Investigations into Cardiopulmonary Diseases of Bovine Calves

By

Dee Church, B.S.

A Thesis

In

Animal Science

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Ryan J. Rathmann
Co-Chair of Committee

Joseph M. Neary
Co-Chair of Committee

Samuel P. Jackson

Mark Sheridan
Dean of the Graduate School

August 2018

Acknowledgements

I would like to acknowledge my committee member, Dr. Samuel Jackson. I thank you for your support and understanding attitude toward the often-changing ideas throughout the graduate school process. Your friendship, flexibility and encouragement has helped me along my path, and I appreciate your support.

I am extremely grateful for my committee chairmen and graduate advisors, Dr. Ryan Rathmann and Dr. Joe Neary. In a time when I did not know the path of graduate school and was without a mentor, these two took me under their wing and taught me more than I realized I could learn. I will be forever grateful of their patience and support. To Dr. Rathmann, thank you for showing me how to run a cow-calf operation and giving me the freedom to grow and learn as an assistant manager of the Texas Tech Beef Unit. I will forever draw upon my experiences here as I try to help other producers become successful within the industry. Dr. Neary, thank you for the research experience and showing me how correct research is done. Also for always being by your phone when I had off the wall ideas and needed to be reined in.

I want to thank all the staff at the Texas Tech Beef Unit because we all know how much work went into this project and will always have a special relationship from the experiences we went through. I want to recognize my family; Dad, Mom, and Spencer, thank you for listening to me and always pushing me to be my best. Without support from home I surely could not have completed the goals I have set for myself thus far. It is so amazingly satisfying to know that I have people that love me, believe in me, and always see the best in me. I am truly a blessed man.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Congestive heart failure, secondary to pulmonary hypertension is becoming increasingly problematic to the beef production industry. Historically this disease was known as high altitude disease (HAD), as it was more prevalent in environments with high elevations (>1500 m) (Alexander et al. 1960); however, it is becoming more common in cattle at moderate altitudes (Neary et al. 2015). Typically, identified by swelling within the brisket region of the beef cattle, HAD is due to hypobaric hypoxia causing pulmonary vasoconstriction, leading to pulmonary hypertension. Cattle losses from this disease can result in 3-5% of the calf crop in herds at elevations greater than 1500 m (Alexander et al. 1960; Holt & Callan, 2007). Pulmonary hypertension may cause right-sided congestive heart failure (RHF). The pathophysiological pathways behind the causes of pulmonary hypertension or the effects of subclinical RHF on beef cattle are not completely understood. The pulmonary arterial pressure (PAP) test is the best-known indicator of an animal's susceptibility to HAD (Holt and Callan, 2007). The increase in PAP is directly related to the degree of hypertrophy of the adventitia and media of small pulmonary arteries and ventricular workload leading to right-sided heart failure (Holt and Callan, 2007). Research has shown, however, an association between respiratory disease, environment, diet, age, and other factors with RHF (Neary et al., 2016).

Respiratory disease is the most significant cause of morbidity/mortality in US beef production systems (USDA NAHMS, 2000); costing the industry an annual

estimated economic loss exceeding $2 billion (Powell, 2013). The cost associated with BRD treatment in beef calves has been estimated to be $23.60 per animal, due to expenses related to pharmaceutical products and supplies (APHIS, 2013). Total costs associated with BRD treatment increases cost of production; as it relates to loss of production and decreased carcass value. Along with BRD, liver abscesses are a common reason for decreased profitability in beef cattle at the finishing phase of the production cycle (last 60 days on feed) (Nagara & Lechtenberg 2007). During this phase, cattle are more susceptible to multiple diseases, but the two discussed here are ruminal acidosis and right heart failure. It is currently understood that decreased pH levels within the rumen leads to a decrease in the thickness of mucosal lining along the gut wall (Pederzolli et al., 2018) The less fortified defense mechanism predisposes cattle to increase gut permeability (Pederzolli et al., 2018). This could lead to an increase in pathogen uptake and cause liver abscessation. With more research it has been seen that the time cattle are most vulnerable to liver abscessation co-insides with the same time cattle are at most risk to right heart failure (Neary et al. 2015), which drives interest in understanding more of the inter relationship between the cardio-pulmonary-gut-hepatic axis.

The beginning and end of the production cycle proves to be the greatest economic loss the beef industry. Before the complete formation of the adaptive immune system, newborn calves at are at an elevated risk of the onset of infectious diseases. Calves, in general, have functionally immature lungs, making them particularly susceptible to hypoxemia (Neary et al., 2014). At the end of the feeding stage, prior to harvest, cattle have a heavier weight, then compared to other stages in production, predisposing them to

increased overall health issues. With these phenotypic factors negatively affecting the health of the animal, there may be multiple interrelated causative factors of morbidity/mortality. In fact, the signs of respiratory disease and RHF, including weakness, malaise, dull expression, drooped ears, and fever, often overlap. This poses a potential problem for producers, veterinarians, and researchers attempting to better identify, characterize, and treat this disease (Will et al., 1962; Malherbe et al., 2012; Neary et al., 2013). Research shows respiratory disease including BRD is common risk factor that puts cattle in hypoxic states, potentially resulting in pulmonary hypertension (Angel and Tyler, 1992; Holt and Callan, 2007).

Antibiotics are often used to treat various disease conditions, such as pneumonia, on cow-calf operations. Unweaned calves have the greatest occurrence of disease, followed by weaned calves, and finally cows (NAHMS 2007). Data from 2007 states that 68 % of operations had used oral or injectable antibiotics to treat disease, 7.2 % of unweaned calves, 6.0 % of weaned replacements, and 1.9 % of cows in the U.S. were treated with oral or injectable antibiotics at least once (NAHMS 2007). Consequently, there is a declining proportion of animals treated with antibiotics with increasing age. With the growing antibiotic resistance concerns mounting within consumers, other avenues of disease prevention have been researched. Some examples that are not included in the research section of this thesis but must be mentioned include: direct fed microbials, prebiotics, and change in feed composition. This concern is only heightening the importance of understanding the interrelationships along the cardio-pulmonary-gut-

hepatic axis so we can better manage the health of cattle through different phases of the beef industry.

Risk factors for right heart remain to be determined, but age, genetics, and accelerated fat deposition may influence susceptibility (Holt and Callan, 2007; M.T. Neary et al., 2014; J. Neary et al., 2014; Neary et al., 2015). There is a lack of research explaining the interrelationship of the bovine cardiopulmonary system and gastrointestinal tract. Therefore, in the first study we set out to determine the effects of hypoxic conditions on calves' intestinal permeability. We also wanted to understand the relationship between respiratory disease and pulmonary arterial pressure.

PULMONARY ARTERIAL PRESSURE TESTING

History

Cor pulmonale, congestive right heart failure secondary to pulmonary hypertension, was originally known as high altitude disease (HAD) (Rhodes, 2005). An animal suffering from right ventricular (RV) failure develops dependent and intrathoracic edema, pulmonary edema, plural effusion, passive liver congestion, perirenal and mesenteric edema, and ascites (Holt & Callan, 2007). Cattle losses from this disease can result in 3-5% of the calf crop in herds at elevations greater than 1500 m (Alexander,1960; Holt & Callan, 2007). The initial work done by Newsom and Glover in 1915 concluded that altitude exposure was the root cause of HAD (Glover & Newsom, 1915). They were also the first to realize that cardiac failure was the cause of morbidity and mortality, thus concluding pharmaceuticals could not cure this disease only genetic

changes within the herd (Rhodes, 2005). Further investigation showed the cardiac failure from HAD was specifically related to right ventricular hypertrophy and right sided congestive heart failure (Alexander & Jensen, 1959).

Cause and Response

The primary vascular changes observed in response to hypoxia included increased arteriolar constriction, hypertrophy of the arteriole smooth muscle layer (medial hypertrophy), thickening of the vascular adventitia, decreased diameter of the lumen of pulmonary arteries, and pruning of small pulmonary arteries and arterioles (Alexander & Jensen, 1963; Stenmark et al. 2006). These changes occur secondary to hypoxia and lead to RV hypertrophy, pulmonary hypertension, right sided heard failure and death (Holt & Callan, 2007). Research performed in 1962 found that the degree of the pulmonary arterial pressure (PAP) measurement was directly related to the degree of medial hypertrophy of the small pulmonary arteries (Will et al., 1962). Under hypoxic conditions pulmonary vascular shunting is seen in all animals. Shunting is seen in cattle to a much greater extent than any other animal (Heath et al. 1969). Shunting is the vasoconstriction mechanism of distributing pulmonary blood flow away from poorly oxygenated lung tissue to more oxygenated areas. This mechanism, the anatomic pattern of the bovine lung, and the small lung-size/body-weight ratio all contribute to severe loss of functional pulmonary capacity (Kainer & Will et al. 1981). Hypertrophy and thickening of the medial layers of the pulmonary arterioles and adventitial tissues occur with chronic hypoxic exposure (Alexander & Jensen, 1963; Stenmark et al. 2006). The ensuing

pulmonary arterial hypertension can cause cor pulmonale (heart disease secondary to pulmonary hypertension). Animals that are clinically affected, show pathologic changes of an increase in hepatic enzymes; such as aspartate transaminase. The complete blood count is generally normal unless there is pulmonary inflammation (Holt & Callan 2007). The liver is generally enlarged and has the characteristic nutmeg appearance of passive hepatic congestion. Transudate is common along with an enlarged heart due to hypertrophy and dilation of the right ventricle. Right ventricular failure in calves is often mistaken for acute viral or bacterial pneumonia due to the rapid onset of clinical signs; alternatively, acute respiratory disease can exacerbate the pulmonary hypoxia of high altitude, resulting in rapid heart failure in susceptible calves or adult cattle (Holt & Callan 2007).

PAP Testing

Pulmonary arterial pressure testing can be used to confirm the presence of pulmonary hypertension. It is also useful in differentiating cor pulmonale from other causes of congestive heart failure (Holt & Callan 2007). This test has clinical usefulness for the individual animal with signs of cor pulmonale but is most useful for screening animals for pulmonary hypertension. With this information, managers will be able to make better breeding management decisions to decrease the likelihood that pulmonary hypertension leads to RHF within the herd. With the optimal equipment and facilities, a veterinarian can PAP test up to 200 animals daily and provide a valuable service to clients with cattle at high altitude (Holt & Callan 2007). Facilities and equipment needed

to perform a PAP measurement include a cattle squeeze chute with a head catch, datascope capable of invasive blood pressure monitoring, pressure transducer to convert fluid pressure to an electric signal, catheter tubing, 12 or 13 gauge needle, disinfectants, isotonic NaCl solution, 3-way stop cocks, and a recording system.

Interpretation

In healthy cattle at elevations over 1,500 m, the jugular pressure should be 6 to 12 mm Hg, the mean RV pressure should be 18 to 30 mm Hg, and mean PAP within the pulmonary artery should typically be less than 44 mm Hg (Holt & Callan, 2007). Cattle with mean PAP >49 mm Hg may develop clinical signs of right sided heart failure (Holt & Callan, 2007). In general, cattle older than 12 months that have a 41mm Hg mean PAP and are located at high altitude, will likely maintain acceptable PAP at high elevations. Cattle with a mean PAP (mPAP) greater than 49 mm Hg at any altitude are at risk for developing right sided heart failure.

Factors affecting pulmonary arterial pressure tests

Breed

Based on research done on more than 150,000 head of cattle, research shows no one breed is resistant to the effects of high-altitude hypoxia (Holt & Callan, 2007). High PAP animals (>50 mm Hg) have been found in all breeds tested. Any animal originating from a low altitude herd has a greater probability to experience high-altitude effects when transferred to high altitudes than those raised in higher elevations. In a study conducted in

the 4-Corners Bull Test center, breed was found to be a significant source of variation influencing PAP scores for bulls developed at high altitude (Crawford et al., 2017). Angus x Gelbvieh bulls were found to have the lowest adjusted PAP scores of all breeds. Angus had relatively high PAP scores (> 44 mm Hg), which may increase their susceptibility to HAD (Crawford et al., 2017). Since Angus cattle are heavily used in the United States, crossing with other cattle with lower mean PAP scores may lead to lower susceptibility to HAD, one such breed being the Gelbvieh. However, high PAP scores have been found in all breeds tested and not one breed of cattle appears to be resistant to high-altitude hypoxia (Holt and Callan, 2007). With this, further research must be conducted in order to gain a better understanding the effect breed has on PAP and HAD.

Gender

Currently no physiologic basis exists for a difference in PAP measurements between male and female cattle. If any differences are seen it is likely related to composition differences, with heifers fattening sooner than their male counterparts. However, pregnant cattle have been reported to have greater mean PAP measurements than non-pregnant cattle (Moore et al. 1979). Animals with lower PAP before pregnancy had reduced arterial PCO_2 tension during pregnancy, suggesting a compensatory hyperventilatory mechanism that could help offset pulmonary hypoxia (Holt & Callan, 2007). The specific mechanism for the increased pulmonary vascular resistance during pregnancy in susceptible cows is not fully understood.

Age

Age should always be considered when PAP testing because the accuracy of the PAP test is lower for those animals tested younger than 12 months of age. Research shows testing animals at 16 months of age and older seems to be the most consistent and accurate point when trying to predict which cattle are more susceptible to develop pulmonary hypertension (Holt & Callan, 2007). An elevated PAP measurement (> 49 mm Hg) regardless of age or elevation is extremely accurate at predicting high PAP later in life. Animals that have a mean PAP between 30 and 40 mm Hg when tested younger than 12 months should remain at acceptable PAP levels as they mature. However, if measurements are between 41 and 49 mm Hg there will be a large variability as they mature, and if the measurement is above 49 mm Hg the animal should be considered high-risk for right-sided cardiac failure.

