.0001$) during the first 7 d post MH challenge. After the spike in RR at 12 h, calves did not exhibit any further increase in RR from 48 h to 168 h (d 7). It should be noted that all measurements from 48 h to 168 h were taken at 0700 h.

Serum minerals

Serum mineral data are presented in Figures 4a, 4b, 4c, and 4d. There were no time × treatment interactions ($P \geq 0.54$) for any of the serum mineral concentrations examined. Calves on the CON treatment had greater serum Cu concentrations ($P = 0.05$). Serum Cu was also effected by time ($P < 0.003$) with serum Cu levels for both treatments increasing over time. Serum Mn levels were not affected by experimental treatment ($P = 0.21$) or time ($P = 0.66$). Serum Zn concentrations increased with time ($P < 0.003$)

similarly to serum Cu levels, but were not affected ($P = 0.36$) by mineral treatment. Calves on the CON treatment also had greater serum Fe concentrations ($P = 0.04$) and serum Fe was effected by time ($P < 0.005$).

Muscle minerals

The data for muscle mineral concentrations are presented in Figures 5a, 5b, 5c, and 5d. There were no time × treatment interactions ($P \geq 0.57$) for any of the muscle mineral concentrations examined. In addition, mineral treatment did not impact the muscle mineral concentrations for any of the minerals evaluated ($P \geq 0.38$). The muscle concentrations of Cu ($P < 0.001$), Mn ($P = 0.05$), and Zn ($P = 0.001$) were all effected by time, with all 3 of the TM having lower concentrations within the muscle on d 28 compared to d -46.

Liver minerals

The data for muscle mineral concentrations are presented in Figures 6a, 6b, 6c, and 6d. There was a significant time × treatment interaction observed ($P < 0.0001$) for liver Cu levels. This resulted from a rapid increase in liver Cu concentrations of MIN calves after mineral supplementation began, while calves on the CON treatment had no change in liver Cu over time. This resulted in calves on the MIN treatment having significantly higher liver Cu concentrations ($P = 0.0001$) on d -4 and d 28. There were no time × treatment interactions ($P \geq 0.31$) for Mn, Zn, or Fe liver mineral concentrations.

Calves on the MIN treatment had greater liver Mn concentrations ($P < 0.008$). Liver Mn was also effected by time ($P < 0.003$) with liver Mn levels for both treatments increasing from d -46 to d -4 then decreasing from d -4 to d 28. Mineral supplementation did not affect liver Zn or Fe concentrations ($P \geq 0.39$). However, liver Zn concentrations decreased with time ($P = 0.01$), while liver Fe concentrations increased with time ($P < 0.004$).

DISCUSSION

The supplementation of TM including Cu, Mn, and Zn has been a longstanding management practice within the feedlot industry primarily to avoid unwanted deficiencies and promote maximum animal performance. While these TM make up a very small percentage of a calf's body mass and are required in extremely small amounts within the diet, it has been well established that certain TM are essential for overall performance, health, and immune function. In addition to general health and immune function mechanisms, the supplementation of TM has been demonstrated to alter specific immune function measurements and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have demonstrated no improvements in performance or health variables from the supplementation of TM.

The published research justifying the supplementation of Cu, Mn, or Zn at levels greater than published requirements is lacking and has produced inconsistent results to date. Regardless, the inclusion of these 3 TM in feedlot diets at concentrations 2 to 3 times greater than published requirements is common by feedlot nutritionists according to

Vasconcelos and Galyean (2007). The role of TM in immune function, combined with the unknown TM status of newly received calves, and expectation of depressed DMI early in the receiving period may serve as a possible explanation for the reported increase in ration TM inclusion.

The challenge employed in this experiment was the same model described by Burciaga-Robles et al. (2010) with slight modifications. This challenge model was selected due to the ability of this model to successfully simulate natural BRD pathogenesis. Additionally this model is able to induce a controlled, simulated BRD event, while simultaneously allowing for accurate determination of antibody responses due to the challenge with known viral and bacterial pathogens. The pathogenesis of BRD typically involves compromised respiratory immune mechanisms and a primary infection with one or more respiratory viruses. The viral infection and the calf's impaired immune response to the virus further compromise the immune system and allow for the colonization of lung tissues by bacteria (Hodgins et al., 2002). Most of the pathogens associated with increased BRD incidence are well documented within the literature. However, many of these pathogens are also frequently isolated from the respiratory tract of clinically healthy cattle making the analysis of BRD in field studies difficult.

The decision to use the specific pathogens in this model are justified by the published literature and previous experiences. It has been reported that PI BVDV calves are a principal source of disease transmission in feedlots (O'Connor et al., 2005). In addition, the presence of a PI BVDV animal within a feedlot pen has been reported to increase the risk for antimicrobial treatment for clinical BRD by 43% compared to non-exposed cattle (Loneragan et al., 2005). In multiple experiments, BVDV 1b has been the

predominant BVDV subtype isolated from calves diagnosed with BRD (Fulton et al. 2002a; Fulton et al. 2002b). The most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD is MH serotype 1, and this serotype has been shown to be responsible for characteristic BRD infection in calves (Hodgins and Shewen, 2004; Booker et al., 2008; Griffin et al., 2010).

The objective of this experiment was to determine the influence of Cu, Mn, and Zn supplementation on the performance, clinical signs, and mineral balance of calves following a BVDV and MH immune challenge. In the current experiment, calves had average TEMP of 40.3°C by h 6 and had average CS of 1 by h 18. The CS observed for calves in this experiment would have dictated that the calves would have been pulled for exhibiting clinical signs of BRD and evaluated for subsequent antimicrobial treatment under the standard operating protocols in place at our facility. The TEMP observed in these calves would have dictated that the calves would have received an antimicrobial treatment for clinical BRD under the same standard operating protocols. At our facility, a calf can meet treatment criteria for clinical BRD and receive an antimicrobial via 2 standard treatment protocols. The first being that an animal can be pulled with a CS of 1 or 2 and had a TEMP of 40°C or greater. The second being that an animal can be pulled with a severe CS (CS = 3 or 4) regardless of TEMP. The increased TEMP and CS of calves challenged in combination with increased BVDV antibody titers and MH WC and LKT antibody titers validated the immune challenge in the current experiment. The increases observed for these 4 response variables would have been similar to those observed by Burciaga-Robles et al. (2010) using a similar challenge model.

In the present experiment, no measures of calf performance or efficiency were affected by the supplementation of additional Cu, Mn, or Zn. A major reason for this could be due to the nature of the basal diet feed to all calves. All nutrients aside from Cu, Mn, and Zn were formulated to exceed NRC (2000) requirements. While no additional Cu, Mn, or Zn were included in the common diet, the analyzed values of the common ration indicate that the diet contained more than adequate in Mn and Zn concentrations. When samples were analyzed by an independent laboratory, the concentration of Mn in the diet was found to be over 2.5 times the NRC (2000) requirement and the concentration of Zn in the diet was found to be over 1.5 times the NRC (2000) requirement. The only mineral deficiency present in the basal diet was Cu.

When analyzed, the common ration contained only 60% of Cu recommended by the NRC (2000). The reason for the high Mn and Zn levels in the diet result primarily from the inclusion of by-product feeds. To accurately reflect production settings, it was important to use commercially relevant ingredients rather than feed a semi-purified diet to control for mineral concentration. With the diet ingredients at our disposal, it was not possible to formulate a Mn or Zn deficient diet. While the diet fed to the calves in this experiment was Cu deficient, the calves used in this experiment were not determined to be Cu deficient prior to the initiation of the experiment as indicted by liver Cu concentrations (Dias et al., 2013). On d -46, prior to any mineral supplementation, liver Cu concentrations were 99.1 mg/kg (dry weight basis) across all calves. The lack of an induced Mn or Zn deficiency at any time during the experiment, combined with a lack of Cu deficiency prior to the initiation of the experiment likely explains the lack of performance difference observed between the mineral treatments.

Calf performance was severely impacted during the challenge portion of the experiment. The ADG of all calves was reduced by approximately 60% from d -4 through d 28 compared to d -46 through d -4. While a portion of this reduction in performance is certainly the result of moving calves from individual pens into metabolism stanchions, the calves were only in the stanchions for 7 d during this period. If it is assumed that stanchions were solely responsible for this lack of performance, the stanchions would have to account for average loss of 20.8 kg of BW over this 7 d period. While it is likely that the stanchions did negatively impact performance, they are unlikely responsible for the majority of the performance loss. There are several probable reasons for this justification. First, the DMI of calves was also depressed during this period, but it was only reduced by 7.8%. In addition, calves were adapted to the stanchions for 5 d prior to the initiation of the experiment, and as a result the stress of simply being placed in a stanchion should have been minimized. Finally, a BW was obtained on d 14 (not reported) at the time of sampling. Based on the BW taken on d 14 and d 28, the calves only gained an average of 0.37 kg/d for the last 14 d of the experiment. This would have allowed the calves 7 d to adjust and pick up any losses in fill that occurred while in the stanchions. This amounted to 43% reduction in pre challenge ADG for the calves from d 14 to d 28.

Not observing a performance response to TM supplementation is not uncommon unless a severe deficiency is induced. In a classical experiment, Ivan and Grieve (1975) evaluated the effects of Cu, Mn, and Zn supplementation alone or in all possible combination on the performance of Holstein calves. These authors found that the

supplementation of the basal ration with the individual TM or combinations had no effect on calf performance.

Some experiments that have induced or attempted to induce TM deficiencies have also failed to see a performance response. Genther and Hansen (2014) observed the effects of dietary TM supplementation on growth performance. Calves either received a control diet supplemented with Cu, Mn, Se, and Zn to meet or exceed NRC (2000) recommendations or a mineral depleted diet containing no supplemental Cu, Mn, Se, and Zn, but additional Fe (300 mg/kg) and molybdenum (5 mg/kg). The authors found no significant differences ($P \geq 0.32$) in overall BW, ADG, DMI, or G:F between calves in control or mineral depleted treatments during an 84 d period. After the depletion period, the calves were finished. No differences ($P \geq 0.13$) in live performance existed between the dietary treatments for the finishing portion of the experiment as well.

It is also common to not observe a performance response to TM supplementation when a stressor, such a transportation, is applied to calves. Arthington et al. (2014) examined the effects of multiple TM injections on the performance and trace mineral status of beef calves both before and after weaning. Treated calves were injected with 60 mg/mL of Zn, 10 mg/mL of Mn, 15 mg/mL Cu, and 5 mg/mL of Se. The control calves were administered an equivalent volume of sterile saline solution. The experimental treatments were then readministered at 100 and 200 d of age. The TM injection had no impact ($P \geq 0.40$) on overall pre weaning (birth to d 250) performance. After weaning, a subset of heifers were selected and transported 1,600 km. Heifers were administered an additional dose of injectable TM or saline following transport. The ADG for heifers receiving the TM injection was actually reduced ($P = 0.05$) compared to control heifers in

the first 14 d following transport. In an additional experiment heifers that were not previously exposed to TM injection were enrolled in a 177-d heifer development experiment. Heifers again received 3 TM injections or 3 injections of sterile saline. The final BW of heifers was not different ($P = 0.64$) between treatments while heifers receiving the TM injections had a tendency ($P = 0.06$) for improved overall ADG.

In the current experiment, there was a tendency for a time × treatment interaction in CS during the first 24 h following the MH challenge. This was a result of CON calves having higher CS at h 12, while MIN calves had numerically higher CS at h 18 and h 24. The greatest CS for MIN calves was observed at h 18. For CON calves, the greatest clinical score was observed at both h 18 and h 24. Based on CS, the majority of these calves would have been pulled between 12 and 24 h by a trained evaluator if they were in a pen environment. When the extended effect (d 0 to d 7) of mineral supplementation on CS following an immune challenge was observed, no interaction or treatment effect was observed, but time significantly impacted CS. Most calves appeared clinically normal within 48 h of the MH challenge and all calves had returned to a CS of 0 by d 7. No calves in the present experiment received a CS of 3 or 4 at any time during the experiment.

In the experiment conducted by Burciaga-Robles et al. (2010), CS were only reported for h 72. The values (CS = 0.45) reported for calves exposed to both BVDV and MH at this time point in their experiment would be higher than those in the present experiment. Corrigan et al. (2007) examined the effects of melengestrol acetate on heifers challenged with MH. The subject measure used in this experiment to assess the clinical severity of the MH challenge was termed total observational score (TOS). The authors

defined TOS as the sum of the clinical scores for respiratory index, activity level, hydration index, appetite index, fecal score, nasal discharge, and ocular discharge. This value would account for many of the same things as CS in the current experiment, but also took RR into account. While a direct comparison between the scores reported in the 2 experiments cannot be made, there is merit in comparing the time points in which calves exhibited elevated CS or TOSs. In the experiment by Corrigan et al. (2007), the highest TOS for all calves occurred at 12 h post MH inoculation. This is in agreement with the present experiment.

Theurer et al. (2013) observed the effects of MH pneumonia on the clinical behavior and physiologic responses of calves during exposure to high environmental temperatures. The authors used a clinical illness score (CIS) using a 1 to 4 scale similar to the system used in the present experiment. All calves inoculated with MH had a CIS of 2 (slight illness, mild depression, and/or cough) at 24 h after MH inoculation. The percentage of calves inoculated with MH with a CIS of 2 varied between 10% and 70% on subsequent evaluation periods with no calf being classified as greater than CIS 2 during the experiment. These results would also be similar to the present experiment with a slightly longer duration to achieve elevated CIS.

These results for CS in the present experiment would be expected for this type of challenge model in our experience. The overall challenge is mild by commercial production standards as it only consists of the exposure to 1 respiratory virus and a controlled dose of 1 bacterial pathogen. The calves in this experiment had previously received all standard vaccinations except for the 2 pathogens for which they were challenged. In addition, these calves had been weaned for approximately 8 wk prior to the

challenge and should not have been experiencing many of the stressors common for newly received calves in commercial settings. These calves would have also had positive energy balances prior to entering the challenge portion of this experiment, and would not have been deficient in any nutrients other than potentially Cu in the case of the CON calves.

In the current experiment, there was no time × treatment interaction or treatment effect for TEMP during the first 24 h or over the course of the first wk. However, time significantly impacted TEMP during both periods. The greatest TEMP for MIN calves occurred at h 6, while the greatest TEMP for CON calves occurred at h 12. By h 24 TEMP were below 40°C for both treatments. After falling below 40°C by 24 h, calves did not exhibit any increase in TEMP from 48 h to 168 h (d 7).

These results would be similar to those observed by Burciaga-Robles et al. (2010). The highest TEMP for calves that were exposed to BVDV and then challenged with MH was observed at h 6 in that experiment. The length of time calves demonstrated a fever was also similar between the 2 experiments. In their experiment, the average TEMP of calves challenged fell below 40°C by 24 h and the calves did not have increased TEMP out to 96 h. The last TEMP taken in their experiment were at h 96. The TEMP results of the present experiment are also supported by the results of Confer et al. (2008), where cattle that were challenged with MH demonstrated an increased TEMP compared to control animals. In addition, Corrigan et al. (2007) reported that heifers challenged with MH had increased TEMP (> 40°C) between 0 to 12 h post challenge and returned to normal TEMP by 24 h. Finally, Theurer et al. (2013) stated that calves challenged with MH had greater TEMP compared to unchallenged calves from 6 h

through 24 h post MH challenge. The calves challenged with MH in their experiment had TEMP above 40°C beginning at h 6 and the average TEMP of challenged calves fell below 40°C by 20 h.

In the current experiment, there was no time × treatment interaction for RR during the first 24 h or over the course of the first wk and mineral treatment did not affect RR over either interval. However, RR was significantly impacted by time during both periods. The greatest RR for all calves occurred at h 12. This increase in RR would follow the trend for elevated TEMP in the calves at this time as well. However, it should be noted that this time coincided with 1900 h and the environmental temperature and humidity were both high at this time. The facility was not environmentally controlled, and the majority of this spike in RR is likely a result of the increased environmental stress rather than the immune challenge. After the spike in RR at 12 h, the calves did not exhibit any further increase in RR from 48 h to 168 h (d 7). Again it should be noted that all measurements from 48 h to 168 h were taken at 0700 h.

In the experiment conducted by Burciaga-Robles et al. (2010), RR were only reported for h 72. The values reported for this time point in that experiment would be considerable lower than those in our experiment. The authors did state that there was a BVDV × MH interaction for RR where calves exposed to BVDV and then challenged with MH had greater RR, while calves challenged with MH but not exposed to BVDV had lower RR compared to those calves not challenged with MH. Interestingly, Burciaga-Robles et al. (2010) noted that RR was greatest for the calves that received no exposure to BVDV and were not challenged with MH compared to calves exposed to BVDV and then challenged with MH or calves challenged with either of the pathogens alone. In

addition, the authors noted that a decreased RR was present for all calves that had increased TEMP compared the unchallenged calves. Since Corrigan et al. (2007) only reported TOS, which would include many measures equivalent to our CS, but also took RR into account, we cannot be certain when the highest RR occurred in that experiment. However the highest TOS occurred at 12 h post MH inoculation. This would be in agreement with the peak CS and RR observed in the present experiment. Theurer et al. (2013) found no differences in RR between MH challenged and unchallenged calves. The authors noted that the calves were housed in extremely high ambient temperatures throughout the experiment and that increases in RRs in response to heat stress potentially obscured their ability pick up differences in RR due to the MH challenge. It is believed that the much of the elevation in RR observed in the present experiment resulted from high ambient temperatures as well.

There were no time × treatment interactions for any of the serum mineral concentrations evaluated in the present experiment. Experimental treatment did not affect serum Mn or Zn concentrations. Calves on the CON treatment had greater serum concentrations of Cu and Fe. Serum Cu and Zn was effected by time with serum levels for both minerals increasing over time for both experimental treatments. Serum Fe was also effected by time with serum Fe levels being somewhat erratic over time. The results in the literature concerning serum TM concentrations are highly varied, and extremely inconsistent from experiment to experiment. Upon reviewing the data, multiple sources were found that both agreed and disagreed with the results in the current experiment. Mineral metabolism within the body is extremely complex, and changes in mineral stores within the body may or may not result in changes in serum mineral concentrations.

Orr et al. (1990) evaluated the effects of BRD and infectious bovine rhinotracheitis (IBR) on serum Cu and Zn. The authors collected serum samples at specific intervals measured serum mineral concentrations for several experiments. Serum Zn was decreased by 34%, 57%, 29% and 15% for calves that were morbid or challenged with IBR across 4 experiments when measured at peak morbidity. In contrast, serum Cu was increased by 5%, 15%, 40%, and 33% for calves that were morbid or challenged with IBR when measured across 4 experiments at peak morbidity. These results for Cu would be similar to those found for Cu in the current experiment where serum Cu increased over time for both experimental treatments. However the results for Zn concentrations observed by Orr et al. (1990) would be in stark contrast to what was observed in the current experiment where Zn concentrations also increased over time.

Nockels et al. (1993) examined the effects of induced stress on the Cu and Zn balance of calves fed organic and inorganic TM. The authors reported that serum Cu was decreased for 4 of the 5 time points sampled and that multiple stress events or mineral removals from the diet caused a decrease in serum Cu. In contrast, serum Zn was increased for both of the induced stress events within the experiment. These results would directly contradict those reported by Orr et al. (1990) and would be similar to the response observed with serum Zn over time in the current experiment, but be in stark contrast to what was observed in the current experiment for serum Cu.

Arthington and Havenga (2012) investigated the effects of injectable TM on calves receiving a viral vaccination. Serum mineral concentrations were evaluated on d 0 prior to vaccination and TM administration and on d 14. No differences existed in serum Cu, Mn, or Zn between the treatments on d 0. Control steers had decreased serum Zn

concentrations on d 14 compared to d 0. This resulted in TM injected steers having greater serum Zn concentrations compared with Control steers on d 14. Steers injected with TM had an increase in serum Cu levels on d 14 relative to d 0 levels. Serum Cu was not different between treatment groups at d 0 or d 14. In addition, serum Mn was not effected by time or mineral treatment. The results for Mn presented in this experiment would be similar to the results found for serum Mn in the present experiment. The results for the other TM would contradict our results except for the time effect on serum Cu concentrations.

Rhodes et al. (2003) studied the effects of TM supplementation level and source on plasma Cu and Zn concentrations of steers. There were no differences in plasma Cu or Zn due to source or concentration of TM supplementation at the end of the evaluation period (d 198). The authors did find that both plasma Cu and Zn concentrations increased over time which would support the results observed for serum Cu and Zn in the present experiment. Richeson and Kegley (2011) examined the effect of injectable TM on the health and performance of highly stressed heifers and found similar results to Rhodes et al. (2003). While the authors found no differences in plasma Cu and Zn concentrations between treatments, the plasma concentrations of both minerals increased over time. These results would be in complete agreement with those in the present experiment. The authors reported that plasma Zn was marginal (0.59 mg/L) on d 0, but increased to adequate levels (1.72 mg/L) by d 28. Similarly, plasma Cu increased from adequate levels on d 0 (0.84 mg/L) to what the authors termed as toxic levels (1.42 mg/L) on d 28.

No time × treatment interactions were observed for any of the muscle mineral concentrations examined in the current experiment. In addition, mineral treatment did not

impact muscle mineral concentrations for any of the TM evaluated. The muscle concentrations of Cu, Mn, and Zn were all effected by time, with all 3 of the TM having lower concentrations within the muscle at the end of the experiment compared to pre supplemented levels. The literature concerning the effects of TM supplementation or immune challenges on muscle TM concentrations is limited. The values reported in this experiment would be similar to those reported by Standish et al. (1971).

There was a significant time × treatment interaction observed for liver Cu levels in the current experiment. This interaction was the result of a rapid increase in liver Cu concentrations of MIN calves after TM supplementation began, while CON calves had no noticeable change in liver Cu over time. In addition, mineral treatment did effect liver Cu levels with calves on the MIN treatment having significantly higher liver Cu concentrations on d -4 and d 28. Time also significantly impacted liver Cu when averaged across treatments. There were no time × treatment interactions for Mn, Zn, or Fe liver mineral concentrations. Mineral supplementation did effect liver Mn concentrations with calves on the MIN treatment having greater liver Mn levels. Liver Mn was also effected by time with liver Mn levels for both treatments initially increasing from d -46 to d -4 then decreasing from d -4 to d 28. Treatment did not affect liver Zn or Fe concentrations, however both TM were effected by time. Liver Zn concentrations decreased over time. In contrast, liver Fe concentrations increased over time.

The liver TM concentrations in the current experiment were compared to critical values reported by McDowell (1985) and standardized reference values reported by Kincaid (1999). According to Kincaid (1999), liver Cu levels for all calves in the current experiment would have been categorized as marginally deficient (< 125 mg/kg) on d -46.

Without any additional Cu supplementation the calves on the CON treatment continued to be considered marginally Cu deficient throughout the experiment. Calves on the MIN treatment had increased liver Cu stores by d -4 due to mineral supplementation, and were considered to have adequate liver Cu concentrations (125 to 600 mg/kg) on d -4 and d 28. Liver Zn concentrations were not affected by mineral supplementation, and were considered to be adequate (25 to 200 mg/kg) for all calves throughout the experiment. In contrast, liver Mn levels were increased for calves on the MIN treatment, but all calves were still considered to be marginally deficient (7 to 13 mg/kg) in liver Mn throughout the experiment. While the liver Cu concentrations for CON calves and liver Mn concentrations for all calves were classified as marginally deficient, it should be noted that the liver concentrations of all 3 TM would not have been considered clinically deficient or below critical levels at any time during the experiment (McDowell, 1985; Kincaid, 1999).

Similar to what was found when reviewing the literature for serum and mineral TM concentrations, the published results concerning liver TM levels are somewhat varied though the results are more consistent than those found for other body TM concentrations. There does still seem to be inconsistency from experiment to experiment, but the sheer number of studies evaluating liver mineral concentrations allows for adequate comparisons to be made. There are multiple experiments evaluation liver TM status, but no 2 are exactly alike, and multiple studies can be found that both agree and disagree with the results presented in the current experiment. That said, multiple classical and recent research experiments support the findings of the current experiment in regard to liver TM concentrations. The liver is the primary storage organ for TM within the

body, therefore liver TM concentrations give the best indication of the true TM status of an animal.

Arthington et al. (2014) examined the effect TM injections on liver TM concentrations when averaged across 3 sampling times. The authors found that the administration of injectable TM resulted in greater liver Cu concentrations (34% increase) and a decreased liver Fe concentrations (13% decrease). Injectable TM did not affect liver Mn or Zn concentrations. After transport stress, a treatment × time interaction was found for liver Cu and Zn concentrations. The liver concentrations of Cu and Zn increased following the TM injection, and were greater for injected heifers on d 13 compared to heifers injected with saline. Concentrations of Fe within the liver tended to be reduced for TM injected heifers. The results found by Arthington et al. (2014) would support our findings for liver Cu in the present experiment where liver Cu increased over time for supplemented calves, but remained static for unsupplemented calves. However, the results reported for Zn would contradict those in the current experiment.

Genther and Hansen (2014) examined the effect of previous TM status and TM mineral injection on mineral balance in calves. Calves were assigned to either a control diet including supplemental Cu, Mn, Se, and Zn or a deficient diet containing no supplemental Cu, Mn, Se, or Zn plus additional Fe and Mo as antagonists to the other TM for 84 d. After the depletion period, raw liver Cu and concentrations for steers on the deficient diet were less than those for steers on the control diet (79.0 ± 11.60 mg/kg DM versus 228.8 ± 11.60 mg/kg DM, respectively). In addition, liver Mn concentrations tended to be greater for steers on the control diet compared to steers on the deficient diet (9.37 ± 0.261 mg/kg DM versus 8.71 ± 0.261 mg/kg DM, respectively. Liver Zn

concentrations were not affected by diet TM inclusion. These values reported by Genther and Hansen (2014) would support the findings in the current experiment for liver TM.

Rhodes et al. (2003) did not have any TM deficiencies in their experiment, but rather examined organic and inorganic TM supplementation at varying concentrations in the diet. For all experimental mineral treatments, liver Cu numerically increased throughout the experiment. Similarly, the concentration of Mn within the liver increased for all experimental treatments for the duration of the experiment. In contrast, liver Zn numerically decreased for all experimental mineral treatments compared to the initial levels observed at the beginning of the experiment. These results while not while not statistically significant, would support the liver TM data in the current experiment.

Stabel et al. (1993) examined the effects of a Cu deficiency by feeding a semi-purified diet (1.5 mg/kg Cu) supplemented with 0 mg/kg or 10 mg/kg of Cu on mineral balance and immune function of calves challenged with IBR and MH. The results observed in their experiment would be in agreement with our results. The authors found that Cu supplementation resulted in increased liver Cu levels. The basal diet contained adequate Mn and Zn, and no differences were observed in liver Mn or Zn levels between treatments. The experimental treatments in their experiment and the present experiment would have been very similar in that the only deficiency present would have been for Cu within the unsupplemented diet.

Finally, Ivan and Grieve (1975) evaluated the effects of supplemental Cu, Mn, and Zn on the tissue TM content of calves. Increased dietary Cu inclusion resulted in increased liver Cu levels. The inclusion of Cu in the diet did not impact liver Mn or Zn

levels. Increased dietary Mn concentration resulted in increased concentrations of all TM (Cu, Mn, and Zn) within the liver. Increased dietary Zn concentrations resulted in decreased liver Cu concentrations but did not affect liver Mn or Zn concentrations. The number of calves used in this experiment was small, but the results demonstrate many aspects of classical TM metabolism. In addition, the results help to explain those observed in the current experiment.

Care must be taken in interpreting serum and muscle TM concentrations. The most accurate predictor of TM status in calves remains the liver, and the results of liver TM concentrations in the current experiment are supported by much of the published literature. Within the body the liver is the primary storage organ for TM and it functions to maintain appropriate blood concentrations of TM. When a diet deficient in Cu is fed, as was the case for calves on the CON treatment in this experiment, Cu will be released from the liver in order to maintain proper blood Cu levels. As a result, serum Cu levels can be inflated, and may not accurately predict Cu status within the body. This would help to explain serum Cu was increased in CON calves compared to MIN calves, even though the MIN calves had significantly improved liver Cu stores.

In addition, when dietary Cu is adequate or excessive, the storage of Cu by the liver should exceed the mobilization of Cu resulting in net buildup of liver Cu stores. However, since serum Cu concentrations are maintained within such narrow ranges, an increase in liver Cu may not be reflected by a representative increase in serum Cu. This would help to explain how serum Cu was increased only slightly for MIN calves overtime, even though the MIN calves had significantly improved liver Cu stores.

Conclusions

The TM Cu, Mn, and Zn are frequently included at levels well in excess of published requirements in feedlot diets. There are certainly multiple reasons for this, but the role of TM in immune function, combined with the unknown TM status of newly received calves likely serves as a plausible explanation even though documented benefits of this practice are lacking. Previous nutrition, stressors, and immune challenge events all can certainly impact the TM balance of calves. The immune challenge in this experiment impacted the CS, TEMP, and mineral balance of calves. With the increased by-product inclusion levels in all feedlot diets, it is unlikely that severe dietary Mn or Zn deficiencies exist in practical production settings even if supplemental Mn and Zn are not included in the diet. However, most diets will be deficient in Cu if additional Cu is not added to the diet. In addition, many regions of the U.S. including the Southern Plains and over 40% of the cattle in the country according to USDA reports are at least marginally Cu deficient. When Cu is supplemented to calves receiving a marginally Cu deficient diet, Cu status within the body can be improved.

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Table 5-1. Composition of common receiving diet[1]

Ingredient (%)[1]	
Prairie hay	35.0
Dry-rolled corn	28.0
Wet corn gluten feed[2]	15.0
Dried distillers grains plus solubles	15.0
Dry supplement B-278[3]	3.5
Liquid supplement[4]	3.5
Nutrient Composition[1,5]	
NE_m, Mcal/kg	1.62
NE_g, Mcal/kg	1.03
TDN, %	69.83
Crude protein, %	16.20
Crude fat, %	3.13
NDF, %	44.47
ADF, %	23.00
Calcium, %	0.80
Phosphorus, %	0.51
Magnesium, %	0.26
Potassium, %	1.03
Sulfur, %	0.30
Copper, mg/kg	6.00
Manganese, mg/kg	50.33
Zinc, mg/kg	47.33
Iron, mg/kg	614.00

[1]All ingredient and nutrient values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Dry supplement B-278 was formulated to contain: 38.123% ground corn, 34.705% limestone, 23.477% wheat midds, 2.571% salt, 0.224% vitamin A (30,000 IU/g), 0.134% vitamin E (500 IU/g), 0.002% vitamin D (500,000 IU/g), 0.002% EDDI, 0.496% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.266% Tylan 40 (Elanco Animal Health; Indianapolis, IN) on a DM basis.
[4]Synergy 19-14 (Westway Feed Products; New Orleans, LA).
[5]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Table 5-2. Effects of trace mineral supplementation on the performance of calves exposed to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection

	Experimental treatment[1]			*P*-value[2]		
Variable	CON	MIN	Pooled SEM	Treatment	Time	Time × treatment
Body weight[3], kg						
d -46	223	225	7.85	0.78	< 0.001	0.95
d -4	268	272	10.7			
d 28	281	287	13.4			
Average daily gain[4], kg						
d -46 to d -4	1.06	1.12	0.12	0.78	< 0.001	0.93
d -4 to d 28	0.42	0.46	0.12			
Dry matter intake[5], kg						
d -46 to d -4	5.88	6.23	0.35	0.48	< 0.001	0.82
d -4 to d 28	5.45	5.84	0.39			
Gain:Feed[6]						
d -46 to d -4	0.177	0.176	0.014	0.95	0.001	0.88
d -4 to d 28	0.117	0.120	0.020			

[1]Experimental mineral treatments: CON = ground corn only, no additional Cu, Mn, or Zn included in the diet; MIN = ground corn containing 150 mg of Cu, 130 mg of Mn, and 320 mg of Zn in the form of Cu methionine, Mn methionine, and Zn methionine, respectively (Mintrex Cu, Mn, and Zn; Novus International, Inc., St. Charles, MO).
[2]*P*-values are included for the effects of experimental treatment, time, and the time by treatment interaction.
[3]Treatment BW with a calculated 2% shrink.
[4]Treatment ADG was calculated from the shrunk (2%) BW and DOF between the time periods.
[5]Treatment DMI was calculated by taking the total DMI in kg for a steer for the period divided by the DOF between the time periods.
[6]Treatment G:F was calculated by taking the individual ADG in kg divided by the individual average DMI in kg for the time periods.

Figures

Figure 5-1a. Subjective clinical severity score of calves during the first 24 h following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. Subjective clinical severity scores were assigned by trained personnel and ranged from 0 to 4: 0 = clinically normal appearing calf, 1 = mild clinical signs, 2 = moderate clinical signs, 3 = severe clinical signs, and 4 = extreme clinical signs or a moribund animal. There was a tendency for a time × treatment interaction ($P = 0.09$). Treatment was not significant ($P = 0.87$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. Within time points, the slice output option of SAS was used to perform mean separations. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 5-1b. Subjective clinical severity score of calves during the first 7 d following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. Subjective clinical severity scores were assigned by trained personnel and ranged from 0 to 4: 0 = clinically normal appearing calf, 1 = mild clinical signs, 2 = moderate clinical signs, 3 = severe clinical signs, and 4 = extreme clinical signs or a moribund animal. There was no time × treatment interaction ($P = 0.15$) and treatment was not significant ($P = 0.53$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-2a. Rectal temperature of calves during the first 24 h following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection.

