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Efficacy of a Novel Intranasal Zinc Solution on the Microbiome, Health, and Growth Performance of High-risk, Newly Received Stocker Cattle

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Efficacy of a Novel Intranasal Zinc Solution on the Microbiome, Health, and Growth
Performance of High-risk, Newly Received Stocker Cattle

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

By

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Bachelor of Science in Animal Science, 2014

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Abstract

The objective of this study was to determine if using an intranasal zinc (**Zn**) solution would impact health and growth performance of high-risk stocker cattle. Male beef calves (n = 239; 3 arrival dates [block]; initial BW = 276 ± 2.4 kg) were stratified by arrival gender and BW and assigned to 1 of 2 treatments: 1) treated with 3 ml of a Zn solution containing 36.24 mg of Zn administered intranasally, or 2) control, in which calves were not treated. Calves were observed daily and if exhibiting signs of morbidity and a rectal temperature $\geq 40^{\circ}\text{C}$ they were treated with an antibiotic. If rectal temperature $\geq 40^{\circ}\text{C}$ persisted cattle were re-treated according to pre-planned protocol. Body weights did not differ ($P \geq 0.22$) across treatments throughout the duration of the study. Calves treated with Zn had a lesser ($P < 0.01$) ADG from d 7 to 28 and d 14 to 28 compared to the control. Control calves tended ($P = 0.06$) to be treated with 3 antibiotics more often than Zn treated calves. Overall treatment antibiotic costs did not differ ($P = 0.64$) across treatments. There were no differences ($P \geq 0.10$) for rectal temperature of calves across treatment. Overall, Zn treated calves were similar in growth performance parameters and minimally different in percentage morbidity compared to the control. With the exception of *Bacillus* spp. and *Pseudomonas aeruginosa* the prevalence of bacterial pathogens were not different ($P \geq 0.14$) across treatments. The presence of *Pseudomonas aeruginosa* was greater ($P \leq 0.04$) in the control compared to the Zn calves, and *Bacillus* spp. tended to be greater ($P = 0.09$) in control calves. There were no differences due to Zn treatment in microbiome analysis; however, differences were found in healthy versus sick cattle.

Keywords: Bovine Respiratory Disease, Zinc, Morbidity

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CHAPTER I

INTRODUCTION

Bovine respiratory disease (BRD) is a major problem in every facet of the cattle production system. Bovine respiratory disease can decrease total economic output due to increased medical costs and labor, decreases in production, and increased death loss (Guzel et al., 2010). Newly received cattle are at a high risk for contracting BRD due to stress associated with weaning, marketing, and transport (Duff and Galyean, 2007). Comingling calves negatively affects the immune system and exposes them to new bacterial and viral agents associated with BRD (Blecha et al., 1984). Highly stressed calves are more likely to experience decreased feed intake (Galyean and Hubbert, 1995; Cole, 1996), which is negatively correlated with growth performance.

The study of microbial health in humans has demonstrated evidence for species-dependent health consequences (Penner et al., 2014). Many of the pathogens associated with BRD are considered to be opportunistic as many reside in the upper respiratory tract of both healthy and sick cattle (Allen et al., 1991; Timsit et al., 2016a). Understanding the nasopharyngeal microbiota as it evolves and changes upon arrival to the feedlot is vital to the health of calves susceptible to contracting BRD (Timsit et al., 2016a). Previous research correlates disrupting the microbiome with an increase in susceptibility to disease (Timsit et al., 2016a).

Zinc is an essential dietary mineral that is necessary for overall growth and health (Todd et al., 1934). The functions of Zn are numerous; it is required for over 2,000 transcription factors for structural and functional integrity (Prasad, 2007). Zinc can be found in either bone or tissue; however, there is no homeostatic mechanism available to mobilize the body supply of Zn.

Therefore, a steady intake of Zn must be available within the diet (Vruwink et al., 1993; Wintergerst et al., 2006). Previous research in humans by Wintergerst et al. (2006) suggests that adequate intake of Vitamin C and Zn ameliorate symptoms and shorten the duration of respiratory tract infections such as the common cold.

Bovine respiratory disease in the upper respiratory tract is similar to the common cold in humans with symptoms such as coughing, difficulty breathing, ocular discharge, fever, and decreased appetite (Bagley, 1997). The common cold in humans is often caused by a rhinovirus infection, which enters via the nasal mucosa and can potentially proceed to become a more widespread infection (Cohen, 2006). For a rhinovirus to enter into the nasal epithelium, the virus must bind to the cellular receptor intracellular adhesion molecule-1 (ICAM-1; Cohen, 2006). Zinc acts as a competitive inhibitor of ICAM-1, which disrupts the virus' ability to penetrate the cell wall and replicate (Cohen, 2006).

Drug companies have developed an alternative method to help mitigate the common cold, including throat lozenges and intranasal sprays aiming to reduce severity and duration of the cold by applying Zn ions directly to the site of the rhinovirus infection (Cohen, 2006). In the cattle industry, the best option to control BRD is to vaccinate against viruses that can initiate the syndrome (Richey, 1994) and administer antibiotics to combat secondary bacterial infections. Alternatively, applying Zn solutions in addition to antibiotics could have a positive effect on immune response and growth performance. The objectives of these studies were to evaluate the impact of a mucosal application of Zn on health and growth performance in high-risk, newly received stocker calves.