Concurrent Illness

Because PAP is an indirect measure of pulmonary blood flow resistance, any pulmonary hypoxia can cause a change in PAP measurement (Angel & Tyler, 1992). Multiple infectious and noninfectious respiratory diseases (examples listed earlier in "Respiratory Disease") can predispose animals to pulmonary hypertension. Hypoxia caused by a respiratory disease can result in pulmonary hypertension and cor pulmonale syndrome, even at low altitudes (<1500 m). Animals at higher altitudes have an increased possibility to compound respiratory disease and pulmonary hypoxia to develop acute heart failure. If PAP measurement is thought to be obstructed by temporary pulmonary

disease, then retesting the animal should be done once animal is perceived to have overcome the illness. Gram-negative sepsis may also cause an increase in PAP. Calves within an experiment were treated with endotoxin and showed increased pulmonary vascular resistance and elevated PAP (Reeves, Daoud, Estridge, 1972-73). This test shows that naturally occurring gram-negative sepsis can affect PAP measurements from animals with concurrent bacterial infections and should be tested at a later date. Gram-negative sepsis can potentiate pulmonary hypertension in susceptible cattle at a high altitude increasing the risk of an individual animal to have RV cardiac failure. Treatment with flunixin meglumine may be clinically helpful in blocking the effect of the endotoxin on pulmonary hypertension (Holt & Callan 2007).

Elevation at Test

High altitude exposure will increase PAP. As the testing site altitude lowers in elevation, so will PAP. Animals should stay at high altitudes a minimum of 3 weeks to increase the accuracy of their PAP. It is not uncommon to see clinical HAD in animals with measurements above 49 mm Hg when the test is taken at any elevation. Based on repeated research it has been observed that PAP measurements increase 1 to 2 mm Hg per 300 m increase in elevation (SD+3) (Holt & Callan 2007). It is recommended that cattle be tested at an elevation above 1500 m. The higher the elevation the more accurate and reliable test results will be.

Environmental conditions

Cold environmental temperature can cause pulmonary hypertension. Cattle in cold environments showed decreased arterial pO_2 and increased arterial pCO_2 indicating hypoventilation-induced hypoxia is partly responsible for pulmonary hypertension. (Will & McMurtry et al. 1978).

At the end of the finishing phase, two aspects of the growth and development of cattle may contribute to the development of subclinical RHF. The first is a rapid increase in body mass (Neary, 2013; Neary, 2014). The second is the high percentage of body fat accumulation (Owens et al., 1995; Neary, 2013). Weight gain during the finishing period increases the metabolic demands for oxygen. To meet the oxygen requirements of the body, the cardiac output must be increased (Neary, 2014). Ironically, the ability of the cardiovascular system to meet the needs of the growing body size may be limited by the accumulation of fat during the finishing period. There is speculation, based on human medical research, that obesity in cattle can cause hypoventilation (Piper and Grunstein, 2011; Neary et al., 2015). Cattle that display higher temperaments, "defined as the reactivity of cattle to humans and novel environments" reportedly have suppressed immune responses and increased levels of stress-related hormones (Burdick et al., 2011). Cattle that are easily stressed are more prone to sympathetic nervous system activation, which can include hypertension (Burdick et al., 2011).

RESPIRATORY DISEASE

Respiratory disease in cattle is the number one cause of mortality within the cattle industry in 2015 (NAHMS, 2015). Overall 3.9 million cows and calves were lost to predator and non-predator causes in 2015. Non-predator deaths accounted for 98 percent of all deaths in adult cattle and 89 percent of deaths in calves. Respiratory problems accounted for the most non-predator deaths in cows at 24 percent and calves at 27 percent of deaths (NAHMS, 2015). Multiple innovations have been developed to combat respiratory disease with modest improvements achieved. Bovine respiratory disease (BRD) can result from multiple interactive causes. Many viral and bacterial pathogens involved with BRD are commensal organisms that may inhabit the nasopharyngeal tract of healthy cattle (Kilma et al, 2014). Following BRD, acute interstitial pneumonia is one of the most costly diseases confronted by feedlots (Amosson et al., 2006).

Bovine Respiratory Disease

Causative agents

Viral agents, including infectious bovine rhinotraceitis (IBR), parainfluenza-3 (PI3), bovina viral diarrhea virus (BVDV Type 1 & 2), and bovine respiratory syncytial virus (BRSV), are the primary culprits which cause respiratory tract disease in feedlot calves (Plummer et al., 2004). The opportunistic pneumonia causing bacterial species of primary concern include: Pasteurella (Mannheimia) haemolytica, Pasteurella multocida, and Histophilus somni. However, Mannheimia haemolytica serotype 1 is the organism most commonly associated with BRD (Pandheretal.,1998). In Europe, Mycoplasma bovis

is responsible for at least 25 to 33% of all pneumonia cases in calves suffering from BRD (Gevaert, 2006).

Thurmond (2005) described factors associated with the mode of transmission of BVD. Transmission of BVD can be vertical (fetal infection) or horizontal (postnatal transmission). When an infection with a nonpathogenic strain occurs before d 42 to 125 in utero, calves can become persistently infected (PI; McClurkinetal.,1984). Persistent infection is constant throughout the infected calf's life. Persistently infected cattle constantly shed the virus, giving it an easy means of transmission. Other viruses associated with BRD, particularly PI3 and BRSV often occur in combination with BVDV (Fulton et al., 2000a). At weaning and early in the marketing process, stressed calves are highly susceptible and are at a greater risk of infections by IBR, BVDV types 1 and 2, and BRSV (Fulton et al., 2000a). Bovine herpesvirus 1(BHV-1, commonly known as IBR) has been shown to predispose cattle to pneumonic pasteurellosis (Patel, 2005). Prevention of predisposing viral infections via preconditioning programs that include vaccination for viral and bacterial agents known to cause BRD decreases the risk of BRD.

Clinical Diagnoses

It is generally accepted that a variable, but relatively high percentage of animals will succumb to BRD; thus, accurate diagnosis is critical in practical situations. Traditional methods for detecting morbid cattle include visual appraisal once or twice daily, with animals displaying various signs including nasal or ocular discharge, depression, lethargy, emaciated body condition, labored breathing, or any combination of

these, being removed from pens for quantitative evaluation. Symptomatic animals with a rectal temperature ≥39.7°C are usually considered morbid and given therapeutic treatment (Duff & Galyean, 2006).

Mode of Action

It is understood that an initial pathogen (ex. a virus) may supress the animal's defense mechanisms, allowing increased colonization of the lower respiratory tract by opportunistic bacteria. There are two types of virus-bacteria disease causing interactions: indirect interactions that aid bacteria and direct interactions that aid viruses. It is thought that virus promoting direct interactions occur when the virus exploits a bacterial component to facilitate penetration into the host cell. Indirect interactions, however, result in increased bacterial pathogenesis because of viral infection. These indirect types of interactions are thought to be how respiratory viruses largely affect bacteria in an indirect fashion (Almand and Moore, 2017). According to Almand and Moore (2017), bacteria-virus interactions are complex, and much is still unknown about these interactions. The BRD complex is thought to be stimulated by indirect interactions in which the viral component makes the host cell types more susceptible to bacterial colonization. The host cell becomes more susceptible to colonization by bacteria due to the virus weakening the host cells immune response, thus rendering the cell vulnerable to colonization. There are four major mechanisms to indirect interactions listed by Almand and Moore (2017): virus-induced increase of bacterial cell receptor concentrations; virus damage to underlying epithelial cells; virus displacement of commensal bacterial; and virus suppression of the host immune system (Almand and Moore, 2017). This indirect

relationship that exists between viruses and bacteria could be one-way pathogens normally found to cause BRD break down the hosts immune system and cause the disease.

Acute Interstitial Pneumonia

Acute interstitial pneumonia (AIP) is a respiratory disease that primarily affects feedlot cattle, especially during dry and hot weather patterns (Woolums et al, 2005). Over the years this disease has been known by other names, including atypical interstitial pneumonia, pulmonary adenomatosis, or dust pneumonia (Woolums, 2015). AIP is a pathological definition that can only be confirmed by microscopic (histopathologic) evaluation of lung tissue from an infected individual. In live cattle, symptoms of AIP are similar to clinical signs of BRD but are not pathognomonic (Woolums, 2015). While AIP is a pathologic diagnosis, acute respiratory distress syndrome (ARDS) is a clinical diagnosis. Experienced feedlot staff may be able to accurately diagnose some AIP cases, but even professional individuals may misdiagnose some AIP cases (Woolums, 2015). Cattle affected by AIP show clinical signs that include extension of the neck to facilitate breathing, excessive salivation, grunting, panting, breathing through the mouth and refusal to travel (Blood, 1962; Doster, 2010). Cattle that display AIP like symptoms tend to respond poorly to treatments (Woolums et al. 2005). In the 2011 US Department of Agriculture National Animal Health Monitoring System survey, 72% of all feedlots reported having cattle with AIP, with AIP affecting 2.8% of cattle placed (USDA, 2011). They also reported 97% of feedlots reported having cattle with BRD, with BRD affecting

16.2% of cattle placed (USDA, 2011). In feedlots where AIP occurs, heifers may be disproportionately affected, but feedlots placing large numbers of heifers do not always see AIP.

Pathology of AIP

Lungs of cattle with AIP remain expanded when the chest is opened as if they are inflated. Most abnormalities are prominent within the dorsocaudal lung (Jensen et al. 1976). A mixture of normal pink lobules among affected purple lobules is discernible with some lobules also containing areas of visible alveolar emphysema (Jensen et al. 1976). They found that in 5 cases the dilated lymphatics contained plugs of fibrin filled with erythrocytes. The change produced dark red reticulation conspicuous through intact pleura and on cut surfaces. Lobules within the lung are firm, movable, and purple-brown in cattle diagnosed with AIP (Jensen et al. 1976). Microscopic lesions show hemorrhage appearing to result from rupture of alveolar septums, including capillaries. Nonfibrinous protein, presumably from plasma, forms acidic homogeneous residue and membranes that line alveolar ducts and individual alveoli. In many lungs, alveoli of entire or parts of lobules were emphysematous (Jensen et al. 1976). Neutrophils, macrophages, and sometimes eosinophils infiltrate into alveoli and airways causing edema and hemorrhage; this may be the only lesions seen in cattle that die soon after disease onset (Woolums, 2015).

Causes of AIP

Digestive problems may predispose feedlot cattle to AIP; cattle with feedlot AIP have been found to have higher ruminal pH values than expected for cattle adapted to a high concentrate diet (Woolums, 2015). Proteins are relatively basic; therefore, the high ruminal pH could be related to abnormal protein metabolism (Loneragan & Gould, 1999). A pneumotoxic compound, 3-methylindole (3-MI), produced by ruminal metabolism of L-tryptophan in green forage is known to cause injury to alveolar epithelial cells leading to AIP (Woolums, 2015). Metabolites of 3-MI bind to cellular proteins and nucleic acids, leading to cellular dysfunction and death.

Monensin may help decrease metabolism of tryptophan to 3-MI by *Lactobacillus* sp in the rumen, but cattle fed monensin in feedlot rations are still at risk to develop AIP (Stanford et al. 2007). Free radical scavengers can reduce the toxicity of metabolites of 3-MI, so both Vitamin E and cysteine have been administered to decrease rates of AIP or increase health in cattle at risk for AIP (Stanford et al. 2007).

GASTROINTESTINAL DISEASES

Calf diarrhea is a commonly reported disease in young animals, and a major cause of productivity and economic loss to cattle producers (Cho & Yoon, 2014). Many pathogens are known to cause calf diarrhea, as it is a multifactorial disease. Calf diarrhea is caused by infectious as well as non-infectious factors (Bartels, 2010). The infectious agents involved in this disease are found to be viruses, bacteria, as well as protozoa. Non-infectious causes of this disease are known to be nutrition, herd size, herd management,

and animal facilities. Calf diarrhea works similarly to that of BRD, in that pathogen load and environmental factors weaken and overwhelm the calf's immunity creating an opportunity for other microbes to infect the host.

An enteric cause of diarrhea in calves could be E. coli, which calves are most susceptible to during the first four days after birth (Foster, 2009). Once the E. coli is ingested, the bacteria infects the epithelium of the gut and multiplies in enterocytes of intestinal villi, which causes villous atrophy and alters permeability. Bovine rotavirus is a primary etiological agent of calf diarrhea (Cho, 2014). There are numerous serogroups of the rotavirus, however three of these function in interfering with cellular homeostasis by elevating calcium ion influx into the cytoplasm (Ball, 2005). This interference changes the movement of nutrients and water across the intestinal epithelium and are more important for viral pathogenesis (Cho, 2014). Cryptosporidium parvum is the most common protozoan associated with gastrointestinal tract disease in calves. These protozoa enter enterocytes and induce changes in intestinal cytoskeleton structures, such as loss of microvilli and shortening of columnar epithelial cells, leading to severe villous atrophy (Heine, 1984). The damage caused by this colonization of epithelial cells may cause prolonged malnutrition and reduced growth rates in affected calves due to malabsorption of milk in the intestinal lumen (Nydam, 2005).

Another gastrointestinal disease similar to calf diarrhea, in that it causes diarrhea, is Coccidiosis. Clinical Coccidiosis is commonly seen in cattle under one year of age, but prevalence quickly declines as cattle grow older and develop an immunity to the disease (Nambiar and Devada, 2002) This disease is caused by host specific protozoa called

coccidia that belong to the genous, *Eimeria spp*. Which parasitizes the epithelium lining of the digestive tract of cattle (Reddy, 2013).

Within the first few weeks of life, the most common cause of morbidity/mortality is neonatal calf diarrhea, commonly called calf scours (National Animal Health Monitoring System, 2007). The most common pathogens associated with calf scours are rotavirus, coronavirus, cryptosporidium, *salmonella spp*, and K99 *E. Coli* (Smith, 2015). These pathogens may adhere/colonize within the gastrointestinal tract and destroy cells that are responsible for the digestion/absorption of nutrients. In addition, bovine viral diarrhea (BVD) can also result in immunosuppression that predisposes calves to contracting respiratory infections.