There was no time × treatment interaction ($P = 0.16$) and treatment was not significant ($P = 0.78$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-2b. Rectal temperature of calves during the first 7 d following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.12$) and treatment was not significant ($P = 0.66$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-3a. Respirations per 30 s of calves during the first 24 h following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.65$) and treatment was not significant ($P = 0.35$). However, time was significant ($P = 0.001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-3b. Respirations per 30 s of calves during the first 7 d following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.31$) and treatment was not significant ($P = 0.52$). However, time was significant ($P = 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-4a. Serum copper (Cu) concentrations of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.83$) for serum Cu levels. However, treatment did effect serum Cu ($P = 0.05$). Time was also significant ($P <$

0.003) for serum Cu. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-4b. Serum manganese (Mn) concentrations of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.54$) and treatment was not significant ($P = 0.21$) for serum Mn levels. Time also did not significantly impact serum Mn ($P = 0.66$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-4c. Serum zinc (Zn) concentrations of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.89$) and treatment was not significant ($P = 0.36$) for serum Zn levels. However, time was significant ($P < 0.003$) for serum Zn concentrations. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-4d. Serum iron (Fe) concentrations of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.70$) for serum Fe levels. However, treatment did effect serum Fe levels ($P = 0.04$). Time was also significant for serum Fe concentrations ($P < 0.005$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-5a. Muscle copper (Cu) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent

Mannheimia haemolytica infection. There was no time × treatment interaction ($P = 0.76$) for muscle Cu levels and treatment did not affect muscle Cu ($P = 0.94$). However, time was significant ($P < 0.001$) for muscle Cu. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-5b. Muscle manganese (Mn) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.57$) and treatment was not significant ($P = 0.38$) for muscle Mn levels. However, time did significantly impact muscle Mn ($P = 0.05$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-5c. Muscle zinc (Zn) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.70$) and treatment was not significant ($P = 0.82$) for muscle Zn levels. However, time was significant ($P = 0.001$) for muscle Zn concentrations. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-5d. Muscle iron (Fe) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.61$) for muscle Fe levels and treatment was not significant for muscle Fe levels ($P = 0.58$). Time was also not significant for muscle Fe concentrations ($P = 0.25$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-6a. Liver copper (Cu) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was a significant time × treatment interaction ($P < 0.0001$) for liver Cu levels. Treatment did effect liver Cu levels ($P = 0.0001$). Time was also significant for liver Cu ($P = 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. Within time points, the slice output option of SAS was used to perform mean separations. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 5-6b. Liver manganese (Mn) concentrations of calves (dry weight basis) preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.89$). Treatment did effect liver Mn levels ($P < 0.008$). Time was also significant for liver Mn ($P < 0.003$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-6c. Liver zinc (Zn) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica* infection. There was no time × treatment interaction ($P = 0.53$) and treatment was not significant ($P = 0.39$) for liver Zn levels. However, time was significant ($P = 0.01$) for liver Zn concentrations. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 5-6d. Liver iron (Fe) concentrations (dry weight basis) of calves preceding and following exposure to bovine viral diarrhea virus type 1b and subsequent *Mannheimia haemolytica*

infection. There was no time × treatment interaction ($P = 0.31$) for liver Fe levels and treatment did effect liver Fe levels ($P = 0.86$). Time was significant for liver Fe concentrations ($P < 0.004$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

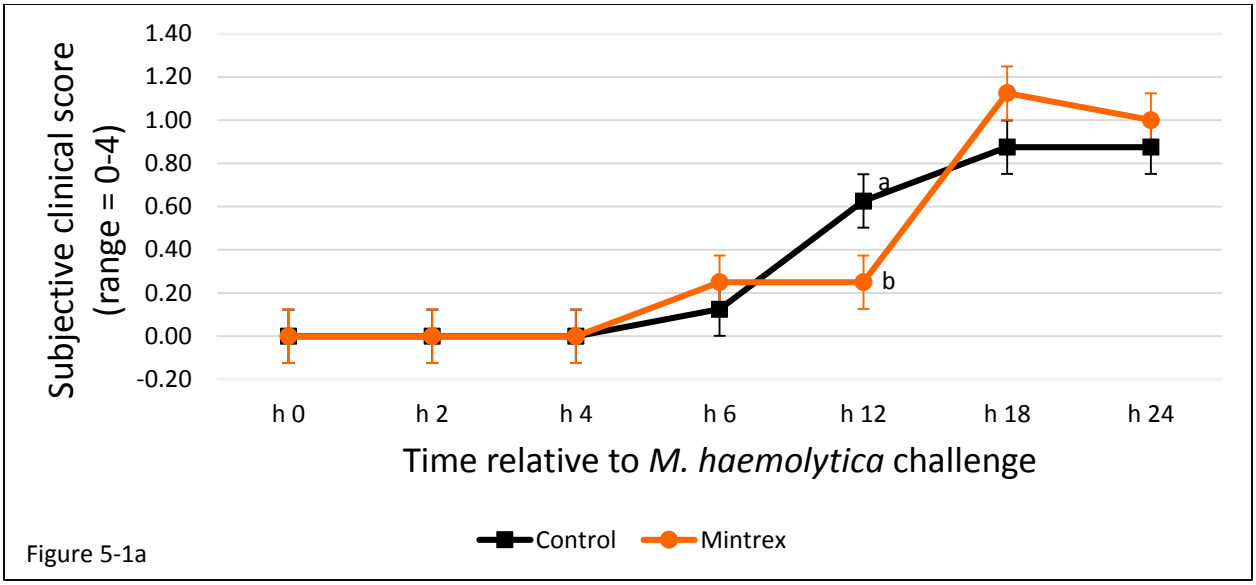


Figure 5-1a

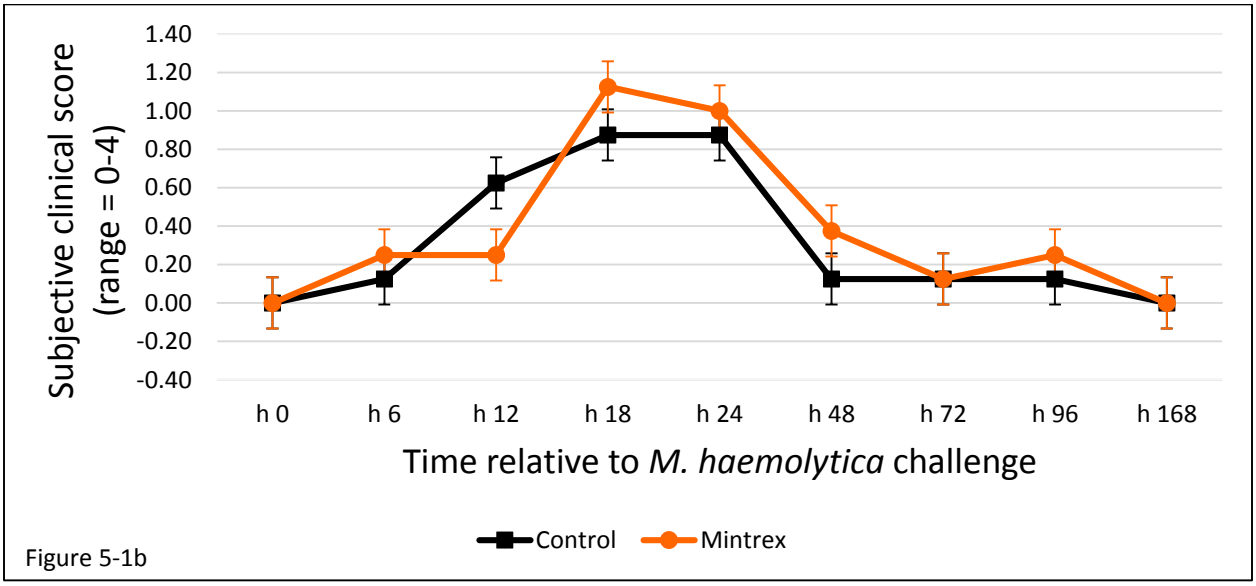
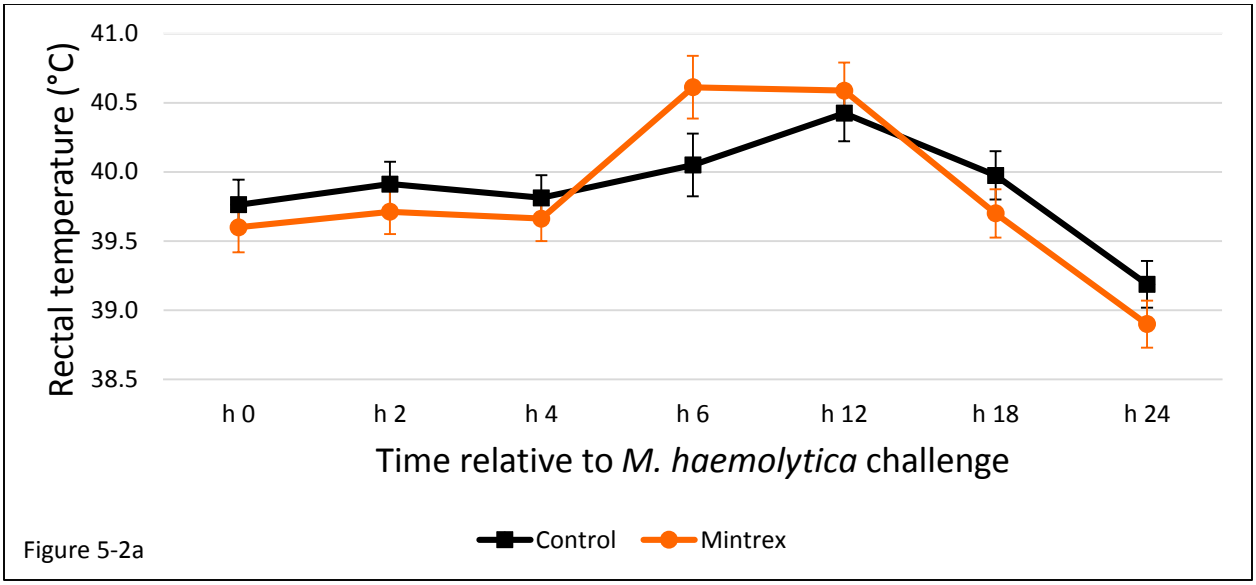


Figure 5-1b



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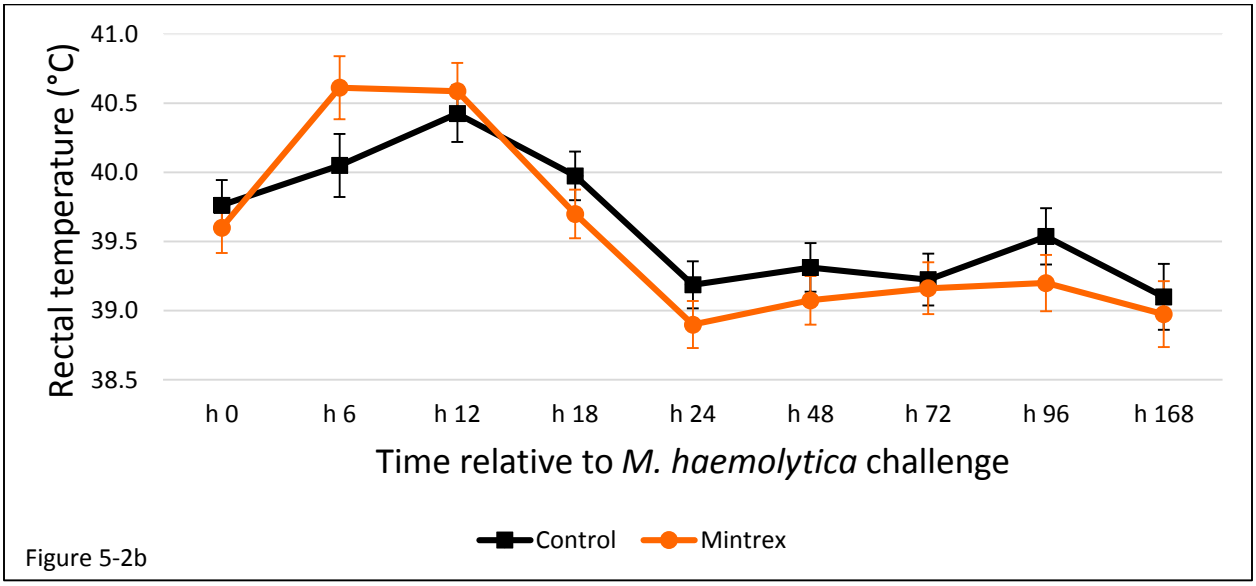

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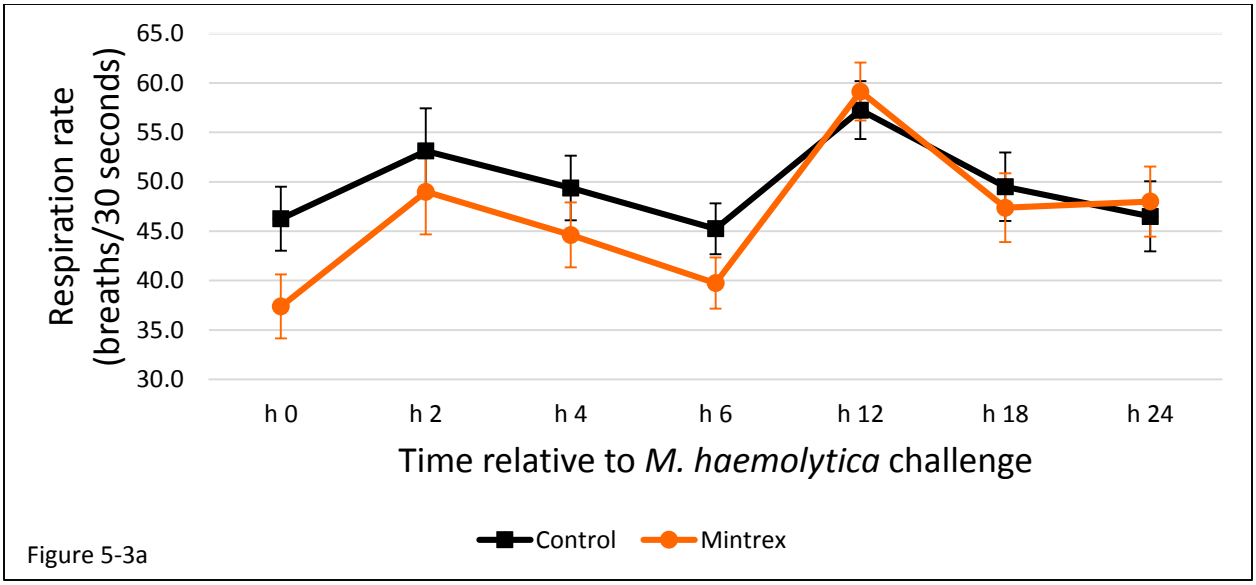

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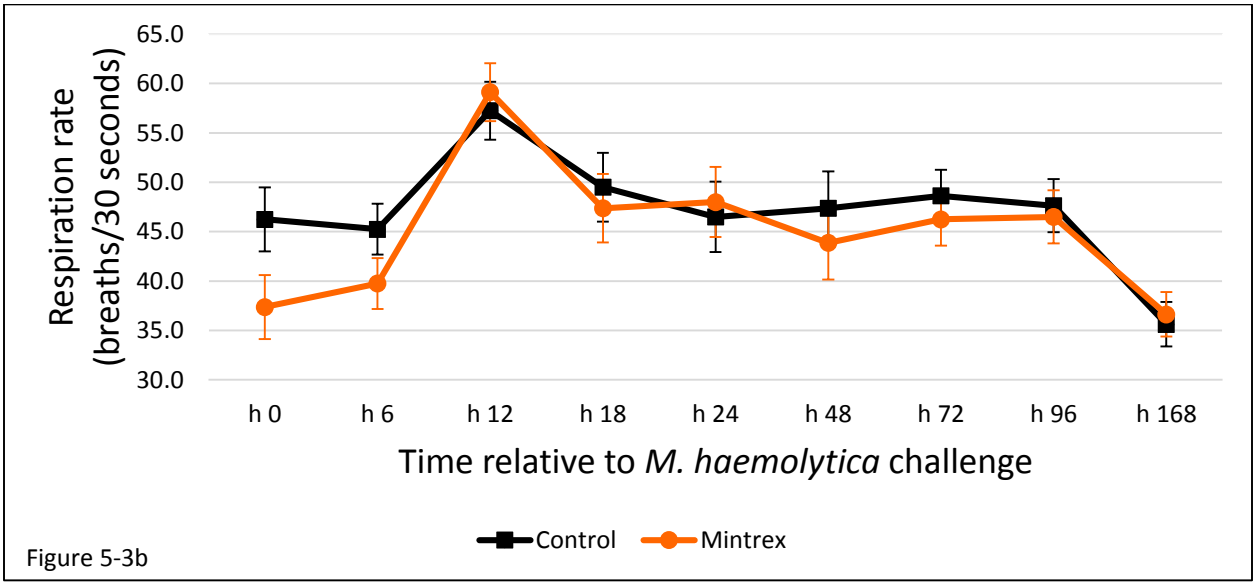

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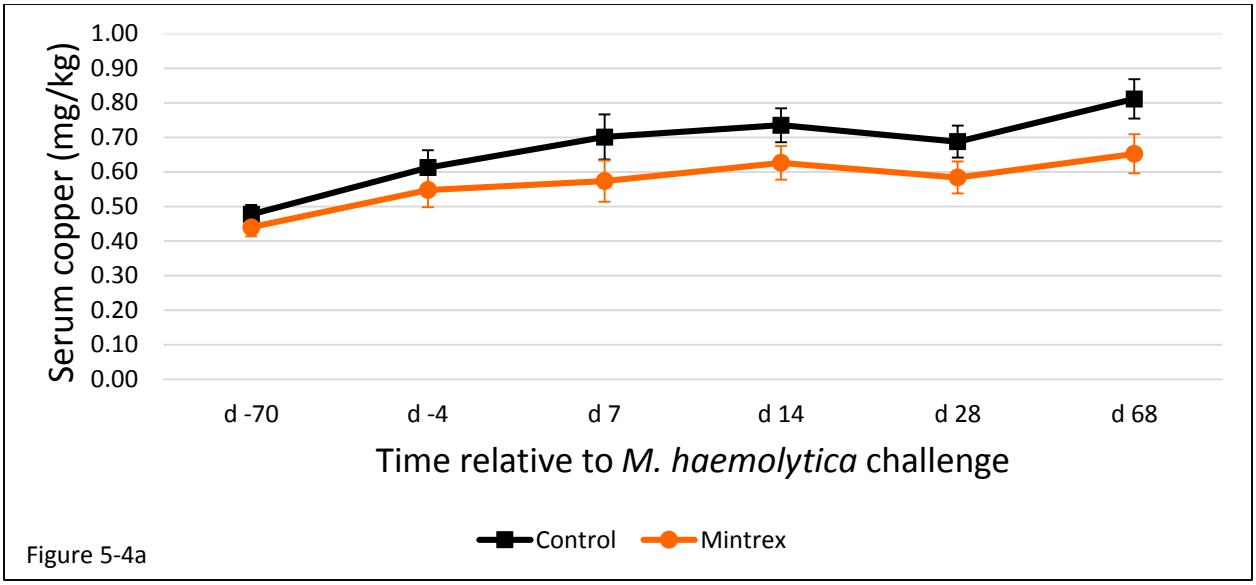


Figure 5-4a

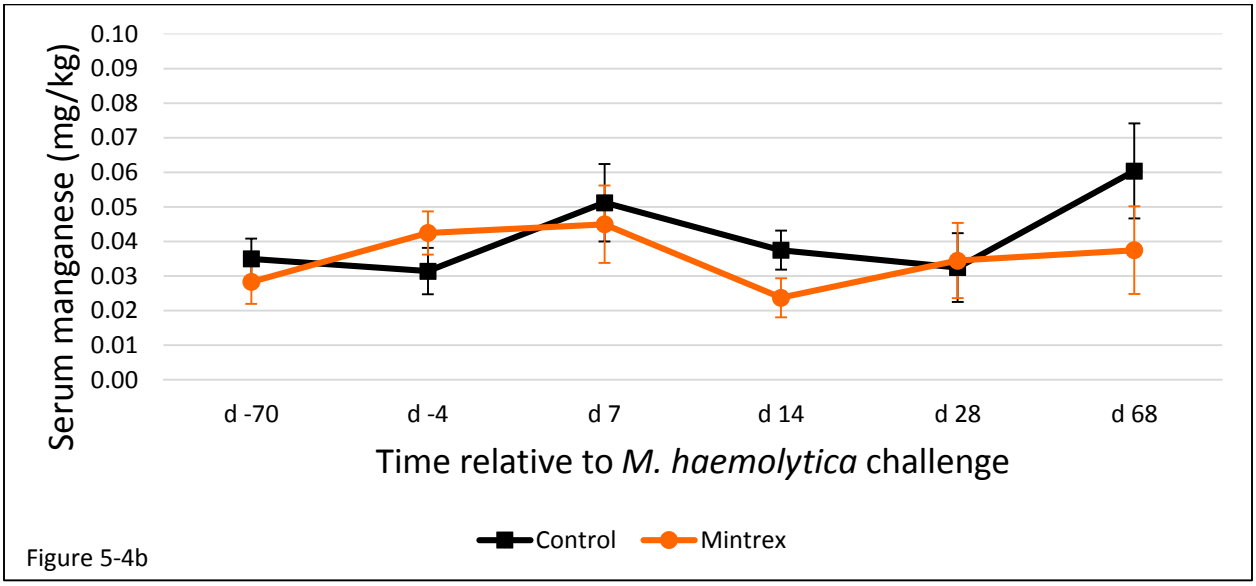
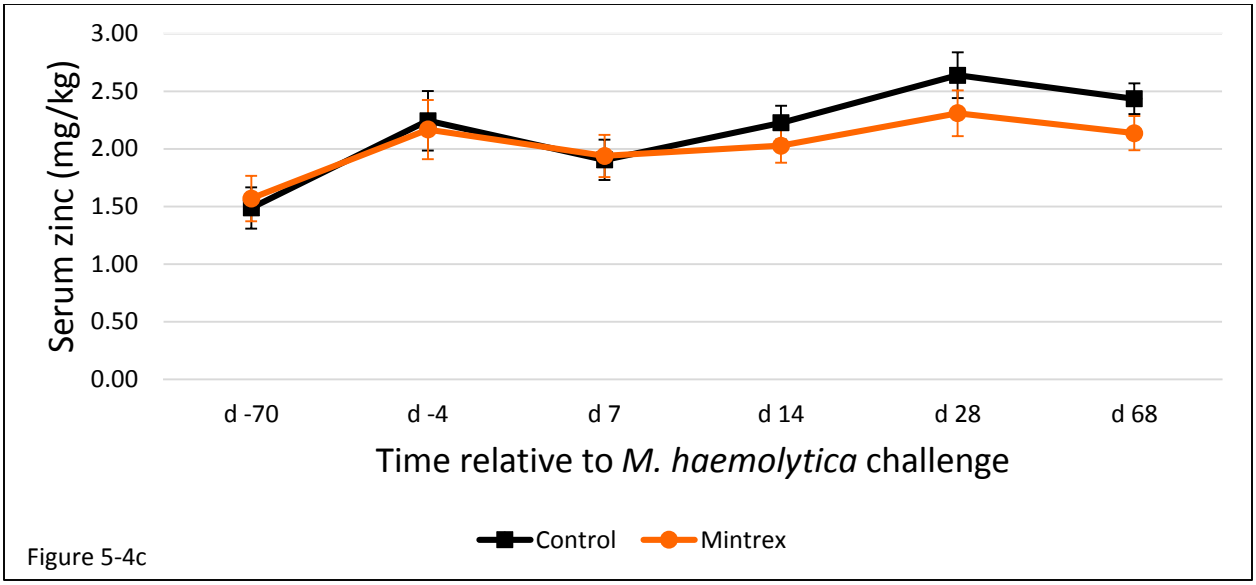


Figure 5-4b



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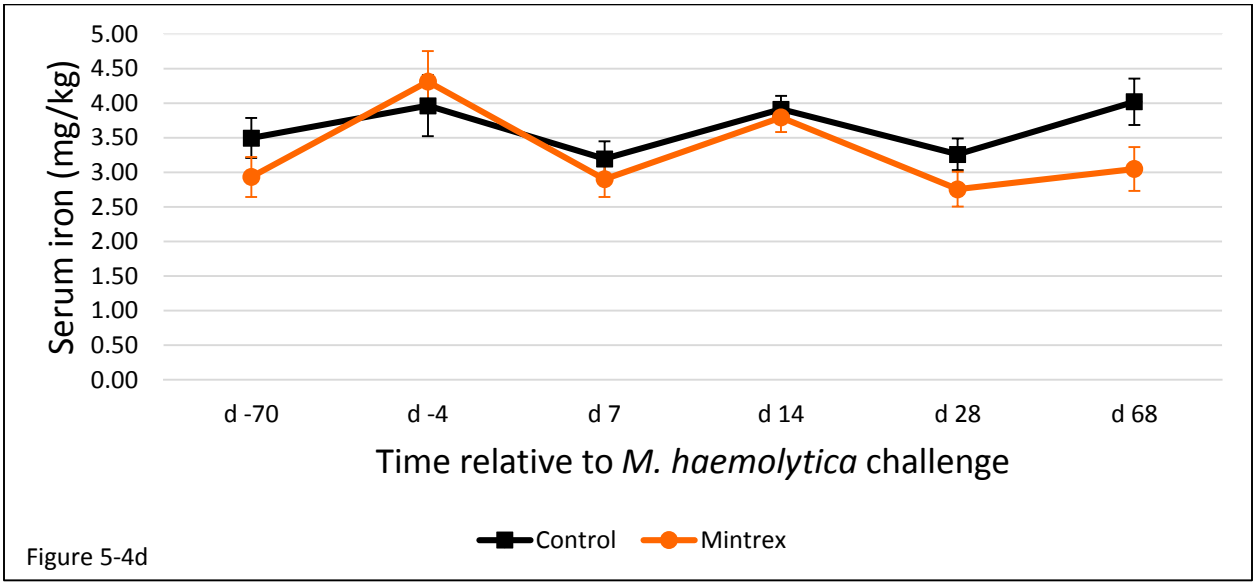
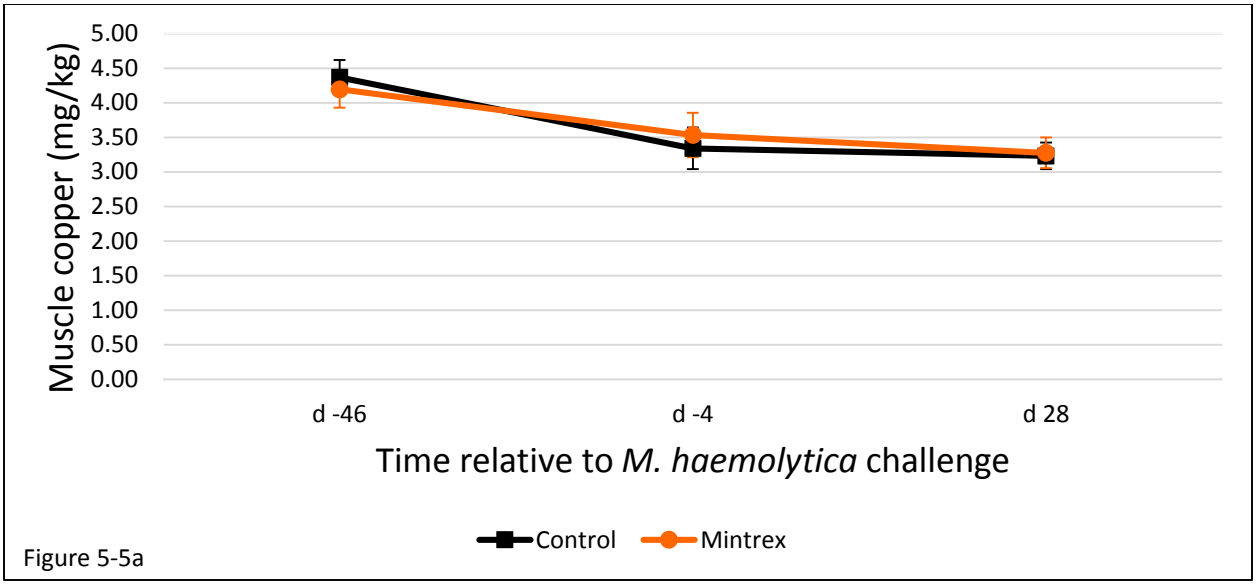


Figure 5-4d



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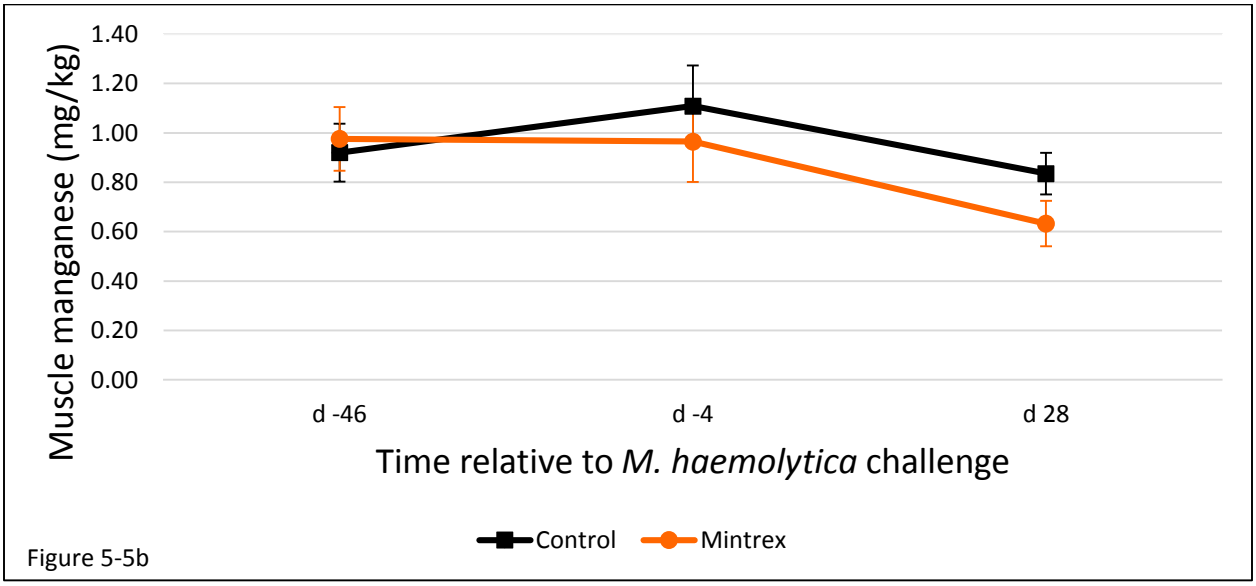