CHAPTER II

REVIEW OF LITERATURE

Overview of Bovine Respiratory Disease

For both stocker and feedlot operations, BRD is a great health concern reducing profit margins in the cattle industry. The high cost of management and treatment of BRD is a contributing factor to producers deeming BRD one of the largest health issues facing the industry (Schneider et al., 2009). Treatment costs along with production losses derived from BRD are estimated to cost producers up to \$750 million annually (Griffin, 1997). Morbidity and mortality are the largest economic loss due to BRD; however, production losses occur from reduction in ADG and feed conversion rates, and increases in metaphylaxis and antibiotic costs therefore increasing labor costs. Although there have been new vaccines and antibiotics that have entered the marketplace, BRD mortality rates have remained unchanged over the past 30 years (Miles, 2009) potentially due to low adoption rates of preconditioning programs by cow-calf operations (Babcock et al., 2006). Between 70 and 80% of the morbidity and between 40 to 50% of the mortalities in feedlot cattle are attributed to BRD (Griffin, 1997; Smith, 1998). A USDA (2013) report estimated that 16.2% of feedlot cattle developed BRD with treatment costs estimated to average \$23.60.

Bovine respiratory disease can occur in any beef production system or in cattle at any age, but it is most prevalent in animals that are physiologically stressed, recently weaned, comingled, transported or transitioned to a new diet. With many sectors encompassing the beef industry, ownership frequently changes hands which differs from many other protein based production systems; therefore, there are often times of disconnect in terms of the background of cattle regarding their prior performance or health as they move from one segment to another.

Communication between buyer and seller in cattle transactions are rare, thus sellers move the health risks of the cattle including the incidence of BRD to the buyer. The number of cattle operations in the United States is increasing; however, with that increase comes a decrease in the average herd size (USDA, 2015). This large percentage of small cow-calf operations has increased the number of cattle entering the market via a commodity-based auction system; therefore, most of the cattle are sold individually and buyers assemble truckloads from multiple cow-calf operations thus comingling cattle and increasing stress and BRD morbidity (Step et al., 2008) because a re-establishment of the social hierarchy is necessary and exposure to novel pathogens increases.

According to a NAHMS (2008) report, 61% of cow-calf operations chose not to vaccinate calves for respiratory disease prior to marketing. Of the total number of calves sold in the United States, 31% were not vaccinated at all, increasing the prevalence of BRD in stocker and feedlot cattle operations. A USDA 2007-08 survey of cow-calf producers estimated the adoption rates of animal health management factors including castration (49.5%), weaning (50.2%), and respiratory vaccination (39.4%) remains relatively low, all of which coincide with an increase in BRD and other health issues (USDA, 2008; USDA, 2010).

Viral Pathogens

When the onset of BRD occurs, new pathogens have been introduced to the calves. The primary viral pathogens associated with BRD include but are not limited to: bovine herpes virus-1 (commonly known as infectious bovine rhinotracheitis virus, BHV1), parainfluenza-3 virus (PI3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV). Other viruses such as bovine adenovirus and bovine coronavirus (BCV) could be involved in the transmission of BRD but are underestimated in comparison to the primary 4 pathogens (Hodgins

et al., 2002). Immune suppression caused by viral pathogens is an important factor for the onset of BRD by increasing susceptibility to secondary bacterial infections (Woolums, 2015).

Viral pathogens may only produce mild clinical signs, but when combined with bacterial pathogens and other stress factors, the onset of BRD may become severe. Cattle often carry many of these pathogens in their upper respiratory tract with no apparent signs, and bacteria are often inactive within the system. Under stressful situations, the pathogens become active, and an infection is established within the animal (Bagley, 1997).

Infectious bovine rhinotracheitis (IBR) is a classical upper respiratory infection often observed following an infection with BHV1 subtypes 1 and 2a in herds with either no prior infection history, calves that have not been vaccinated, or those with low levels of immunity (Wentink et al., 1993; Bagley, 1997). During times of stress, shedding of the virus will begin to cause infection and when combined with other bacterial pathogens, it can cause severe cases of pneumonia and shipping fever (Bagley, 1997). In herds where vaccination is a common practice, clinical respiratory disease is often mild or unapparent and is most commonly observed in young animals (Van Nieuwstadt and Verhoeff, 1983).

Parainfluenza-3 infection often coincides with BHV1 and/or BVDV infection and is associated with both acute and chronic pneumonia in cattle (Hodgins et al., 2002). Parainfluenza-3 is a relatively mild disease by itself and symptoms displayed are similar to infection with BHV1 including: fever, cough, and nasal and ocular discharge. Animals infected with both viruses appeared more depressed and displayed greater rectal temperatures than those infected with PI3 alone (Ghram et al., 1989; Hodgins et al., 2002).

Bovine respiratory syncytial virus is a recognized disease agent that is identified nationally in respiratory infections (Bagley, 1997) and is most often seen in calves prior to

weaning (Pirie et al., 1981). Transmission most often occurs through contaminated nasal secretions (Hodgins et al., 2002), which can be easily transmitted from animal to animal in auction markets and during transportation to feedlots. The spread of this virus occurs rapidly within naïve herds and is known to produce high morbidity rates from 60 to 80% (Van der Poel et al., 1995).