Intestinal Permeability

It is now thought that alterations to gut permeability may be due to pathogens "hijacking" different components of tight junctions as a way to complete their life cycle. Tight junctions (TJs) are highly specialized membrane domains involved in many important cellular processes such as the regulation of the passage of ions and macromolecules across the paracellular space and the establishment of cell polarity in epithelial cells (Flores, 2015). Once thought to be a static parameter, is now known to be dynamic and can respond to a variety of stimuli such as dietary state, humoral or neuronal signals, inflammatory mediators, mast cell products, and a variety of cellular pathways that can be usurped by microbial or viral pathogens (Fasano, 2004). In one study, Wang discovered that Zonula occludens toxin derived from Vibrio cholerae interacts with a specific intestinal epithelial surface receptor, with ensuing activation of a complex

intracellular cascade of events that regulate tight junction permeability (Wang, 2000). While we do not know if this is exactly how the pathogens described in this article work, we now have a better understanding of how luminal organisms can modulate the state of the tight junction through multiple mechanisms and while opening tight junctions may be of benefit for the microflora, it may be deleterious to the host (Arrieta, 2006).

IMMUNE FUNCTION

Cattle are exposed to a variety of environmental, nutritional, and management stressors that could potentially interact with functions of the immune system. In general, stressors that are imposed by management practices and the environment (weaning, shipping, thermal stress, etc.) lead to immunosuppression through disruption of the normal somatotropic axis and altered endocrine secretions (Carroll and Forsberg, 2007). During times of stress, cattle produce highly coordinated biological responses within the body that are designed to maintain homeostasis (Carroll and Forsberg, 2007). For example, glucocorticoids stimulate cytokine expression/secretion, immune cell proliferation/differentiation, and regulation of effector cell function; these compounds can also be inhibitory to certain immune functions (Carroll and Forsberg, 2007). Catecholamines are also known to modulate the immune system by reducing phagocytosis and impairing lymphocyte proliferation, decreasing antibody secretion, and inhibiting production of proinflammatory cytokines (Carroll and Forsberg, 2007).

In times of stress, optimizing the balance of dietary nutrients provided to cattle offers a tremendous opportunity to improve immune function. Dietary energy, protein,

vitamins, and minerals (in addition to adequate intake of good quality water) are essential to support immune responsiveness. Providing adequate dietary energy is critical to support immune function. The development and functionally of immune cells depend upon total energy balance and the energetic fuel sources available. With glucose being critical for proliferation, differentiation, and activation of phagocytic cells and lymphocytes, it is the preferred metabolic fuel during an immune challenge (Ingvartsen and Moyes, 2012).

Essential amino acids are required for synthesis and function of immune cells. When there is an immune response, proteins and amino acids may be diverted from normal functions to support the synthesis of immunoglobulins, T cells, and B cells (Scrimshaw and Sangiovanni, 1997). Additionally, proteins stored in lean tissue can be broken down for energy production and serve as substrates to support immune function (Scrimshaw and Sangiovanni, 1997). Dietary deficiency of protein inhibits humoral and cell-mediated immune responses (Scrimshaw and Sangiovanni, 1997).

Micronutrients also play an essential role in supporting the immune system. Vitamins are crucial for maintenance and function of lymphocytes, natural killer cells, and neutrophils (Carroll and Forsberg, 2007). Vitamins also benefit antibody production and serves as antioxidants in eliminating harmful reactive oxidative species (Carroll and Forsberg, 2007). Minerals control immune responses by serving as cofactors for key enzymes along with supporting antibody responsiveness, cell-mediated immune cell function, and natural killer cell activity (Carroll and Forsberg, 2007).

CONCLUSION

Multiple factors breakdown the immune system causing an array of negative impacts on the health of cattle. The success of a beef producer is ultimately driven by good management practices and the ability of animals to overcome stress. Selecting animals that have the ability to overcome adversity and thrive in their environment will need to be the goal for future producers. The understanding of factors causing morbidity/mortality within the beef cycle along with how the animal overcomes stressors, drives research to better understand the connection of the cardio-pulmonary-gut-hepatic axis.

LITERATURE CITED

Alexander AF, Will DH, Grover RF, et al. Pulmonary hypertension and right ventricular hypertrophy in cattle at high altitude. Am J Vet Res 1960;21:199.

Alexander AF, Jensen R. Gross cardiac changes in cattle with high mountain (brisket) disease in experimental cattle maintained at high altitudes. Am J Vet Res 1959;20:680.

Alexander AF, Jensen R. Pulmonary vascular pathology of high altitude-induced pulmonary hypertension in cattle. Am J Vet Res 1963;24:1112.

Almand EA, Moore MD, Jaykus L-A. Virus-Bacteria Interactions: An Emerging Topic in Human Infection. Kuhn JH, ed. *Viruses*. 2017;9(3):58. doi:10.3390/v9030058.

Amosson, S.H., B. Guerrero and L.K. Almas, 2006. Economic analysis of solid-set sprinklers to control dust in feedlots. Proceedings of the 38th Southern Agricultural Economics Association Annual Meeting, Feb. 5-8, Orlando, pp:1-20.

Angel KL, Tyler JW. Pulmonary hypertension and cardiac insufficiency in three cows with primary lung disease. J Vet Intern Med 1992;6:214.

Animal and Plant Health Inspection Service. 2013. Types and costs of respiratory disease treatments in U.S. feedlots. Center for Epidemiology and Animal Health extension, Washington, D.C.

Arrieta, M. C., Bistritz, L., & Meddings, J. B. (2006). Alterations in intestinal permeability. Gut, 55(10), 1512–1520.

Ball JM, Mitchell DM, Gibbons TF, Parr RD Viral Immunol. 2005; 18(1):27-40.

Bartels CJ, Holzhauer M, Jorritsma R, Swart WA, Lam TJ Prev Vet Med. 2010 Feb 1; 93(2-3):162

Blood, D.C., 1962. A typical interstitial pneumonia of cattle. Can. Vet. J., 3:40-47.

Burdick, N. C., R. D. Randel, J. A. Carroll, and T. H. Welsh. 2011. Interactions between Temperament, Stress, and Immune Function in Cattle. Int. J. Zool. 2011:1–9.

Carroll, J.A. and N.E. Forsberg. 2007. Influence of stress and nutrition on cattle immunity. Vet. Clin. Food Anim. Pract. 23:105-149.

Cho, Y., & Yoon, K.-J. (2014). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science*, *15*(1), 1–17.

Crawford, N. F., R. M. Enns, S. E. Speidel, B. LaShell, T. N. Holt, and M. G. Thomas. 2017. Case Study: Factors influencing pulmonary arterial pressure in cattle of the San Juan Basin Research Center 4-Corners Bull Test data. Prof. Anim. Sci. 33:387–392.

Doster, A.R., 2010. Bovine atypical interstitial pneumonia. Vet. Clin. North Am. Food Anim. Pract., 26:395-407.

Ellis, J.A. 2001. The immunology of the bovine respiratory disease complex. Vet. Clin. North Am. Food Anim. Pract. 17(3): 535-550.

Fasano A, Nataro J P. Intestinal epithelial tight junctions as targets for enteric bacteria-derived toxins. Adv Drug Deliv Rev 200456795–807.

Flores EF, Ridpath JF, Weiblen R, Vogel FS, Gil LH Virus Res. 2002 Jul; 87(1):51-60.

Glover GH, Newsom IE, Brisket disease (dropsy of high altitude). In: Colorado Agricultural Experiment Station, 204 preliminary report; 1915. p. 3-24.

Heath D, Castillo Y, Arias-Stella J, et al. The small pulmonary arteries of the llama and other domestic animals native to high altitudes. Cardiovasc Res 1969;46:184.

Heine J, Pohlenz JF, Moon HW, Woode GN. Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species. J Infect Dis. 1984;150:768–775.

Hohenboken, W., T. Jenkins, J. Pollak, D. Bullock, and S. Radakovich. 2005. Genetic improvement of beef cattle adaptation in America. In: Proc. 37th Beef Improvement Federation Research Symposium and Annual Meeting. p. 115-120.

Holt, T. N., & Callan, R. J. (2007). Pulmonary Arterial Pressure Testing for High Mountain Disease in Cattle. Veterinary Clinics of North America - Food Animal Practice.

Jensen R, Pierson RE, Braddy PM, et al. Atypical intersitial pneumonia in yearling feedlot cattle. J Am Vet Med Assoc 1976; 169(5):507-10.

Kainer, R. A., & Will, D. A. (1981). Morphophysiologic bases for the predisposition of the bovine lung to bronchial pneumonia. Prog Clin Biol Res, 59B, 311–317.

Kilma, C.L., R. Zaheer, S.R. Cook, C.W. Booker, S. Hendrick, T.W. Alexander, and T.A. McAllister. 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J. Clin. Micro. 52(2): 438-48.

Loneragan GH, Gould DH. Atypical interstitial pneumonia in U.S. feedlots. Proc Acad Vet Consult. Winter Meeting :1. Denver, December 2-4, 1999.

McAllister. 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J. Clin. Micro. 52(2): 438-48.

Moore LG, Reeves JT, Will DH, et al. Pregnancy-induced pulmonary hypertension in cows susceptible to high mountain disease. J Appl Physiol 1979;150:564.

Nagaraja, T. G., & Lechtenberg, K. F. (2007). Liver abscesses in feedlot cattle. The Veterinary Clinics of North America.Food Animal Practice, 23(2), 351–69, ix.

Nambiar KS, Devada K. Prevalence of bovine coccidiosis in Trissur. Indian Vet Med J. 2002;26:211–214.

National Animal Health Monitoring System. 2008. Beef 2007-08. United States Dept. of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System, Fort Collins, CO.

National Animal Health Monitoring System. 2015. Death Loss in U.S. Cattle and Calves Due to Predator and Nonpredator Causes. United States Dept. of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System, Fort Collins, CO.

Neary, J., F. Garry, and S. Raabis. 2014. Age-related changes in arterial blood-gas variables in Holstein calves at moderate altitude. Open Access Anim. Physiol.:13.

Neary, J. M. 2013. Pre-weaned beef calf mortality on high altitude ranches in Colorado. Colorado State University. Libraries.

Neary, J. M. 2014. Epidemiological, physiological and genetic risk factors associated with congestive heart failure and mean pulmonary arterial pressure in cattle. Colorado State University. Libraries.

Neary, J. M., C. W. Booker, B. K. Wildman, and P. S. Morley. 2016. Right-Sided Congestive Heart Failure in North American Feedlot Cattle. J. Vet. Intern. Med. 30:326–334.

Neary, J. M., F. B. Garry, T. N. Holt, A. P. Knight, D. H. Gould, and D. A. Dargatz. 2013a. Pulmonary arterial pressures, arterial blood-gas tensions and serum biochemistry of beef calves born and raised at high altitude. Open Access Anim. Physiol. 5:1–8.

Neary, J. M., F. B. Garry, T. N. Holt, M. G. Thomas, and R. M. Enns. 2015. Mean pulmonary arterial pressures in Angus steers increase from cow–calf to feedlot–finishing phases. J. Anim. Sci. 93:3854.

Neary, J. M., D. H. Gould, F. B. Garry, A. P. Knight, D. A. Dargatz, and T. N. Holt. 2013b. An investigation into beef calf mortality on five high-altitude ranches that selected sires with low pulmonary arterial pressures for over 20 years. J. Vet. Diagn. Invest. 25:210–8.

Neary, M. T., J. M. Neary, G. K. Lund, T. N. Holt, F. B. Garry, T. J. Mohun, and R. A. Breckenridge. 2014. Myosin heavy chain 15 is associated with bovine pulmonary arterial pressure. Pulm. Circ. 4:496–503.

Nydam DV, Mohammed HO. Quantitative risk assessment of *Cryptosporidium* species infection in dairy calves. J Dairy Sci. 2005;88:3932–3943.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. W. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim. Sci. 73:3152–72.

Pederzolli, Rae-Leigh, Van Kessel A.G., Campbell J., Hendrick S., Wood K.M., Penner, G.B. 2018. Effect of ruminal acidosis and short-term low feed intake on indicators of gastrointestinal barrier function in Holstein steers. J. Anim. Sci., Volume 96, Issue 1, 15 February 2018, Pages 108–125.

Piper, A. J., and R. R. Grunstein. 2011. Obesity hypoventilation syndrome: mechanisms and management. Am. J. Respir. Crit. Care Med. 183:292–8.

Powell, J. 2013. Livestock health series: Bovine respiratory disease. Div. of Agri. Fact Sheet FSA3082. University of Arkansas. Fayetteville, AR.

Reddy, B., Sivajothi, S., & Rayulu, V. C. (2015). Clinical coccidiosis in adult cattle. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, *39*(3), 557–559.

Reeves JT, Daoud FS, Estridge M. Pulmonary hypertension caused by minute amounts of endotoxin in calves. J Appl Physiol 1972;33:739.

Reeves JT, Daoud FS, Estridge M. Endotoxin: a cause of spontaneous pulmonary hypertension in cattle? Am J Vet Res 1973;34:1573.

Rhodes J. Comparative physiology of hypoxic pulmonary hypertension: historical clues from brisket disease. J Appl Physiol 2005;98:1092.

Sanford K, McAllister TA, Ayroud M, et al. Acute interstitial pneumonia in feedlot cattle: effects of feeding feather meal or vitamin E. Can J Vet Res 2007;71(2): 152-6.

Scrimshaw, N.S. and J.P. Sangiovanni. 1997. "Synergism of nutrition, infection, and immunity: an overview." Am. J. Clin. Nutr. 66: 464S-77S.