Figure 5-5b

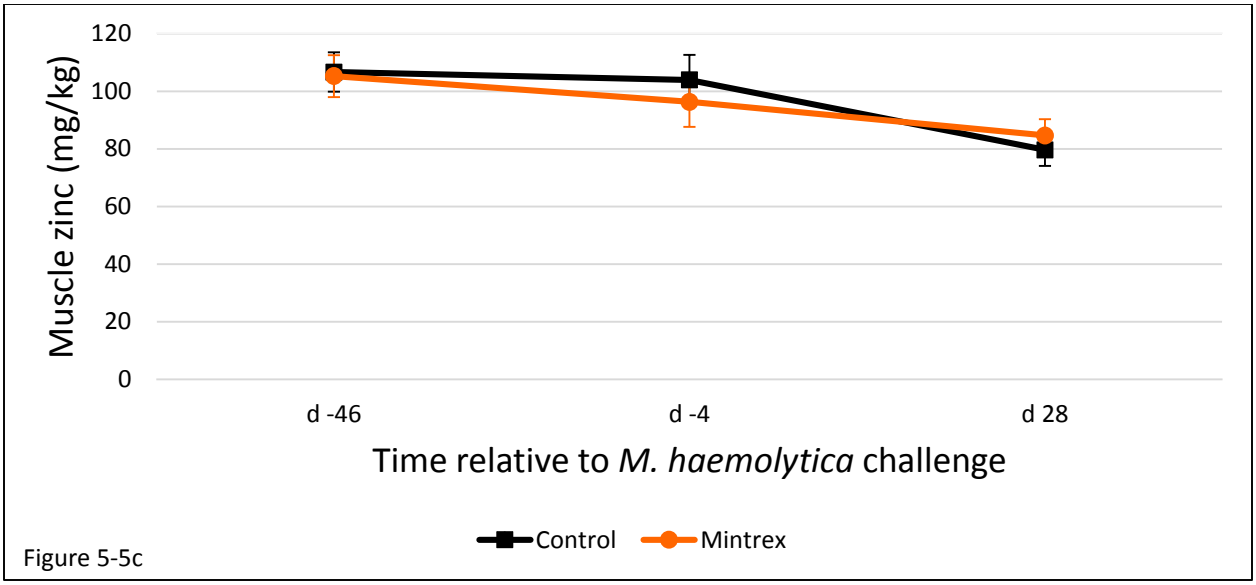

Figure 5-5c

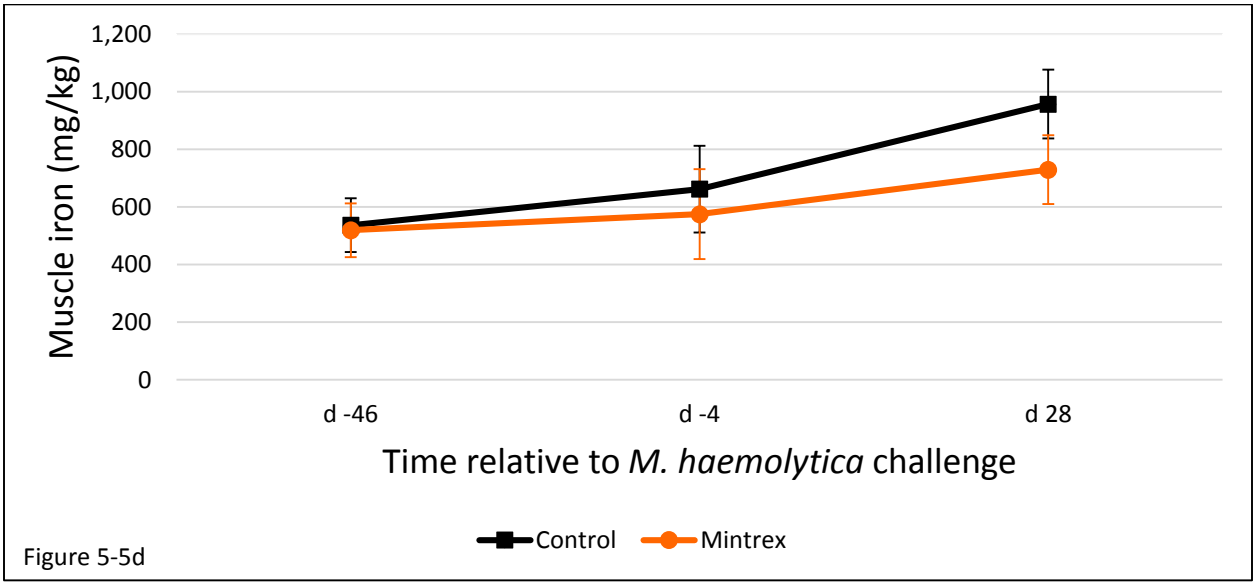

Figure 5-5d

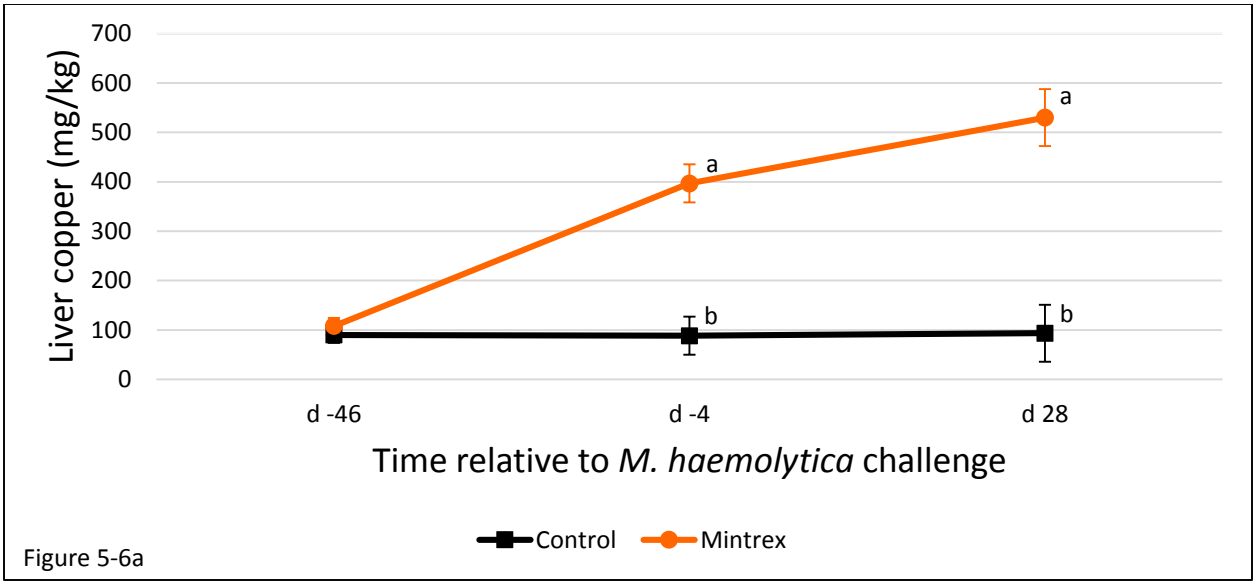

Figure 5-6a

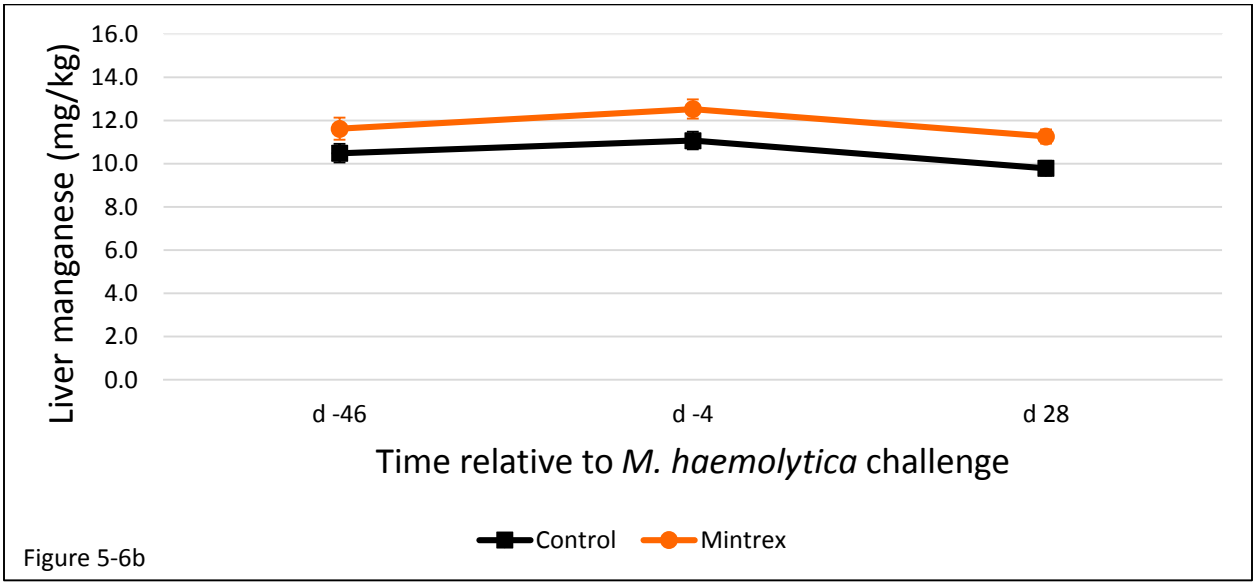

Figure 5-6b

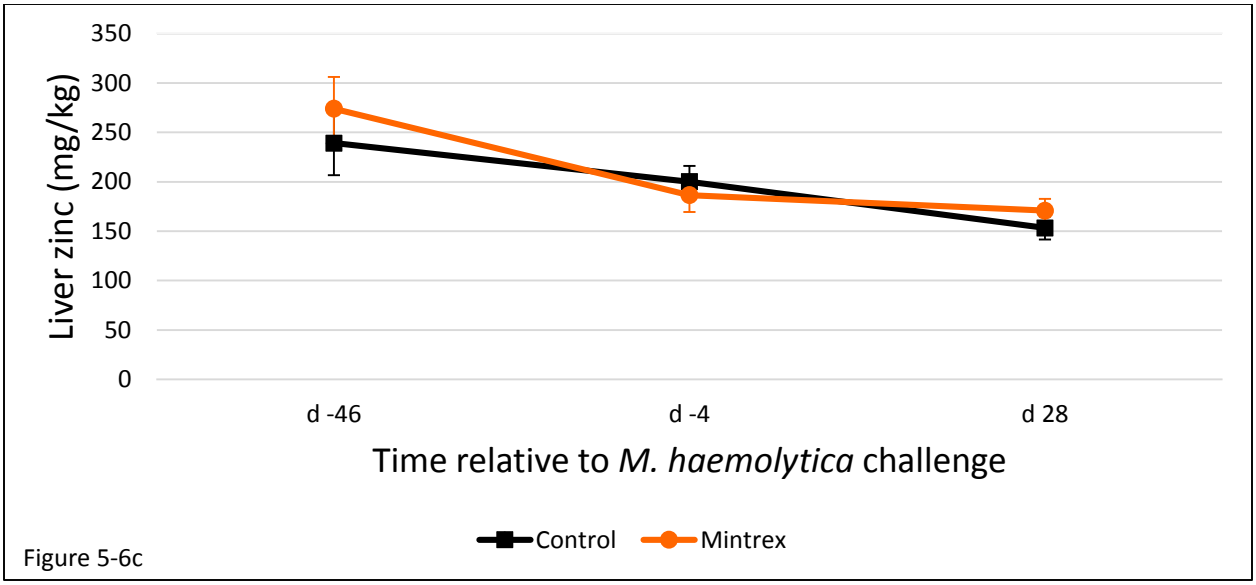

Figure 5-6c

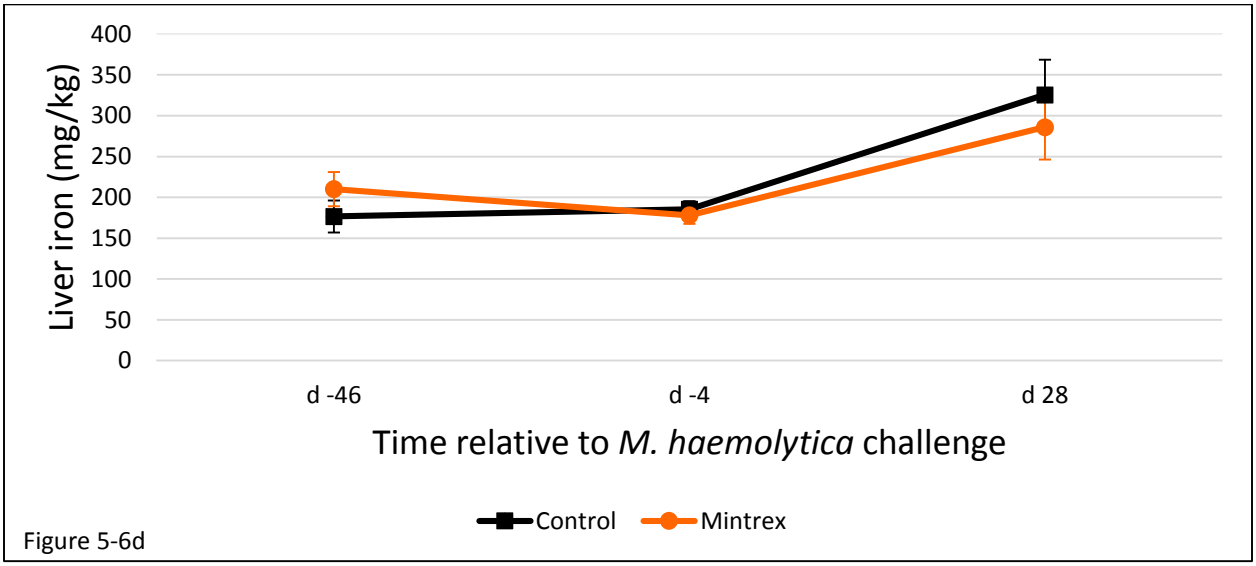

Figure 5-6d

CHAPTER VI

EFFECT OF COPPER, MANGANESE, AND ZINC SUPPLEMENTATION ON ANITBODY TITERS AND MULTIPLE IMMUNE RESPONSE VARIABLES OF CALVES FOLLOWING EXPOSURE TO BOVINE VIRAL DIARRHEA VIRUS TYPE 1B AND SUBSEQUENT MANNHEIMIA HAEMOLYTICA INFECTION

ABSTRACT

Trace mineral (TM) supplementation has been demonstrated to alter immune function in some experiments (Galyean et al., 1999). The objective of this experiment was to determine the influence of dietary copper (Cu), manganese (Mn), and zinc (Zn) supplementation on serum antibody titers and multiple immune response variables of calves following a bovine viral diarrhea virus (BVDV) and *Mannheimia haemolytica* (MH) immune challenge. Steers (n = 16; BW = 225 ± 20 kg) from a single ranch were processed, weaned, and randomly pairwise assigned to either mineral supplemented (MIN) or control (CON) experimental treatments. The MIN calves received 150 mg of Cu, 130 mg of Mn, and 320 mg of Zn daily while the CON calves received the basal diet with no additional Cu, Mn, or Zn supplementation. The basal diet contained sufficient Mn and Zn, but inadequate Cu based on NRC (2000) nutrient requirements. After 46 d on the experimental treatments, all calves were naturally exposed to heifer persistently infected (PI) with BVDV type 1b for 4 d and then subsequently intratracheally

challenged with MH. Data were analyzed using the GLIMMIX procedure of SAS with sampling time serving as a repeated measure and calf serving as the experimental unit. The immune challenge was validated via increased BVDV 1b antibody titers, MH whole cell (WC) and leukotoxin (LKT) antibody titers, rectal temperatures (TEMP), and subjective clinical scores (CS). A time by treatment interaction was observed for BVDV 1b antibody titers ($P \leq 0.003$). Calves receiving MIN tended to have reduced ($P = 0.07$) BVDV antibody titers, but had increased ($P = 0.02$) MH WC antibody titers compared to CON calves. Mineral supplementation did not impact MH LKT antibody titers ($P \geq 0.48$). Calves on the CON treatment tended ($P = 0.09$) to have increased serum cortisol during the first 24 h following the MH challenge. Total leukocytes counts were not different among experimental treatments ($P \geq 0.39$). However, CON calves had a higher percentage of neutrophils from d -4 to d 28 ($P = 0.03$). In contrast, MIN calves had a higher percentage of lymphocytes over the same interval ($P = 0.01$). Calves on the MIN treatment tended ($P \leq 0.10$) to have higher levels of base excess in both blood and extracellular fluid. The supplementation of Cu, Mn, and Zn has the potential to impact serum antibody titers and multiple immune response variables in calves challenged with BVDV and MH.

Key Words: bovine respiratory disease, mineral supplementation, immune challenge, copper, manganese, zinc, bovine viral diarrhea virus, *Mannheimia haemolytica*

INTRODUCTION

Bovine respiratory disease (BRD) is the most significant production problem for the feedlot industry, accounting for the majority of morbidity, mortality, and decreased production in feedlots with estimated annual economic losses in excess of $2 billion (Powell, 2013). The supplementation of trace minerals (TM) has been demonstrated to alter immune function and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have shown no improvements in performance or health variables from the supplementation of TM. Overall, TM research has been very inconsistent when investigating the ideal concentrations and sources of TM supplementation needed for optimum results.

Calves that are persistently infected (PI) with bovine viral diarrhea virus (BVDV) have been reported as a principal source of disease transmission in feedlots (O'Connor et al., 2005). In addition, the presence of a PI BVDV animal within a feedlot pen has been reported to increase the risk for antimicrobial treatment for clinical BRD by 43% compared to non-exposed cattle (Loneragan et al., 2005). In multiple experiments, BVDV 1b has been the predominant BVDV subtype isolated from calves diagnosed with BRD (Fulton et al. 2002a; Fulton et al. 2002b). The initial viral infection combined with a calf's previously compromised immune system allows for the rapid colonization of lung tissues by bacteria (Hodgins et al., 2002). The most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD is *Mannheimia haemolytica* (MH) serotype A1, and this serotype has been shown to be responsible for characteristic BRD infection in calves (Hodgins and Shewen, 2004; Booker et al., 2008; Griffin et al., 2010). The objective of this experiment was to determine if copper (Cu), manganese (Mn), and

zinc (Zn) supplementation effected serum BVDV and MH antibody titers and multiple immune response parameters of calves following a BVDV and MH immune challenge.

MATERIALS AND METHODS

All procedures for the present experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Animal Care and Use Protocol AG-12-5).

Cattle description and initial processing

An Angus-based commercial cow herd was identified as a potential source to supply calves for this experiment. An initial pool of 18 bull calves were selected from a single pasture. Eighty days prior to the initiation of the experiment, all bull calves were tagged with an individually numbered tag, given an initial vaccination for clostridial pathogens (Covexin 8; Merck Animal Health, Summit, NJ), and surgically castrated at the ranch of origin. Blood samples were collected and analyzed as described by Burciaga-Robles et al. (2010) to ensure calves were seronegative to the pathogens to be used in the challenge portion of this experiment. On 24 d prior to initiation of the experiment, a second blood sample collected and analyzed to confirm steers remained seronegative to the pathogens to be used in the challenge portion of this experiment. Ear notches were also obtained and tested via immunohistochemistry (IHC) by a commercial diagnostic lab to ensure no calves were persistently infected (PI) with BVDV.

Eleven days prior to the experiment, calves received a second vaccination for clostridial pathogens (Covexin 8; Merck Animal Health, Summit, NJ), were vaccinated for viral pathogens excluding BVDV (Inforce; Zoetis, Florham Park, NJ), received a vaccine for infectious bovine keratoconjunctivitis (IBK) (Autogenous Bacterin; Newport Laboratories, Worthington, MN), and were treated for the control of internal and external parasites (Ivermax Plus; Norbrook Laboratories, Lenexa, KS). Calves also received a prophylactic dose of tilmicosin phosphate (300 mg per mL) administered at the rate of 1.5 mL per 45.4 kg of BW (Micotil; Elanco Animal Health, Indianapolis, IN) and a fly tag (Corathon; Bayer, Shawnee Mission, KS). After processing, all steers were transported 97 km to the Animal Science Equine Center at Oklahoma State University. This facility was chosen to prevent exposure of the recently weaned calves to any other cattle at the other University research facilities. Calves remained at the Equine Center for a 6 d weaning period.

Challenge model and facility management

Five days prior to initiation the experiment, the steers were gathered and transported 6 km to the Nutrition and Physiology Research Center (NPRC) at Oklahoma State University. Calves were weighed, and BW in combination with initial antibody titers were used to allocate 16 steers to experimental treatments. Calves were then placed in metabolic stanchions with headlocks for a 5 d adaptation period. Each stanchion had an individual automatic water bowl and each calf was provided with an individual feed trough. Calves were placed in metabolic stanchions prior to the experiment to allow for

adaption of calves to the stanchions and automatic water bowls. Metabolic stanchions were only used during this experiment during the adaptation period (5 d) and the intense sampling period (d 0 through d 7).

After the adaptation period, the experiment was initiated. All time will be referenced in relation to the MH challenge as d 0. All calves were randomly assigned individual 3.05 × 3.66 m slatted floor pens on d -46. Each pen had access to 2 automatic water bowls and each calf was provided with an individual feed bunk. Calves remained in these individual pens for the first 42 d of the experiment.

The BVDV and MH challenge model described by Burciaga-Robles et al. (2010) was utilized in this experiment with slight modifications. Pre BVDV challenge samples were collected on d -4. After this sampling, all calves were comingled in a common pasture for 4 d with an animal previously confirmed as being PI positive with BVDV1b via IHC and genotyping as described by Fulton et al. (2006). During this time, all animals shared a single common water tank, and were fed together in 3 portable 3 m feed bunks.

On d 0, calves were gathered and placed in the metabolic stanchions. Pre-MH challenge sampling occurred at h 0. Immediately after h 0 sampling, all steers received 10 mL of a solution containing 6×10^9 CFU of MH serotype 1 that was reconstituted and grown prior to the challenge as described by Mosier et al. (1998). The MH was delivered via intratracheal bronchoalveolar lavage by a licensed veterinarian as described by Dowling et al. (2002), with slight modifications. Briefly, steers were restrained and a broncheoalveolar lavage tube (Bivona Medical Technology, Gary, IN) was gently inserted into the ventral meatus of a nostril, passed on into the trachea, and positioned

within 2 to 3 cm of the tracheal bifurcation. The MH challenge solution was then delivered in a way such that the challenge solution would be allowed to enter both lungs. No adverse effects of the challenge procedure itself were observed.

For the next 7 d, calves remained in the stanchions and were monitored several times daily. Intensive sampling occurred during this time. After the 7 d intensive sampling period, calves were returned to their respective individual 3.05 × 3.66 m slatted floor pens for the duration of the experiment. On d 14 and d 28, calves were gathered, briefly restrained in a manual squeeze chute, and sampled again. The calves did remain in our possession after the experiment was terminated, and an additional sampling occurred on d 68. All calves were comingled and received the same diet from d 28 to d 68.

Common diet and feeding

While on pasture with their dams prior to weaning, cows and calves received no mineral supplementation. The only TM calves would have had received would have been provided through the milk of their dams or forage consumed on the ranch. Upon arrival to the Horse Unit, calves were given ad libitum access to water, Bermuda grass hay, and the common receiving ration minus the dry supplement (Table 1). After transportation to the NPRC and being placed in stanchions, the Bermuda hay was removed, and the calves continued to receive ad libitum access to water and the common receiving ration minus the dry supplement. On d -46, a common dry supplement (Table 1) was included in the ration. This supplement was formulated to meet or exceed NRC (2000) nutrient

requirements except for Cu, Mn, and Zn. The common supplement contained no additional Cu, Mn, or Zn from organic or inorganic sources.

The calves continued to receive ad libitum access to the common receiving ration, with supplement now included, and water for the duration of the experiment. Feed was delivered twice daily at 0800 h and 1500 h. Bucket feeders were cleaned and orts were weighed back weekly. To ensure that the calves still received their respect experimental mineral treatments during the 4 d BVDV challenge, calves were gathered each morning at 0700 h and sorted into their respective experimental treatments. Each group then received 11.3 kg of the common receiving ration and their respective experimental top dress. After all 11.3 kg was consumed, all calves were returned to the common pasture with the PI animal. Ration samples were collected daily, and dried in a forced air oven for 48 h at 60ºC to determine dry matter. All daily ration samples were composited gravimetrically and analyzed at a commercial laboratory (Servi-Tech Inc., Dodge City, KS) for nutrient composition (Table 1).

Experimental mineral treatments

Starting on d -46 calves on the MIN experimental treatment received a ground corn top dress daily containing 150 mg of Cu, 130 mg of Mn, and 320 mg of Zn in the form of Cu methionine, Mn methionine, and Zn methionine, respectively (Mintrex Cu, Mn, and Zn; Novus International, Inc., St. Charles, MO). Calves on the CON experimental treatment received a top dress daily that only contained ground corn. Top dresses were batched daily and weighed out on a gram scale to the nearest 0.01 g. The

kitchen mixer was cleaned daily before mixing the CON top dress. The CON top dress consisted of 1000 g of ground corn mixed for 5 min and 125 g of the top dress was placed in 8 identical plastic color coded containers.

The mixer was cleaned again prior to making the MIN top dress. The MIN top dress consisted of 968 g of ground corn and 32 g of Mintrex mineral (8 g of Mintrex Cu, 8 g Mintrex Mn, and 16 g Mintrex Zn) on an AF basis. This mixture was mixed for 5 min and 125 g of the top dress was placed in 8 identical plastic color coded containers. This resulted in 121 g of ground corn with 1 g Mintrex Cu, 1 g of Mintrex Mn, and 2 g of Mintrex Zn per MIN container. Top dresses were then delivered to each individual pen or stanchion immediately after delivery of the common ration and gently mixed in. All feed bunks, pens, stanchions, and containers were color coded to match their respective experimental treatment.

Serum collection and preparation

Serum was required for multiple analyses in this experiment including: antibody titers, cytokines, haptoglobin, and hormones. For all serum analyses conducted, whole blood was collected via the jugular vein using an 18 gauge needle and serum separator tube (Corvac Serum Separator Tube; Tyco Healthcare Group LP, Mansfield, MA) at multiple time points throughout the experiment. The whole blood samples were allowed to clot for 12 to 24 h at 4°C. After the clotting time, chilled blood samples were centrifuged at 2,500 × g for 20 min. Serum was then aliquoted to multiple 2 mL

microcentrifuge tubes and immediately frozen at -20°C until further analyses could be completed.

Bovine viral diarrhea virus antibodies

Serum samples from d -70, -3, 0, 3, 7, 14, and 28 were submitted to a research laboratory at Oklahoma State University, Department of Veterinary Pathobiology, for BVDV serology using a virus neutralization test in Madin-Darby bovine kidney cell monolayers in 96-well microtiter plates as described by Fulton et al. (2002a). The virus used as challenge virus in the virus neutralization test were CP BVDV1b (TGAC 8HB). A 1:4 dilution was the lowest tested, and titers of less than 1:4 were considered negative (0).

Mannheimia haemolytica antibodies

Serum samples from d -70, -3, 0, 3, 7, 14, and 28 were submitted to a research laboratory at Oklahoma State University, Department of Veterinary Pathobiology, to determine MH antibodies to whole bacterial cell (WC) and leukotoxin (LKT). The procedure utilized a formalin killed MH S1 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.

Hormones

Serum samples from d -4, h 0, h 2, h 4, h 6, h 12, h 18, h 24, d 2, d 3, d 4, d 7, d 14, d 28 relative to the MH challenge were submitted to a research laboratory at New Mexico State University for IGF-I analysis. Serum IGF-I values were determined via a double-antibody RIA as described by Berrie et al. (1995) with modifications described by Camacho et al. (2012). The detection limit for the IGF-I assay was 4 ng/mL.

Serum samples from d -4, h 0, h 2, h 4, h 6, h 12, h 18, h 24, d 2, d 3, d 4, d 7, d 14, d 28 relative to the MH challenge were submitted to a research laboratory at New Mexico State University for cortisol analysis. Serum cortisol concentrations were quantified by solid-phase RIA using components of a commercial kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with the modifications described by Kiyma et al. (2004).

Hematology

Whole blood was collected via the jugular vein using an 18 gauge needle and EDTA tube (Monject EDTA Tube; Tyco Healthcare Group LP, Mansfield, MA) at multiple time points throughout the experiment. Whole blood samples that were collected on d -4, h 0, h 2, h 4, h 6, h 12, h 18, h 24, d 2, d 3, d 4, d 7, d 14, d 28 relative to the MH challenge were used for hematology analysis. The samples were immediately analyzed using an automated hematology analyzer (ProCyte Dx Hematology Analyzer, IDEXX Laboratories Inc., Westbrook, ME) for multiple blood cell parameters. Response variables measured included: erythrocytes, hemoglobin, hematocrit percentage, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin

concentration, reticulocytes, platelets, leukocytes, neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage, and basophil percentage.

Blood gas, pH, and metabolites

Whole blood was collected via the jugular vein using an 18 gauge needle and sodium heparin tube (Monject Sodium Heparin Tube; Tyco Healthcare Group LP, Mansfield, MA) at multiple time points throughout the experiment. Whole blood samples that were collected on d -4, h 0, h 2, h 4, h 6, h 12, h 18, h 24, d 2, d 3, d 4, d 7, d 14, d 28 relative to the MH challenge were used for blood gas, pH, glucose, and lactate analysis. Samples were immediately analyzed using a blood gas analyzer (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA). Response variables measured included: pH, partial pressure of carbon dioxide, partial pressure of oxygen, sodium, potassium, calcium, glucose, lactate, bicarbonate ion, base excess in extracellular fluid (ECF), base excess in blood, and oxygen saturation.

Data collection, calculations, and statistical analysis

Data were analyzed using repeated measures analysis with the GLIMMIX procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) with steer serving as the experimental unit. Various covariance structures within models were compared. The covariance structure that best fit the data in the present experiment was a non-structured covariance. In the few instances where model optimization could not be completed, a

Toeplitz or banded Toeplitz covariance structure was employed. Sampling time served as the repeated measure and *P*-values are reported for the effect of treatment, time, and the interaction of time and treatment. If a time × treatment interaction was present, a slice output option was used to determine the time points at which treatments were different.

RESULTS

Effectiveness of BVDV and MH challenge model

The immune challenge model described by Burciaga-Robles et al. (2010) was validated in this experiment. The BVDV and MH challenges were both proved successful via increased antibody titers for BVDV as well as for MH WC and MH LKT. In addition, TEMP and CS (data not presented) both significantly increased during the challenge, then returned to normal levels. The responses observed to the immune challenge were consistent with previous experiments by Burciaga-Robles et al. (2010) and others using this challenge model.

Bovine viral diarrhea virus antibodies

Serum BVDV 1b antibody titers are shown in Figure 1. Average BVDV 1b antibody titers less than 1:4 were considered negative (0) for all calves prior to the initiation of the experiment. No change in BVDV 1b titers was detected for any of the calves until d 14 and at d 14, no difference existed between experimental treatments. However, by d 28, the BVDV 1b antibody titers of calves receiving no additional TM

supplementation (CON) had increased at a more rapid rate than calves receiving MIN. This resulted in a time × treatment interaction ($P \leq 0.003$) being observed for BVDV 1b antibody titers.

Mannheimia haemolytica antibodies

Serum MH WC and LKT antibody titers are presented in Figure 2a and 2b, respectively. Average MH WC and LKT antibody titers were near (0) and considered negative for all calves prior to the initiation of the experiment. There were no time × treatment interactions ($P \geq 0.27$) observed for MH WC or MH LKT antibody titers. Time was significant ($P < 0.0001$) for both MH WC and LKT antibody titers. Serum MH WC antibody titers increased slightly during the pre-challenge portion of the experiment and then remained fairly static until d 3 post MH challenge. This same pattern was observed for serum MH LKT antibody titers. Calves on the MIN treatment exhibited greater ($P = 0.02$) MH WC antibody titers compared to calves on the CON treatment, while the MH LKT antibodies of MIN calves were numerically increased ($P = 0.48$) compared to CON calves. Serum MH WC and LKT antibodies appeared to plateau by d 28 for both treatments.

Hormones

Serum IGF-I concentrations are presented in Figures 3a and 3b. There were no time × treatment interactions ($P \geq 0.15$) observed for serum IGF-I concentrations during

the first 24 h following the MH challenge or over the length of the experiment. Time was significant for serum IGF-I concentrations during the first 24 h following the MH challenge ($P = 0.02$) and over the length of the experiment ($P < 0.004$). Serum IGF-I decreased until 2 d post MH challenge and then slowly increased for the duration of the experiment. Mineral supplementation did not affect ($P \geq 0.46$) IGF-I concentrations.

Serum cortisol concentrations are presented in Figures 4a and 4b. There were no time × treatment interactions ($P \geq 0.28$) observed for serum cortisol concentrations during the first 24 h following the MH challenge or over the length of the experiment. Time was significant for serum cortisol concentrations during the first 24 h following the MH challenge ($P = 0.0002$) and over the length of the experiment ($P = 0.01$). Serum cortisol decreased until 1 d post MH challenge, was relatively constant from d 1 to d 4, and increased again during the latter part of the experiment. During the first 24 h following the MH challenge, CON calves tended ($P = 0.09$) to have elevated serum cortisol concentrations compared to MIN calves. Mineral supplementation did not affect ($P \geq 0.31$) cortisol concentrations over the length of the experiment.

Hematology

Hematology analysis is presented in Figures 5 through 18. Response variables reported include: erythrocytes (Figures 5a and 5b), hemoglobin (Figures 6a and 6b), hematocrit percentage (Figures 7a and 7b), mean corpuscular volume (Figures 8a and 8b), mean corpuscular hemoglobin (Figures 9a and 9b), mean corpuscular hemoglobin concentration (Figures 10a and 10b), reticulocytes (Figures 11a and 11b), platelets

(Figures 12a and 12b), leukocytes (Figures 13a and 13b), neutrophil percentage (Figures 14a and 14b), lymphocyte percentage (Figures 15a and 15b), monocyte percentage (Figures 16a and 16b), eosinophil percentage (Figures 17a and 17b), and basophil percentage (Figures 18a and 18b). Significant time × treatment interactions ($P \leq 0.05$) were observed for erythrocytes, hemoglobin, hematocrit, and eosinophil percentage during the first 24 h following the MH challenge. There was a tendency ($P \leq 0.10$) for a time × treatment interaction observed for mean corpuscular volume and mean corpuscular hemoglobin concentration, over the length of the experiment.

Time significantly impacted multiple hematology parameters. Time was significant ($P \leq 0.05$) for erythrocytes, hemoglobin, hematocrit percentage, mean corpuscular volume, mean corpuscular hemoglobin concentration, reticulocytes, leukocytes, eosinophil percentage, and basophil percentage during the first 24 h following the MH challenge. There was also a tendency ($P \leq 0.10$) for time to impact lymphocyte percentage during the first 24 h following the MH challenge. Time was significant ($P \leq 0.05$) for erythrocytes, hemoglobin, hematocrit percentage, mean corpuscular volume, mean corpuscular hemoglobin concentration, reticulocytes, platelets, leukocytes, monocyte percentage, and eosinophil percentage, over the length of the experiment. There was also a tendency ($P \leq 0.10$) for time to impact mean corpuscular hemoglobin, lymphocyte percentage, and basophil percentage over the length of the experiment.

When looking at the general trends for hematology over the length of the experiment, both mineral treatments were similarly affected and the results are quite variable. However, erythrocytes, hemoglobin, hematocrit percentage, mean corpuscular

volume all behaved in a similar fashion. In relation to the MH challenge, these variables all decreased from d -4 to d 1, remained fairly constant from d 1 to d 4, and then increased from d 4 through the end of the experiment. Mean corpuscular hemoglobin concentration followed the exact opposite trend by increasing, leveling off, and then decreasing over essentially the same intervals. Reticulocytes were highly variable over time, and platelets increased with time. Total leukocytes increased until 18h post challenge, decreased until d 4, and then increased again toward the end of the experiment. The percentage of lymphocytes decreased until d 1 and then slowly increased prior to leveling off later in the experiment. The percentage of monocytes remained relatively steady until d 1, increased to d 3 or d 4 and then decreased toward the end of the experiment. Eosinophil percentages exhibited multiple peaks, first decreasing until d 1, then increasing to d 3, decreasing again until d 7, and finally increasing until the end of the experiment. Basophil percentages were effected by time, but were highly variable.