Bovine viral diarrhea virus is a common pathogen present in the majority of cattle herds and is known to cause respiratory, digestive tract, and reproductive problems (Bagley, 1997). Bovine viral diarrhea virus was the virus most commonly isolated from pneumonic lungs found in cattle (Reggiardo, 1979). In previous studies, inoculation of calves susceptible to BVDV, typically leads to a mild or moderately severe respiratory disease (Baule et al., 2001; Brodersen and Kelling, 1998; Potgieter et al., 1984) while those calves infected with both BVDV and *Mannheimia haemolytica* following inoculation resulted in severe pneumonic disease (Potgieter et al., 1985; Potgieter et al., 1984). Infection of susceptible cows with BVDV within 2 to 4 months of gestation (Peterhans et al., 2010) can lead to the birth of a persistently infected (PI) and immunotolerant calf (McClurkin et al., 1984). The PI calf will then begin to shed the BVDV strain it was exposed to *in utero* throughout its life (Kahrs, 2001). Calves infected with BVDV subgenotype 1b are often PI-BVDV calves (Fulton et al., 2005), and this particular strain is not included in commercial vaccines used to help mitigate BVDV infection (Richeson, 2011). Calves with PI-BVDV can give way to the onset of the BRD complex due to being a primary respiratory pathogen or due to decreased immune function in exposed calves (Bagley, 1997; Welsh et al., 1995). Testing for PI-BVDV upon arrival is recommended to producers to quickly identify infected calves and remove them from the herd immediately. One calf infected with PI-BVDV,

could have the potential to infect an entire pen and adjoining pens due to the constant shedding of the strain of BVD carried by the calf (Kahrs, 2001).

Bacterial Pathogens

Bacterial pathogens are also prevalent in high-risk cattle susceptible to BRD. The most common bacterial pathogens associated with BRD are *Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni*. *Mannheimia haemolytica* serotype 1 is the pathogen most commonly associated with BRD (Pandher et al., 1998). The majority of pathogens associated with BRD are known to be present in the respiratory tract of the animal and then become opportunistic colonizers within the lung after the onset of a viral infection (Woolums, 2015). *Mannheimia haemolytica* and *Pasteurella multocida* are known to be a major cause of “shipping fever” in combination with a viral pathogen as well as increased stress (Bagley, 1997).

Mannheimia haemolytica, a gram negative bacteria, is commonly found in the respiratory tract of healthy cattle (Frank, 1989), but in conjunction with an active viral infection, added stress factors, or both it migrates to the lungs where it can multiply rapidly (Rehmtulla and Thompson, 1981; Confer et al., 1990). *Mannheimia haemolytica*'s greatest impact occurs in recently weaned beef calves upon arrival to feedlots (Jubb and Kennedy, 1970; Mosier et al., 1989; Wilson, 1989). Symptoms associated with *Mannheimia haemolytica* respiratory infections are similar to those of an upper respiratory tract infection: fever, nasal discharge, cough, respiratory distress, inappetence, and weight loss (Friend et al., 1977). Prior research has proven *Mannheimia haemolytica* commonly resides in the respiratory tract; however, it has been identified that tonsillar tissue as well as the nasopharynx is a reservoir for the pathogen (Frank and Briggs, 1992; Frank et al., 1995). Therefore, nasal swabs could test negative for *Mannheimia haemolytica* yet cattle could test positive when tonsils are swabbed (Frank et al., 1994). When

stress is introduced to the animal, the calf may not maintain the commensal relationship with the bacteria leading to the onset of a respiratory infection in the calf (Rice et al., 2008).

Pasteurella multocida is a gram-negative bacterium that is classified into different subspecies, serogroups, and serotypes (Dabo et al., 2008). Bovine respiratory disease induced by *Pasteurella multocida*, is associated with environmental and stress factors including: comingling, transportation, weaning, and overcrowding (Dabo et al., 2008). The presence of the bacteria in the upper respiratory tract is common in healthy animals therefore the presence of *Pasteurella multocida* does not equal disease, confirming the ubiquitous nature of the pathogen as well as the multifactorial nature of BRD (Virtala et al., 1996). Although, *Mannheimia haemolytica* is the most common pathogen isolated from shipping fever, the proportion of fatalities in feedlots attributable to *Pasteurella multocida* seems to be increasing (Welsh et al., 2004). This increase could be attributed to changes in virulence factors among the pathogens, changes in management of cattle or identification of sick cattle, and efficacy of antimicrobial agents used by producers (Welsh et al., 2004). Hodgins et al. (2002) states that very little is known about the virulence factors contribution to pulmonary pathogenicity, but *Pasteurella multocida* is considered to be less virulent in comparison to *Mannheimia haemolytica*. Epidemiologic investigation of *Pasteurella multocida* in association to BRD is scant due to the ubiquitous nature of the bacterium (Dabo et al., 2008). Since the pathogen normally resides within the animal, confirmation of its involvement in BRD requires post-mortem cultures, which are not practical for producers (Dabo et al., 2008).