Smith, D.R. 2015. Health Management of Confined Cows and Calves. Proc. of Confined Cow-Calf Symposium. Dr. Kenneth and Caroline McDonald Eng Foundation. pp 17-22.

Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. Circ Res 2006;99:675.

USDA. Feedlot 2011 Part IV: health and health management on U.S. feedlots with a capacity of 1,000 or more head. Ft Collins (CO): USDA-APHIS-VS- CEAH-NAHMS; 2011.

Wang W, Uzzau S, Goldblum S E. *et al* Human zonulin, a potential modulator of intestinal tight junctions. J Cell Sci20001134435–4440.

Will, D. H., A. F. Alexander, J. T. Reeves, and R. F. Grover. 1962. High Altitude Induced Pulmonary Hypertension in Normal Cattle. Circ. Res. 10:172– 177.

Will, D. H., McMurtry, I. F., Reeves, J. T., & Grover, R. F. (1978). Cold-induced pulmonary hypertension in cattle. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 45(3), 469–73.

Woolums, AR, Loneragan GH, Hawkins LL, et al. A survey of the relationship between management practices and risk of acute interstitial pneumonia at U.S. feedlots. Bov Pract 2005;39:125.

Woolums, A. R. (2015). Feedlot Acute Interstitial Pneumonia. The Veterinary Clinics of North America. Food Animal Practice, 31(3), 381–9.

CHAPTER 2

SUCCESSFUL TREATMENT OF SUCKLING RED ANGUS CALVES FOR BOVINE RESPIRATORY DISEASE IS NOT ASSOCIATED WITH INCREASED MEAN PULMONARY ARTERIAL PRESSURES AT WEANING

ABSTRACT

The purposes of this study were to determine if the successful treatment of bovine respiratory disease (BRD) in suckling calves was associated with a long-term increase in mean pulmonary arterial pressure (mPAP) and, to screen for associations between blood leukogram variables and mPAP. A cohort of Red Angus calves (n=74) were followed from birth to weaning at an altitude of 975 m. Calves were weaned at 172 ± 14 days when their mPAP was measured and whole blood collected. Thirty calves that had been treated for BRD (34 to 45 days prior) and 30 calves that had not required treatment for BRD were sampled. Treatment for BRD had no effect on mPAP (P = 0.37). Mean mPAP was 48 ± 8 mm Hg (± SD) with a minimum of 34 mm Hg and a maximum at 69 mm Hg. Weaning weight and sex tended to be associated with mPAP, but they explained just 5% of the variation in mPAP (P = 0.08; Adj. r^2 = 0.05). Fibrinogen (P = 0.008) and absolute lymphocyte count (P = 0.06) were negatively associated with mPAP, whereas absolute monocyte count was positively associated with mPAP (P = 0.01). The findings of this study suggest that pre-weaning treatment for BRD does not increase a calves' post-weaning risk of congestive right heart failure. Further, components of the immune and acute phase response system may play a role in the development and progression of pulmonary hypertension.

INTRODUCTION

Cattle are highly susceptible to congestive right heart failure secondary to pulmonary hypertension or cor pulmonale. Mean pulmonary arterial pressure (mPAP) is a product of steady-state vascular resistance. The most commonly recognized cause of pulmonary hypertension in cattle is hypoxia-induced pulmonary arterial vasoconstriction and remodeling associated with high altitude exposure. Respiratory disease, however, may also lead to increased mPAP by predisposing affected individuals to alveolar hypoxia (Holt and Callan, 2007) and, in severe cases, reducing pulmonary vascular capacity through fibrosis and obliteration of pulmonary vessels. Feedlot cattle treated for bovine respiratory disease (BRD) were 3 times more likely to die from congestive right heart failure than cattle that did not require treatment (Neary et al., 2016). It has also been determined that cattle with the greatest mPAP as suckling calves, typically have the greatest mPAP through the confined feeding phase (Neary et al., 2015a); consequently, interventions that reduce mPAP in the cow-calf phase may have beneficial carryover health effects as cattle enter the next phase of production.

The purposes of this study were to determine if healthy calves that were successfully treated for BRD during the suckling phase had significantly greater mPAP than healthy herdmates that did not require treatment for BRD and, to screen for associations between the blood leukogram and mPAP. It was hypothesized that calves treated for BRD would have greater mPAP than calves that did not require treatment.

MATERIALS AND METHODS

Study site and husbandry

Seventy-four purebred Red Angus calves were born in a pasture setting from January 9, 2017 to February 22, 2017 at the Texas Tech Beef Center in New Deal, TX. The calves were raised, and the study conducted, at an altitude of 975 m. These calves were a result of a fresh in vitro and conventional embryo transfer program that were transferred into commercial black-hided dams. All recipients were second-calf heifers. Calving took place on a dormant pasture containing WW-B.Dahl Old World Bluestem (*Bothriochloa bladhii*) and Bermuda (*Cynodon dactylon*) grasses. Cows were monitored every 3 hours for signs of calving difficulty and assistance provided as necessary. Once all cows had calved, the cow-calf pairs were moved to corn stalks until March 30, 2017. Cows were transported to a dry-lot setting for 7 d so calves could be processed and resorted prior to being placed on non-irrigated wheat pasture. Cows were rotated to multiple wheat pastures as forage availability dictated. In general, conditions were fairly dry during the pre-weaning, foraging phase.

On June 5, 2017, when the calves were 127 ± 14 (±SD) days of age, the cow-calf pairs were moved permanently into a drylot setting (27.4 m x 36.5 m with a 3.6 m concrete apron adjacent to feed bunks). In the drylot, cows were fed a total mixed ration consisting of corn gluten feed, cotton burrs, cracked corn, and a custom mineral supplement that met their lactating nutritional requirements (NRC, 2016). Each pen had shade and a creep area where calves could have access to free choice Bermuda grass hay

along with a molasses-based lick tub at 12% crude protein (Purina Stress Tub; Gray Summit, MO). The cattle remained in the dry-lot setting until July 24, 2017, when PAP was measured immediately prior to weaning. All procedures were approved by the Texas Tech University Animal Care and Use Committee (Protocol 17016-01) prior to commencing the study.

Health management

Prior to calving, cows were vaccinated for the following pathogens: infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD Types I and II), parainfluenza-3 virus (PI3), bovine respiratory syncytial (BRSV), and *Leptospira canicola* bacteria (Bovi-Shield Gold FP5 VL5 HB; Zoetis, Florham Park, NJ), rotavirus, coronavius, *Clostridium* type C, *Escherichia coli* Bacterin (Scour Guard 4KC; Zoetis, Florham Park, NJ), and *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* types C and D bacterin-toxoid (Ultrabac 7; Zoetis, Florham Park, NJ). The cows also received doramectin (Dectomax Injectable; Zoetis, Florham Park, NJ) for internal parasites and albendazole (Valbazen Oral Drench; Zoetis, Florham Park, NJ) for external parasites.

Within 24 hours of birth, calves received a unique numbered ear tag, were weighed using hand held digital scale (Cabela's 330-lb. Digital Scale, Cabela's, Sidney, NE), vaccinated against BRSV, IBR and PI3 (Inforce 3; Zoetis, Florham Park, NJ), and rotavirus and coronavirus (Calf-Guard; Zoetis, Florham Park, NJ). All calves were observed nursing within 24 hours of birth.

All calves received a second series of vaccinations on March 30th, 2017, when the cow-calf pairs were brought into a dry-lot temporarily before moving to wheat pasture. Calves were revaccinated against BRSV, IBR and PI3 (Inforce 3; Zoetis, Florham Park, NJ), and given new vaccinations that included: *Mannheimia haemolytica*, BVD Types I and II (One Shot BVD; Zoetis, Florham Park, NJ), *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* types c and d bacterin-toxoid (Ultrabac 7; Zoetis, Florham Park, NJ), d-Apha-Tocopherol with vitamins A and D (Vitamin E-AD-300; VetOne, Boise, ID), zinc, manganese, selenium and copper (Multimin 90; Multimin USA, Fort Collins, CO). Calves were also injected with the anthelmintic doramectin (Dectomax; Zoetis, Florham Park, NJ).

Calves were checked twice daily (morning and evening) for clinical signs of illness. Calves showing signs of illness were caught and treated, as necessary, by the manager of the Texas Tech University Beef Center. The manager had extensive experience and training in identifying ill cattle within a feedlot setting. Visual signs of respiratory disease included lethargic movement, drooping ears, cough, labored breathing, nasal discharge, and diminished appetite. If the calf showed signs consistent with respiratory disease and had a rectal temperature greater than 39.4 ºC (Handheld Thermistor Rectal Thermometer, Cooper-Atkins, Middlefield, CT), the handler treated the calf with tulathromycin (2.5 mg/kg) (Draxxin; Zoetis, Florham Park, NJ). If no improvement in clinical signs occurred within 7 d, calves were retreated for respiratory disease with ceftiofur crystalline free acid (6.6 mg ceftiofur equivalents/kg) (Excede; Zoetis, Florham Park, NJ). No calves were treated more than twice.

Pulmonary arterial and right atrial pressure measurement

On July 24, 2017, the day of weaning, pulmonary arterial pressures (PAP) and right atrial pressures (RAP) were obtained from 60 calves: 30 that had been treated for respiratory disease and 30 calves that had not required any treatment for respiratory disease. Blood samples were collected during the procedure. The first treated and untreated calves to be processed in the chute were sampled. All calves, including those not sampled, were clinically healthy. Calves were restrained in a hydraulic chute and individually weighed on an electronic scale certified by the Texas Department of Agriculture (accuracy ± 0.454 kg). Calves were housed in a pen without food or water for 3 hours to get an accurate 4 % shrink in gross weight.

Once adequately restrained within the chute, a hydraulic neck bar was used to expose the right side of the calf's neck. The neck was then liberally sprayed with chlorhexidine solution before a 12 gauge, 8.9 cm hypodermic needle was inserted into the jugular vein. Blood was collected in 10 mL glass tubes containing the anti-coagulant EDTA. The tubes were inverted 5-times before storage in an insulated container cooled with ice packs. Flexible, saline-filled polyethylene catheter tubing (1.3 m length and external and internal diameters of 17 and 12 mm, respectively) was then fed through the needle and into the jugular vein. A pressure transducer (TranStar DPT, Smiths Medical ASD, Inc., Dublin, OH) connected the catheter and oscilloscope (BM5Vet, Bionet America, Inc. Tustin, CA, U.S.A.). The change in the pressure waveform that occurred as

the catheter tip was advanced through the right atrium, right ventricle, and finally into the pulmonary artery was monitored on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms. Blood samples were shipped overnight in an insulated container lined with icepacks and a complete blood count performed the next day (West Texas A & M Diagnostic Laboratory, Amarillo, TX). Plasma protein was assessed by refractometry. Plasma fibrinogen was estimated by using an established rapid heat precipitation micromethod (Millar et al., 1971). The calves remained in dry-lot setting for one-month post-procedure.

STATISTICAL ANALYSES

Statistical analyses were performed using a commercially available software (Stata version 12.1, College Station, TX). Summary statistics are presented as mean ± SE unless otherwise specified. Between group differences were evaluated using Student's t-test with equal variances or Kruskal-Wallis equality of populations rank test, depending on the distribution of the data assessed graphically. Two backwards step-wise linear regression models that screened phenotypic and blood leukogram variables for association with the outcome variable mean pulmonary arterial pressure (mPAP) were performed. Variables were screened for a univariable association with mPAP. Those that had a probability of a Type I error of 0.20 or less were included in the full regression model. The phenotypic variables included treatment for respiratory disease, weaning weight (calculated with a 4% shrink), sex (bull or heifer), and age. Multiple BRD

treatments were not accounted for in the analyses due to the small number of calves that this involved. The blood leukogram variables included concentrations of leukocytes (neutrophils, lymphocytes, monocytes), erythrocytes, hemoglobin, platelets, plasma protein, and fibrinogen, and the ratio of neutrophils-to-lymphocytes. The marginal means were calculated and plotted with the observed values against mPAP. Evaluations of model fit included graphical and statistical assessments of residual error normality (Shapiro-Wilk test), heteroskedasticity (Breusch-Pagan/Cook-Weisberg test), and linearity.

RESULTS

Descriptive

Calves weighed 33.3 ± 4.5 kg (± SD) at birth. Birth weight was not associated with future BRD treatment status ($P = 0.27$). Three cows required calving assistance; the calves were pulled without mechanical assistance. The mean age of the calves tested was 172 ± 14 days (± SD). The mean body mass of calves treated for BRD (177 ± 4.5 kg) tended to be less than calves that had not been treated (189.3 ± 4.9 kg, $P = 0.09$). Average daily gain from birth to weaning was 0.88 ± 0.13 kg (± SD) and was not affected by BRD treatment status ($P = 0.26$). Calves were treated for BRD from 45 to 34 days prior to weaning. The majority (75%) of calves were treated between 40 and 45 days prior to weaning. Three of the calves sampled were treated twice for BRD with a one-week interval between treatments. Mean mPAP was 48 ± 8 mm Hg (± SD) with a minimum of 34 mm Hg and a maximum at 69 mm Hg. An increase in mPAP by 1 mm Hg was

associated with a 0.5 ± 0.1 mm Hg increase in mean right atrial pressure ($P < 0.001$; Adj. $r^2 = 0.40$).