Mineral supplementation did not affect many of the hematology variables examined. However, calves receiving MIN demonstrated greater ($P = 0.04$) SD of platelet distribution widths compared to CON calves during the first 24 h following the MH challenge. For the length of the experiment, MIN calves tended ($P = 0.07$) to have increased platelet large cell ratios than CON calves. Calves receiving MIN had an increased ($P = 0.01$) percentage of lymphocytes, while calves receiving CON had an increased ($P = 0.03$) percentage of neutrophils and tended ($P = 0.10$) to have greater total neutrophil numbers.

Blood gas, pH, and metabolites

Blood gas variables, pH values, and blood metabolites are presented in Figures 19 through 30. Response variables reported include: pH (Figures 19a and 19b), partial pressure of carbon dioxide (Figures 20a and 20b), partial pressure of oxygen (Figures 21a and 21b), sodium (Figures 22a and 22b), potassium (Figures 23a and 23b), calcium (Figures 24a and 24b), glucose (Figures 25a and 25b), lactate (Figures 26a and 26b), bicarbonate ion (Figures 27a and 27b), base excess in ECF (Figures 28a and 28b), base excess in blood (Figures 29a and 29b), and oxygen saturation (Figures 30a and 30b). There were no time × treatment interactions ($P \geq 0.11$) observed for blood gas variables, pH values, or blood metabolites during the first 24 h following the MH challenge or over the length of the experiment. Time significantly impacted multiple blood gas parameters. Time was significant ($P \leq 0.05$) for blood pH, partial pressure of oxygen, sodium, glucose, lactate, bicarbonate, base excess in ECF, and base excess in blood during the first 24 h following the MH challenge. There was also a tendency ($P = 0.07$) for time to impact partial pressure of carbon dioxide during the first 24 h following the MH challenge. Time was significant ($P \leq 0.05$) for partial pressure of carbon dioxide, partial pressure of oxygen, calcium, glucose, lactate, bicarbonate, base excess in ECF, base excess in blood, and oxygen saturation over the length of the experiment. There was also a tendency ($P \leq 0.08$) for time to impact sodium and potassium over the length of the experiment.

When looking at the general trends for blood gas variables, pH, and blood metabolites over the length of the experiment, the results are also quite variable. Partial pressure of carbon dioxide changed over time, but only fluctuated approximately 5

mm/Hg within treatment. Partial pressure of oxygen increased until d 1, decreased until d 7, and then increased toward the end of the experiment. Calcium within the blood increased until d 3, and then decreased for the duration of the experiment. Sodium followed an opposite pattern, decreasing until d 4, then increasing for the duration of the experiment. Potassium slowly increased through d 14 and then had decreased by d 28. Glucose and lactate both initially decreased, leveled off, and then increased until the end of the experiment. Bicarbonate and both measurements of base excess were variable, but increased approximately to d 0, leveled off for a few days, and then increased to the end of the experiment. The oxygen saturation of blood increased until d 1, decreased slightly until d 7, and then increased toward the end of the experiment. Mineral supplementation did not affect many blood gas parameters. Calves receiving MIN tended ($P \leq 0.10$) to have greater base excess in ECF and base excess in blood compared to CON calves over the length of the experiment.

DISCUSSION

The role of TM including Cu, Mn, and Zn in immune function has received attention from researchers for many years. It is well established that certain TM are essential for overall performance, health, and immune function. In addition to general health and immune function mechanisms, the supplementation of TM has been demonstrated to alter specific immune function measurements and reduce morbidity associated with BRD in some cases (Galyean et al., 1999). However, other experiments have demonstrated no improvements in performance or health variables from the

supplementation of TM. Limited published research justifying the supplementation of Cu, Mn, or Zn at levels greater than published requirements has produced inconsistent results. However, the role of TM in immune function, combined with the unknown TM status of newly received calves and expectation of depressed DMI early in the receiving period may serve as a possible explanation for increased TM inclusion even though justification of the practice is not necessarily supported by the published literature.

The pathogenesis of BRD typically involves compromised respiratory immune mechanisms and a primary infection with one or more respiratory viruses. The viral infection and the calf's impaired immune response to the virus further compromise the immune system and allow for the colonization of lung tissues by bacteria (Hodgins et al., 2002). Most of the pathogens associated with increased BRD incidence are well documented within the literature. However, many of these pathogens are also frequently isolated from the respiratory tract of clinically healthy cattle making the analysis of BRD in field studies incredibly difficult. The challenge employed in this experiment was the same model described by Burciaga-Robles et al. (2010) with slight modifications. This challenge model was selected due to the ability of this model to successfully simulate natural BRD pathogenesis. Additionally, this model is able to induce a controlled, simulated BRD event, while simultaneously allowing for accurate determination of antibody responses due to the challenge with known viral and bacterial pathogens.

The decision to use the specific pathogens in this model are justified by the published literature and previous experiences. It has been reported that PI BVDV calves are a principal source of disease transmission in feedlots (O'Connor et al., 2005). In multiple experiments, BVDV 1b has been the predominant BVDV subtype isolated from

calves diagnosed with BRD (Fulton et al. 2002a; Fulton et al. 2002b). With great repeatability, the most common bacterial pathogen isolated from the respiratory tract of calves treated for BRD is MH serotype 1, and this serotype has been shown to be responsible for characteristic BRD infection in calves (Hodgins and Shewen, 2004; Booker et al., 2008; Griffin et al., 2010).

The immune challenge model was validated in this experiment. The BVDV and MH challenges were both proved successful via increased antibody titers for BVDV as well as for MH WC and MH LKT. In addition, TEMP and CS both significantly increased during the immune challenge, then returned to normal levels. In the current experiment, calves had average TEMPs of 40.3°C by h 6 and had average CS of 1 by h 18. The CS observed for calves in this experiment would have dictated that the calves would have been pulled for exhibiting clinical signs of BRD and evaluated for subsequent antimicrobial treatment under the standard operating protocols in place at our facility. The TEMP observed in these calves would have dictated that the calves would have received an antimicrobial treatment for clinical BRD under the same standard operating protocols. At our facility, a calf can meet treatment criteria for clinical BRD and receive an antimicrobial via 2 standard treatment protocols. The first being that an animal can be pulled with a CS of 1 or 2 and had a TEMP of 40°C or greater. The second being that an animal can be pulled with a severe CS (CS = 3 or 4) regardless of TEMP. The increased TEMP and CS of calves challenged in combination with increased BVDV antibody titers and MH WC and LKT antibody titers validated the immune challenge in the current experiment. The increases observed for these response variables would have been similar to those observed by Burciaga-Robles et al. (2010) using a similar challenge

model. The objective of this experiment was to determine the influence of copper (Cu), manganese (Mn), and zinc (Zn) supplementation on serum BVDV and MH antibody titers and multiple immune response parameters of calves following a BVDV and MH immune challenge.

In the current experiment, average BVDV 1b antibody titers were less than 1:4 and were considered negative (0) for all calves prior to the initiation of the experiment. Even after exposure to the PI BVDV animal on d -4, no change in BVDV 1b titers was detected for any of the calves until d 14 (18 d after BVDV exposure). This time interval was longer than that reported by Burciaga-Robles et al. (2010). In that experiment, an increase in BVDV 1b antibody titers was observed on d 7 (10 d after BVDV exposure). In addition, Burciaga-Robles et al. (2010) observed exponentially greater BVDV 1b titers on d 14 and d 28 compared to those observed on d 14 and d 28 in the present experiment. The reasons for the longer delay lower levels observed for BVDV antibody titer response in the present experiment, could result from the calves in the current experiment being more naïve. It could also be a result of other experimental conditions, or a less severe challenge in the current experiment.

Arthington and Havenga (2012) did not challenge calves directly, but examined the effects of injectable TM on the humoral immune response to multivalent vaccine administration in seronegative calves. The authors found that neutralizing BVDV antibody titers increased following vaccination and were greater than baseline antibody titers on d 14. In addition, the most rapid increase in BVDV antibody titers was observed from d 14 through d 30. These results would closely resemble those of the current experiment. Kegley et al. (2012) found similar results when investigating supplemental

Cu, Mn, Zn, and cobalt (Co) fed in organic or inorganic from to stressed calves. When only observing naïve calves, the authors found an initial increase in BVDV antibody titers by d 14 and then a rapid increase in BVDV antibody titers until d 42.

In the present experiment, no differences existed among experimental treatments until d 28. By d 28, the BVDV 1b antibody titers of CON calves had increased at a more rapid rate than calves receiving MIN. This resulted in a time × treatment interaction being observed for BVDV 1b antibody titers. This rapid increase in the BVDV 1b antibody titers of CON calves resulted in CON calves having greater BVDV 1b antibody titers on d 28 and a tendency for CON calves to have increased BVDV 1b antibody titers for the duration of the experiment. These results unfortunately cannot be compared to the findings of Arthington and Havenga (2012) who only reported pooled treatment means for BVDV antibody titers. In addition the results in the present experiment could not be compared to the results from Kegley et al. (2012) who did not utilize a non-supplemented control group.

Stabel et al. (1993) challenged Cu deficient and Cu supplemented calves with Infectious Bovine Rhinotracheitis (IBR) and MH (formally known as *Pasteurella hemolytica*). While the authors used a different viral challenge than we used in the current experiment, they noticed similar responses in viral antibody titers for calves that received adequate dietary Cu and calves that were fed a Cu deficient diet. The authors found no difference in serum IBR antibody titers from d 0 to d 7 among Cu supplemented or Cu deficient calves. However, those calves that were Cu deficient had significantly higher IBR antibody titers by d 10 and numerically higher IBR antibody titers on d 14

and d 21 compared to Cu supplemented calves. Time significantly impacted BVDV 1b antibody titers in the present experiment and all other experiments discussed.

In the current experiment, average MH WC and LKT antibody titers were near (0) and considered negative for all calves prior to the experiment initiation. In addition, there were no time × treatment interactions observed for either MH WC or MH LKT antibody titers. Time was significant for both MH WC and LKT antibody titers. Serum MH WC and LKT antibody titers increased slightly during the pre-challenge portion of the experiment and then remained fairly static until d 3 post MH challenge. This response would be consistent with that found by Burciaga-Robles et al. (2010) who also found no significant increase in MH WC or LKT antibody titers from d -3 to d 3 post MH challenge. However in contrast to what was observed for BVDV 1b antibody titers, the calves in the current experiment exhibited greater MH WC and LKT antibody titers on d 7, d 14, and d 28 compared to the calves challenged with MH in the experiment by Burciaga-Robles et al. (2010).

The results in the present experiment for MH WC and LKT antibody titers would also be supported by those found by Confer et al. (1997). The authors found that vaccination with 50 or 100 µg of LKT or a challenge with live MH stimulated significant increases in LKT neutralizing antibody titers between d 7 and d 14. The vaccination with 100 µg of LKT or a challenge with live MH stimulated also resulted in significant increases in MH WC antibody titers to by d 14. For calves challenged with MH, the antibody responses remained significantly greater than the d 0 values for the duration of the experiment.

Calves on the MIN treatment exhibited greater MH WC antibody titers compared to CON calves. A similar pattern was also observed in the MH LKT antibodies of calves, however there was no statistical difference among experimental mineral treatments. Serum MH WC and LKT antibodies appeared to plateau by d 28 for both treatments. Stabel et al. (1993) noticed similar responses in MH antibody titers for calves that received adequate dietary Cu and calves that were fed a Cu deficient diet. The authors noted that measurable increases in MH antibody titers were observed by 14 d after inoculation. By 23 d post MH challenge, Cu supplemented calves had numerically higher MH antibody titers than Cu deficient calves (85.1 versus 37.3, respectively). The authors noted that there was a large amount of variation within the Cu supplemented group, which caused them to not detect a significant difference in MH antibody titers among the treatments.

Burciaga-Robles et al. (2010) reported a BVDV × MH × time interaction for MH LKT antibodies and suggested that calves exposed to PI BVDV steers had decreased production of MH LKT antibodies. These results are similar to those reported by Zhang et al. (1997) in which calves that were challenged with bovine immunodeficiency virus (BIV) had decreased and delayed BVDV antibody production after vaccination. In addition, calves in that experiment challenged with BIV also had decreased BHV1 antibody production after inoculation with the virus compared to calves that were not previously challenged with BIV. These data would suggest that the exposure of healthy calves to an initial viral challenge results in a suppression of the immune system thus predisposing calves to a secondary infection due to decreased antibody production toward the secondary pathogen. In addition, these data could explain the antibody titer

differences between the two experimental treatments in the current experiment. The decrease in MH WC antibody titers observed in the CON calves in the current experiment is likely the result of increased BVDV antibody titers in the same calves indicating a more severe immune suppression resulting from the BVDV challenge in those calves.

In the present experiment there were no time × treatment interactions observed for serum IGF-I or cortisol concentrations and mineral supplementation did not affect serum concentrations of either hormone over the length of the experiment. However, CON calves had elevated serum cortisol concentrations at h 12 and tended to have elevated serum cortisol concentrations at h 18. This resulted in CON calves tending to have elevated serum cortisol concentrations compared to MIN calves during the first 24 h following the MH challenge. Time affected both hormones during the first 24 h following the MH challenge and over the course of the experiment.

Serum IGF-I decreased rapidly from pre BVDV challenge sampling until post MH challenge then increased slowly from 2 d post MH challenge until the end of the experiment. Numerous studies have demonstrated reduced IGF-I concentrations independent of animal DMI during an immune challenge (Elsasser et al., 1987; Elsasser, 1988; Spurlock, 1997). These reduced concentrations of blood IGF-I associated with immune challenge events probably are the result of decreased IGF-I synthesis in multiple tissues (Spurlock, 1997). An endotoxin challenge administered to rats by Fan et al. (1994) caused a decrease in plasma, liver, skeletal muscle, and pituitary IGF-I, and liver and skeletal muscle IGF-I compared to control animals. Spurlock (1997) stated that the reduction of circulating IGF-I seemed to be an important part of necessary bodily function to support an immune response. Elsasser et al. (2008) reported decreased plasma

IGF-I concentrations for 24 h following a LPS challenge. The authors stated that the reduction in plasma IGF-I likely reflects a mechanism to spare nutrients necessary for immune defense and that the metabolic functions of IGF-I must be redirected. Elsasser et al. (2008) stated that this was not only true for IGF-I message transcription and tissue and plasma IGF-I concentrations but also through the redistribution IGF-I to tissues affected by the pro-inflammatory response.

Serum cortisol also decreased rapidly from pre BVDV challenge until post MH challenge. Serum cortisol increased slightly at d 7 and was then highly elevated on d 14 and d 28. Cortisol response to an immune challenge have proven somewhat inconsistent, and are potential influenced by multiple variables unrelated to the actual immune challenge. Arthington et al. (1997) found that supplemental chromium and a bovine herpesvirus-1 (BHV1) immune challenge did not influence cortisol levels of calves. In another chromium supplementation experiment by Burdick et al. (2011), a cortisol response was detected in calves subjected to a LPS challenge. Peak cortisol concentrations were measured at 1 h post challenge and returned to baseline levels within 4 h. It should be noted that this was an acute LPS challenge, where 0.5 mg/kg body weight of *Escherichia coli* O111:B4 was administered intravenously.

The highest measurements for serum cortisol in the present experiment coincided with sampling periods where calves were gathered, handled and briefly restrained in a manual squeeze chute. When calves were maintained in stanchions during the intensive sampling portion of the experiment (d 0 through d 7) serum cortisol levels remained relatively constant after the first 12 h. There was a slight increase in serum cortisol from h 0 until h 6, but it is impossible to determine if that slight increase is due to the immune

challenge, or initial brief restraint and being placed in the stanchions. When the data are examined closely, it would seem that the spikes in cortisol observed in the present experiment are related to periods of animal handling and restraint rather than to the BVDV and MH immune challenge. Experiments by Lay et al. (1992), Wohlt et al. (1994), and Apple et al. (2005) have all demonstrated similar increases cortisol responses to physical restraint and handling.

With the multitude of hematology variables examined in the current experiment, multiple time × treatment interactions and tendencies were observed. Time × treatment interactions existed for erythrocytes, hemoglobin, hematocrit, the SD of platelet distribution width, eosinophils, and eosinophil percentage during the first 24 h following the MH challenge and for the SD of erythrocyte distribution width over the duration of the experiment. There was also a tendency for a time × treatment interaction observed for the CV of erythrocyte distribution width during the first 24 h following the MH challenge and for mean corpuscular volume and mean corpuscular hemoglobin concentration over the length of the experiment.

The effect of the challenge was significant with multiple hematology parameters being significantly impacted by time. This was true both for the 24 h period following the MH challenge and for the duration of the experiment. These results were similar to those found by Burciaga-Robles et al. (2010). The authors in that experiment found multiple hematology parameters that were affected by either the exposure to PI animals, the MH challenge, or both. Burciaga-Robles et al. (2010) noticed that haptoglobin concentrations were increased for steers challenged with MH from h 18 to h 96 following the MH challenge. Interestingly, Burciaga-Robles et al. (2010) found that the exposure of steers

to a PI BVDV animal resulted in less total leukocytes, while the MH challenge resulted in greater total leukocytes. Neutrophils were also increased in steers challenged with MH. The exposure of calves to both a PI animal and the MH challenge resulted in decreased lymphocytes. Similar to what was observed with total leukocytes, the authors found that PI BVDV exposure resulted in decreased eosinophils while the MH challenge resulted in increased eosinophils. Basophils followed the same pattern with PI exposure reducing basophil numbers while a challenge with MH increased basophil counts. Hematocrits tended to be decreased and hemoglobin was decreased for steers challenged with MH in their experiment. Mean corpuscular volume and mean corpuscular hemoglobin concentration were reduced for steers challenged with MH. Total erythrocytes were also reduced for steers challenged with MH.

Other researchers have demonstrated the effects of an immune challenge of various hematology parameters or demonstrated differences in hematology variables for sick versus healthy calves. Martin and Lumsden (1987) sampled calves entering Canadian feedlots and monitored them for subsequent BRD incidence. The hematology values reported in this experiment would also be similar to those found in the current experiment. The authors found that those calves subsequently treated for BRD had significantly lower hematocrits, fewer platelets, and more band cells at arrival compared to calves that did not get sick.

When comparing our hematology results to standard reference ranges for reported for cattle, most erythrocyte parameters fell within normal ranges (Radostits et al., 2000; Merck, 2012). The one exception would be mean corpuscular volume, which was slightly lower at multiple time points than the reference values reported. However, the mean

corpuscular volumes in the current experiment would be similar to those reported by Burciaga-Robles et al. (2010) and Martin and Lumsden (1987). Most leukocyte variables in the current experiment would also fall within normal reference ranges if averaged across all sampling times. However, multiple leukocyte variables peaked outside the standard reference ranges (Radostits et al., 2000; and Merck, 2012).

Total peak leukocytes (CON = 11.7 × 10^3/μL; MIN = 13.6 × 10^3/μL) and lymphocytes (CON = 7.12 × 10^3/μL; MIN = 9.32 × 10^3/μL) both occurred at 18 h post MH challenge. Peak neutrophils (CON = 6.13 × 10^3/μL; MIN = 5.61 × 10^3/μL) occurred slightly later at 24 h post MH challenge. Monocytes were highly variable and exhibited multiple peaks thought the experiment. While they occurred on different days, the maximum monocyte values observed for CON and MIN calves were 1.17 × 10^3/μL and 1.10 × 10^3/μL, respectively. All of these peak values for leukocytes, lymphocytes, neutrophils, and monocytes exceed the standard reference ranges for cattle. The values for lymphocytes also exceed those reported by Burciaga-Robles et al. (2010). Eosinophils and Basophils both were within the same standard reference ranges for cattle throughout the experiment.

Mineral supplementation only affected limited hematology variables examined. Calves receiving MIN demonstrated greater SD of platelet distribution widths during the first 24 h following the MH challenge and tended to have increased platelet large cell ratios for the duration of the experiment compared to CON calves (data not shown). Interestingly, while total leukocytes were not different among the TM treatments, there were some differences in specific leukocyte percentages. Calves receiving MIN had an

increased percentage of lymphocytes, while calves receiving CON had an increased percentage of neutrophils.

The increased neutrophil percentages observed in the CON calves in the current experiment are in conflict with those commonly found in both rats and humans. Typically, circulating neutrophils are reduced in the blood of humans or rats in cases of Cu deficiency (Percival, 1998). In addition to being reduced in number, the ability of neutrophils to generate superoxide anion and kill ingested bacteria is also decreased in cases of even marginal Cu deficiency (Percival, 1998). Boyne and Arthur (1986) and Xin et al. (1991) have reported similar decreases in neutrophil function in cattle where Cu deficient diets were fed. In contrast, Arthington et al. (1995) found that Cu depletion or repletion did not affect neutrophil function in beef heifers. We did not examine neutrophil function the current experiment.

The decreased lymphocyte percentages observed in the non-supplemented calves in our experiment would be supported by previous research. Multiple researchers have demonstrated that a Cu deficiency is associated with reduced lymphocyte counts. Blakley and Hamilton (1987), Koller et al. (1987), and Bala et al. (1991) demonstrated that a lower Cu levels are associated with a reduced T lymphocytes in rodents. Cerone et al. (1998) found that the number of B lymphocytes was significantly decreased by about 40% in the Cu deficient calves. However, Cerone et al. (1998) reported only a 7% reduction in T lymphocytes in Cu deficient calves. Total lymphocytes were reduced in the Cu deficient calves in that experiment, but the difference was not statistically significant.

In the current experiment, there were no time × treatment interactions observed for blood gas variables, pH values, or blood metabolites during the first 24 h following the MH challenge or over the length of the experiment. Similar to what was observed with the hematology variables, the effect of the challenge was significant and time impacted multiple blood gas variables, pH values, and blood metabolites for the 24 h period following the MH challenge and for the duration of the experiment. These results were also similar to those found by Burciaga-Robles et al. (2010). The authors in that experiment found multiple blood gas variables, pH values, and blood metabolites parameters that were affected by either the exposure to PI animals, the MH challenge, or both. The concentration of certain metabolites, namely glucose, and lactate were higher in the present experiment when compared to the results reported by Burciaga-Robles et al. (2010).

In the current experiment, Ca slowly increased until 72 h following the MH challenge, then slowly decreased for the remainder of the experiment returning to baseline levels by d 14. These results are similar to those reported by Orr et al. (1990). The authors evaluated the effects of BRD and IBR on serum Ca levels of calves. In that experiment, serum Ca increased for the first 4 d following the IBR challenge, then decreased prior to returning to baseline levels for the remainder of the experiment. Orr et al. (1990) stated that the increases in serum Ca were not due to a decrease in DMI, and that they must be a result of market-transit stress and BRD or the IBR infection. In the current experiment, glucose and lactate both sharply decreased early in the challenge period, leveled out during the first wk after the MH challenge, and then increased through the end of the experiment.

Stress associated with transportation and disease has been shown to impact blood metabolite concentrations of calves (Galyean et al., 1981; Montgomery et al., 2009). Mitchell et al. (1988) reported increased plasma lactate concentrations for cattle that were handled and subjected to transport stress compared to cattle that were not handled and transported. Montgomery et al. (2009) took samples at the time of initial processing and then monitored heifers for the incidence of BRD. The authors observed a linear decrease in both plasma glucose and lactate concentrations as the number of BRD treatments increased. The authors attributed the decrease in plasma glucose concentrations for those heifers subsequently treated for BRD to a possible disease challenge prior to processing. Steiger et al. (1999) and Kushibik et al. (2000) both observed biphasic responses in plasma glucose in calves infused bacterial lipopolysaccharides or injected recombinant tumor necrosis factor-α (TNF-α), respectively. These data would indicate that an immune challenge causes an initial period of hyperglycemia followed by a subsequent period of hypoglycemia and may explain some of the changes in glucose concentrations observed in the steers in the present experiment.

It should be noted that these studies are observing glucose concentrations at a single time point or over a duration of a few hours, where we observed glucose concentrations across larger intervals of time. Glucose concentrations are highly variable and fluctuate rapidly with metabolism and after biological events. We attempted to control for as much variation as possible for all blood variables and metabolites in the current experiment by maintaining a tight sampling schedule. Samples on d -4 and d 28 would have been taken over a window of several hours as calves were biopsied on those

days as well. However, sampling at all other time points for the daily values occurred at 0700 h and would have been completed for all claves within a 15 to 30 min span of time.

It should also be noted that while the challenge did effect multiple blood gas variables, pH values, and blood metabolites, it did not necessarily cause them to be abnormal. When comparing our results to standard reference ranges for reported for cattle, most blood gas variables, pH values, and blood metabolites fell completely within normal ranges (Gokce et al., 2004; Cornell, 2012). Mineral supplementation had a minimal affect blood gas parameters and blood metabolites. Calves receiving MIN tended to have greater base excess in ECF and base excess in blood compared to CON calves over the length of the experiment. These differences are likely the result of numerical increases in bicarbonate at the beginning and end of the challenge period for calves on the MIN treatment.

Conclusions

The supplementation of TM has been shown to alter immune function in some experiments, however, the effects on specific immune responses in cattle have been somewhat minimal and highly variable except in cases of extreme TM deficiency. The BVDV and MH immune challenge employed in this experiment impacted antibody titers, and multiple hematology, blood gas, and blood metabolite variables. However, most of the hematology, blood gas, and blood metabolite variables measured remained within normal ranges, so the clinical relevance of most of these changes is likely minimal except as validation of a successful immune challenge. The supplementation of Cu, Mn, and Zn

has the potential to impact serum antibody titers and leukocyte populations in calves fed a Cu deficient diet and experiencing a controlled short term immune challenge. These results would serve as evidence of an important role for TM, especially Cu, in the normal immune function of calves.

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Table 6-1. Composition of common receiving diet[1]

Ingredient (%)[1]	
Prairie hay	35.0
Dry-rolled corn	28.0
Wet corn gluten feed[2]	15.0
Dried distillers grains plus solubles	15.0
Dry supplement B-278[3]	3.5
Liquid supplement[4]	3.5
Nutrient Composition[1,5]	
NE_m, Mcal/kg	1.62
NE_g, Mcal/kg	1.03
TDN, %	69.83
Crude protein, %	16.20
Crude fat, %	3.13
NDF, %	44.47
ADF, %	23.00
Calcium, %	0.80
Phosphorus, %	0.51
Magnesium, %	0.26
Potassium, %	1.03
Sulfur, %	0.30
Copper, mg/kg	6.00
Manganese, mg/kg	50.33
Zinc, mg/kg	47.33
Iron, mg/kg	614.00

[1]All ingredient and nutrient values are presented on a DM basis.
[2]Sweet Bran® (Cargill; Dalhart, Texas).
[3]Dry supplement B-278 was formulated to contain: 38.123% ground corn, 34.705% limestone, 23.477% wheat midds, 2.571% salt, 0.224% vitamin A (30,000 IU/g), 0.134% vitamin E (500 IU/g), 0.002% vitamin D (500,000 IU/g), 0.002% EDDI, 0.496% Rumensin 90 (Elanco Animal Health; Indianapolis, IN), and 0.266% Tylan 40 (Elanco Animal Health; Indianapolis, IN) on a DM basis.
[4]Synergy 19-14 (Westway Feed Products; New Orleans, LA).
[5]Feed samples were analyzed for nutrient composition by an independent laboratory (Servi-Tech Laboratories; Dodge City, KS).

Figures

Figure 6-1. Serum bovine viral diarrhea virus (BVDV) type 1b neutralization antibody titers of calves following exposure to BVDV 1b and subsequent *Mannheimia haemolytica* (MH) infection. Treatment tended to be significant ($P = 0.07$) and time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There was a time × treatment interaction ($P < 0.003$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-2a. Serum *Mannheimia haemolytica* (MH) whole cell (WC) antibody titers of calves following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent MH infection. There was no time × treatment interaction ($P = 0.48$). Treatment ($P = 0.02$) and time ($P < 0.0001$) effects were significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-2b. Serum *Mannheimia haemolytica* (MH) leukotoxin (LKT) antibody titers of calves following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent MH infection. There was no time × treatment interaction ($P = 0.27$) or treatment effect ($P = 0.48$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-3a. Serum IGF-I concentrations of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.43$) or treatment effect ($P = 0.46$). However, time was significant ($P = 0.02$). Values plotted

represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-3b. Serum IGF-I concentrations of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.15$) or treatment effect ($P = 0.99$). However, time was significant ($P < 0.004$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-4a. Serum cortisol concentrations of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.28$). Treatment tended to be significant ($P = 0.09$) and time was significant ($P = 0.0002$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-4b. Serum cortisol concentrations of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.87$) or treatment effect ($P = 0.31$). However, time was significant ($P = 0.01$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-5a. Total erythrocyte counts of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica*

(MH) infection. Treatment was not significant ($P = 0.49$). However, time was significant ($P < 0.008$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There was a time × treatment interaction ($P = 0.03$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-5b. Total erythrocyte counts of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.64$) or treatment effect ($P = 0.30$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-6a. Hemoglobin concentrations of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. Treatment was not significant ($P = 0.54$). However, time was significant ($P < 0.007$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There was a time × treatment interaction ($P = 0.05$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-6b. Hemoglobin concentrations of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.48$) or treatment effect ($P = 0.40$). However, time was significant ($P = 0.0004$). Values plotted

represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-7a. Hematocrit percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. Treatment was not significant ($P = 0.60$). However, time was significant ($P < 0.006$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There was a time × treatment interaction ($P = 0.04$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-7b. Hematocrit percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.56$) or treatment effect ($P = 0.44$). However, time was significant ($P = 0.0004$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-8a. Mean corpuscular volumes of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.17$) or treatment effect ($P = 0.84$). However, time was significant ($P = 0.0009$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-8b. Mean corpuscular volumes of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia*

haemolytica (MH) infection. Treatment was not significant ($P = 0.85$). However, time was significant ($P < 0.007$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There tended to be a time × treatment interaction ($P < 0.08$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-9a. Mean corpuscular hemoglobin amounts of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.14$). Treatment ($P = 0.87$) and time ($P = 0.29$) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-9b. Mean corpuscular hemoglobin amounts of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.12$) or treatment effect ($P = 0.77$). However, time tended to be significant ($P = 0.07$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-10a. Mean corpuscular hemoglobin concentrations of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.17$) or treatment effect ($P = 0.84$). However, time was significant ($P < 0.005$). Values

plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-10b. Mean corpuscular hemoglobin concentrations of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. Treatment was not significant ($P = 0.99$). However, time was significant ($P < 0.003$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There tended to be a time × treatment interaction ($P = 0.10$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-11a. Total reticulocyte counts of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.55$) or treatment effect ($P = 0.99$). However, time was significant ($P = 0.05$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 11b. Total reticulocyte counts of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.47$) or treatment effect ($P = 0.81$). However, time was significant ($P = 0.05$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-12a. Total platelet counts of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica*

(MH) infection. There was no time × treatment interaction ($P = 0.82$). Treatment ($P = 0.91$) and time ($P = 0.16$) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-12b. Total platelet counts of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.24$) or treatment effect ($P = 0.98$). However, time tended to be significant ($P = 0.06$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-13a. Total leukocyte counts of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.66$) or treatment effect ($P = 0.78$). However, time was significant ($P < 0.002$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-13b. Total leukocyte counts of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.12$) or treatment effect ($P = 0.39$). However, time was significant ($P < 0.002$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-14a. Neutrophil percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.98$). Treatment ($P =$

0.38) and time (P = 0.12) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-14b. Neutrophil percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.93). Treatment was significant (P = 0.03). Time was not significant (P = 0.35). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-15a. Lymphocyte percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.98) or treatment effect (P = 0.25). However, time tended to be significant (P = 0.07). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-15b. Lymphocyte percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.94). Treatment was significant (P = 0.01) and time tended to be significant (P = 0.07). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-16a. Monocyte percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.61). Treatment (P =

0.49) and time ($P = 0.34$) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-16b. Monocyte percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.46$) or treatment effect ($P = 0.51$). However, time was significant ($P < 0.005$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-17a. Eosinophil percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. Treatment was not significant ($P = 0.41$). However, time was significant ($P < 0.0001$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group. There was a time × treatment interaction ($P = 0.01$). A slice output option of SAS was used to perform mean separations within time points. Means with different superscripts (a,b) differ ($P \leq 0.05$).