Histophilus somni is a gram-negative bacterium incapable of survival outside of the body, however, it has been shown to remain viable in the nasal mucus at 23.5° C for at least 70 days (Dewey and Little, 1984; Harris and Janzen, 1989). Calves can become infected with *Histophilus*

somni by inhaling aerosolized particles that contain the bacteria (Harris and Janzen, 1989). The respiratory tract is a site of entry for the bacteria, producing the septicemic form of *Histophilus somni* which is known to cause both upper and lower respiratory infections (Harris and Janzen, 1989). Pneumonia associated with *Histophilus somni* is more of a subacute or chronic infection than those of *Pasteurella multocida* and *Mannheimia haemolytica*, with necrotizing bronchiolitis and alveolitis lesions (Potgieter et al., 1988; Stephens, 1990; Hodgins et al., 2002). Previous research has established that weaned calves inoculated with *Histophilus somni* intrabronchially developed more severe clinical disease and lesions when combined with BRSV or BHV1 both of which are viral pathogens associated with BRD (Potgieter et al., 1988).

Prevention Strategies for Bovine Respiratory Disease

Management/Preconditioning

Management is key in reducing stressful environments for calves susceptible to contracting BRD. Evaluating all possible causes for stress and identifying which factors can be reduced or eliminated helps to mitigate prevalence of BRD (Bagley, 1997). It is important to recognize the critical detection period of the disease after weaning, placing on feed, or shipping of the cattle (Bagley, 1997). Attempting to avoid comingling calves arriving from different sources due to the shedding of potential pathogens (bacterial or viral) along with not overcrowding pens and ensuring new calves have the ability to be in close proximity to food and water could be beneficial in decreasing stress (Bagley, 1997).

Many producers believe that preconditioning cattle is beneficial to reduce morbidity and mortality in calves weighing less than 318 kg (USDA-APHIS, 2000a). Preconditioning programs ensure calves have been weaned for a certain period of time, vaccinated with both viral and clostridial vaccines, treated with an anthelmintic, castrated, dehorned, and are accustomed to

feed bunks and water troughs prior to traveling to a feedlot (Duff and Galyean, 2007). Cow/calf producers using a 45-d preconditioning program earned \$14.00/calf more in net returns compared to weaned calves that were not preconditioned (Dhuyvetter et al., 2005). In addition, feedlot producers also benefited in preconditioning programs and were able to pay premiums for preconditioned calves (Dhuyvetter et al., 2005). Previous research supports preconditioning programs being highly effectiveness in reducing morbidity associated with BRD, however, their application is not widespread (Duff and Galyean, 2007).

Vaccines

Vaccination is an integral part of preconditioning programs, and a sound relationship should be established between veterinarians, nutritionists, and farm managers to ensure an effective health management program is being followed (Duff and Galyean, 2007). Vaccination programs are established within cattle herds to raise the level of resistance to viruses and other potential pathogens prior to the disease challenge (Anonymous, 2005). The use of vaccinations can improve reproductive efficiency by reducing infertility, abortions, and embryonic and fetal deaths (APHIS, 2010). Vaccines contain antigens of disease, which stimulate cattle's immune systems thereby creating an immune response before natural exposure to disease-causing agents (Wenzel and Mathis, 2015). Modified live vaccines (**MLV**) contain live modified microbes that have the antigens for a disease-causing agent but they do not actually cause the disease and killed vaccines contain components of an antigen or just pieces of the disease (Wenzel and Mathis, 2015).

Vaccines are available for several infectious diseases of cattle and vaccines for BHV1, PI3, BVD, and BRSV should be included in preconditioning programs (Bagley, 1997; Duff and Galyean, 2007). The Value Added Calf program recommends calves be vaccinated 4 to 6 wk

prior to weaning with an injectable vaccine and an intranasal vaccine followed by revaccination with MLV to be administered at weaning (Anonymous, 2005). However, if vaccination prior to weaning is not economically feasible for the producer, it is recommended that calves be vaccinated at weaning and again 14 to 21 d later (Duff and Galyean, 2007).

Infectious bovine rhinotracheitis is a viral infection of the upper respiratory tract and is present in almost all cattle herds (Bagley, 1997). Modified live virus vaccines and killed or attenuated products are available for administration against BHV1 (Bagley, 1997). Some of the vaccines available are designed to be administered either intramuscularly (IM) or intranasally (IN), and IN vaccines can be used around or for pregnant cows (Bagley, 1997). Previous research evaluated using a single IN vaccination with BHV1 and suggested that it could provide significant protection in the face of maternal antibodies and in combination with a booster could result in prolonged protection (Patel, 2005). Bagley (1997) suggested IN vaccines would cause an antibody response within 3 d and could be useful in the event of an outbreak of the disease. Parainfluenza-3 virus is often included in BHV1 vaccines and could be used on the same schedule listed for the vaccination (Bagley, 1997).

Bovine viral diarrhea virus is another virus present in almost all herds and a number of MLV and killed vaccines are available; however, killed vaccines require 2 doses in order to stimulate good immunity (Bagley, 1997). Zimmerman et al. (2006) found that a single dose of a MLV of BVDV administered at 4 to 5 wk of age stimulated strong protective immunity to challenge an infection with virulent type 2 BVDV. Bovine respiratory syncytial virus, although relatively new, is identified in respiratory infections nationally and both MLV and killed vaccinations are available for administration (Bagley, 1997).