Table 1.1. Descriptive Statistics

	Untreated	Treated
Mean weight, kg*	189.3 ± 4.9	177 ± 4.9
Mean age, day	172 ± 14	172 ± 14
Sex, male	15	14
Sex, female	15	16
BRD Treatment ,d	--	40 ± 5
mPAP, mm Hg	49.2 ± 6.8	47.4 ± 8.3
Neutrophils, µL	3.85 ± 0.28 $\times 10^3$	4.69 ± 0.35 $\times 10^3$

[1]Treated 40 d prior to weaning.
***Denotes *P* < 0.1**

Effect of BRD on mean pulmonary arterial pressure

Treatment for BRD had no effect on mPAP ($P = 0.37$). Only weaning weight and sex tended to be associated with mPAP, but they explained just 5% of the variation in mPAP ($P = 0.08$; Adj. $r^2 = 0.05$). For every 100 kg increase of weaning weight showed a mPAP increase by 6 ± 4 mm Hg (P=0.08) when controlling for sex. Males tended to have a mPAP 3 ± 2 mm Hg less than females (P=0.13) when controlling for weaning weight.

Effect of BRD on blood leukogram

Only absolute neutrophil count tended to differ according to whether calves had been treated for BRD. Untreated calves had 3.85 ± 0.28 x 10^3 neutrophils per µL of blood and treated calves had 4.69 ± 0.35 x 10^3 neutrophils per µL of blood ($P = 0.06$).

Association between leukogram and mean pulmonary arterial pressure

Fibrinogen, absolute monocyte count, and absolute lymphocyte count were associated with mPAP ($P < 0.001$; Adj. $r^2 = 0.30$). Fibrinogen ($P = 0.008$) and absolute lymphocyte count ($P = 0.06$) were negatively associated with mPAP, whereas absolute monocyte count was positively associated with mPAP ($P = 0.01$) (Fig. 1).

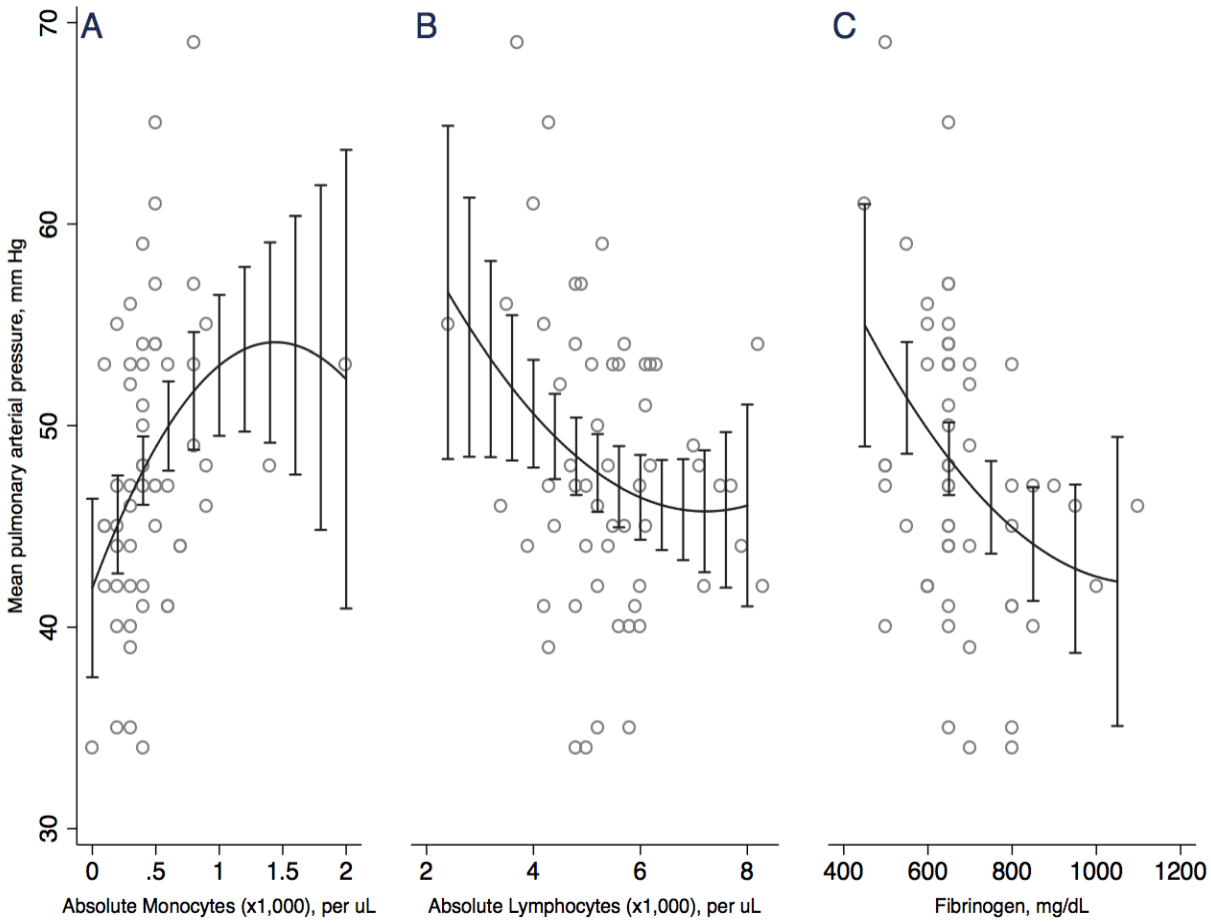

Figure 1. Mean pulmonary arterial pressure mmHg independently related to Absolute Monocyte, Absolute Lymphocytes, and Fibrinogen.

DISCUSSION

The findings of this study indicate that the successful treatment of BRD in suckling Red Angus calves does not lead to an increase in mPAP when measured at weaning. Importantly, this suggests that pre-weaning treatment for BRD does not increase a calves' post-weaning risk of congestive right heart failure. Our study did indicate, however, that components of the immune and acute phase response system may play a role in the development of pulmonary hypertension. Furthermore, the mPAP levels reported in this study were considerably greater than mPAP reported in other mammalian species and even similarly-aged calves located at a higher altitude. These findings are much more than a physiological curiosity; pulmonary hypertension is deleterious to the health and survival of cattle (Neary et al., 2016).

Cattle have a pronounced hypoxia-induced pulmonary pressor response (Tucker et al., 1975), which means that an animal's mPAP increases in association with altitude. We would, therefore, anticipate that the mPAP of calves at the moderate altitude of 975 m to be lower than mPAP in similar aged calves located at a greater altitude. The values recorded in our study, however, are greater than those observed in 6 mo old male black Angus calves at an altitude of 2,170 m (42 mm Hg) (Neary et al., 2015b) and only slightly less than 6 month old crossbed Angus heifers (51 mm Hg) and steers (54 mm Hg) at an altitude of 2,730 m (Neary et al., 2013).

Both environmental and genetic factors are likely etiological factors for the high mPAP observed in our study. Perhaps, the greatest environmental difference is that the calves in our study were managed in a confinement or dry-lot system rather than a

traditional pasture-based system. Research conducted at the same facility on the previous year's calf crop reported that confinement was deleterious to calf health (Burson, 2017). Although the calves in our study appeared healthy when mPAP was measured, the possibility that subclinical disease or long-term sequela of a prior infection contributed to an increase in mPAP cannot be ruled out.

Genetics is also a key determinant of mPAP. Studies of Angus cattle suggest that mPAP is moderately heritable (Shirley et al., 2008a; Crawford et al., 2016). A recent evaluation of mPAP obtained from over 2,400 bulls at an altitude of 2,255 m reported that Red Angus bulls had the second greatest mPAP among the 10 different beef breeds and breed-crosses studied (Crawford et al., 2017). This may be partly attributable to the relatively greater frequency of the hypoxia inducible transcription factor 2A (HIF2A) isoform in the Red Angus breed (Heaton et al., 2016) that has been associated with increased risk for pulmonary hypertension in Angus cattle (Newman et al., 2015). Red Angus and Angus cattle had the second and fifth greatest frequencies of the HIF2A isoform associated with pulmonary hypertension among the 46 breeds evaluated (Heaton et al., 2016).

The HIF-alpha proteins, encoded by the endothelial Per-ARNT-Sim (PAS) domain-containing protein 1 gene (*EPAS1*), are highly conserved regulators of the mammalian hypoxic response. Under normoxic conditions, HIF proteins are degraded, but under hypoxic conditions HIF proteins are protected from degradation and have numerous downstream effects. Missense mutations of EPAS1 affecting the oxygen-dependent degradation domain cause a gain-of-function. There has been speculation that

HIF proteins may be responsible for hematopoietic stem cell activation, from which monocytes are derived (Florentin and Dutta, 2017). If so, the positive association between blood monocyte concentration and mPAP may be attributable to *EPAS1* variants within the calves tested.

Monocytes and macrophages are the main effector cells of lung inflammation, a hallmark of pulmonary hypertension (Frid et al., 2006). In a mouse model, chronic hypoxia led to monocytosis followed by the recruitment of monocytes into the lungs (Amsellem et al., 2017). Here the macrophages predominantly acquired the M2 phenotype, which are conducive to the growth of pulmonary arterial smooth muscle cells (Amsellem et al., 2017). Fibrocytes, mononuclear cells of a monocyte/macrophage lineage, were reported to accumulate in the pulmonary adventitia of neonatal Holstein calves following two weeks of hypoxia that led to the development of pulmonary hypertension (Frid et al., 2006). Whether the monocyte/macrophage infiltration and subsequent inflammation of the lung predispose cattle to other pulmonary diseases remains to be determined.

In agreement with our findings, a decline in blood lymphocyte concentration but stable neutrophil levels has been previously reported human patients with pulmonary hypertension (Yıldız et al., 2013). In contrast to our study, however, most studies of human pulmonary arterial hypertension indicate that the neutrophil to lymphocyte ratio is a better predictor of disease severity than measurements of absolute neutrophils or lymphocytes alone (Özpelit et al., 2015; Harbaum et al., 2017). The reason for the negative association between lymphocyte concentration and mPAP is unclear, but it may

be attributable to a dampening effect of lymphocytes sub-types on pulmonary inflammation and remodeling (Taraseviciene-Stewart et al., 2007; Tamosiuniene et al., 2011).

The negative association between fibrinogen and mPAP suggests that abnormalities of coagulation may play a role in the development or progression of pulmonary hypertension. In one study, patients with primary pulmonary hypertension had impaired fibrinolytic activity (Welsh et al., 1996). In another study of human subjects exposed to high altitude, plasma fibrinogen and fibrinolytic activity increased regardless of whether or not an individual developed pulmonary hypertension; however, individuals with pulmonary hypertension had a significantly greater concentration of other pro-coagulant variables such as platelet adhesiveness, and clotting factors V and VIII (Singh and Chohan, 1972). This suggests that the increase in fibrinogen per se does not predispose to pulmonary thrombi but is facilitated by the presence of other pro-coagulant factors that were not measured in our study. The lower fibrinogen in calves with the greatest mPAP may be attributable to activated coagulation pathways within the pulmonary vasculature and, consequently, the consumption of fibrinogen.

Finally, and in agreement with prior observations, mPAP was found to be positively associated with weaning weight (Shirley et al., 2008b). The strength of association, however, was small and, consequently, of little relevance within the cohort studied.

In conclusion, the successful treatment of BRD in suckling Red Angus calves did not lead to a long-term increase in mPAP. Irrespective of BRD treatment history,

however, mPAP were high. The potential effects of pulmonary hypertension, and associated pulmonary inflammation, on bovine lung health and disease susceptibility need to be more extensively evaluated.

LITERATURE CITED

Amsellem, V., S. Abid, L. Poupel, A. Parpaleix, M. Rodero, G. Gary-Bobo, M. Latiri, J.-L. Dubois-Rande, L. Lipskaia, C. Combadiere, and S. Adnot. 2017. Roles for the CX3CL1/CX3CR1 and CCL2/CCR2 Chemokine Systems in Hypoxic Pulmonary Hypertension. Am. J. Respir. Cell Mol. Biol. 56:597–608.

Burson, W. C. 2017. Confined versus conventional cow-calf management systems: Implications for calf health. Texas Tech University.

Crawford, N. F., R. M. Enns, S. E. Speidel, B. LaShell, T. N. Holt, and M. G. Thomas. 2017. Case Study: Factors influencing pulmonary arterial pressure in cattle of the San Juan Basin Research Center 4-Corners Bull Test data. Prof. Anim. Sci. 33:387–392.

Crawford, N. F., M. G. Thomas, T. N. Holt, S. E. Speidel, and R. M. Enns. 2016. Heritabilities and genetic correlations of pulmonary arterial pressure and performance traits in Angus cattle at high altitude. J. Anim. Sci. 94:4483.

Duff, G. C., & Galyean, M. L. (2007). BOARD-INVITED REVIEW: Recent advances in management of highly stressed, newly received feedlot cattle. Journal of Animal Science, 85, 823–840.

Florentin, J., and P. Dutta. 2017. Origin and production of inflammatory perivascular macrophages in pulmonary hypertension. Cytokine 100:11–15.

Frid, M. G., J. A. Brunetti, D. L. Burke, T. C. Carpenter, N. J. Davie, J. T. Reeves, M. T. Roedersheimer, N. van Rooijen, and K. R. Stenmark. 2006. Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. Am. J. Pathol. 168:659–669.

Harbaum, L., K. M. Baaske, M. Simon, T. Oqueka, C. Sinning, A. Glatzel, N. Lüneburg, K. Sydow, C. Bokemeyer, and H. Klose. 2017. Exploratory analysis of the neutrophil to lymphocyte ratio in patients with pulmonary arterial hypertension. BMC Pulm. Med. 17:72.

Heaton, M. P., T. P. L. Smith, J. K. Carnahan, V. Basnayake, J. Qiu, B. Simpson, and T. S. Kalbfleisch. 2016. Using diverse U.S. beef cattle genomes to identify missense mutations in EPAS1, a gene associated with pulmonary hypertension. F1000Research 5:2003.

Holt, T., and R. Callan. 2007. Pulmonary arterial pressure testing for high mountain disease in cattle. Vet. Clin. North Am. Food Anim. Pract. 23:575–596.