Figure 6-17b. Eosinophil percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.40$) or treatment effect ($P = 0.94$). However, time was significant ($P < 0.002$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-18a. Basophil percentages of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.17$) or treatment effect

(P = 0.86). However, time was significant (P = 0.01). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-18b. Basophil percentages of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.84) or treatment effect (P = 0.76). However, time tended to be significant (P = 0.08). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-19a. Blood pH of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.25) or treatment effect (P = 0.36). However, time was significant (P < 0.006). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-19b. Blood pH of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.40). Treatment (P = 0.27) and time (P = 0.13) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-20a. Partial pressure of carbon dioxide within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.33) or treatment effect (P = 0.22). However, time tended to be

significant (P = 0.07). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-20b. Partial pressure of carbon dioxide within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.45) or treatment effect (P = 0.14). However, time was significant (P = 0.02). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-21a. Partial pressure of oxygen within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.88) or treatment effect (P = 0.34). However, time was significant (P = 0.02). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-21b. Partial pressure of oxygen within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.81) or treatment effect (P = 0.67). However, time was significant (P < 0.005). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-22a. Sodium concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent

Mannheimia haemolytica (MH) infection. There was no time × treatment interaction (P = 0.57) or treatment effect (P = 0.93). However, time was significant (P = 0.05). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-22b. Sodium concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.41) or treatment effect (P = 0.38). However, time tended to be significant (P = 0.08). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-23a. Potassium concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.26). Treatment (P = 0.96) and time (P = 0.77) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-23b. Potassium concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.92) or treatment effect (P = 0.78). However, time tended to be significant (P = 0.08). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-24a. Calcium concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.33). Treatment (P = 0.72) and time (P = 0.16) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-24b. Calcium concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.62) or treatment effect (P = 0.57). However, time was significant (P = 0.0005). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-25a. Glucose concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.11) or treatment effect (P = 0.46). However, time was significant (P < 0.003). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-25b. Glucose concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.50) or treatment effect (P = 0.20). However, time was significant (P < 0.002). Values

plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-26a. Lactate concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.14) or treatment effect (P = 0.33). However, time was significant (P = 0.03). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-26b. Lactate concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.71) or treatment effect (P = 0.17). However, time was significant (P = 0.0006). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-27a. Bicarbonate concentration within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.24) or treatment effect (P = 0.15). However, time was significant (P = 0.05). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-27b. Bicarbonate concentration within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent

Mannheimia haemolytica (MH) infection. There was no time × treatment interaction (P = 0.14) or treatment effect (P = 0.11). However, time was significant (P = 0.003). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-28a. Base excess in extracellular fluid (ECF) within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.44) or treatment effect (P = 0.20). However, time was significant (P < 0.008). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-28b. Base excess in extracellular fluid (ECF) within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.34). Treatment tended to be significant (P = 0.10) and time was significant (P < 0.005). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-29a. Base excess in blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction (P = 0.41) or treatment effect (P = 0.20). However, time was significant (P = 0.01). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-29b. Base excess in blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.11$). Treatment tended to be significant ($P = 0.09$) and time was significant ($P = 0.002$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-30a. Oxygen saturation percentage within the blood of calves during the first 24 h following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.64$). Treatment ($P = 0.56$) and time ($P = 0.12$) were also not significant. Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

Figure 6-30b. Oxygen saturation percentage within the blood of calves during the first 28 d following exposure to bovine viral diarrhea virus (BVDV) type 1b and subsequent *Mannheimia haemolytica* (MH) infection. There was no time × treatment interaction ($P = 0.73$) or treatment effect ($P = 0.92$). However, time was significant ($P < 0.004$). Values plotted represent least squares means ± SE of the mean, calculated for 8 animals per experimental group.

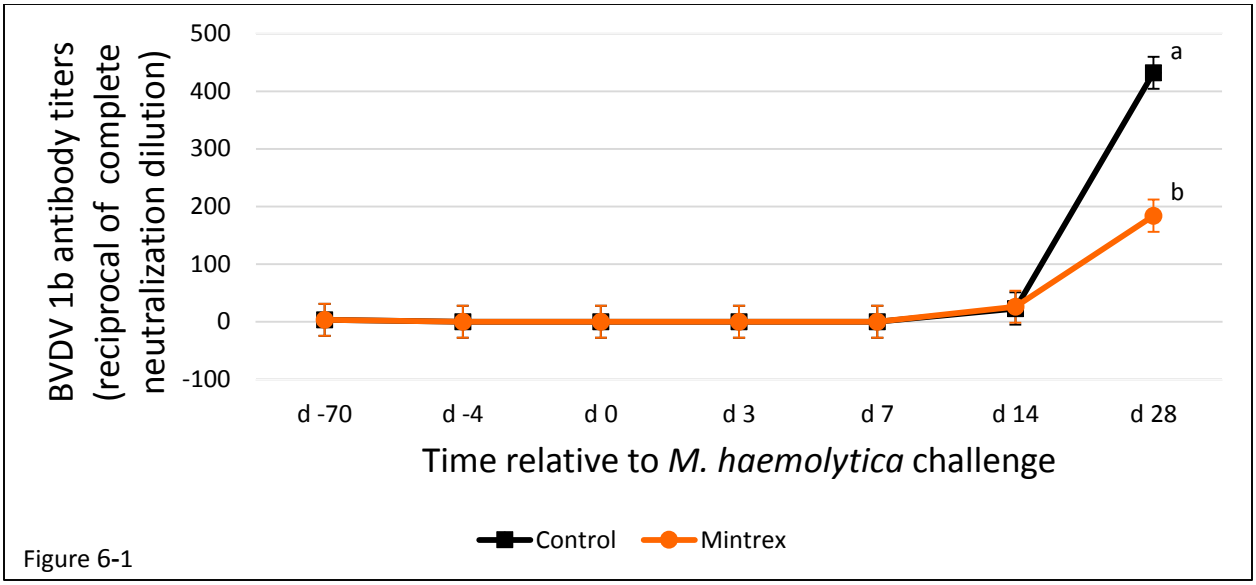


Figure 6-1

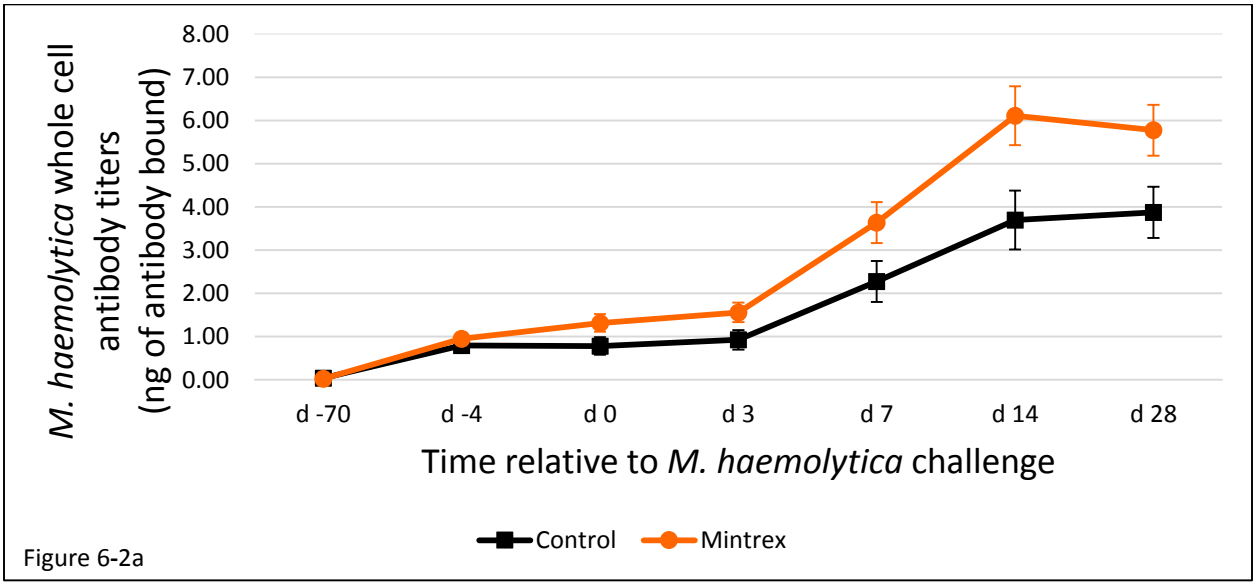


Figure 6-2a

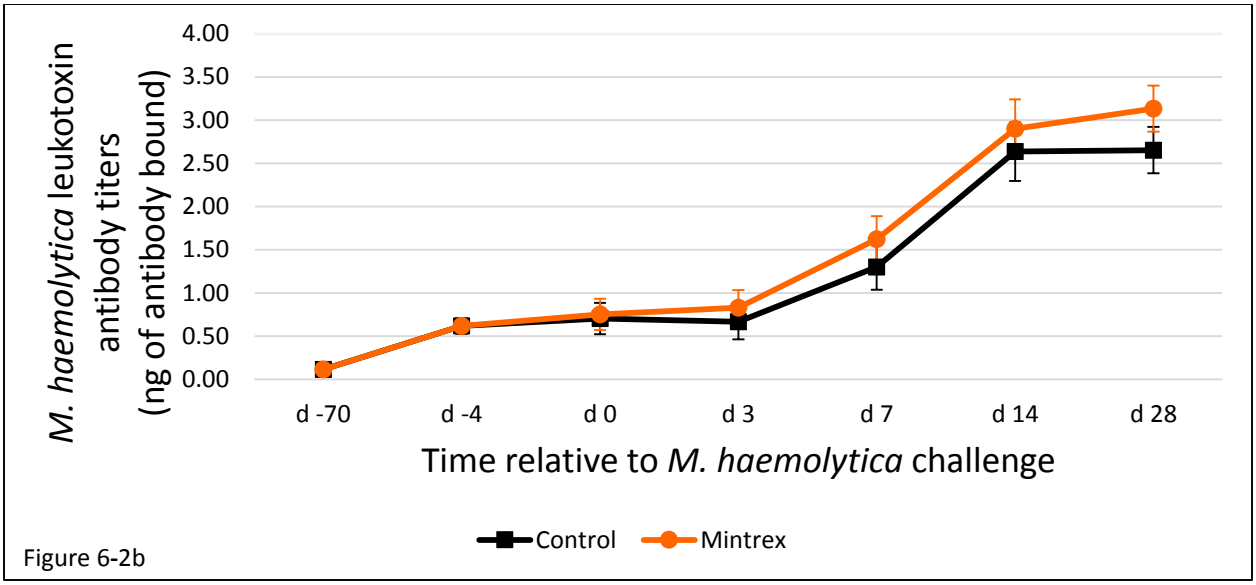


Figure 6-2b

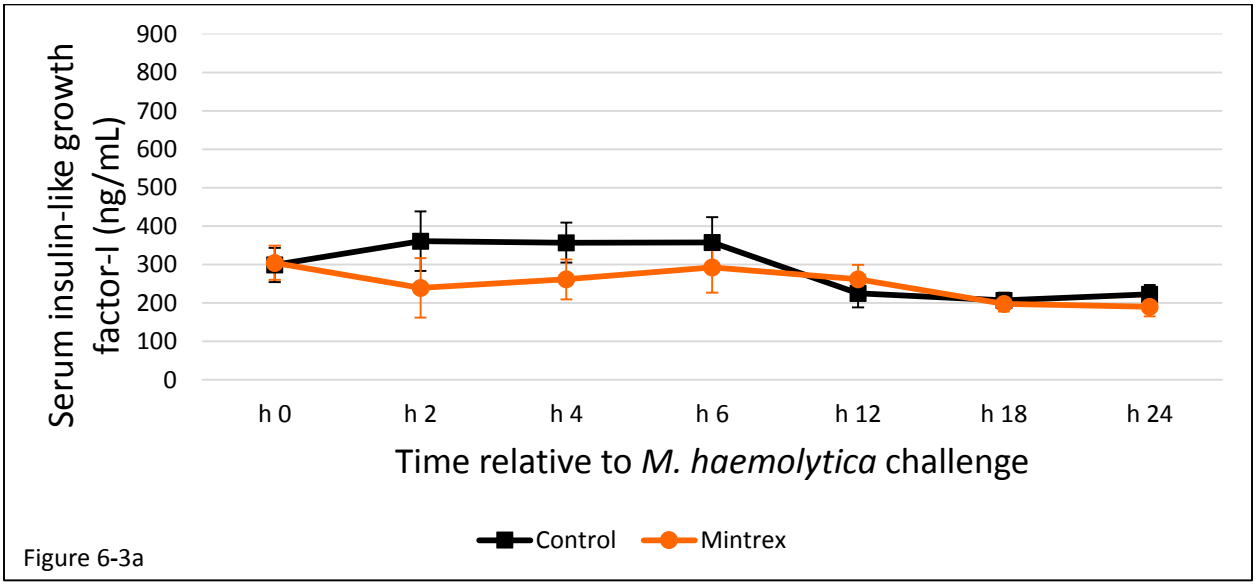


Figure 6-3a

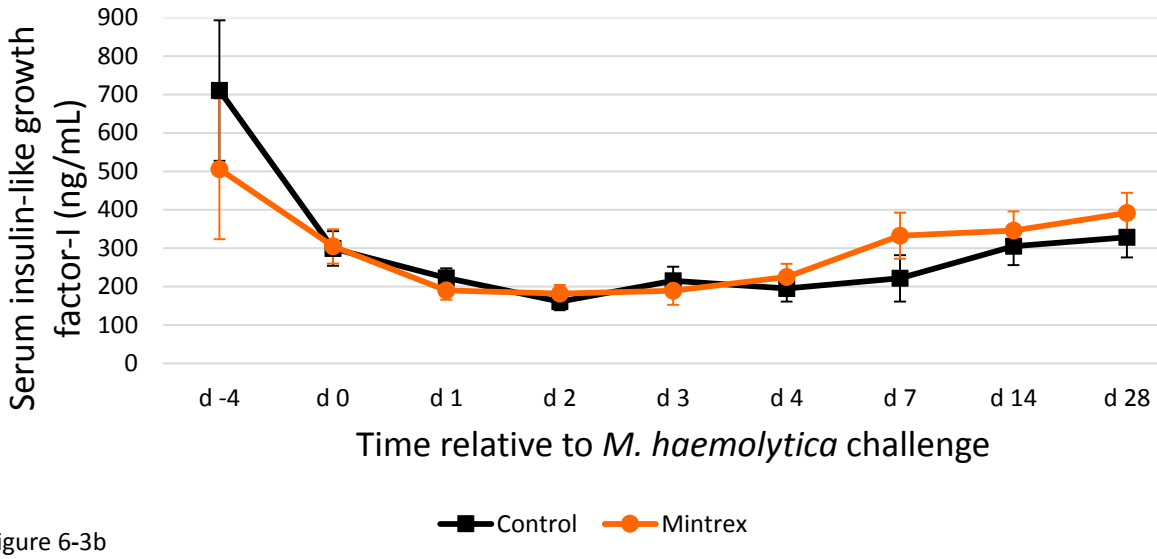


Figure 6-3b

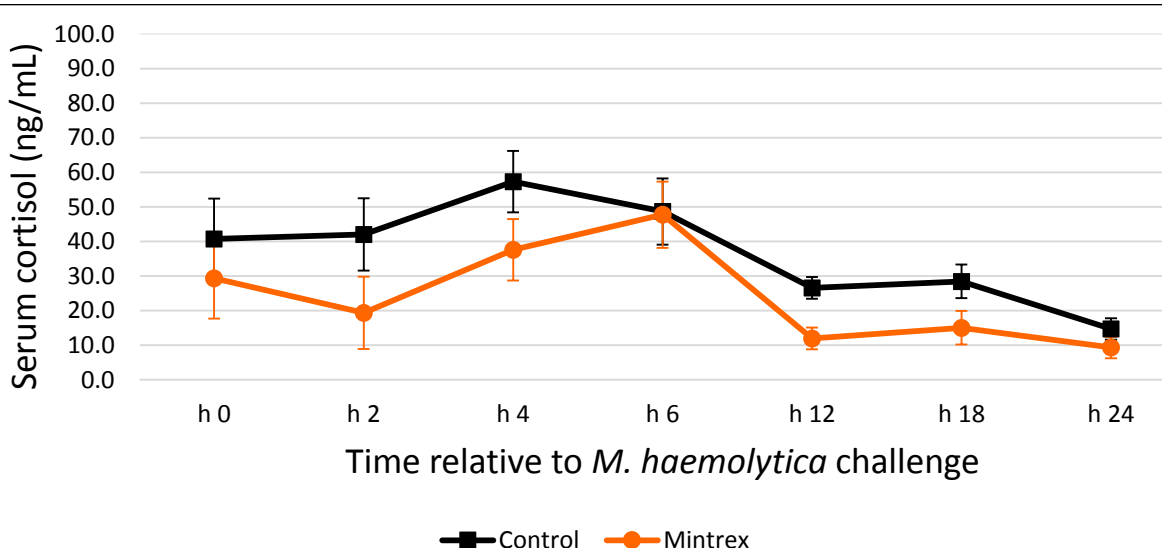


Figure 6-4a

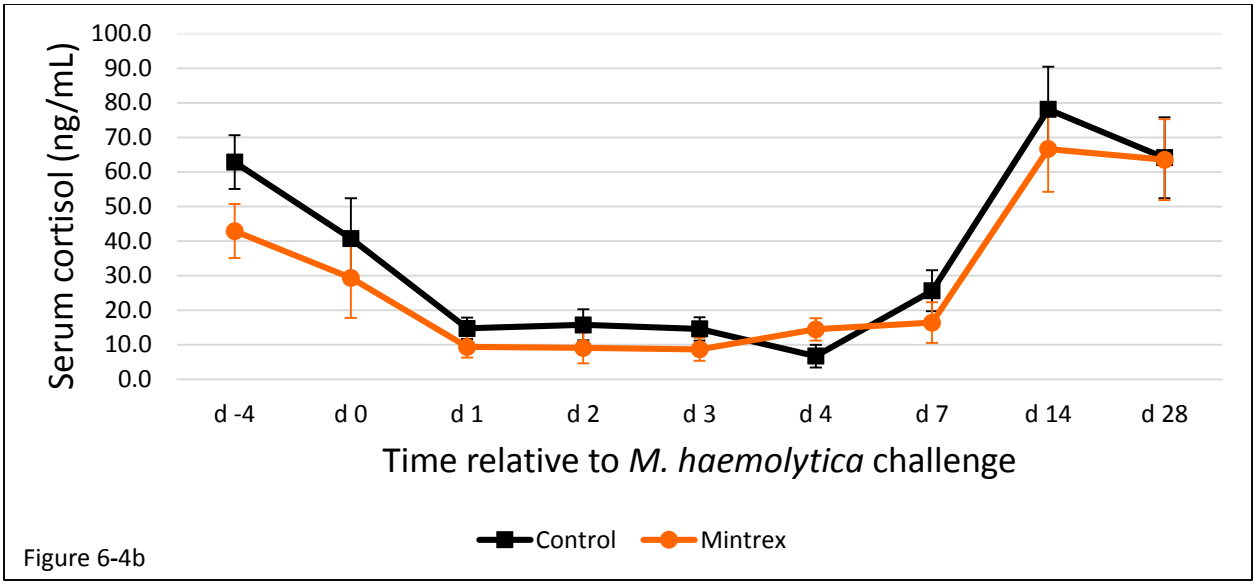


Figure 6-4b

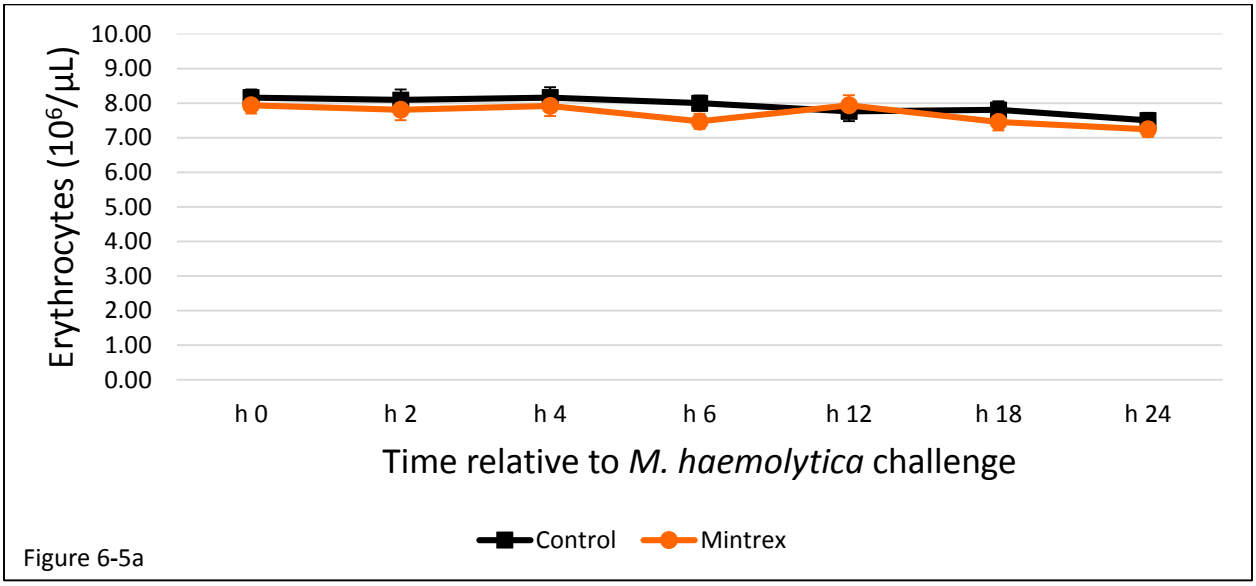


Figure 6-5a

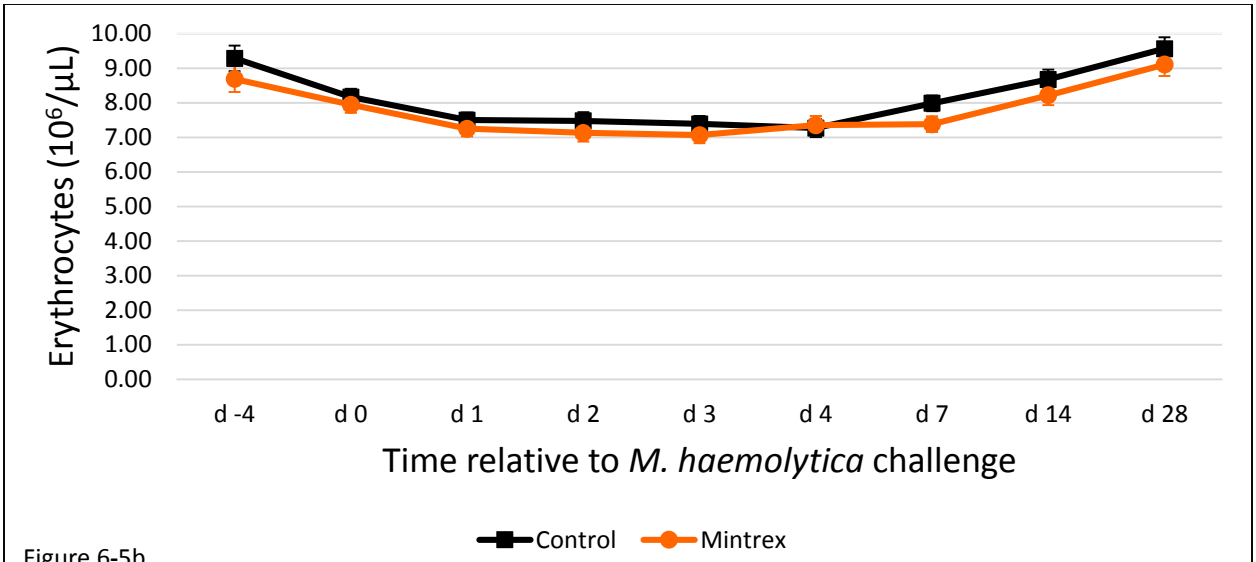


Figure 6-5b

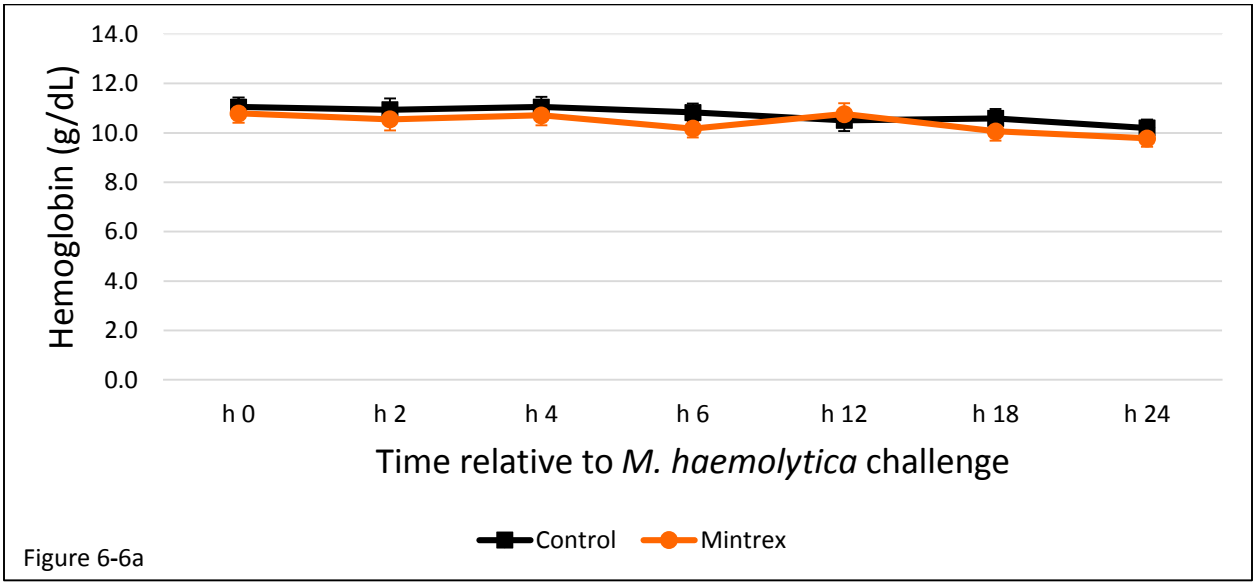
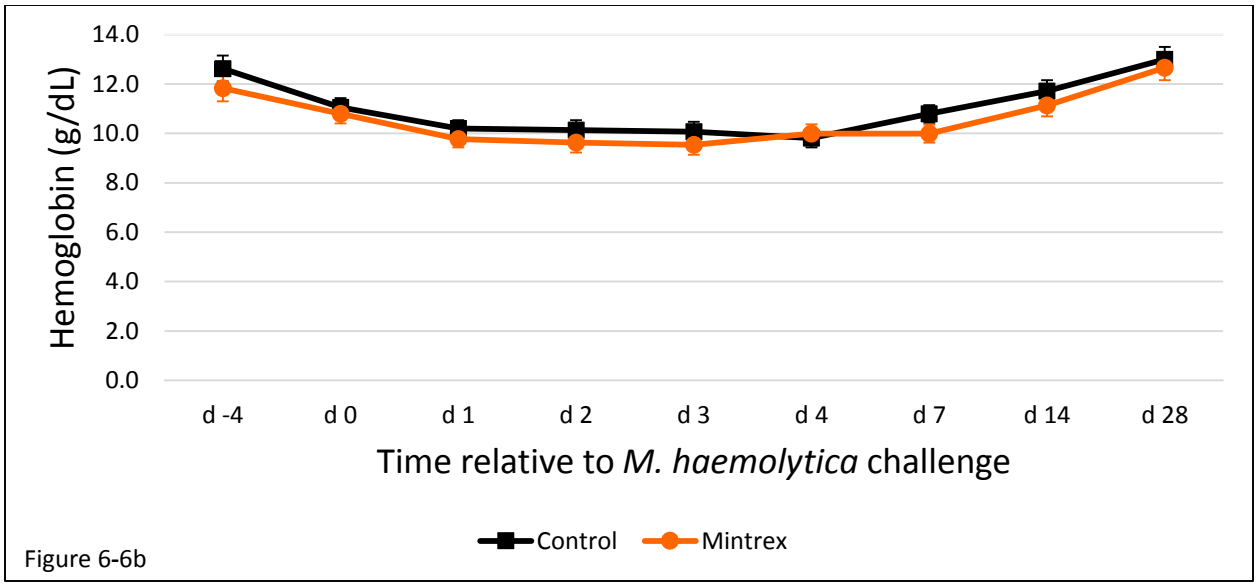


Figure 6-6a



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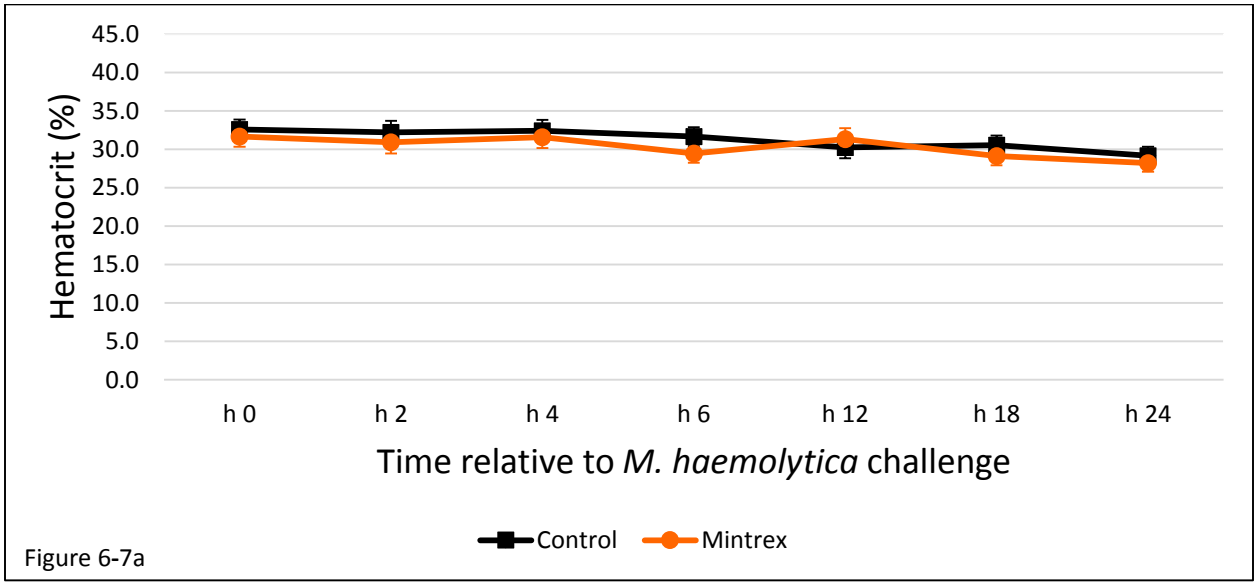

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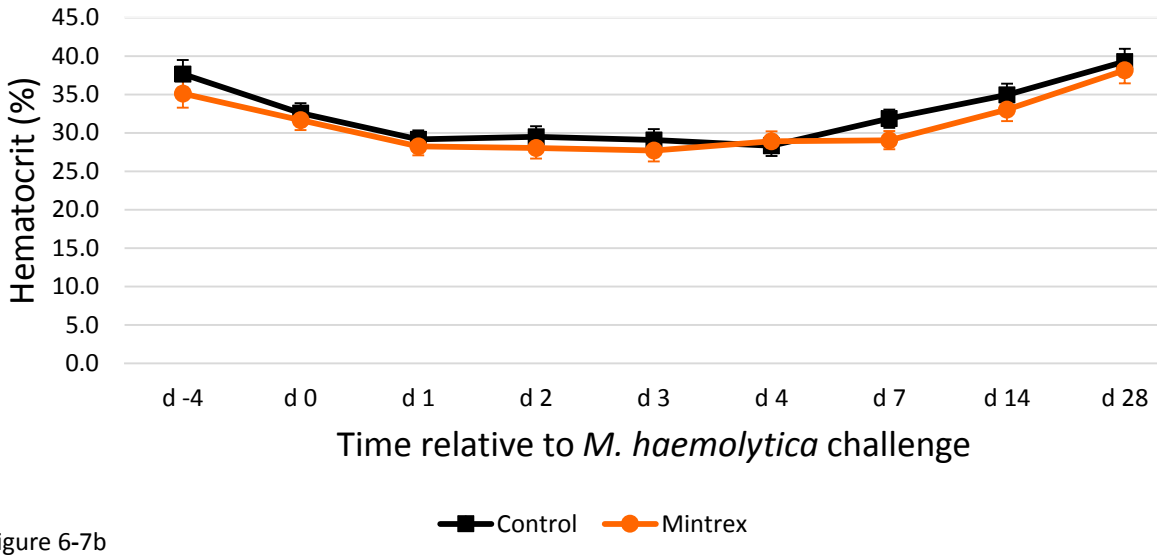

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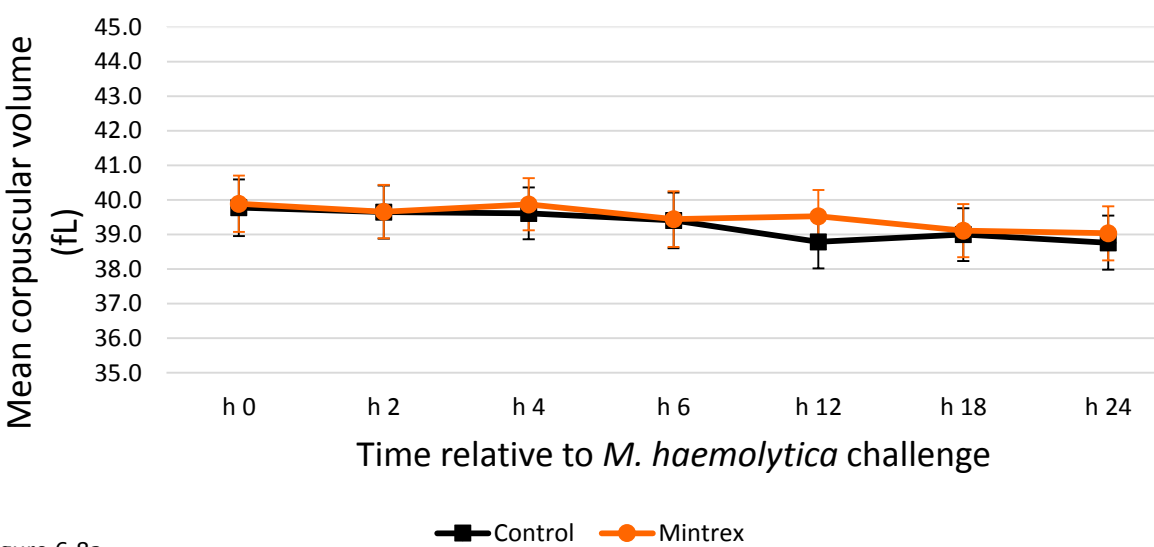