Vaccines intended for prevention of BRD have been manufactured for over a century (Mosier et al., 1989). Hjerpe (1990) found the use of antibiotics in cattle vaccinated with *Mannheimia haemolytica* within 3 d before or 7 d after vaccination is not recommended due to inhibiting replication of vaccine organisms. Therefore, the use of antibiotics in calves upon arrival at feedlots makes it impractical to use live bacterial vaccines (Rice et al., 2008). Recent studies have begun to focus on induction of immunity to *Mannheimia haemolytica* by a mucosal delivery of the antigen (Rice et al., 2008). Plants have been engineered for the expression of *Mannheimia haemolytica* antigens aiming to produce a transgenic edible vaccine (Lee et al., 2001).

Research in vaccinating cattle with non-living *Pasturella multocida* vaccines are limited (Dabo et al., 2008). Vaccines currently available for use in cattle against *Pasturella multocida* are predominantly traditional bacterins as well as a live streptomycin-dependent mutant (Dabo et al., 2008). Vaccination of calves with vaccines containing MLV parainfluenza-3 virus, killed *Mannheimia haemolytica*, and killed *Pasturella multocida* vaccine enhanced resistance against an experimental induced challenge involving stress and aerosol exposure to PI3, *Mannheimia haemolytica*, and *Pasturella multocida* (Matsuoka et al., 1966). However, in recent research neither intratracheal or aerosol vaccination of calves with formalin *Pasturella multocida* in the face of an experimental challenge resulted in protection (Confer et al., 1996; Dowling et al., 2004; Prado et al., 2005).

Diet

Nutritional status of cattle prior to the onset of BRD is critical to the outcome of the challenge of the disease (Duff and Galvayan, 2007). Nutritional background of cattle used in experiments with BRD is typically unknown, therefore, little is known on the effects of the plane

of nutrition prior to a BRD challenge on the health and immunity of beef cattle (Duff and Galyean, 2007). The variation in nutritional status among animals could explain the variation in response to nutritional supplements: protein, minerals, and vitamins, that are evident in published BRD literature (Duff and Galyean, 2007). Energy restriction not resulting in malnutrition is known to increase the life span of rodents as well as benefiting their immune systems (Pahlavani, 2000). Yet, energy restricted mice did not survive an influenza infection possibly due to diminished innate immune function (Ritz and Gardner, 2006). Rivera et al. (2005) reported that a greater concentrate, milled diet is likely to provide the optimal receiving diet for lightweight, newly received calves under high stress with limited effects on BRD.

Studies in mice fed protein-free diets for duration of 2 to 3 wk showed that bactericidal immune defenses were not adversely affected, however adversely affected weight loss (Jakab et al., 1981). However, a protein deficiency in the mice resulted in a failure to eliminate influenza virus from the lungs and the viral infection ultimately suppressed bactericidal defenses (Jakab et al., 1981). Previous research, (Whitney et al., 2006; Galyean et al., 1999), found calves fed diets consisting of greater crude protein (CP) increased the likelihood of being diagnosed with BRD due to elevated body temperatures.

Supplemental Zn has shown beneficial effects for the prevention of pneumonia and diarrhea, or used as an adjuvant to an antimicrobial therapy for treatment of pneumonia, typically seen in Zn deficient children (Hambidge, 2006). Therefore, it is likely that beneficial effects on supplemental nutrients on immunity and challenge of BRD would be most likely seen in cattle with marginal or deficient status of the nutrient (Duff and Galyean, 2007).

Vitamin A plays an important role in immune function and deficiency of vitamin A in humans and rats increased severity of infection (Twining et al., 1997). In regards to a BRD

infection, it is important to supplement vitamin A to calves with a marginal or a known deficiency with an injection of vitamin A as the quickest route to increasing body stores (Duff and Galyean, 2007). However, in the absence of a deficiency, it does not seem that supplementing calves with vitamin A had any major effects on the incidence of a BRD infection (Duff and Galyean, 2007).

Vitamin E in different concentrations in receiving diets did not affect ADG, DMI, or G:F ratio (Rivera et al., 2002). Carter et al. (2005), showed the greatest dose of vitamin E tended to decrease the retreatment rate of BRD. Research is generally supportive of supplemental vitamin E on BRD morbidity (Duff and Galyean, 2007).

Ancillary therapies

Ancillary therapy (ANC) is commonly given in conjunction with an antimicrobial when treating calves susceptible to BRD (Wilson et al., 2015). Standard protocol to combat a BRD infection is to administer an injectable antimicrobial; however, it is common for an ANC to be prescribed and given along with the antimicrobial (Wilson et al., 2015). The intention of the ANC is not only to replace the use of an antimicrobial, but to help improve response to BRD in calves also treated with antibiotics (Wilson et al., 2015). Improvement by ANC can be accomplished by relieving harmful effects of inflammation, blocking histamine activity, and aiding in boosting the immune system in the defense against the pathogens (Apley, 1994).

The most common forms of ANC include antihistamines, B vitamins, corticosteroids, direct-fed microbials, nonsteroidal anti-inflammatory drugs (NSAID), viral vaccines, and vitamin C (NAHMS, 2001; Terrell et al., 2011; NAHMS, 2013). The use of NSAID is the most consistent ANC in decreasing high rectal temperatures in BRD calves; however, the response is often short-lived and there are typically no differences found in rectal temperature at the end of

evaluation (FDA, 2009; Apley, 2010; Francoz et al., 2012; Wilson et al., 2015). In a recent study, Wilson et al. (2015) found that the use of no ANC resulted in greater body weight at time of calves' third treatments, improved ADG between second and third treatments, and calves lived numerically longer than the calves supplemented with ANC: NSAID, vitamin C, and a viral vaccine. This study did not support the use of ANC being administered to calves experiencing a severe natural immune challenge during the receiving period.