Neary, J. M., C. W. Booker, B. K. Wildman, and P. S. Morley. 2016. Right-Sided Congestive Heart Failure in North American Feedlot Cattle. J. Vet. Intern. Med. 30:326–334.

Neary, J. M., R. D. Brown, T. N. Holt, K. R. Stenmark, R. M. Enns, M. G. Thomas, and F. B. Garry. 2016. Static and dynamic components of right ventricular afterload are negatively associated with calf survival at high altitude. J. Anim. Sci. 94:4172.

Neary, J. M., F. B. Garry, T. N. Holt, A. P. Knight, D. H. Gould, and D. A. Dargatz. 2013. Pulmonary arterial pressures, arterial blood-gas tensions and serum biochemistry of beef calves born and raised at high altitude. Open Access Anim. Physiol. 5:1–8.

Neary, J. M., F. B. Garry, T. N. Holt, M. G. Thomas, and R. M. Enns. 2015a. Mean pulmonary arterial pressures in Angus steers increase from cow-calf to feedlot-finishing phases. J. Anim. Sci. 93:3854–61. A

Neary, J. M., F. B. Garry, T. N. Holt, M. G. Thomas, and R. M. Enns. 2015b. Mean pulmonary arterial pressures in Angus steers increase from cow-calf to feedlot-finishing phases. J Anim Sci 93:3854–3861.

Newman, J. H., T. N. Holt, J. D. Cogan, B. Womack, J. A. Phillips 3rd, C. Li, Z. Kendall, K. R. Stenmark, M. G. Thomas, R. D. Brown, S. R. Riddle, J. D. West, and R. Hamid. 2015. Increased prevalence of EPAS1 variant in cattle with high-altitude pulmonary hypertension. Nat Commun 6:6863.

NRC. 2000. Nutrient Requirements of Beef Cattle: Update 2000, 7th ed. Natl. Acad. Press, Washington, DC.

Özpelit, E., B. Akdeniz, M. E. Özpelit, S. Tas, S. Bozkurt, K. C. Tertemiz, C. Sevinç, and Ö. Badak. 2015. Prognostic value of neutrophil-to-lymphocyte ratio in pulmonary arterial hypertension. J. Int. Med. Res. 43:661–671.

Shirley, K. L., D. W. Beckman, and D. J. Garrick. 2008a. Inheritance of pulmonary arterial pressure in Angus cattle and its correlation with growth. J. Anim. Sci. 86:815–819.

Shirley, K. L., D. W. Beckman, and D. J. Garrick. 2008b. Inheritance of pulmonary arterial pressure in Angus cattle and its correlation with growth. J. Anim. Sci. 86:815–819.

Singh, I., and I. S. Chohan. 1972. Blood coagulation changes at high altitude predisposing to pulmonary hypertension. Br. Heart J. 34:611–7.

Tamosiuniene, R., W. Tian, G. Dhillon, L. Wang, Y. K. Sung, L. Gera, A. J. Patterson, R. Agrawal, M. Rabinovitch, K. Ambler, C. S. Long, N. F. Voelkel, and M. R. Nicolls. 2011. Regulatory T Cells Limit Vascular Endothelial Injury and Prevent Pulmonary Hypertension. Circ. Res. 109:867–879.

Taraseviciene-Stewart, L., M. R. Nicolls, D. Kraskauskas, R. Scerbavicius, N. Burns, C. Cool, K. Wood, J. E. Parr, S. A. Boackle, and N. F. Voelkel. 2007. Absence of T Cells Confers Increased Pulmonary Arterial Hypertension and Vascular Remodeling. Am. J. Respir. Crit. Care Med. 175:1280–1289.

Tucker, A., I. F. McMurtry, J. T. Reeves, A. F. Alexander, D. H. Will, and R. F. Grover. 1975. Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude. Am J Physiol 228:762–767.

Welsh, C. H., K. L. Hassell, D. B. Badesch, D. C. Kressin, and R. A. Marlar. 1996. Coagulation and Fibrinolytic Profiles in Patients With Severe Pulmonary Hypertension. Chest 110:710–717.

Table 1.2. Ingredient inclusion and analyzed nutrient composition (DM Basis) of diets

Item	Confined Diet, %	Pasture Composition, %	Pasture Supplement, %
Ingredient			
Wet Corn Gluten Feed	52.00		88.90
Ground Cotton Burrs	30.00		
Corn Grain, Cracked	15.00		
Bermuda grass forage, dormant		40.00	
WW-B. Dahl forage, dormant		60.00	
Limestone	2.00		
Supplement – 1.0[1]	1.00		
Supplement – 5.0[2]			11.10
Nutrient Composition[3]			
Dry Matter, %	71.44	89.20	68.84
Crude Protein, %	16.69	6.34	20.71
Dietary NDF, %	7.75	30.32	11.58
Ether Extract, %	2.07	0.92	2.66
NEm, Mcal/kg	1.61	1.06	1.86
NEg, Mcal/kg	1.00	0.49	1.25

[1]Supplement composition (DM Basis): 55.05% ground corn; 40% salt; 1.97% zinc sulfate; 0.83% Rumensin-90 (Elanco Animal Health; Greenfield, IN); 0.50% manganous oxide; 0.50% Endox (Kemin Industries; Des Moines, IA); 0.39% copper sulfate; 0.31% vitamin E; 0.25% Selenium (premix 0.2%); 0.17% iron sulfate; 0.02% vitamin A; 0.006% EDDI; 0.004% cobalt carbonate.

[2]Supplement composition (DM Basis): 53.76% ground corn; 36.84% limestone; 8% salt; 0.50% Endox (Kemin Industries; Des Moines, IA); 0.39% zinc sulfate; 0.17% Rumensin-90; 0.10% manganous oxide; 0.08% copper sulfate; 0.06% vitamin E; 0.05% Selenium (premix 0.2%); 0.03% iron sulfate; 0.004% vitamin A; 0.001% EDDI; 0.0008% cobalt carbonate.

[3]Nutrient compositions were determined by analyzing composite samples at a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX). Values for DM were determined by averaging weekly samples (forced air oven for 24 h at 100°C).

CHAPTER 3

DEVELOPMENT OF PULMONARY ARTERIAL HYPERTENSION AND DIFFUSE ALVEOLAR DAMAGE IN 2-MONTH OLD DAIRY CALVES FOLLOWING AN ACUTE EPISODE OF BLOODY SCOURS

ABSTRACT

The goal of this study was to evaluate the effect of hypoxia on intestinal permeability and cardiopulmonary physiology in 2-month old calves. Calves were exposed to normoxic (975 m altitude; controls) or hypoxic (4,570 m altitude) conditions for 2-weeks. Pulmonary arterial pressures and intestinal permeability to mannitol and lactulose were assessed on Days 0 and 14. Calves were euthanized on Day 15. Two control calves shed occult fecal blood on Day 3; consequently, all calves were treated for coccidiosis. Control calves tended to have greater mean pulmonary arterial pressure than hypoxic calves at Day 0 ($P = 0.17$), but there was no difference between groups at Day 14 ($P = 0.47$). On average, mean pulmonary arterial pressure increased by 16 ± 2 mm Hg from Day 0 to 14 ($P < 0.001$). Serum lactulose was 0.8 ± 0.4 mg/L greater in the control group than the hypoxic group on Days 0 and 14 ($P = 0.08$). Serum mannitol was 2.0 ± 0.8 mg/L greater in control calves than hypoxic calves on Day 0 ($P = 0.009$) but there was no difference between groups at Day 14 ($P = 0.61$). In summary, hypoxia did not affect intestinal permeability, but the results were confounded by intestinal disease. Interestingly, the two calves that had bloody scours had the greatest pulmonary arterial pressures and diffuse alveolar damage. The findings of this study provide preliminary evidence that intestinal disease may contribute to the development of pulmonary diseases in cattle.

INTRODUCTION

Bovine pulmonary hypertension is associated with arterial hypoxemia, systemic arterial hypotension, and increased central venous pressure. (Hecht, et al. 1962; Will,et al.1962) These physiological changes are conducive to tissue ischemia. This may be particularly deleterious to tissues, such as the intestinal mucosa, that must function in low oxygen environments under normal physiological conditions. (Zheng, et al.1962) Impaired intestinal mucosal barrier function may, therefore, be a sequela of rising mean pulmonary arterial pressures in cattle progressing through the confined feeding period of production. If so, mucosal ischemia may promote the translocation of bacteria and toxins across the intestinal wall and into the mesenteric lymph or portal venous circulations. Reduced mucosal barrier function may be a component cause for numerous diseases of feedlot cattle approaching slaughter weight such as liver abscess formation. The goal of this study was, therefore, to evaluate the effect of hypoxia-induced pulmonary hypertension on intestinal mucosal barrier function in a Holstein calf model. We hypothesized that hypoxia-induced pulmonary hypertension would be associated with increased intestinal permeability.

MATERIALS AND METHODS

Overview

Six, 2-month old male, intact, clinically healthy Holstein dairy calves were collected from a farm in West Texas. Calves were housed for 2-weeks under normoxic (975 m altitude) or hypoxic (4,570 m altitude) conditions. Pulmonary arterial pressures were measured on Days 0 and 14 of the study. Calves were euthanized on Day 15 and

lung tissue was collected for histology. Pulmonary arteriolar remodeling was semi-quantitatively scored. The study was approved by the Texas Tech University Institutional Animal Care and Use Committee (Protocol 16109-12).

Study site, housing, and feed

Male, intact Holstein calves were obtained from one commercial dairy in West Texas (n = 6). Calves were born and raised on the dairy until collection at 2 months of age (Day -5). The calves were clinically healthy, but the farm calf manager reported a recent outbreak of bloody scours among other calves on the farm. Calves were fed four to five liters of colostrum within 24 hours of birth and provided with 2.8 L of milk twice per day until weaning at 60 days of age. From two weeks of age they were provided with ad libitum access to a pelleted complete ration calf starter (≥ 20% crude protein, dry matter basis).

Calves were weighed on arrival at the university farm (altitude: 975 m) and randomly allocated to one of two pens stratified by body mass (Agri-Plastics, Grassie, ON, Canada). The hypoxic group (n = 3) was housed on a raised slatted floor inside a temperature-controlled chamber (temperature 17 ± 3 °C) (dimensions: 1.8 m x 2.3 m). The normoxic control group (n = 3) was housed in a shaded outdoor pen (1.8 m x 3.5 m) with straw bedding on a sloped concrete floor. The pen was moved to a new location every three days, and the inside of the pen was cleaned with a virucidal disinfectant (Virkon S, DuPont, Wilmington, DE). Soiled straw was removed daily and all straw was replaced every 3 days. Maximum and minimum daily temperatures experienced by the

control calves during the study ranged from 11 to 30 °C and from 5 to 15 °C, respectively. After a five day acclimation period, the air within the chamber housing the hypoxic group was reduced to 14% oxygen, simulating an altitude of 4,570 m (Day 1). Calves were provided ad libitum access to water and a pelleted complete ration calf starter (≥ 20% crude protein, dry matter basis).

Two control calves (calves 2 and 3) started shedding occult fecal blood on day three; consequently, all calves were treated for coccidiosis over a 5-day period starting on day five. Calves were given amprolium in drinking water at a rate of 47 mL per 10 gallons of water, providing approximately 10 mg amprolium per kg body mass at the usual rate of water consumption. Fecal shedding of blood ceased on day eight and feces returned to normal color and consistency on day nine. Calves did not show signs of tenesmus and maintained a normal appetite.

Pulmonary arterial pressure measurement

Pulmonary arterial pressure testing was performed on Days 0 and 14. The neck was cleaned with chlorhexidine solution before a 12 gauge, 8.9 cm hypodermic needle was inserted into the jugular vein. Flexible, saline-filled polyethylene catheter tubing (external and internal diameter of 17 and 12 mm, respectively) was then fed through the needle and into the jugular vein. A pressure transducer (TranStar DPT, Smiths Medical ASD, Inc., Dublin, OH) connected the catheter and oscilloscope (BM5Vet, Bionet America, Inc. Tustin, CA, U.S.A.). The change in the pressure waveform that occurred as the catheter tip was advanced through the right atrium, right ventricle, and finally into the

pulmonary artery was monitored on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms. These distinct waveforms help the veterinarian performing the PAP measurement to orientate themselves within each chamber.

Arterial blood-gas analysis

Arterial blood-gas analyses were performed to verify the hypoxic status of the calves exposed to hypoxic and normoxic conditions. Samples were collected from all calves on Days 0 and 14. Approximately one to three mL of blood was collected from the auricular artery using a 20-gauge, 2.5 cm hypodermic needle attached to a pre-heparinized three mL syringe. Air bubbles were immediately expelled and the first several drops of blood discarded before analysis on a portable analyser (VetScan i-STAT 1, Abaxis, Union City, CA, USA). Blood-gas tensions were adjusted according to rectal temperature.

Intestinal permeability evaluation

Intestinal permeability to the synthetic substances D-Mannitol (100%) (Fisher Scientific, Bridgewater, NJ) and lactulose (99%, Alfa Aesar, Ward Hill, MA) were evaluated twice: on Days 0 and 14. Poly-vinyl tube (outer diameter 0.64cm) was used as a nasogastric tube to administer the substances dissolved in 60 mL of warm water (15 g lactulose and 5 g mannitol). The calf's head was restrained to one side before the

nasogastric tube was inserted into the nostril and advanced caudo-ventrally so that it passed along the ventral meatus, through the nasopharyngeal opening and into the esophagus. The lactulose and mannitol were syringed into the tube. Approximately, five mL of air was used to clear all remaining fluid from the nasogastric tube before it was removed.

A 16 gauge, five cm catheter was placed in the jugular vein to facilitate the collected of blood at 0, 2, 4, 6, and 8 hours following the administration of the lactulose and mannitol. Blood was collected in red stopper blood tubes (10 mL) and the serum stored (-20 °C) within approximately one hour of collection.