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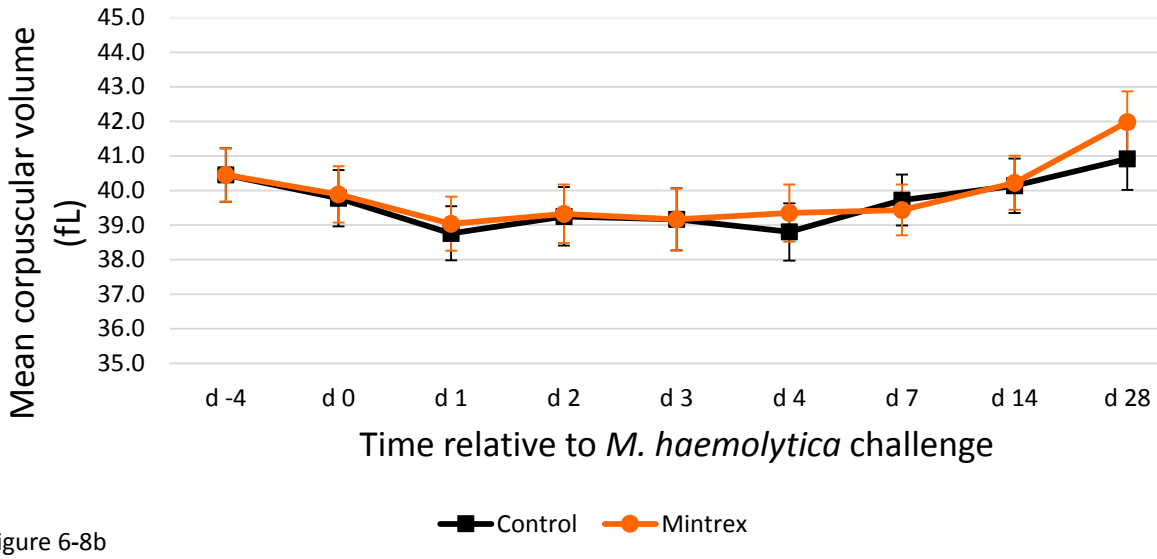

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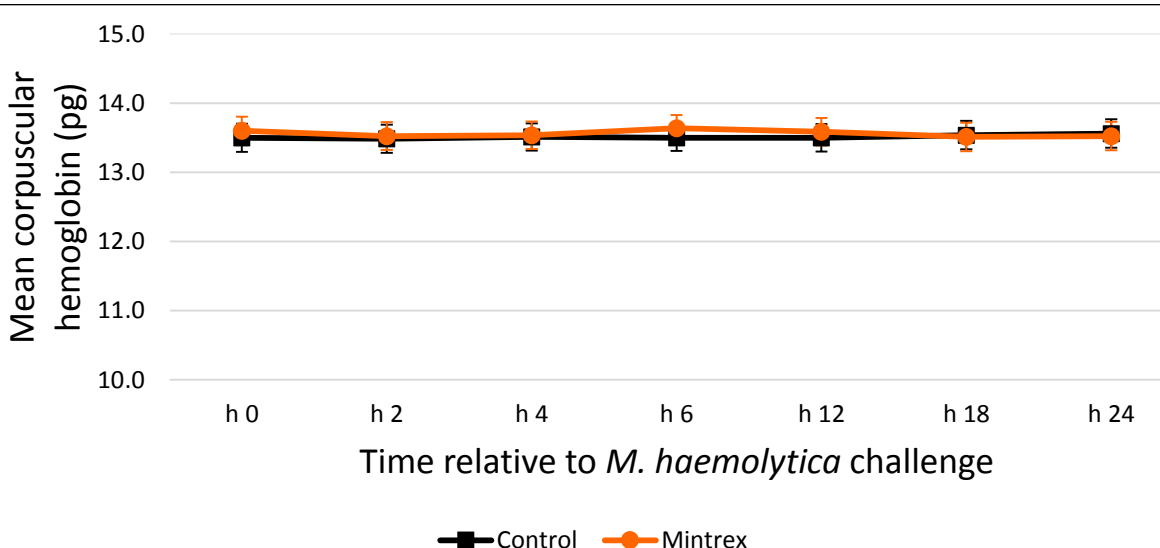

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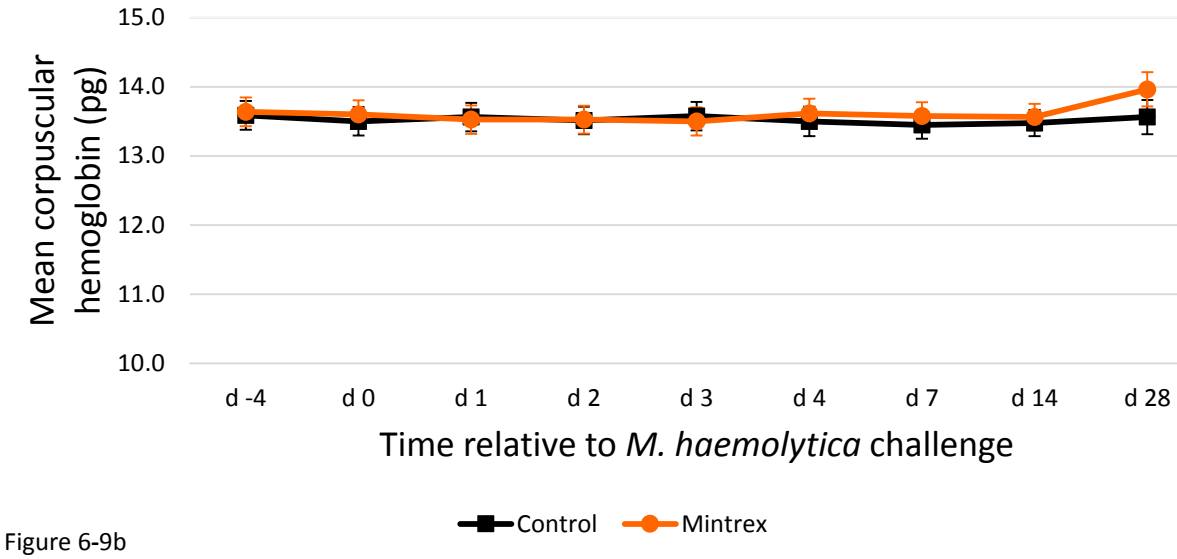

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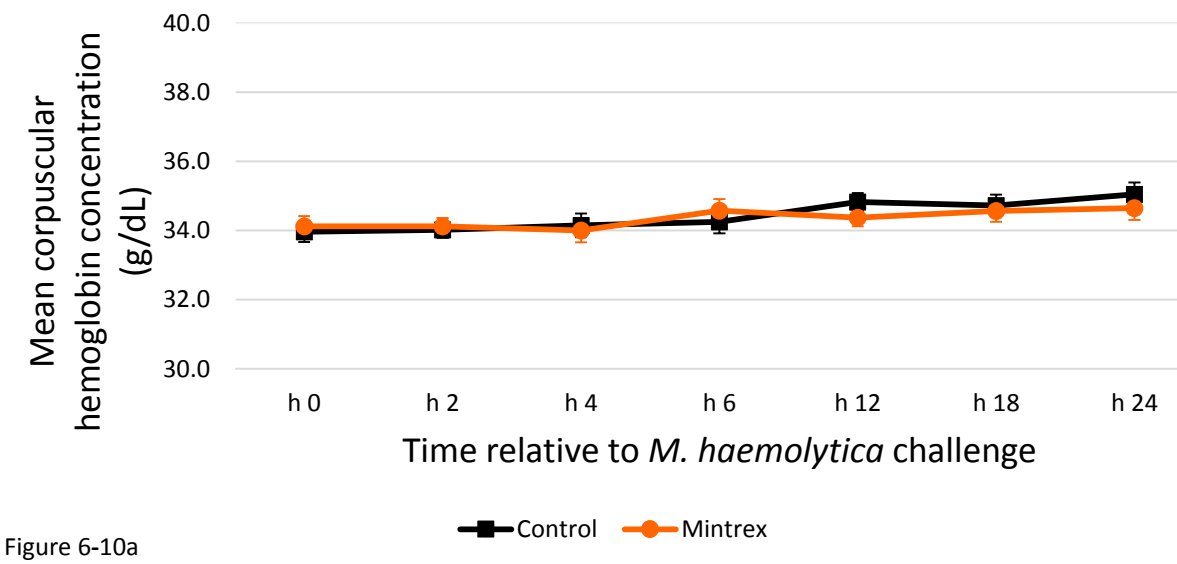

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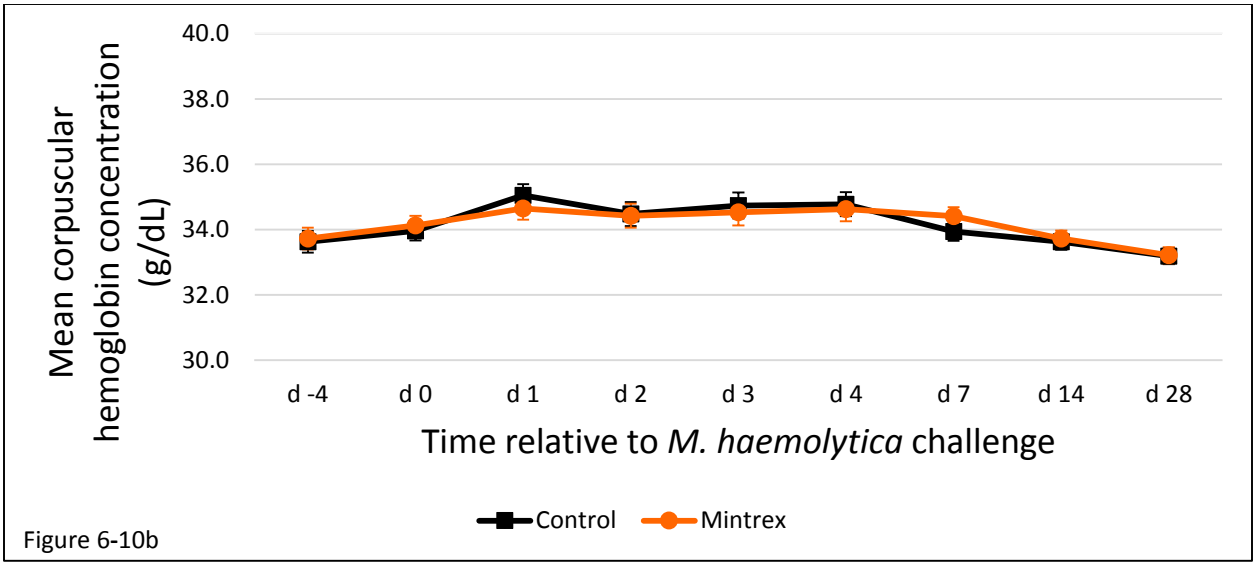

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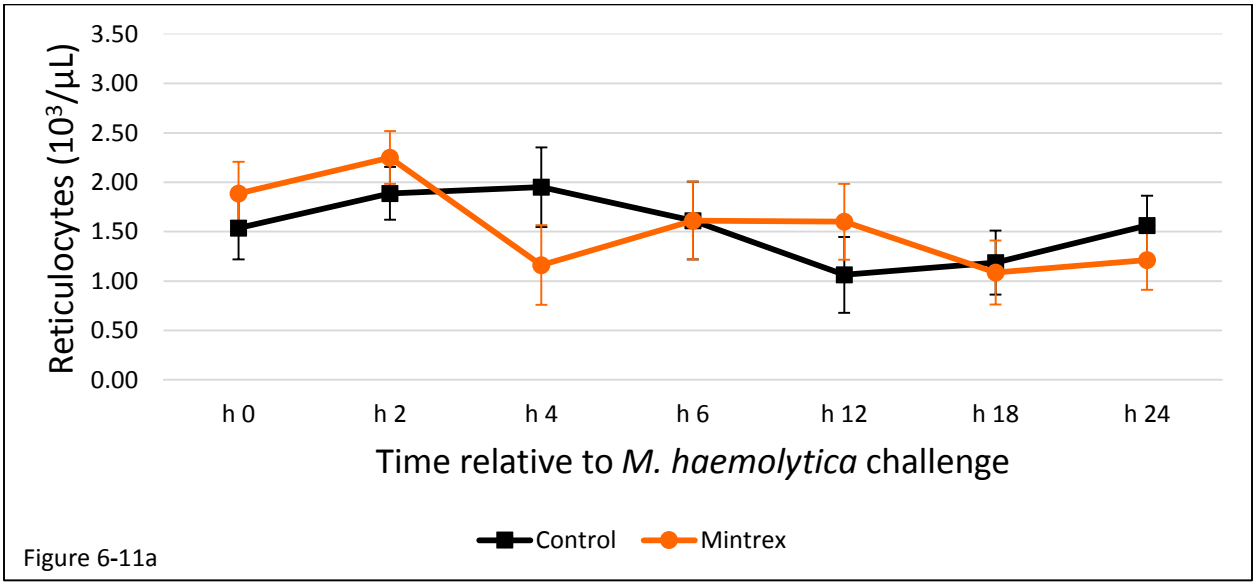

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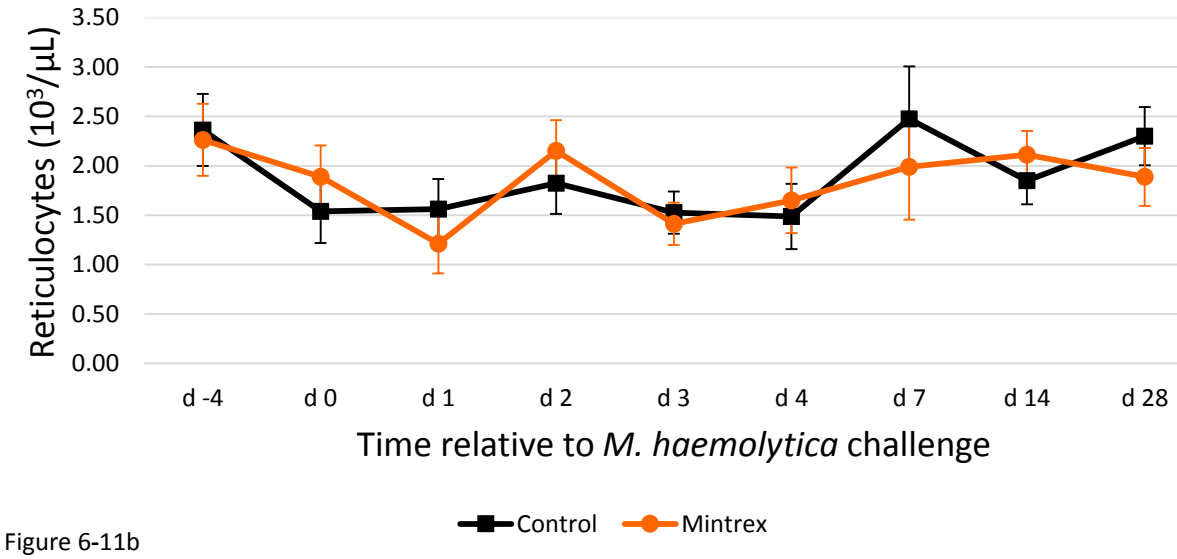

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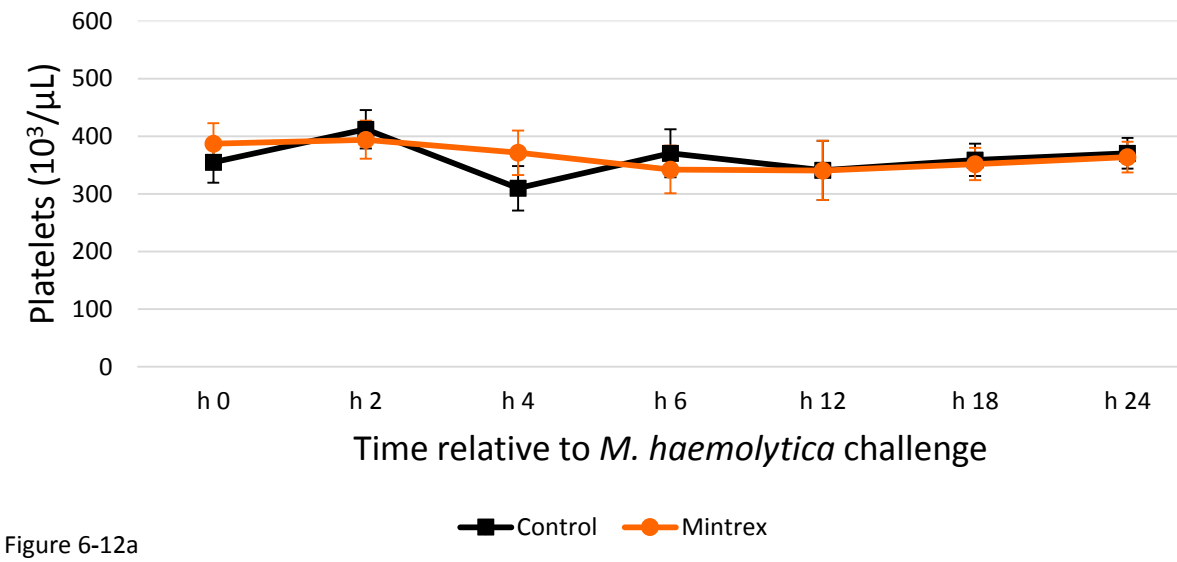

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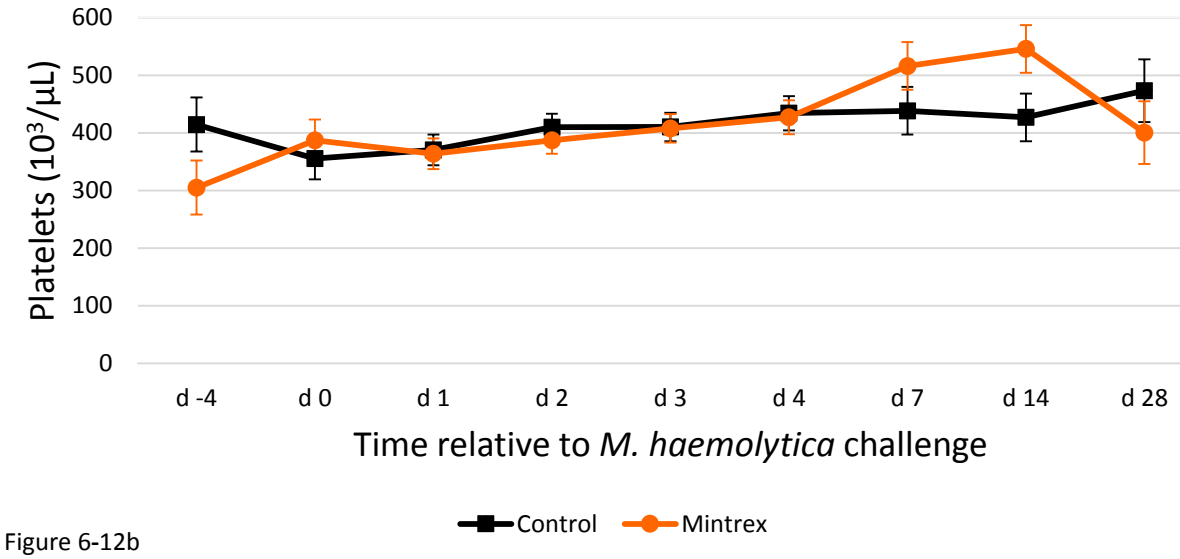

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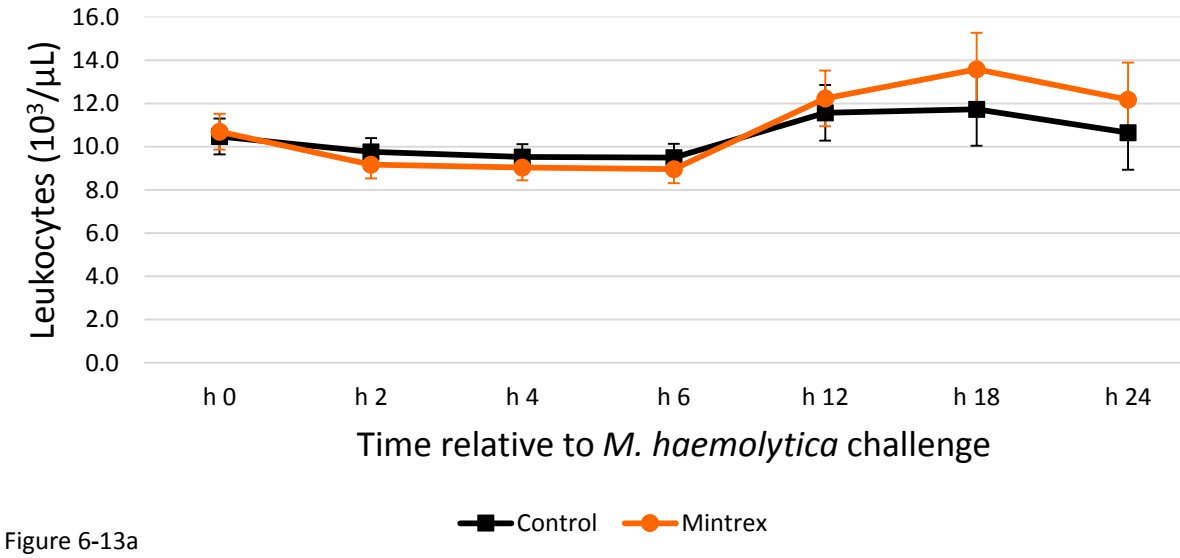

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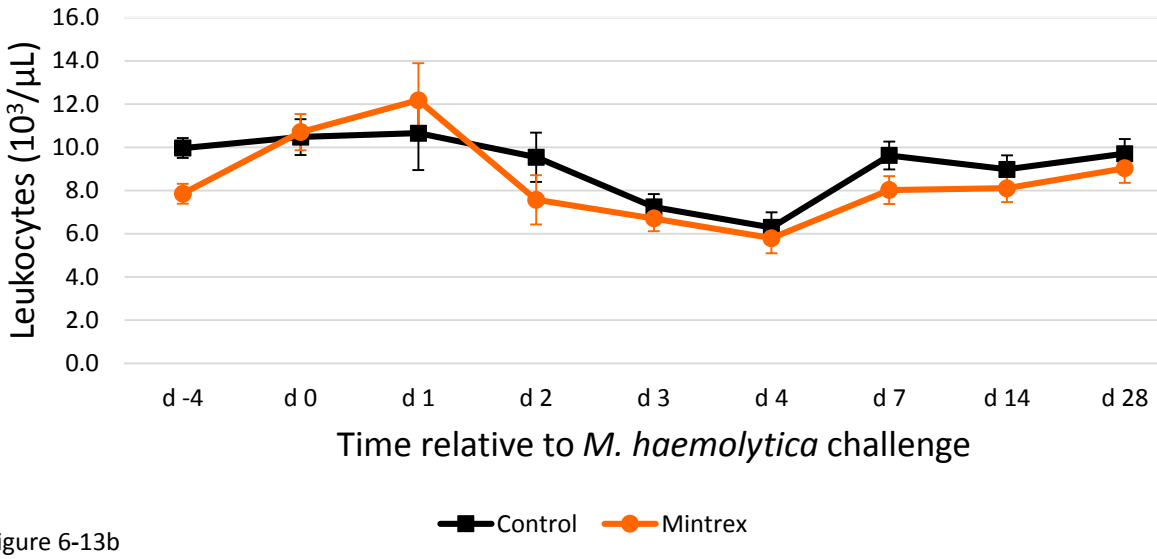

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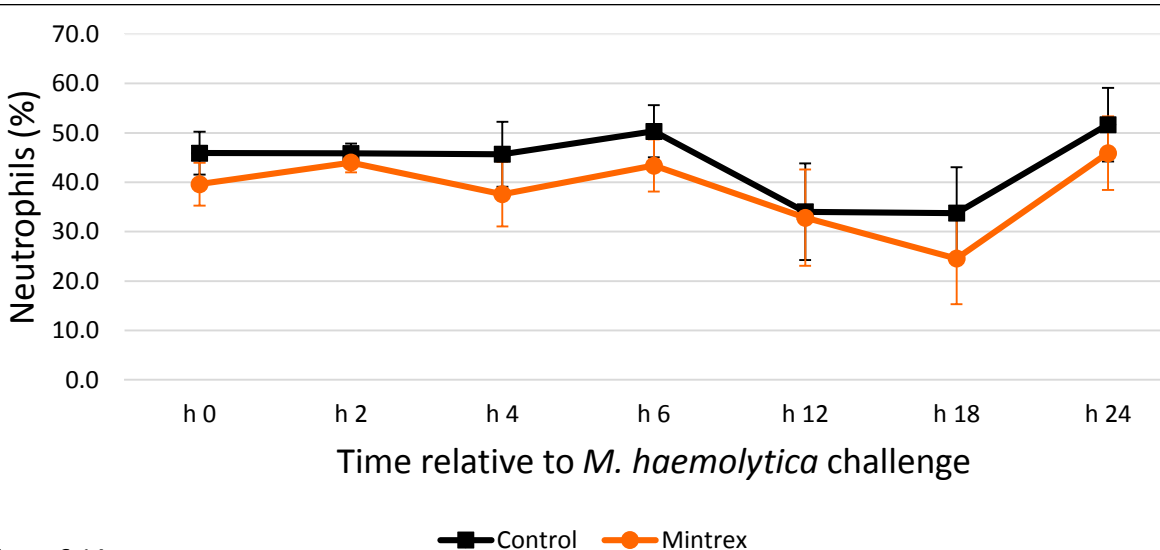

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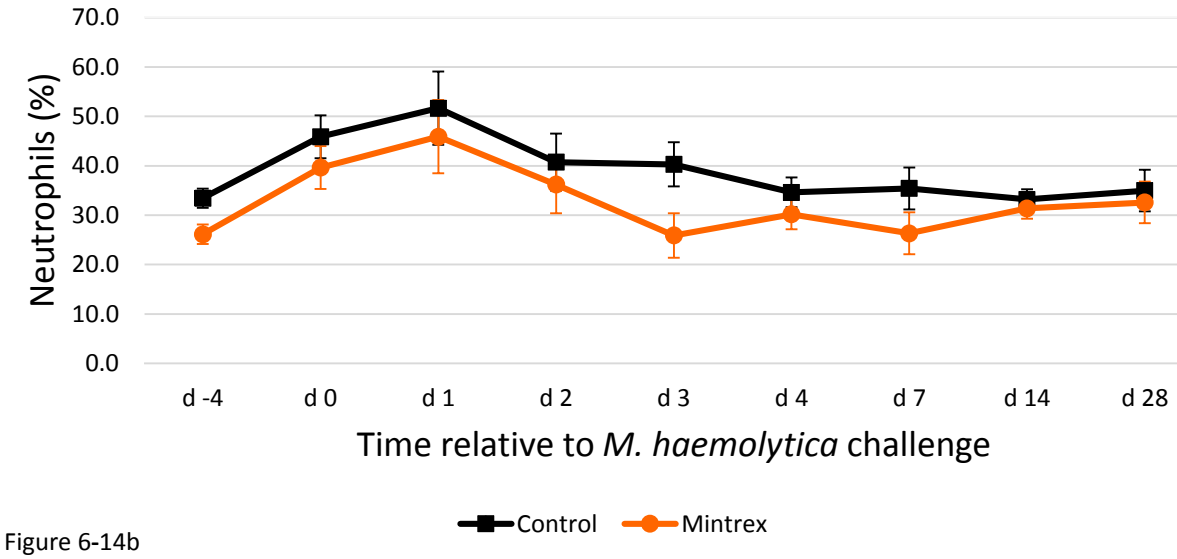

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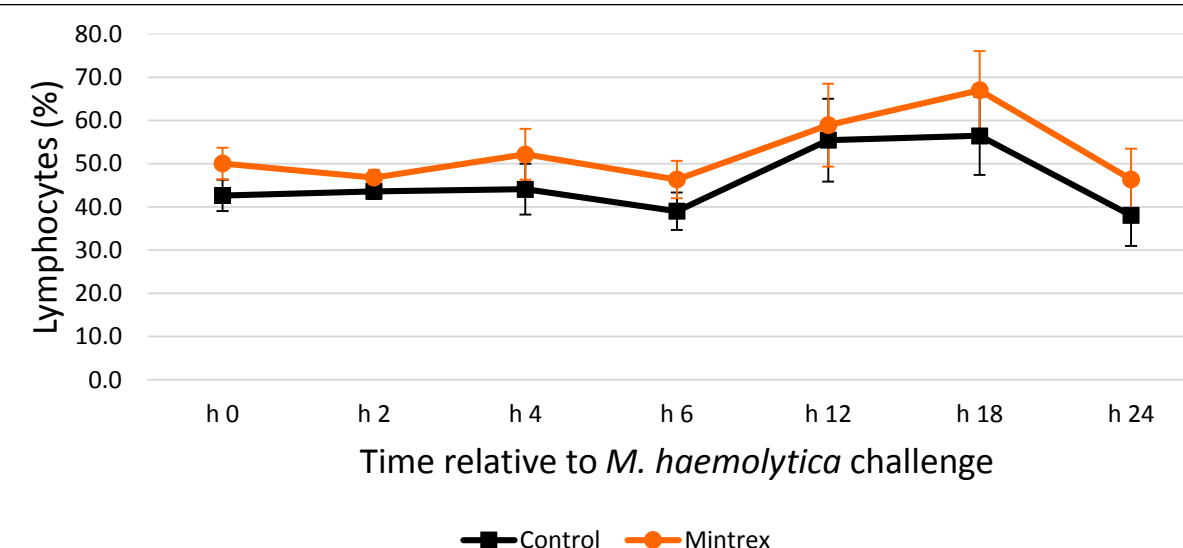

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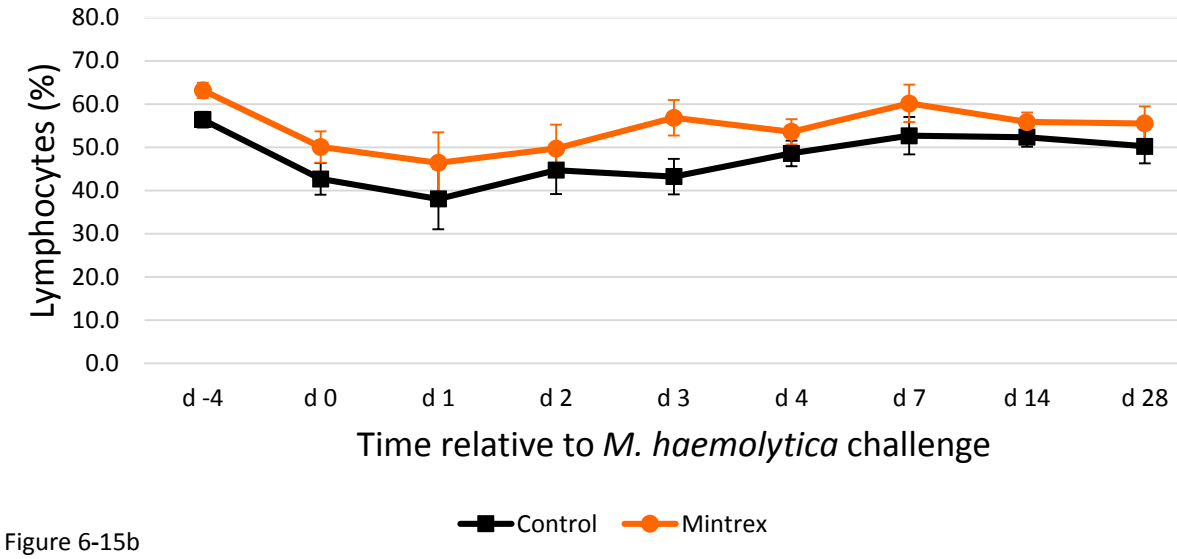

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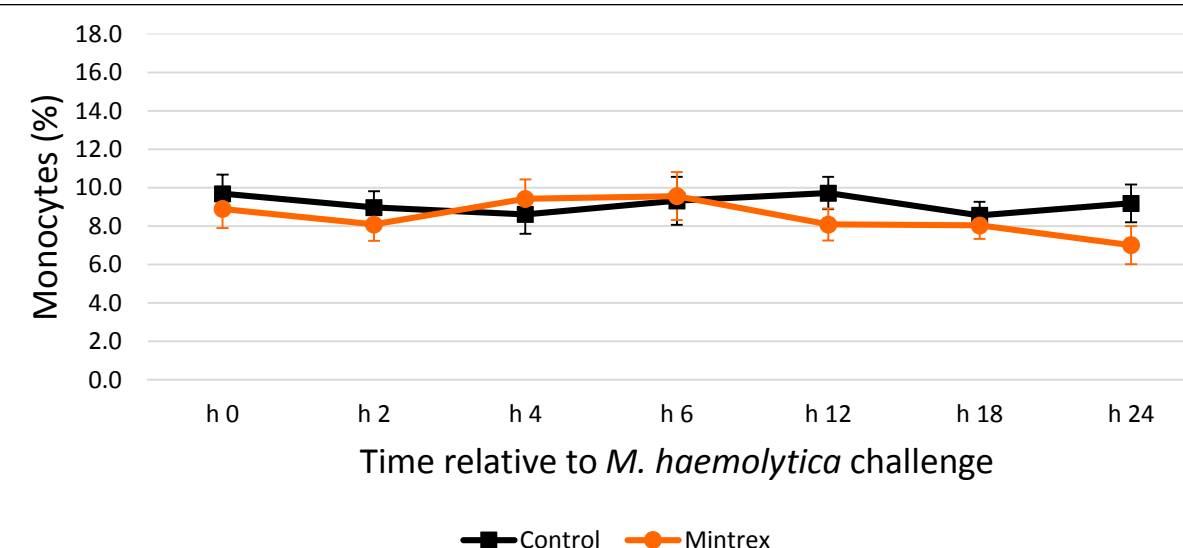