Bovine Microbiome

The microbiome analysis is a fairly new field of study; however, the linkage among the microbial community and health outcomes in monogastrics has been well documented along with demonstrated evidence for species-dependent health consequences (Penner et al., 2014). Microbiome is defined as a community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and the collection of microorganisms living in or on the human body (Merriam-Webster, 2016). Research on the microbiome using next generation non-culture technologies has shown that the microbial species cultured from the rumen over the past several decades represents only a small fraction of the diversity in the rumen (Kohn, 2015).

Although a multitude of microorganisms are found throughout the digestive tract, the microbiota in the rumen possesses a true symbiotic relationship with the apparent host (Church, 1988). Microorganisms including: bacteria, protozoa, and anaerobic fungi depend on the rumen microbial population to provide the physiological conditions necessary for their existence. These microorganisms are essential for digestion and fermentation of the large amounts of fibrous feeds, which ruminants metabolize and utilize while other animals excrete the product without utilization (Church, 1988). More than 200 species of bacteria and 100 species of protozoa have been found to reside in the rumen by culture based techniques; however, culture based

techniques are not fully suitable for characterizing the overall microbial diversity due to species that are not yet culturable (Chaucheyras-Durand and Ossa, 2014). Recent research using quantification by real-time PCR has shown that some uncultured bacteria are as abundant as some of the major known bacteria in the rumen (Kim et al., 2011). The microorganisms within the rumen are affected by diet composition (Chaucheyras-Durand et al., 2012), host genetics (Benson et al., 2010), and by environmental factors (Uyeno et al., 2010). As of 2010, the Ribosomal Database Project found the diversity of bacteria and archaea in the rumen amounts to 13,478 bacterial and 3,516 archaeal sequences (Kim et al., 2011) and from that 7,000 species of bacteria and 1,500 species of archaea were estimated (Chaucheyras-Durand and Ossa, 2014). The rumen microbiota is established very soon after birth and by a precise sequence that is reflected by a decrease in aerobic and facultative taxa and an increase in anaerobic taxa (Chaucheyras-Durand and Ossa, 2014). Some of the rumen bacteria are essential when it comes to mature rumen function (cellulolytic bacteria) and can be detected after birth and up until the arrival of solid plant material in the diet (Chaucheyras-Durand and Ossa, 2014). Understanding the rumen microbial ecology and diversity is important to determine the species present and the roles they play within the ecosystem (Chaucheyras-Durand and Ossa, 2014).

The bovine nasopharynx is a niche for opportunistic pathogens and is also a known site of entry for viral infectious agents prevalent in BRD cattle (Holman et al., 2015b). Microbiome research associated with BRD has focused on pathogens known to be present in the onset of BRD by using culture-based methods. Along with the rumen, the microbiome within the nasopharyngeal tract is home to many bacteria that have not yet been cultured. The pathogens associated with BRD colonize the upper respiratory tract of both healthy and sick cattle, which makes them opportunistic pathogens, having the ability to cause disease under certain conditions

(Allen et al., 1991, Timsit et al., 2016a). Understanding the role of the nasopharyngeal microbiota as it evolves and changes after calves are introduced into the feedlots is vital to cattle susceptible to BRD (Timsit et al., 2016a). Round and Mazmanian (2009) found that disturbances of the human gut such as medical procedures and lifestyle changes have been linked to an increased risk of disease. This could also correlate to the nasopharyngeal microbiota of cattle, and any disturbance could lead to an increase susceptibility of disease (Timsit et al., 2016a). Previous studies with broad isolation and identification from the upper respiratory tract of cattle have reported some common results and with continued research in the microbiome field more will be discovered (Timsit et al., 2016a).

Research looking into the nasopharyngeal microbiota of feedlot calves found that calves diagnosed with BRD had a significantly different microbiota when compared to healthy calves (Holman et al., 2015a). This research suggests that the nasopharyngeal microbiota is severely and negatively affected by the presence of BRD (Holman et al., 2015a), much like disturbances in the gut microbiota has been linked to an increased risk of disease (Round and Mazmanian, 2009). It has been found that the nasopharyngeal microbiota are primarily dominated by two phyla, *Proteobacteria* and *Firmicutes* (Holman et al., 2015a) that also reside in the rumen microbiota (Chaucheyras-Durand and Ossa, 2014). As calves are arriving to feedlots, the nasopharyngeal microbiota is undergoing significant changes from weaning, to feedlot arrival, and throughout feedlot duration (Timsit et al., 2016b). The continuation of research within this field can explore the relevance of the variation in the microbial populations and how it can be used to target BRD susceptibility (Timsit et al., 2016b).

Zinc

Zinc has been known to be an essential dietary mineral for certain lower forms of life for over a century and for higher plants since 1926 (Underwood and Suttle, 1999). Zinc was determined to be necessary for growth and health in rodents; shortly thereafter zinc deficiency was produced experimentally in pigs, poultry, lambs and calves (Todd et al., 1934). Zinc deficiency is associated with severe inappetence and growth depression, impaired reproductive performance, and abnormalities of the skin. Zinc has been determined to cure and prevent parakeratosis (a thickening and hardening of skin) in pigs while a Zn deficiency can occur in commercial type rations from excess calcium (Tucker and Salmon, 1955). Since carbonic anhydrase was discovered to be a zinc metalloenzyme (Keilin and Mann, 1940), wide ranges of zinc-responsive pathological conditions have been discovered in animals and humans.