To extract serum proteins in preparation for Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis, samples were thawed at room temperature and 100 µL of homogenized serum sample transferred to a microcentrifuge tube (2 mL, Eppendorf, Hauppage, NY, USA). Next, 300 µL of solvent mixture containing acetonitrile (LC/MS Optima Grade, Fisher Scientific, Bridgewater, NJ, USA) and water (LC/MS Optima Grade, Fisher Scientific, Bridgewater, NJ, USA) (80:20; v/v) was transferred to the tube. After five minutes at room temperature, the tube, now containing serum and solvent solution, was vortexed for 30 seconds prior to centrifugation at 21,130 x g for 10 min (5424 R, Eppendorf, Hauppage, NY, USA). The supernatant was transferred into a syringeless filter vial (PTFE, 0.45 µm, GE Healthcare UK Ltd., UK) for LC-MS/MS analysis. Standards were prepared using 10 mg of the standard in 10 mL (mannitol) or 15 mL (lactulose) of solvent (50:50; v/v; methanol:water). From these, serial dilutions of 1, 50, 10, 25, 50, 100 µg/mL in acetonitrile/water mixture (80/20; v/v) were prepared.

Ultra High-Pressure Liquid Chromatography-Mass Spectrometry (UPLC-MS) was performed on a liquid chromatograph with triple staged quadrupole mass spectrometer (TSQ-MS) (Ultimate 3000, TSQ Endura, Thermo Fisher Scientific, Waltham, MA). Serum extract (5 µL) was injected into an RP-Amide column (Accentis RP-Amide**;** 5 µm; 50 x 2.1 mm, Sigma-Aldrich, St. Louis, MO, USA). The column and autosampler tray temperatures were 45˚C and 10˚C, respectively. Mobile phases A and B consisted of 0.1% formic acid (LC/MS Optima Grade, Fisher Scientific, Bridgewater, NJ, USA) in water and 0.1% formic acid in acetonitrile, respectively. The flow rate was 0.4 mL/min. Mobile phase gradient information, mass spectrometry parameters, and transitions monitored for lactulose and mannitol analysis are provided (Tables II, II-1, and II-2).

Table 2. Gradient information for UHPLC

Minute	Mobile phase A (%)	Mobile phase B (%)
0	25	75
3	25	75
5	60	40
6	60	40
8	25	75
10	25	75

Table 2.1. Mass spectrometry parameters used

MS parameters	TSQ Endura
Scan method	SRM
MS Run time (min)	10
Polarity	Negative
Spray Voltage, (V)	2500.00
Sheath Gas (Arb) (Nitrogen, N_2)	40
Aux Gas (Arb) (Nitrogen, N_2)	10
Sweep gas (Arb) (Nitrogen, N_2)	1
Ion Transfer Tube Temp (°C)	342
Vaporizer Temp (°C)	358
CID gas (mTorr) (Argon, Ar)	1.5
Q1 Resolution (FWHM)	0.7
Q3 Resolution (FWHM)	0.7

Table 2.2. Transitions monitored for lactulose and mannitol analysis

TSQ MS/MS					
Analyte	Parent Ion (Q1)	Product Ion (Q3)	Collision energy (V)	RF lens (V)	Ion
Lactulose	341.122	161.111	10.25	133.75	Target ion
	341.122	101.169	12.78	133.75	Confirmation ion - I
	341.122	179.040	10.25	133.75	Confirmation ion - II
Mannitol	181.061	101.111	12.38	88.55	Target ion
	181.061	89.151	12.63	88.55	Confirmation ion - I
	181.061	163.097	10.25	88.55	Confirmation ion - II

Postmortem examination and histology

Calves were euthanized with intravenous pentobarbital sodium (85 mg/kg) on Day 15 of the study. The atria were separated from the ventricles at the atrioventricular junction. The right ventricular free wall (RV) was separated from the left ventricle and septum (LVS). The RV and LVS were individually weighed.

The right diaphragmatic lung lobe was perfused with formalin (10%, neutral buffered) at 15 to 20 cm H_2O for approximately five minutes. After five days of formalin fixation, lung sections were collected midway along the dorsal aspect of the lobe for histology. Tissue from the caudate liver lobe was also preserved in formalin (10%).

Tissue sections (4 μm) were stained with hematoxylin and eosin. Pulmonary arterioles (< 500 μm) were semi-quantitatively scored for medial hypertrophy and adventitial fibrosis (0 = no lesion; + 1 = mild; + 2 = moderate; + 3 = severe). The liver was evaluated for congestion, hydropic degeneration, lipidosis, and other miscellaneous lesions.

STATISTICAL ANALYSES

Statistical analyses were performed using a commercially available software (Stata version 12.1, College Station, TX). Summary statistics are presented as mean ± SE unless otherwise specified. Between group differences were evaluated using Student's *t*-test with equal variances. Student's t-test is a suitable statistical method for small sample sizes ($n \leq 5$) even if group sizes are unequal as long as the effect size is expected to be large. (de Winter J, 2013) Generalized estimating equations with an exchangeable correlation structure were used to evaluate the effect of hypoxia (hypoxic versus normoxic) on serum levels of lactulose, mannitol, and the lactulose to mannitol ratio. Two-way interactions were evaluated between group and test and between group and time from lactulose and mannitol administration.

RESULTS

Descriptive

On Day 0, calf body masses ranged from 75.0 to 86.4 kg with a median of 76.4 kg. Mean body masses for hypoxic and control calves were 78.9 ± 2.8 kg and 79.2 ± 3.6 kg, respectively ($P = 0.95$). On Day 14, calf body masses ranged from 82.7 to 94.0 kg

with a median of 90.4 kg. By the end of the study, the body mass of all calves had increased ($P = 0.01$), but the controls (93.1 ± 0.5 kg) were significantly heavier than the hypoxic calves (85.9 ± 1.7 kg) ($P = 0.02$).

Cardiopulmonary pressures

Controls had greater mean right atrial pressures than hypoxic calves at Day 14 ($P = 0.001$) but not at Day 0 ($P = 0.53$) (Table II-3). There was no difference in mean right ventricular pressures at Day 0 ($P = 0.81$) or Day 14 ($P = 0.30$). Control calves tended to have greater mean pulmonary arterial pressures than hypoxic calves on Day 0 ($P = 0.17$) but there was no difference between groups on Day 14 ($P = 0.47$). On average, mean pulmonary arterial pressure increased by 16 mm Hg (± 2 mm Hg) from Day 0 to 14 ($P < 0.001$).

Table 2.3. Cardiopulmonary pressures (systolic/diastolic and (mean)) and heart rate in 2-month old Holstein calves exposed to hypoxia or normoxia for 2-weeks.

		Right atrium		Right ventricle		Pulmonary artery		Heart rate	
Group	Calf	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Control	1	2/-4 (-1)	16/0 (8)	48/-4 (19)	54/0 (23)	40/9 (19)	49/11 (28)	133	120
	2	6/-2 (1)	18/5 (11)	55/-13 (16)	56/4 (29)	41/-1 (15)	48/29 (39)	135	154
	3	10/-4 (0)	16/5 (11)	51/-6 (23)	51/0 (27)	45/6 (19)	51/17 (32)	124	128
Hypoxic	4	5/-10 (-5)	2/-2 (0)	48/-20 (21)	42/-1 (20)	35/4 (14)	36/17 (26)	134	123
	5	20/-14 (0)	25/-10 (-2)	55/-11 (26)	44/-6 (19)	40/6 (17)	36/17 (28)	125	122
	6	14/-5 (1)	5/-3 (0)	39/-23 (6)	45/7 (28)	29/0 (10)	40/27 (35)	152	100

Arterial blood-gas

Arterial pCO_2 did not differ between control and hypoxic calves on Day 0 (P = 0.99) or Day 14 (P = 0.71) of the study (Table II-4). Arterial pO_2 did not differ between control and hypoxic calves on Day 0 (P = 0.99), but pO_2 was significantly lower in hypoxic calves than controls on Day 14 (P = 0.005).

Table 2.4. Arterial oxygen and carbon dioxide tensions in 2-month old Holstein calves at 0 and 14 days of exposure to hypoxic (4,570m altitude) or normoxic (975 m altitude) conditions.

		P_aCO_2 (mm Hg)		P_aO_2 (mm Hg)	
Group	Calf	Day 0	Day 14	Day 0	Day 14
Control	1	35	43	75	81
	2	32	42	46	71
	3	39	27	51	81
Hypoxic	4	33	49	50	58
	5	34	31	49	52
	6	40	41	47	48

Pathology

The ratio of right ventricular mass to total ventricular mass was greater in hypoxic calves than controls indicating greater work hypertrophy ($P = 0.04$). Both hypoxic and control calves showed histologic lesions of mild (1+) to moderate (2+) medial hypertrophy of the pulmonary arterioles and zero to moderate adventitial fibrosis (Table II-5). Two control calves (calves 2 and 3) had gross and histologic lesions consistent with interstitial pneumonia: heavy, wet lungs that failed to collapse (Fig. II) and diffuse alveolar damage (Fig. II-1). One hypoxic calf (calf 4) had extensive (3+) bronchial associated lymphoid tissue (BALT) and two control calves (calves 1 and 3) had moderate (2+) BALT proliferation. In all cases the BALT was only found around large bronchioles.

All calves showed histologic evidence of hepatic congestion, which primarily affected hepatic lobule zones one and two. Two hypoxic calves and one control calf showed hydropic degeneration. Two control calves had hepatic lipidosis. Only one calf, a control (calf 3), had liver micro abscesses.

Table 2.5. Cardiac mass, pulmonary arteriolar remodeling, and hepatic lesions in 2-month old Holstein calves after 14 days of exposure to hypoxic (4,570m altitude) or normoxic (975m altitude) conditions.

			Pulmonary arteriole		
Group	Calf	RV:TV*	Medial hypertrophy	Adventitial fibrosis	Liver
Control	1	0.28	2+	1+	Hepatic lipidosis (2+), congestion (zone 2)
	2	0.27	1+	0	Congestion and sinusoid dilation (zones 1 and 2), hepatic lipidosis (1+)
	3	0.31	1+	1+	Congestion and sinusoid dilation (zones 1 and 2), hydropic degeneration (3+), multi-foci abscessation (2+)
Hypoxic	4	0.33	1+	1+	Congestion and sinusoid dilation (zones 1 and 2)
	5	0.32	2+	2+	Congestion and sinusoid dilation (zones 1 and 2), hydropic degeneration (1+)
	6	0.32	1+	0	Congestion and sinusoid dilation (zones 1 and 2), hydropic degeneration (1+), portal vein dilation (2+)

* Right ventricular mass: total ventricular mass

FIGURE LEGENDS

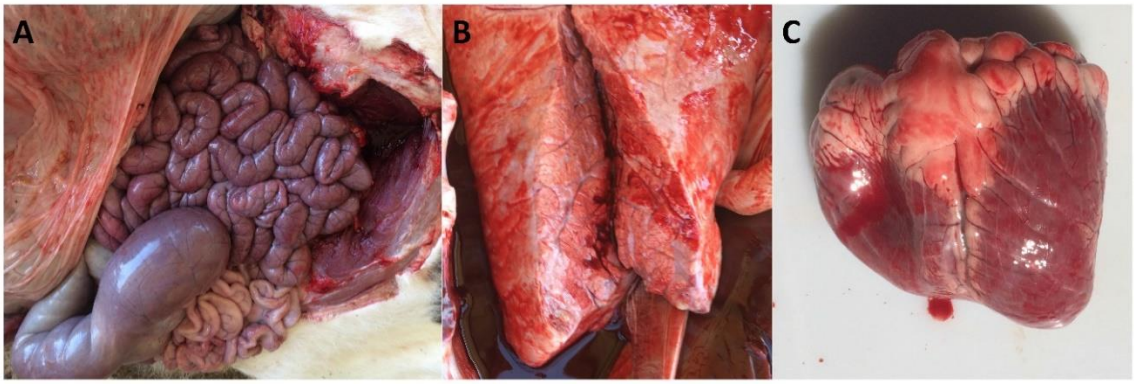

Figure 2. Gross lesions observed in a control calf (calf 2) that developed bloody scours 13 days prior to postmortem examination showing (A) reddened, inflamed intestines next to healthy intestine, (B) wet lungs that failed to collapse, and (C) heart showing a dilated right ventricle.

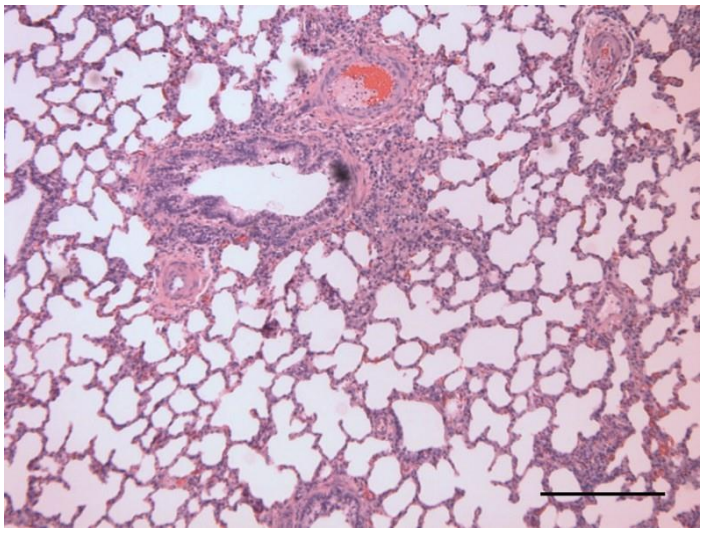

Figure 2.1. Pulmonary arteriolar medial hypertrophy and adventitial fibrosis and diffuse alveolar damage in a 2-month old Holstein calf 1-week after treatment for coccidiosis (calf 2). H&E. Bar = 0.25 mm.