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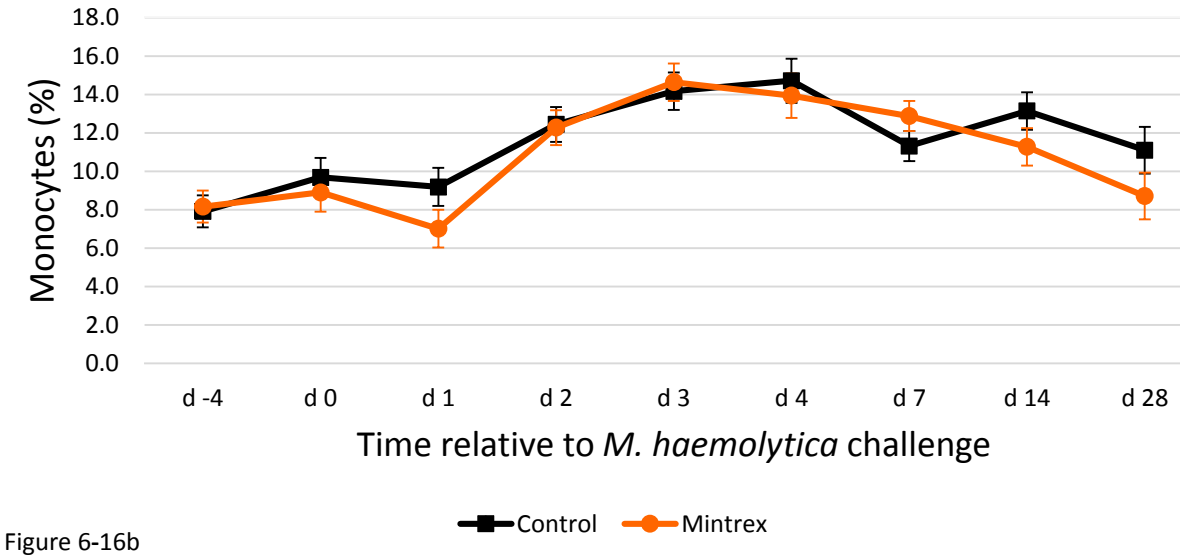

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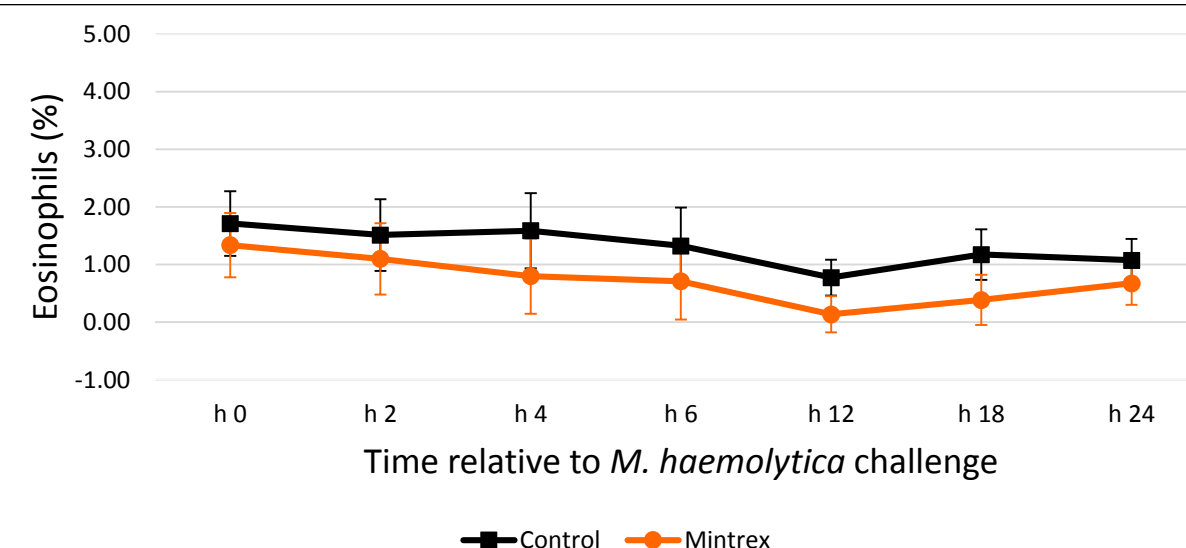

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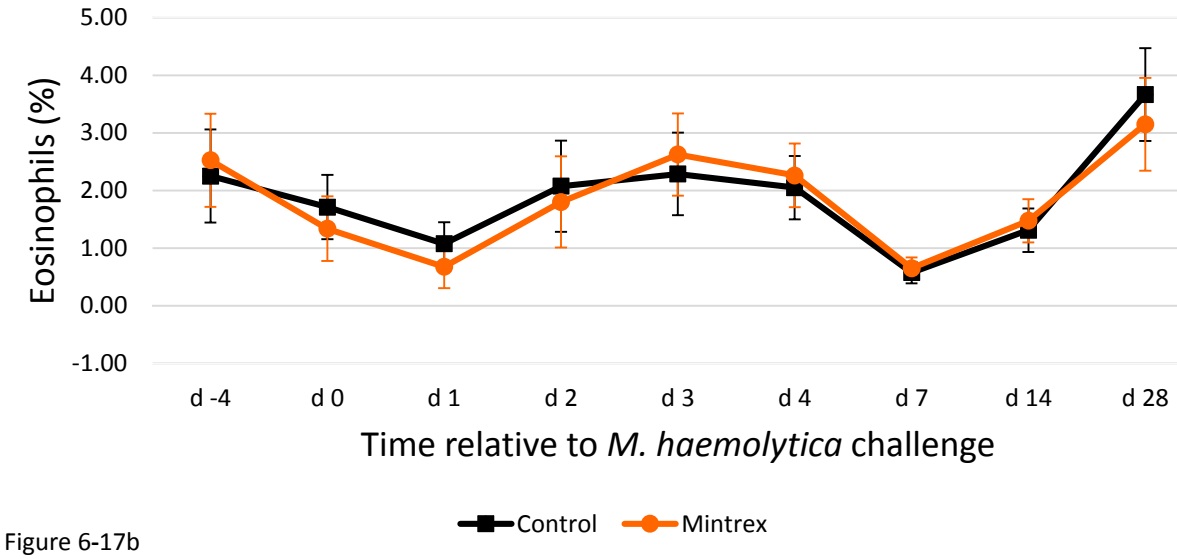

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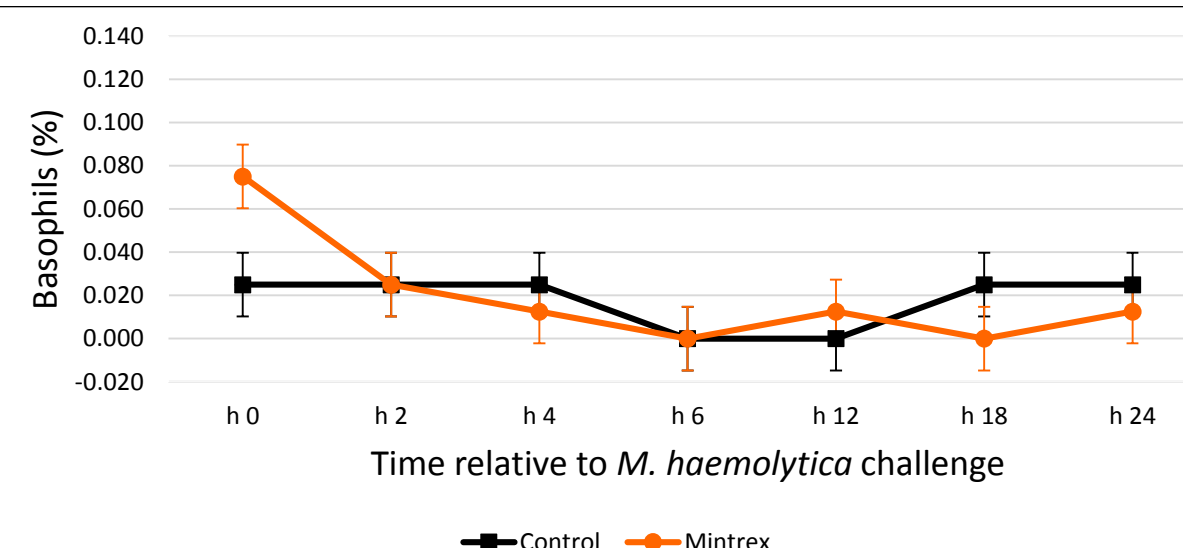

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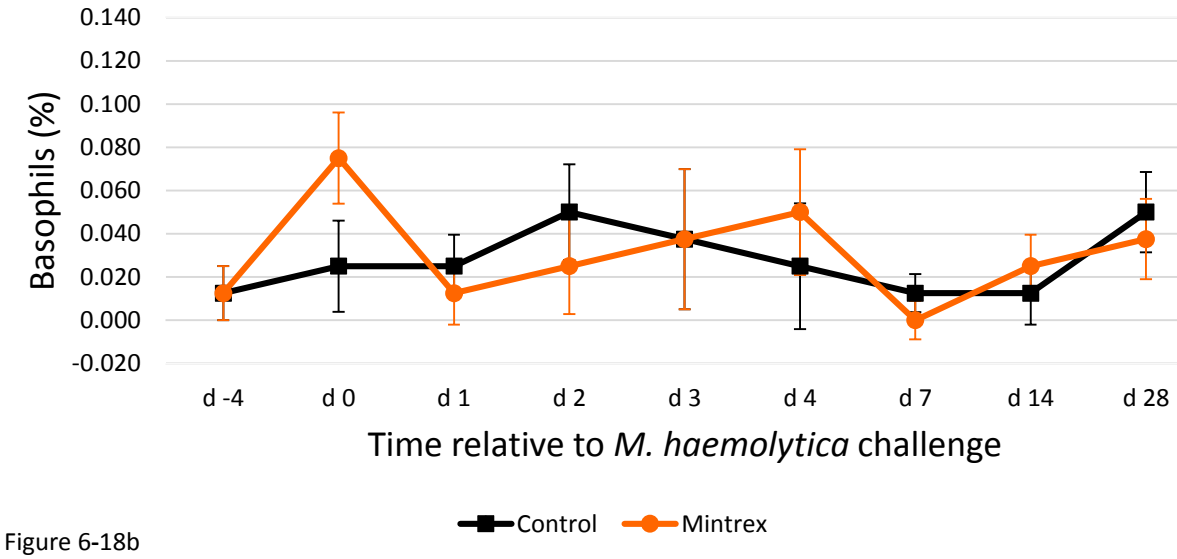

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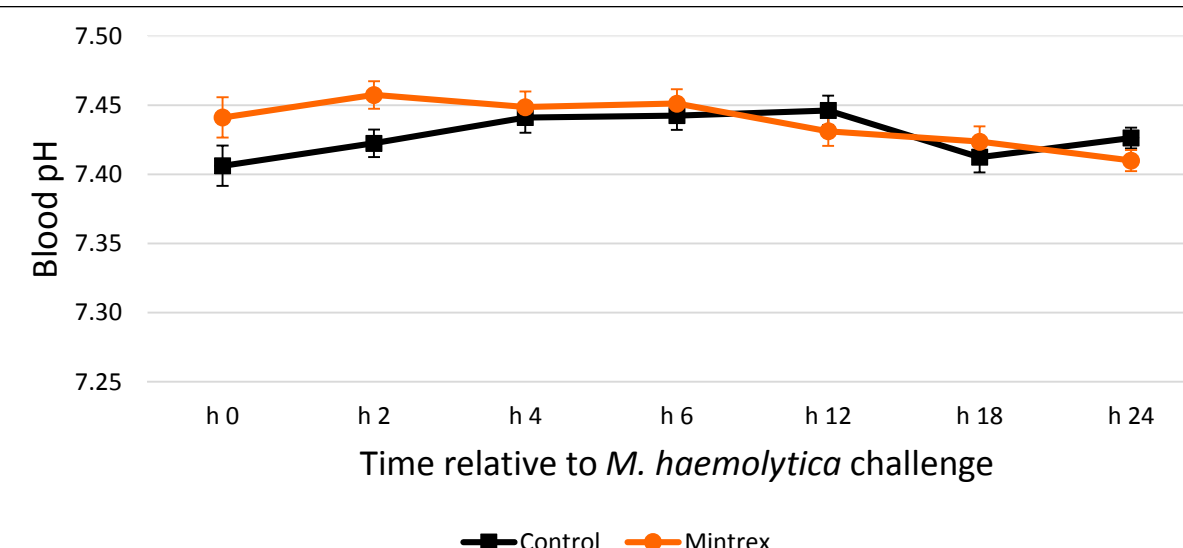

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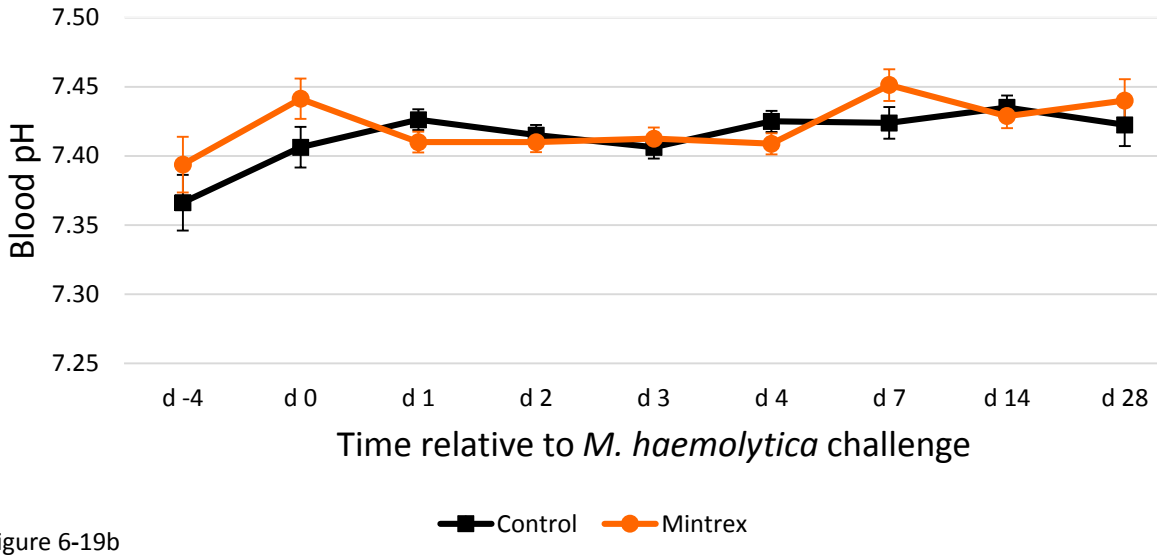

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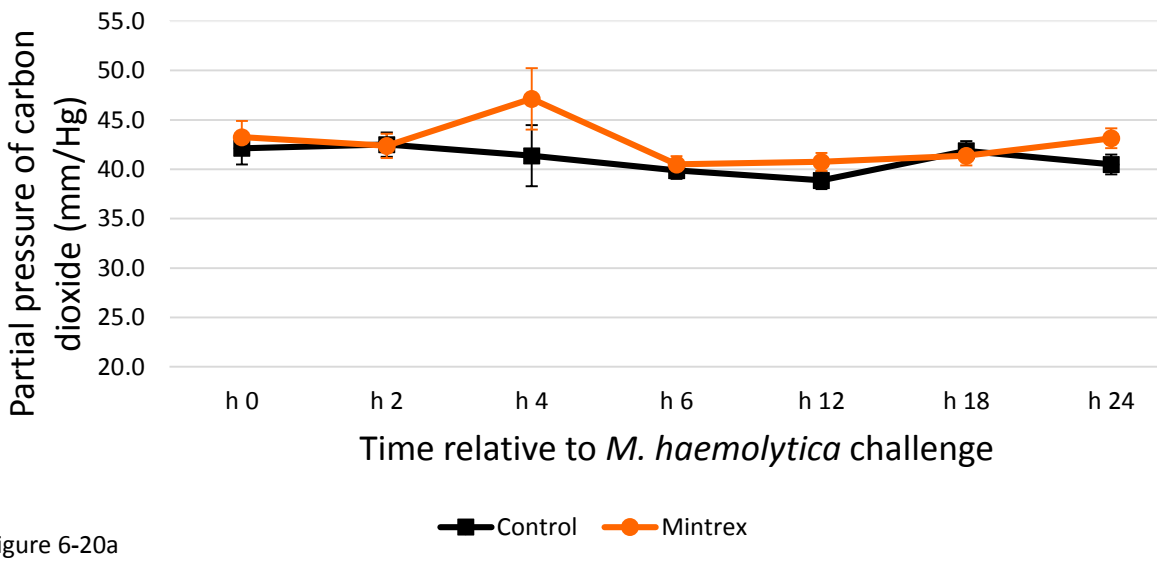

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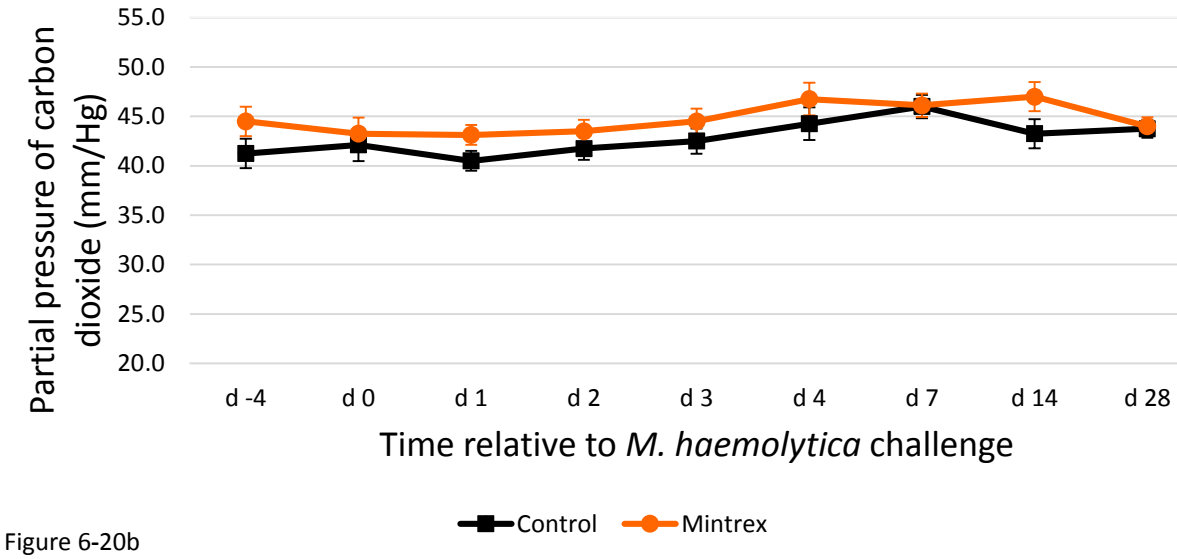

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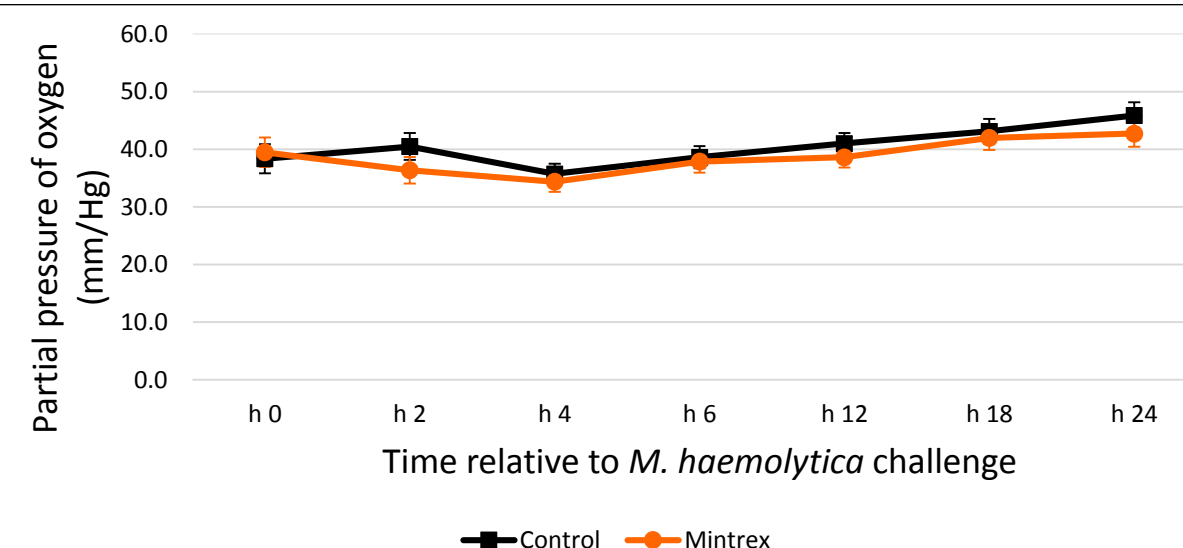

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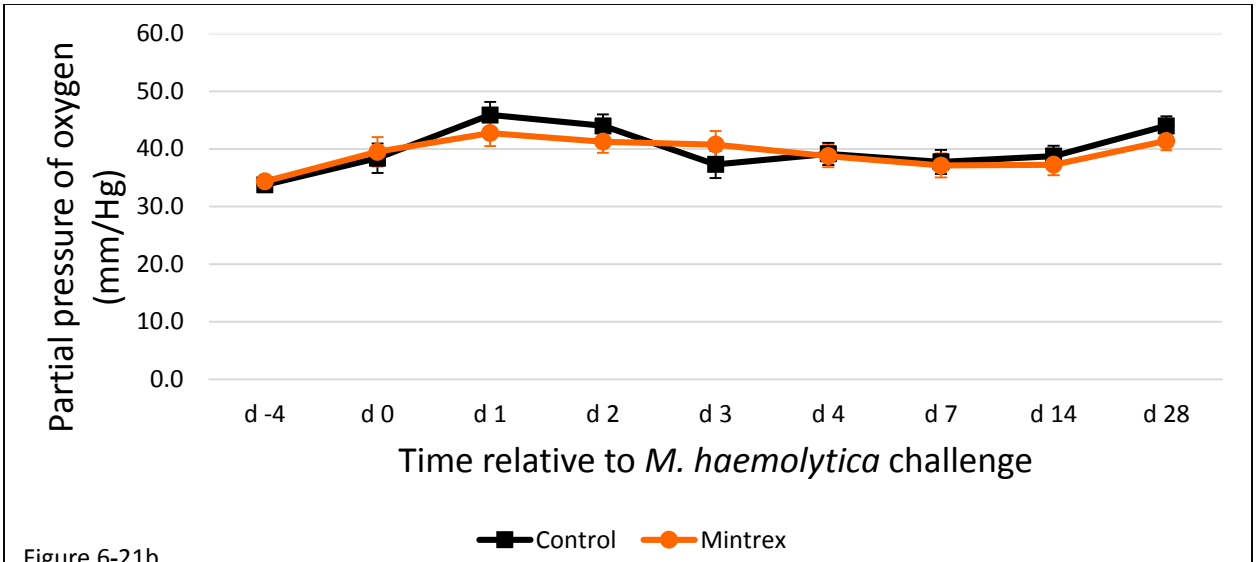

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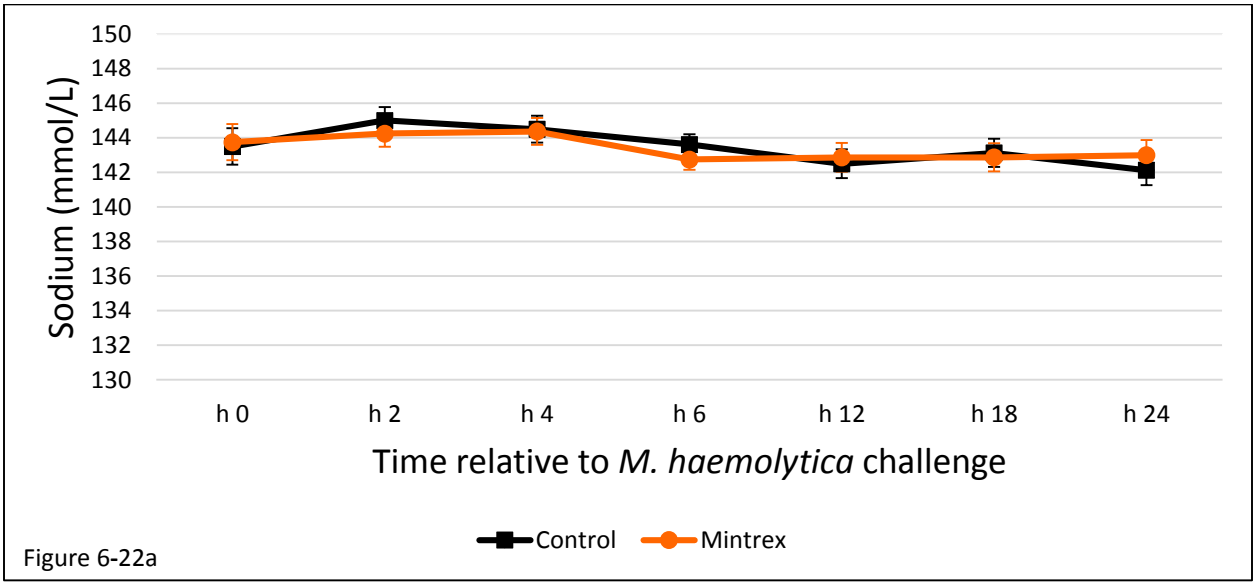

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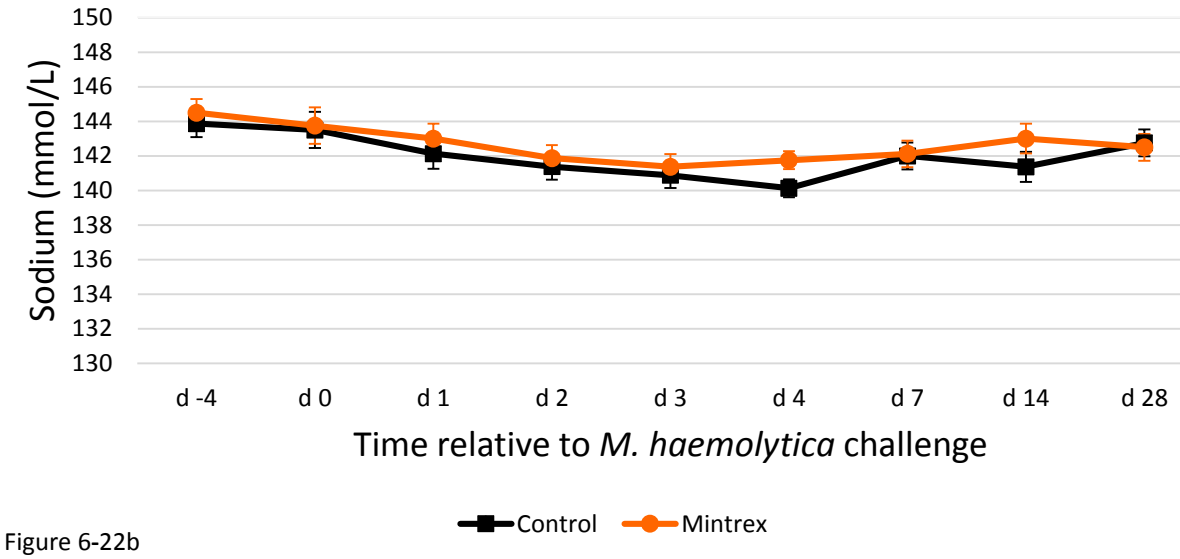

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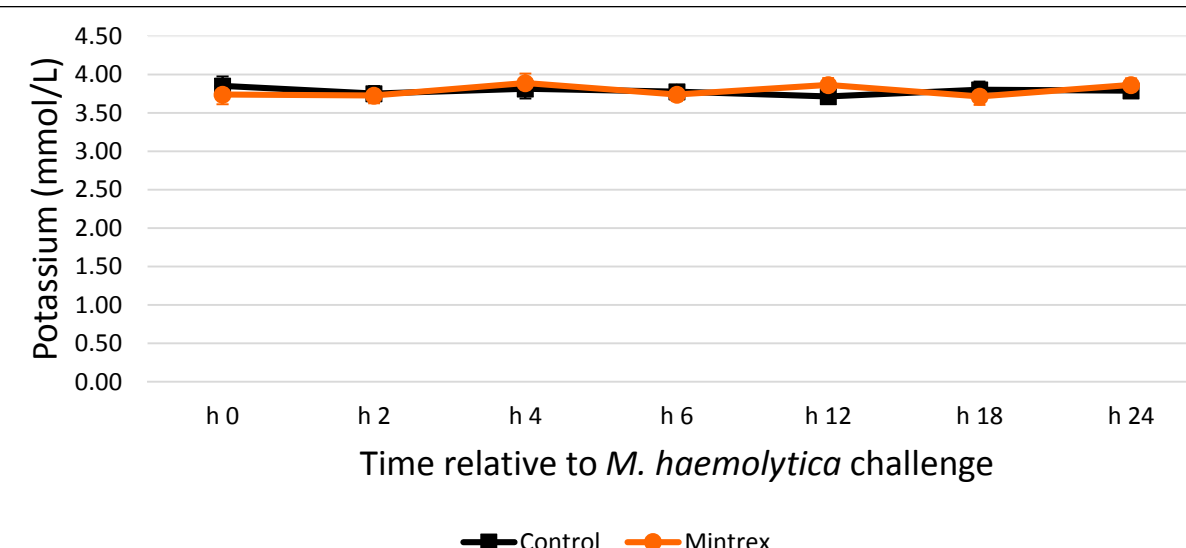

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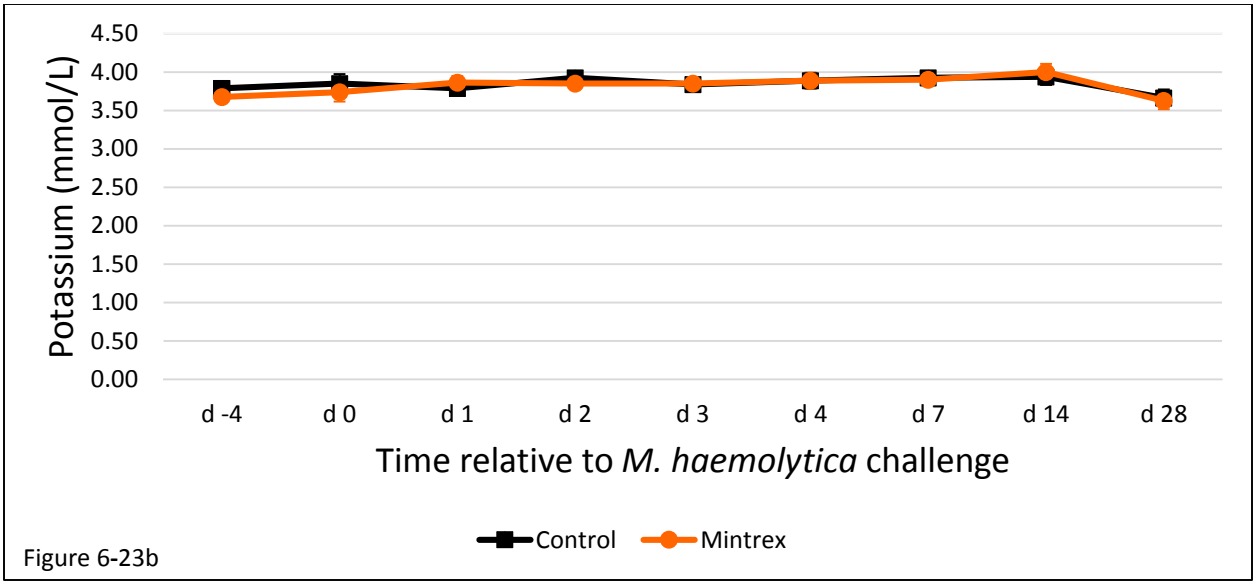