Concentrations of zinc are widely distributed in the body from the retina of the eye, to bone, muscles, and blood. Absorption of Zn in ruminants is an active, carrier-mediated (saturable) process dependent on need (Suttle et al., 1982; Davies, 1980). The small intestine is the primary site of Zn absorption in the small intestine (Davies, 1980). Zinc absorption is limited at high intakes due to mucosal induction of the metal-binding protein metallothionein and other dietary factors can set a ceiling for absorption (Cousins, 1996). This is done by interfering with the process of absorption or limiting the amount that is available to be absorbed (Underwood and Suttle, 1999). Zinc is excreted predominantly through feces and some is excreted through endogenous losses in the saliva, bile, pancreatic and gastric juices. In addition, smaller amounts of Zn are excreted in the urine (Underwood and Suttle 1999). Miller et al. (1968) found that fecal endogenous losses decrease dramatically in calves given a diet low in Zn (2 mg/kg). The most important nutritional functions are those that have the potential to limit health and production when livestock are deprived of Zn.

The effect of supplemental Zn on cattle health has yielded conflicting results in previous research and the mechanism by which the mineral works in conjunction with the immune response is not fully understood (Galyean et al., 1999). When the animal is undergoing a Zn deficiency, the immune system does not function properly, which could cause an increase in susceptibility to disease (Suttle and Jones, 1989).

Influences of zinc on upper respiratory infections

The common cold in humans often begins with one of the 200 types of rhinovirus infection, which enters via nasal mucosa and can potentially proceed to a more widespread infection (Cohen, 2006). For a rhinovirus to enter into the nasal epithelium the virus must bind to the cellular receptor intracellular adhesion molecule-1 (ICAM-1); (Cohen, 2006). In humans, Zn acts as a competitive inhibitor of ICAM-1, which prevents the virus from penetrating the cell wall and replicating (Cohen, 2006). After the recognition of the potential positive effects of Zn supplementation, an alternative method has been developed to help ward off the common cold in humans, such as throat lozenges and intranasal sprays which apply Zn ions directly to the site of the rhinovirus infection (Cohen, 2006).

Bovine respiratory disease complex in its upper-respiratory form is very similar to the common cold in humans with symptoms such as coughing, difficulty breathing, ocular discharge, fever, and decreased appetite (Bagley, 1997). It is known that the cause of bovine respiratory disease is multifaceted with the main factors being a combination of stress, and a viral or bacterial infection (Bagley, 1997). In the case of a viral infection contributing to the presence of BRD, antibiotics administered only combat possible secondary bacterial infections. The use of mucosal applications of Zn solutions could potentially have a positive effect on growth performance and the immune response of cattle that are highly susceptible to bovine respiratory

disease. The application of Zn in concert with antibiotics in BRD susceptible calves could have a positive effect on their immune response and growth performance.

CHAPTER III

Efficacy of a novel intranasal zinc solution on health and growth performance of high-risk,
newly received stocker cattle

Abstract:

The objective of this study was to determine if using an intranasal zinc (**Zn**) solution would impact health and growth performance of high-risk stocker cattle. Male beef calves (n = 239; 3 arrival dates [block]; initial BW = 276 ± 2.4 kg) were stratified by arrival gender and BW into 2 treatments: 1) treated with 3 ml of a Zn solution containing 36.24 mg of Zn administered intranasally on d 0, or 2) control, in which calves were not treated. Calves were observed daily for signs of morbidity and a clinical illness score (1 [normal] to 5 [morbid]) was recorded. Cattle that scored > 1 and had a rectal temperature greater $\geq 40^{\circ}\text{C}$ were treated with an antibiotic. If rectal temperature $\geq 40^{\circ}\text{C}$ persisted past first antibiotic post-treatment interval, cattle were re-treated according to a pre-planned protocol. Body weights were similar across treatments throughout the duration of the study ($P \geq 0.22$). Calves treated with Zn had a lower ADG from d 7 to 28 and d 14 to 28 compared to the control ($P < 0.01$). Control calves tended to be treated with 3 antibiotics more often than Zn treated calves ($P = 0.06$). Overall antibiotic costs did not differ between treatments ($P = 0.64$). There were no differences ($P \geq 0.10$) for rectal temperatures of calves due to treatment. From the results of this study, calves treated upon arrival intranasally with a Zn solution had no differences in overall growth performance and minimal differences in morbidity compared to control calves.

Keywords: Bovine Respiratory Disease, Zinc, Morbidity

INTRODUCTION

Bovine respiratory disease (**BRD**) can decrease total economic output due to increased medical costs and labor, decreases in production, and death loss (Guzel et al., 2010). Newly received cattle are at a high risk for contracting BRD due to stress associated with weaning, marketing, and travel (Duff and Galyean, 2007). Stress negatively affects the immune system (Blecha et al., 1984) at a time when calves are exposed to new bacterial and viral pathogens associated with BRD.