Intestinal permeability

Control calves had greater intestinal permeability to mannitol than the hypoxic calves on Day 0 but had similar intestinal permeability at Day 14 (Fig. II-2). Control calves tended to have greater permeability to lactulose than hypoxic calves throughout the study. Serum lactulose levels were 0.8 ± 0.4 mg/L greater in the control group than the hypoxic group ($P = 0.08$). Serum lactulose levels decreased 1.0 ± 0.4 mg/L from Day 0 to Day 14 ($P = 0.02$). Serum lactulose levels did not significantly vary among sampling time points ($P = 0.29$).

Serum mannitol levels were 2.0 ± 0.8 mg/L greater in control calves relative to hypoxic calves on Day 0 ($P = 0.009$). There was a significant interaction between group and Day ($P = 0.04$). Serum mannitol decreased by 1.4 ± 0.6 mg/L in control calves from Day 0 to Day 14 ($P = 0.01$), but there was no change in serum mannitol in the hypoxic calves ($P = 0.76$). There was no difference in serum mannitol between groups at Day 14 ($P = 0.61$). Mannitol levels tended to decrease by 0.2 ± 0.1 mg/L per hour from administration ($P = 0.09$).

The serum lactulose to mannitol ratio decreased by 2.7 ± 1.1 from Day 0 to 14 in the hypoxic group ($P = 0.01$) but there was no change in the ratio between Days 0 and 14 in the control group ($P = 0.85$). Calves in the hypoxic group had a ratio that was 2.8 ± 0.9 mg/L greater than the control calves on Day 0 ($P = 0.003$). There was a tendency for an interaction between group and Day ($P = 0.10$). The ratio did not significantly vary among over time from administration ($P = 0.86$).

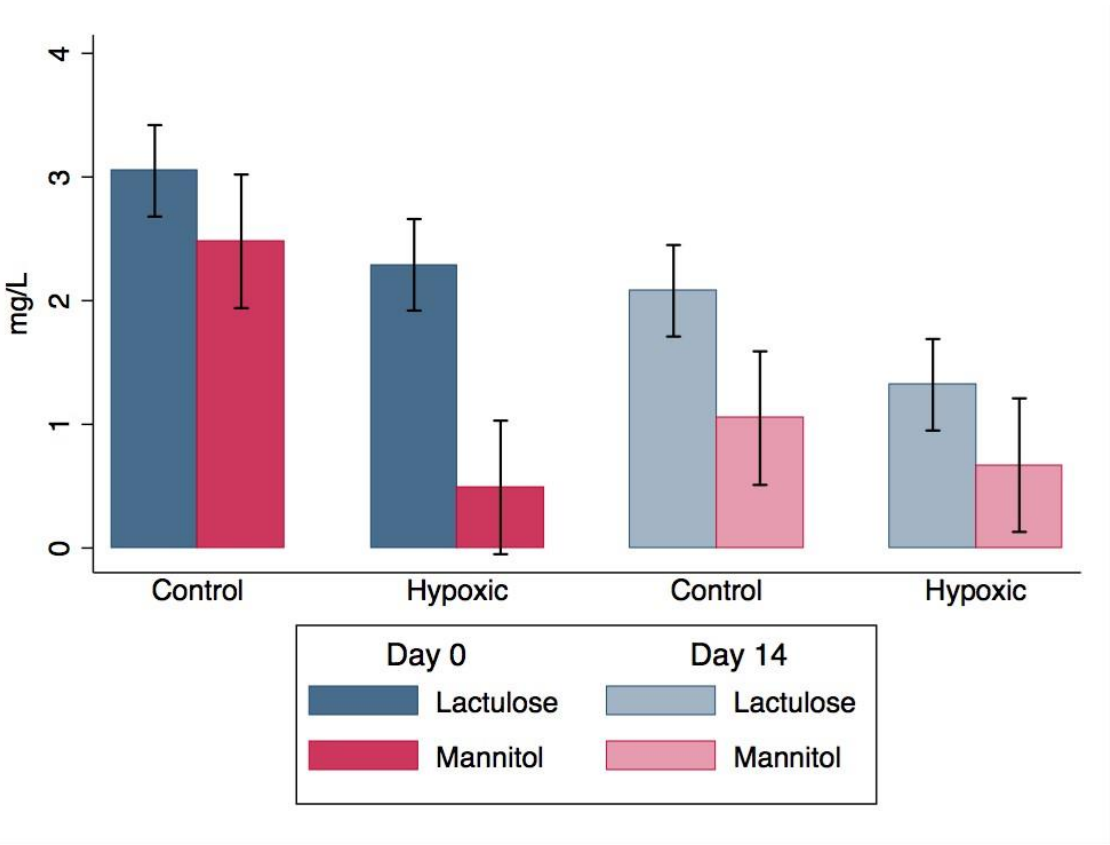

Figure 2.2. Serum concentrations of lactulose and mannitol in 2-month old Holstein calves at 0 and 14 days of exposure to hypoxic (4,570 m altitude) or normoxic (975 m altitude) conditions.

DISCUSSION

The findings of this study provide preliminary evidence that intestinal inflammation may be associated with pulmonary disease in cattle. Unfortunately, we were unable to test our proposed hypothesis because control calves developed bloody scours. Because of this unforeseen event, however, the findings are all the more notable. The control calves showed a similar increase in mean pulmonary arterial pressure as the calves housed under hypoxic conditions, but they had significantly greater arterial oxygen

tensions indicating that the increase in mean pulmonary arterial pressure was not attributable to hypoxia-induced pulmonary hypertension. Furthermore, the two calves that developed bloody scours had gross and microscopic pathology consistent with diffuse alveolar damage, the histologic counterpart of acute lung injury. In concert, these findings provide preliminary evidence that intestinal inflammation may contribute to the development of pulmonary disease in cattle.

Given the small study size, our findings are not robustly supported by statistical analyses. There is, however, considerable supporting evidence for an inter-relationship between the pulmonary and gastrointestinal systems. This is to be expected given that the pulmonary and gastrointestinal systems share a common embryonic origin: the lungs evolved as an outgrowth from the primitive gut. In one study of feedlot cattle, the incidence of acute interstitial pneumonia (AIP) was reported to be 70% greater in pens in which at least one animal had died from a digestive disorder than pens in which digestive disorder death loss did not occur. (Longeran, 2002) There is also accumulating evidence that dietary intervention with probiotics may have a favorable effect on the incidence and recovery of cattle from respiratory diseases. (Keyser et al., 2007; Timmerman 2005) Evidence for a link between inflammatory diseases in the respiratory and gastrointestinal systems in humans is also mounting. (Keely et al., 2012) Crohn's Disease sufferers, for example, are approximately 3-times more likely to die from chronic obstructive pulmonary disease (COPD) than none sufferers. (Duricova et al, 2010; Jess et al, 2006) It is plausible that inflammatory mediators released into the circulation by inflamed bowel mucosa triggers a secondary inflammatory event within the lung. (Wang et al, 2013)

Inflammatory mediators likely contributed to the rise in mean pulmonary arterial pressure in the control calves through either a direct effect of the pulmonary vasculature or indirectly by inducing diffuse alveolar damage. Alveolar hypoxia was unlikely the primary because arterial oxygen tensions were significantly greater in the controls than the calves housed under hypoxic conditions. Gut-derived gram-negative sepsis likely contributed to the development of pulmonary hypertension in the control calves in our study. Injection of calves with endotoxin was reported to increase pulmonary arterial resistance and pressure mediated, in part, by prostaglandin F. (Anderson et al,1975; Reeves et al, 1973; Reeves et al, 1972; Tikoff et al,1966) Most notably, calves with large pressor responses had increased pulmonary arterial wedge pressures, which may have reflected pulmonary venous hypertension and interstitial edema formation. (Reeves et al, 1972) Furthermore, pulmonary edema was observed on gross and histological examination of calves given heat killed *Pseudomonas aeruginosa* organisms (Reeves et al, 1972) or endotoxin. (Tikoff et al, 1966) Similarly, studies of broiler chickens intravenously (Wideman et al, 2009) or intratracheally (Lorenzoni et al, 2008) injected with lipopolysaccharide (LPS) reported a significant but short-lived increase in mean pulmonary arterial pressure. In humans, pulmonary hypertension is commonly reported in association with acute lung injury; however, it is unknown if the hypertension is merely a consequence of increased arterial resistance secondary to diffuse alveolar damage, if the increased arterial pressure contributes to the development of alveolar damage, or if they share a common etiology. (Ryan et al, 2014)

The etiologic agent of the bloody scours was not investigated in our study, but we believe that it was most likely attributable to coccidiosis due to the age of the calves, the positive response to treatment, and the potential for parasite accumulation in the straw bedding. Given that the calves were not housed under identical conditions there are other potential confounding factors, such as environmental temperature and straw bedding that need to be considered; however, other than the development of bloody scours, there is no evidence, to our knowledge, that any of these factors could have contributed to the findings of this study.

In conclusion, we report the development of pulmonary arterial hypertension and diffuse alveolar damage in 2-month old Holstein calves following an acute episode of bloody scours at an altitude of 975 m. The findings of this study provide preliminary evidence that inflammatory gastrointestinal-pulmonary cross-talk may contribute to pulmonary arterial remodeling and hypertension in cattle.

CONCLUSION

The main purpose of this study was to investigate the factors that influence cardiopulmonary disease in cattle. With a basic understanding of what factors cause these diseases in cattle, the study focused on how a hypoxic environment may influence pulmonary arterial pressure. Although the study resulted in inconclusive results, it provided the authors with preliminary evidence that intestinal inflammation may be associated with pulmonary disease in cattle. Additional research should be conducted in order to verify the authors hypothesis that intestinal inflammation, as well as hypoxia, is in fact associated with pulmonary disease. In future studies, researchers should increase

the number of calves studied as well as confirm the animals do not have any underlying illnesses that may affect the results. In addition to increasing sample size and verifying health, future research may consider different bedding due to the possibility of sickness from the bedding. Researchers should also investigate the effect gut permeability has on cardiopulmonary diseases. Testing gut permeability will allow researchers insight on the affect hypoxia has on the permeability of the gut.

Although this study was not conclusive, some valuable information was gained. Since the control calves showed a similar increase in mean pulmonary arterial pressure as the calves housed under hypoxic conditions, this indicated the increase in mean pulmonary arterial pressure was not attributable to hypoxia-induced pulmonary hypertension, thus giving some indication that intestinal inflammation may contribute to higher pulmonary arterial pressures. Though this is not definitive evidence, it is a step in determining the viability of this hypothesis and gives enough information to continue researching this topic.

LITERATURE CITED

Anderson FL, Tsagaris TJ, Jubiz W, Kuida H: 1975, Prostaglandin F and E levels during endotoxin-induced pulmonary hypertension in calves. Am J Physiol 228:1479-1482.

de Winter J: 2013, Using the Student's t-test with extremely small sample sizes. Practical Assessment, Research & Evaluation 18:1-12.

Duricova D, Pedersen N, Elkjaer M, et al.: 2010, Overall and cause-specific mortality in Crohn's disease: a meta-analysis of population-based studies. Inflamm Bowel Dis 16:347-353.

Hecht HH, Kuida H, Lange RL, et al.: 1962, Brisket disease. II. Clinical features and hemodynamic observations in altitude-dependent right heart failure of cattle. Am J Med 32:171-183.

Jess T, Loftus EV, Jr., Harmsen WS, et al.: 2006, Survival and cause specific mortality in patients with inflammatory bowel disease: a long term outcome study in Olmsted County, Minnesota, 1940-2004. Gut 55:1248-1254.

Keely S, Talley NJ, Hansbro PM: 2012, Pulmonary-intestinal cross-talk in mucosal inflammatory disease. Mucosal Immunol 5:7-18.

Keyser SA, McMeniman JP, Smith DR, et al.: 2007, Effects of Saccharomyces cerevisiae subspecies boulardii CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J Anim Sci 85:1264-1273.

Loneragan GH: 2002, Epidemiological characteristics of AIP in feedlot cattle. *In*: Academy of Veterinary Consultants. Colorado Springs.

Lorenzoni AG, Wideman RF, Jr.: 2008, Intratracheal administration of bacterial lipopolysaccharide elicits pulmonary hypertension in broilers with primed airways. Poult Sci 87:645-654.

Reeves JT, Daoud FS, Estridge M: 1973, Endotoxin: a cause of spontaneous pulmonary hypertension in cattle? Am J Vet Res 34:1573-1576.

Reeves JT, Daoud FS, Estridge M: 1972, Pulmonary hypertension caused by minute amounts of endotoxin in calves. J Appl Physiol 33:739-743.

Ryan D, Frohlich S, McLoughlin P: 2014, Pulmonary vascular dysfunction in ARDS. Ann Intensive Care 4:28.

Tikoff G, Kuida H, Chiga M: 1966, Hemodynamic effects of endotoxin in calves. Am J Physiol 210:847-853.

Timmerman HM, Mulder L, Everts H, et al.: 2005, Health and growth of veal calves fed milk replacers with or without probiotics. J Dairy Sci 88:2154-2165.

Wang H, Liu JS, Peng SH, et al.: 2013, Gut-lung crosstalk in pulmonary involvement with inflammatory bowel diseases. World J Gastroenterol 19:6794-6804.

Wideman RF, Bowen OT, Erf GF: 2009, Broiler pulmonary hypertensive responses during lipopolysaccharide-induced tolerance and cyclooxygenase inhibition. Poult Sci 88:72-85.

Will DH, Alexander AF, Reeves JT, Grover RF: 1962, High altitude-induced pulmonary hypertension in normal cattle. Circ Res 10:172-177.

Zheng L, Kelly CJ, Colgan SP: 2015, Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A Review in the Theme: Cellular Responses to Hypoxia. Am J Physiol Cell Physiol 309:C350-360.