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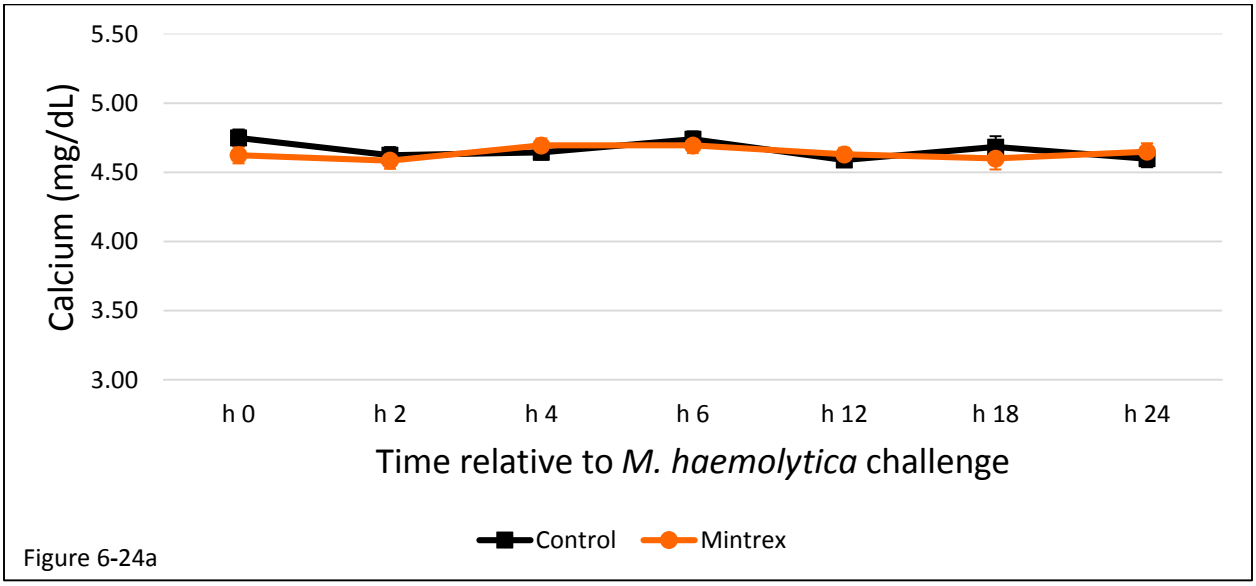

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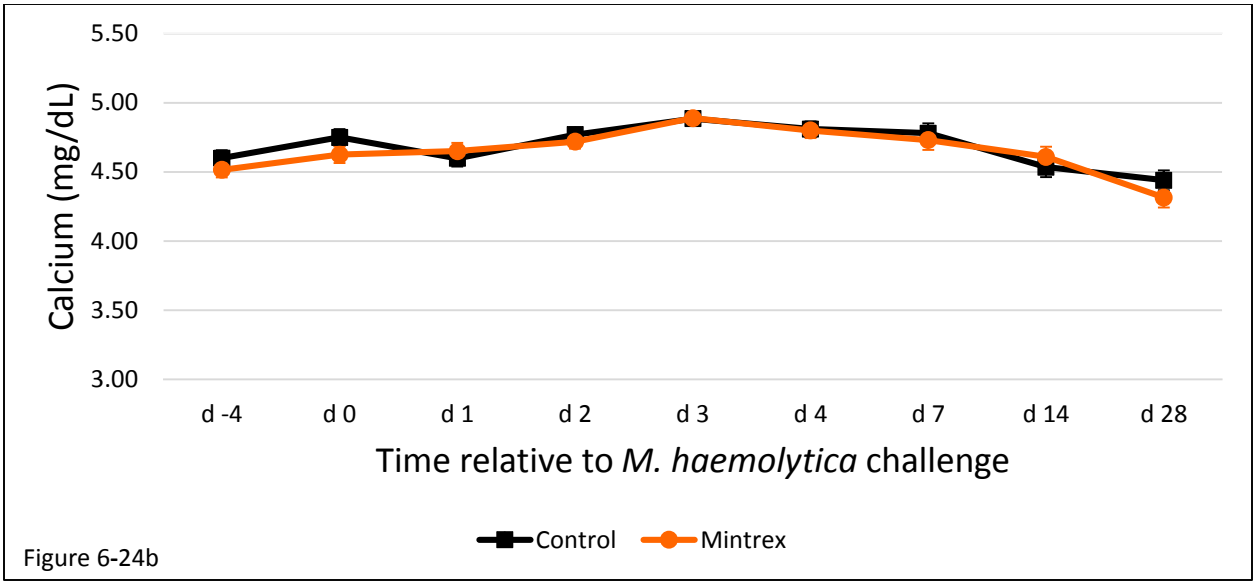

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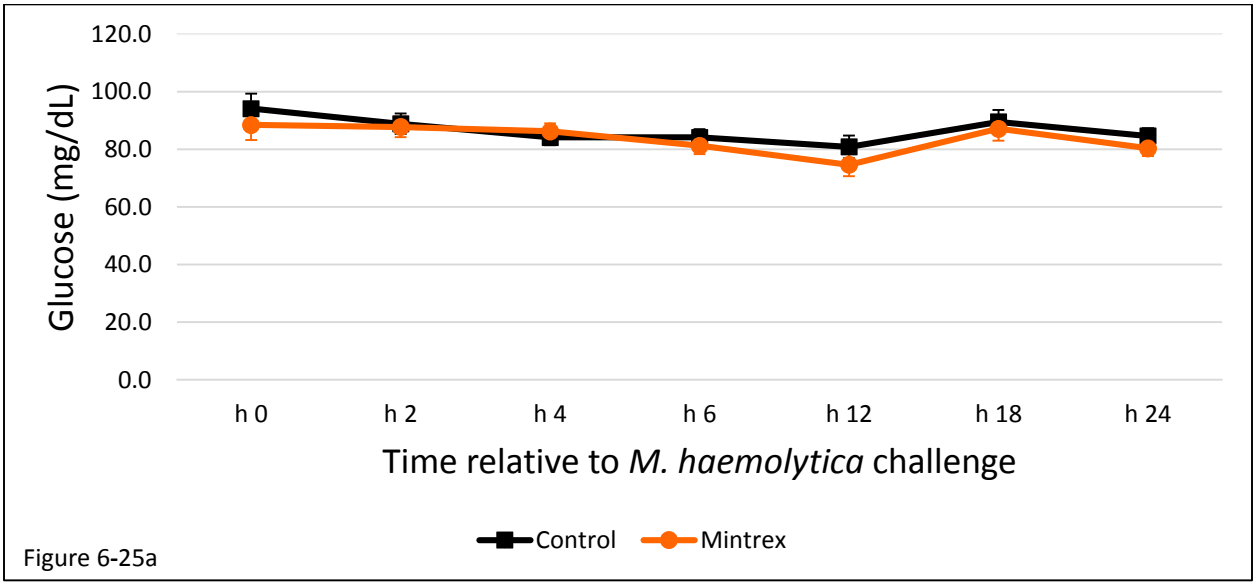

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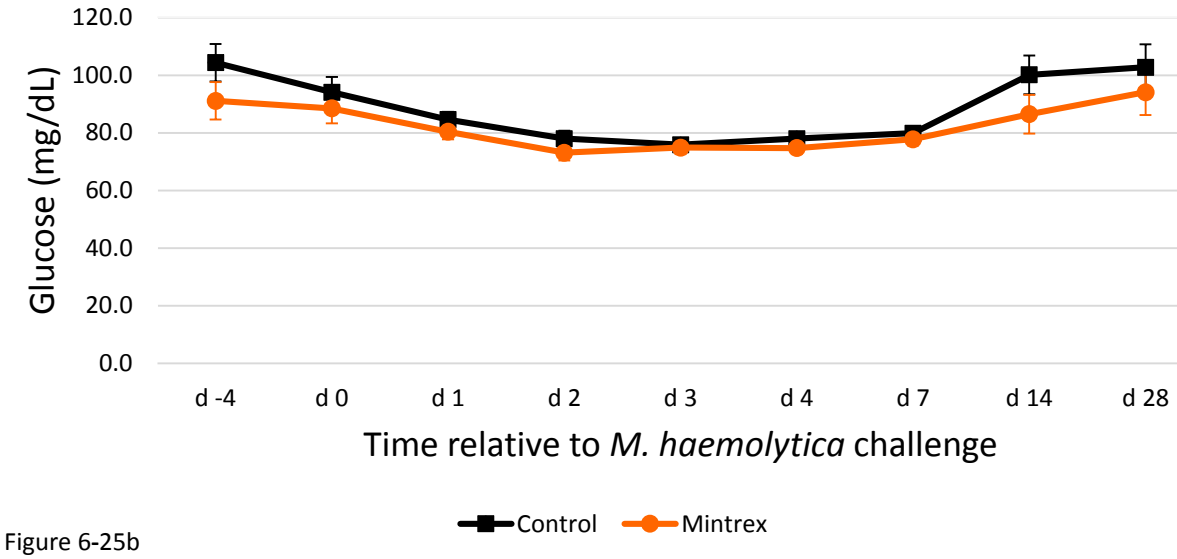

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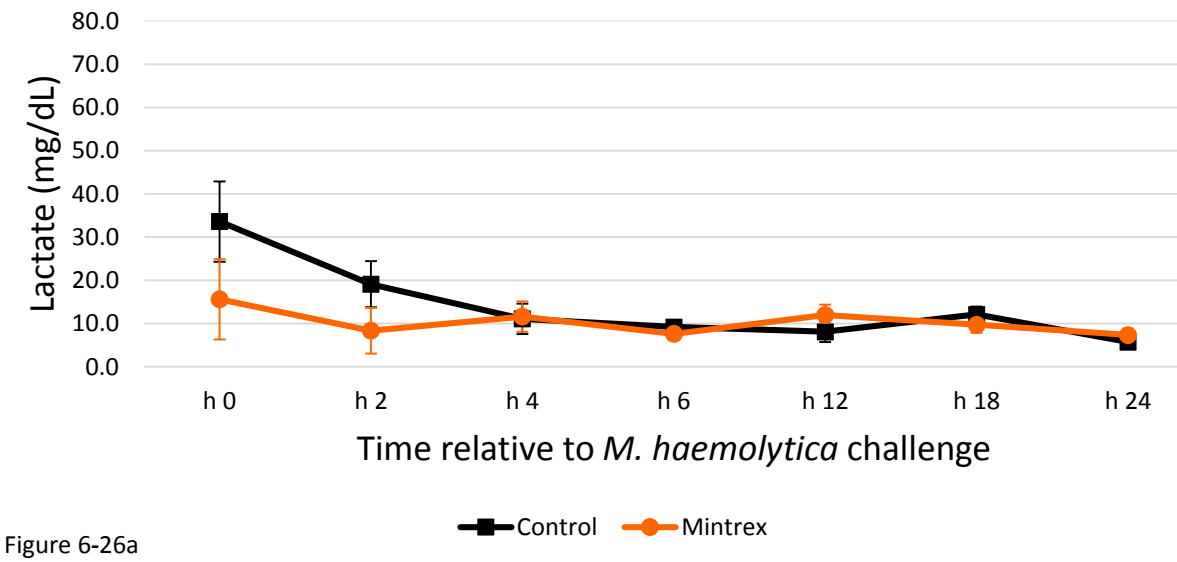

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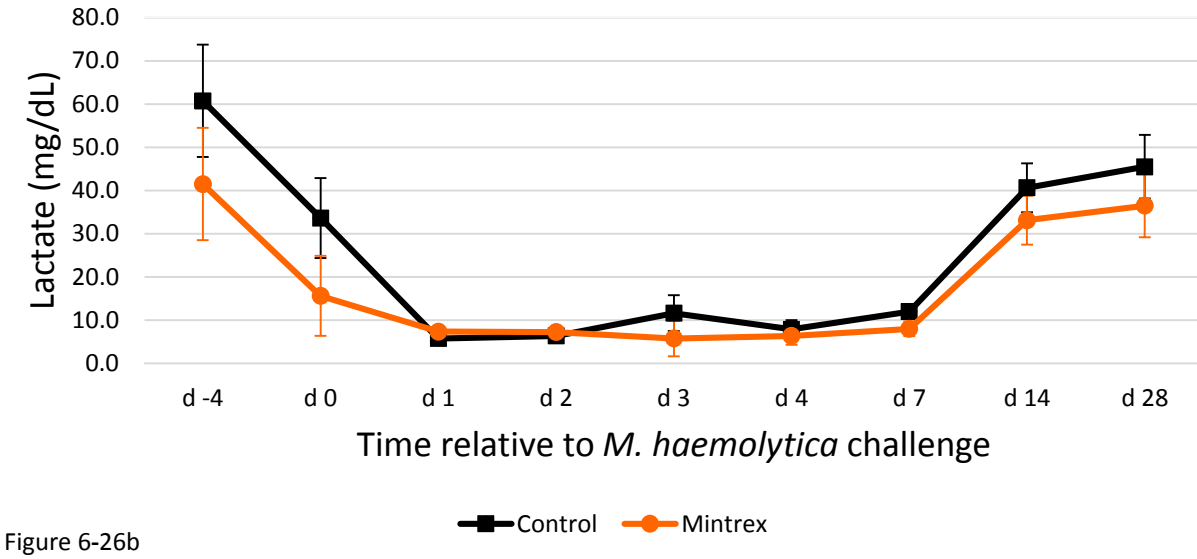

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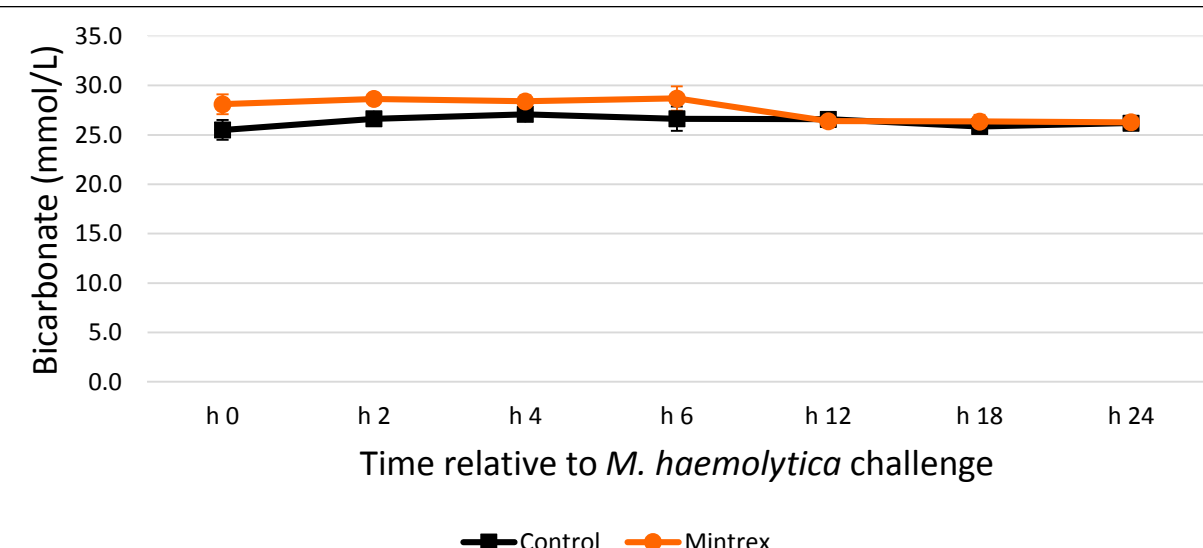

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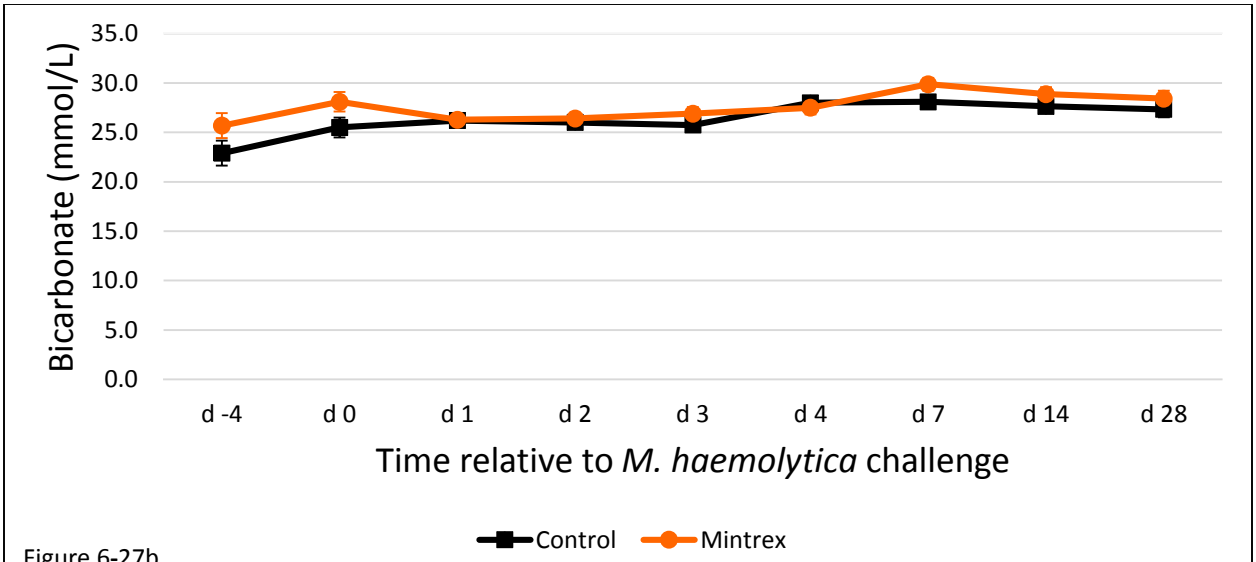

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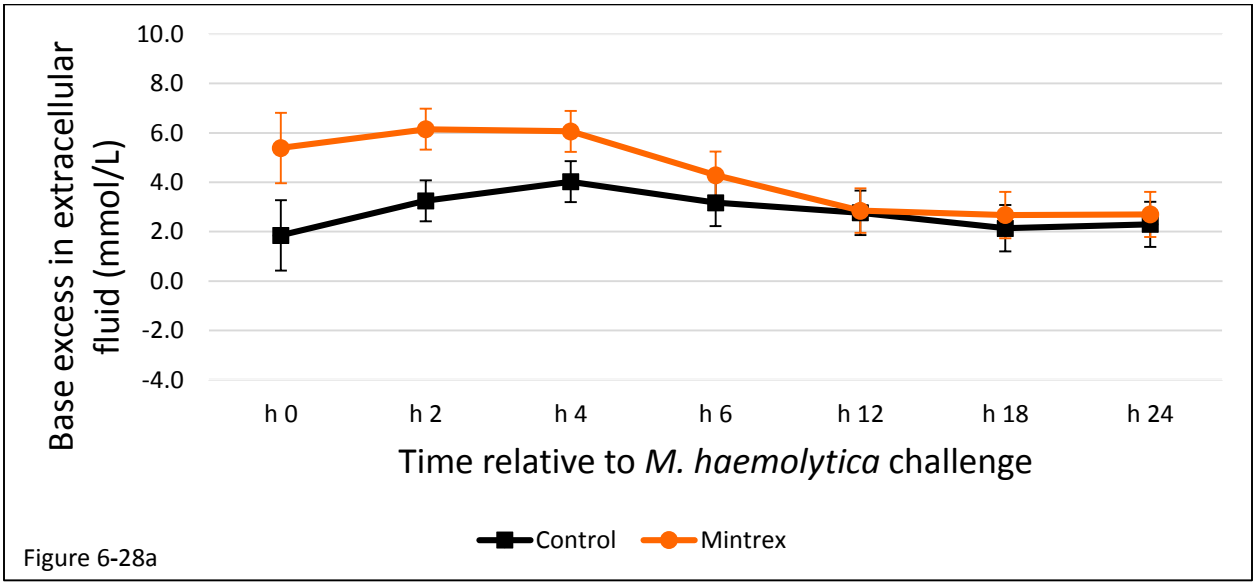

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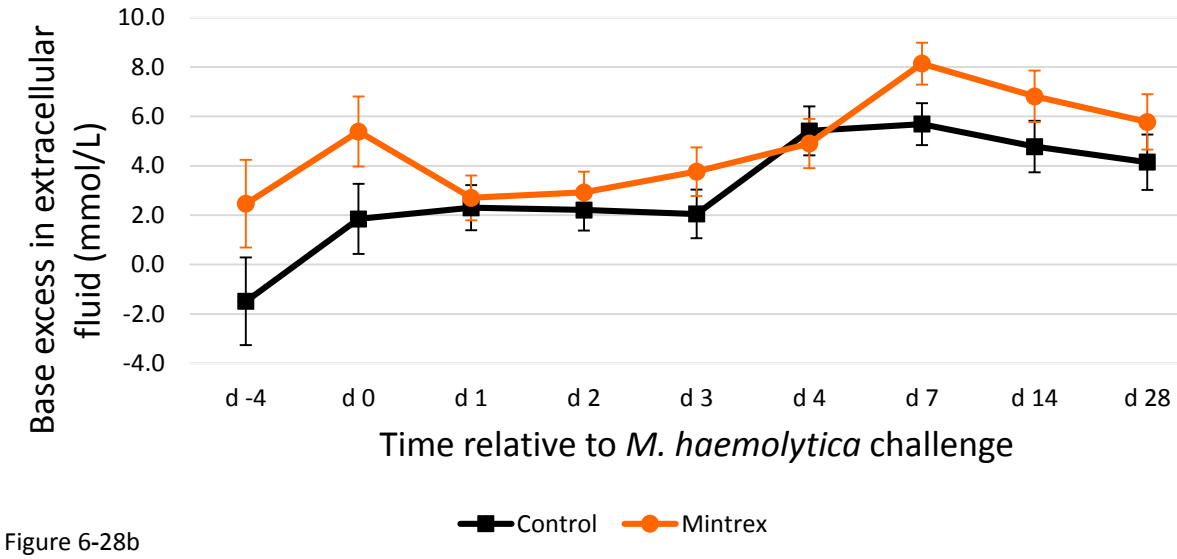

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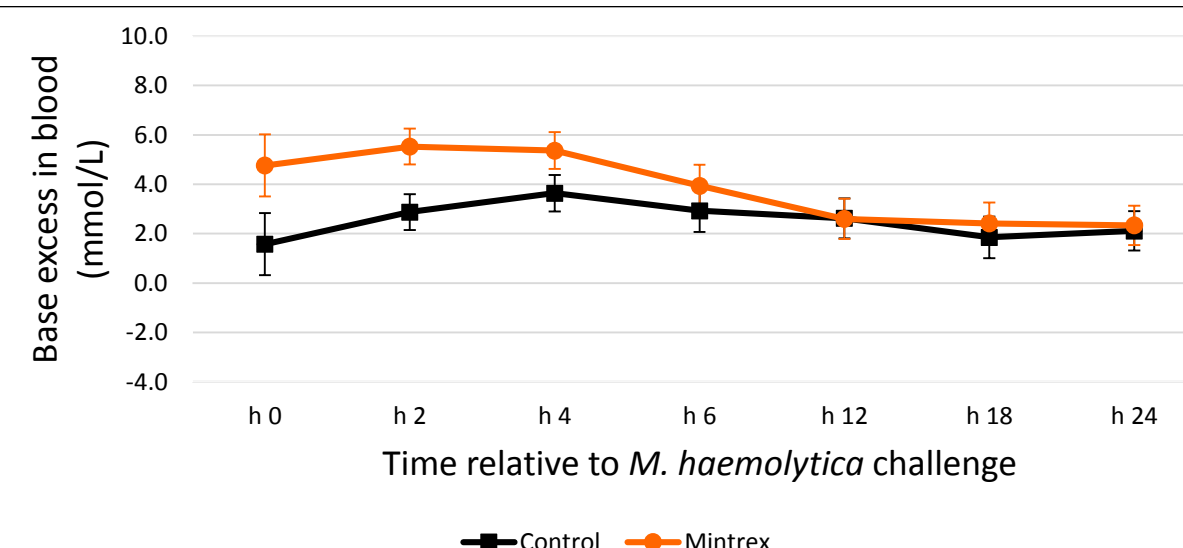

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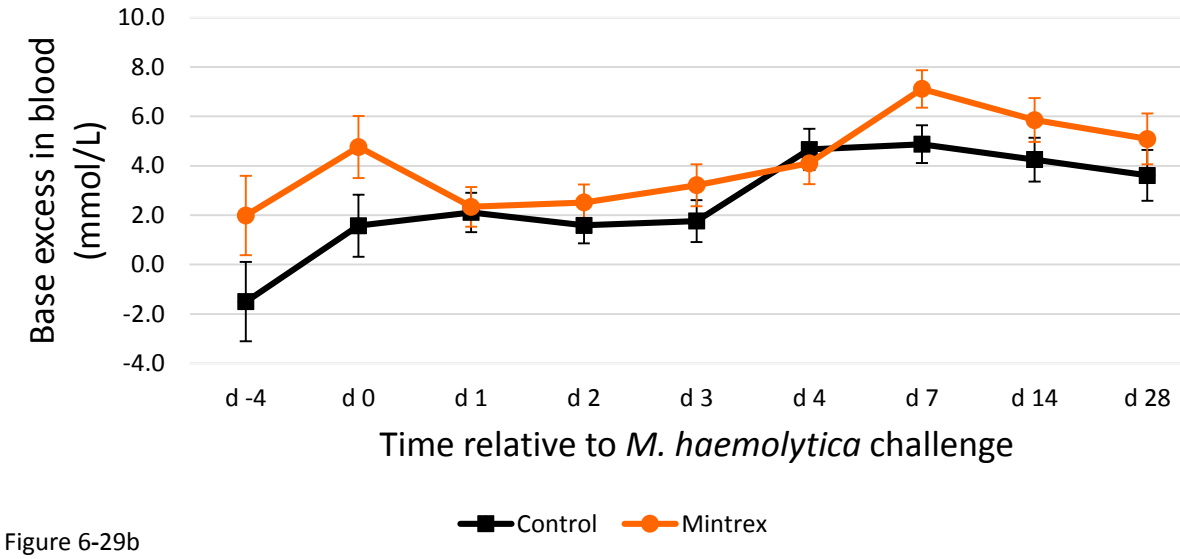

Figure 6-29b

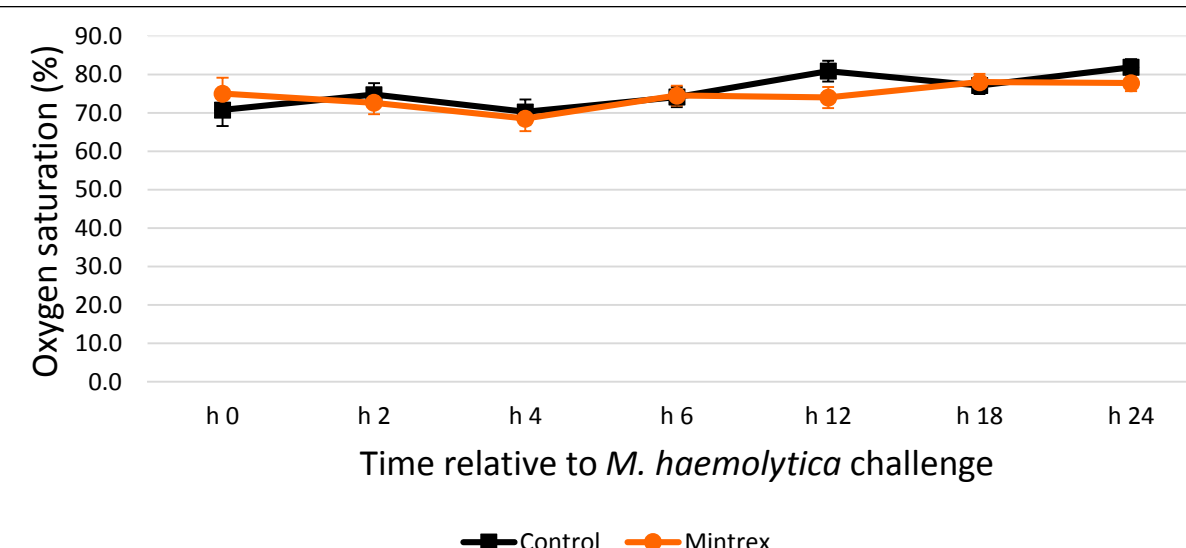

Figure 6-30a

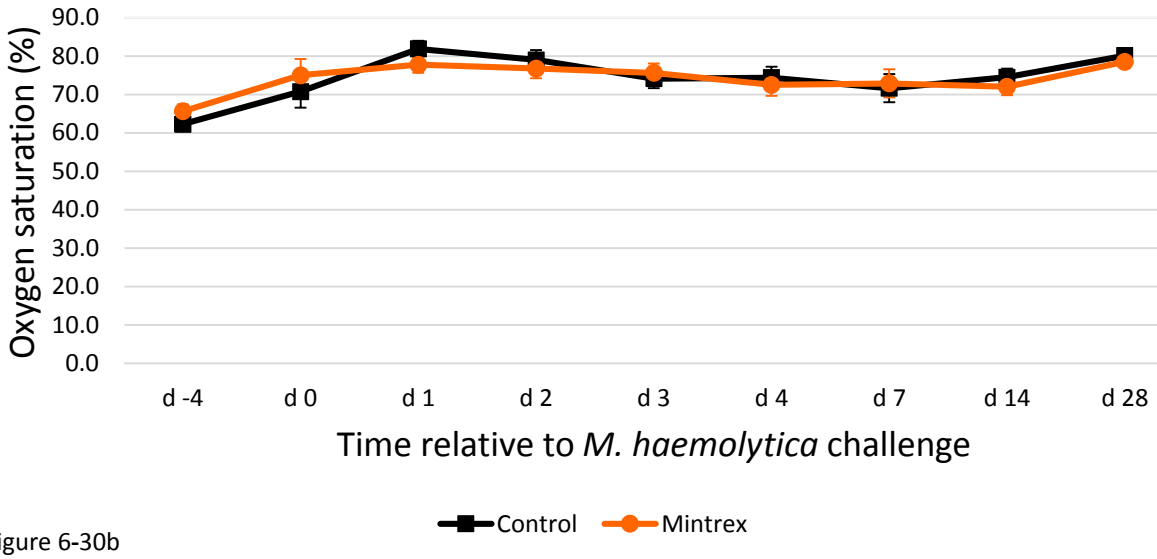
Oxygen saturation (%)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
d -4
d 0
d 1
d 2
d 3
d 4
d 7
d 14
d 28
Time relative to *M. haemolytica* challenge
Control
Mintrex

Figure 6-30b

APPENDICES

All procedures for the experiments presented in this dissertation were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 10/8/2015

Date : Tuesday, October 09, 2012 Animal Care and Use Protocol (ACUP) No : AG125

Proposal Title: Evaluation of Mintrex® Beef chelated trace minerals on clinical signs, immune response variables, and mineral balance in calves following natural exposure to bovine viral diarrhea virus type 1 and subsequent Mannheima haemolytica infection

Principal Investigator:

Christopher Richards
Animal Science
201 An. Science
Campus

Reviewed and Processed as: Full Committee

Approval Status Recommended by Reviewer(s) : Approved

The revised protocol and Appendix H are approved. You are approved to use a maximum of 20 cattle for the next three years.

Signatures :

Charlotte L. Ownby

Charlotte Ownby, IACUC Chair

Tuesday, October 09, 2012
Date

cc: Department Head, Animal Science
Director, Animal Resources

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 10/11/2015

Date : Friday, October 12, 2012 Animal Care and Use Protocol (ACUP) No : AG1211

Proposal Title: Evaluation of Multiple Ancillary Therapies Utilized in Combination with an Antimicrobial for the Treatment of Bovine Respiratory Disease in Newly Received High-risk Calves

Principal Investigator:

Clint Krehbiel
Animal Science
208 Ani Sci
Campus

Reviewed and Processed as: Special Review

Approval Status Recommended by Reviewer(s) : Approved

The revised protocol is approved. You are approved to use a maximum of 1500 cattle for the next three years.

Signatures :

Charlotte L. Ownby

Charlotte Ownby, IACUC Chair

Friday, October 12, 2012
Date

cc: Department Head, Animal Science
Director, Animal Resources

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

VITA

Blake Kenyon Wilson

Candidate for the Degree of

Doctor of Philosophy

Thesis: ANCILLERY THERAPY USE AND TRACE MINERAL SUPPLEMENTATION IN BEEF CATTLE: IMPACTS ON CLINICAL HEALTH, IMMUNE RESPONSE VARIABLES, ANIMAL PERFORMANCE, AND CARCASS TRAITS

Major Field: Animal Science

Biographical:

Personal Data: Born in Okmulgee, Oklahoma, on September 20, 1985, the son of Andy and Catherine Wilson

Education: Completed the requirements for the Doctor of Philosophy in Animal Science at Oklahoma State University, Stillwater, Oklahoma in July, 2014. Completed the requirements for the Master of Science in Animal Science at Oklahoma State University, Stillwater, Oklahoma in July, 2010. Completed the requirements for the Bachelor of Science in Animal Science with a minor in Agricultural Economics and Agribusiness from Oklahoma State University, Stillwater, Oklahoma in May, 2008.

Experience: Employed as: Graduate Research Coordinator, Willard Sparks Beef Research Center, Department of Animal Science, Oklahoma State University, 2010-2014; President of the Animal Science Graduate Student Association, Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, 2011-2012; Secretary of the Animal Science Graduate Student Association, Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, 2010-2011; Graduate Research and Teaching Assistant, 2008-2010, Department of Animal Science, Oklahoma State University; Undergraduate Research and Teaching Assistant, Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, 2006-2008; Assistant Ranch Manager, Hillier Angus Ranch, Stillwater, Oklahoma, 2004;

Professional Memberships: American Society of Animal Science, American Registry of Professional Animal Scientists, Gamma Sigma Delta Honor Society, Oklahoma State University Animal Science Graduate Student Association, Plains Nutrition Council