Zinc is an essential dietary mineral necessary for overall growth and health (Todd et al., 1934). Zinc holds antiviral properties and evidence suggests adequate intake of vitamin C and Zn can ameliorate symptoms shortening respiratory tract infections (Wintergerst et al., 2006). Zinc deficiencies can lead to impaired immune response resulting in an altered resistance to infection (Wintergerst et al., 2006), negatively affecting growth performance. Previous research has shown supplementing Zn in the diet can increase ADG in calves (Spears and Kegley, 2002). Supplemental Zn (10 to 30 mg) could be an adjuvant therapy for treating infectious diseases in Zn deficient children (Wintergerst et al., 2006).

Bovine respiratory disease in the upper respiratory tract is similar to the common cold in humans with symptoms such as coughing, difficulty breathing, ocular discharge, fever, and decreased appetite (Bagley, 1997). For a rhinovirus to enter into the nasal epithelium, the virus must bind to an intracellular adhesion molecule-1 (ICAM-1), which is a cellular receptor (Cohen, 2006). Zinc acts as a competitive inhibitor of ICAM-1, which disrupts the virus's ability to penetrate the cell wall and replicate (Cohen, 2006).

Drug companies have developed alternative methods to help mitigate the common cold, including throat lozenges and intranasal sprays aiming to reduce severity and duration of the cold

by applying Zn ions directly to the site of the rhinovirus infection (Cohen, 2006). Alternatively applying Zn solutions in addition to antibiotics could have a positive effect in cattle afflicted with BRD immune response and growth performance. The objective of this study was to evaluate whether a mucosal application of Zn would impact health and growth performance in high-risk, newly received stocker calves.

MATERIALS AND METHODS

The University of Arkansas Animal Care and Use Committee approved protocols and procedures used in this study, approval # 15030. A total of 240 male beef calves (initial BW = 276 ± 2.4 kg) were obtained from regional auction markets on 3 different dates: January 22, 2015 (Block 1, n = 82), March 9, 2015 (Block 2, n = 76), and March 30, 2015 (Block 3, n = 82). One calf within block 2 was removed from the study due to refusing to drink from the waterer. Upon arrival, cattle were identified with individual ear tags, weighed (unshrunk), arrival castrate status recorded, rectal temperature taken, and an ear was notched for detection of persistent infection of bovine viral diarrhea (PI-BVDV), with ear notches sent to Cattle Stats, LLC (Oklahoma City, OK) for analysis. All cattle were negative for PI-BVDV. Cattle were vaccinated for clostridial (Covexin 8, Merck Animal Health, Madison, New Jersey) and respiratory viruses including: infectious bovine rhinotracheitis (IBR), parainfluenza virus 3 (PI3) and bovine viral diarrhea (BVD); (Titanium 5+ PH-M, Elanco, Greenfield, Indiana). An anthelmintic was administered to control internal and external parasites (Dectomax Pour-on, Zoetis, Florham Park, New Jersey). Bull calves within block 1 and 2 were castrated via banding (California bander, InoSol Co. LLC, El Centro, CA); bull calves in block 3 were surgically castrated.

Within block, calves were stratified by arrival castrate status and initial BW for allotment to 1 of 8 0.4-ha grass paddocks with 4 pen replications per treatment. Administration of

treatments occurred on d 0. Zinc treated calves were given 3 ml per nostril of a solution containing 36.24 mg of Zn administered intranasally using a single use nasal atomizer (Mucosal Atomization Device; Wolfe Tory Medical, Inc., Salt Lake City, UT) and a control with calves not given anything intranasally. The Zn solution consisted of 3.62% zinc acetate (**ZnAc**) and 0.9% sodium chloride (**NaCl**) in deionized water solution. Pens were arranged such that no fence-line contact occurred between treatments. The nasal atomizer device helped to provide exact dosing and volume to the area with the optimal particle size (30-100 microns) for proper application to the mucosal membrane. Cattle in block 1 (n = 82) were weighed on d 0, 1, 7, 14, 28, 45, and 46. Cattle in block 2 (n = 75) were weighed on d -1, 0, 1, 7, 14, 28, 48 and 49, and cattle in block 3 (n = 82) were weighed on d -1, 0, 1, 7, 14, 27, and 28. Variations between the end dates were associated with the poor weather in the spring of 2015.

Rectal temperatures were recorded from all calves on d 0, 1, 7, and 14 using a digital thermometer (Model No. M216, GLA Agricultural Electronics, San Luis Obispo, CA). Calves were given ad libitum access to bermudagrass hay and water. They were offered a grain supplement up to 1.8 kg/d on an as-fed basis of 68% corn, 26% dried distillers' grain, 1% salt, 2% limestone, and 2% molasses. The grain supplement also included a vitamin and trace mineral premix and provided 160 mg monensin/d (Rumensin, Elanco Animal Health; Table 1). The diet met and or exceeded all nutritional requirements (NRC, 1996).

Calves were observed at 0800 each morning to assess morbidity and a clinical illness score (1 [normal] to 5 [morbid]; Elanco Animal Health) was recorded. Calves were observed for the following symptoms: coughing, depression, ocular and nasal discharge. If a calf displayed 2 symptoms they were pulled from their pens and their rectal temperatures were taken. If calves scored > 1 on the illness score and had a rectal temperature greater than $\geq 40^{\circ}\text{C}$, calves were

treated with florfenicol (Nuflor, Merck Animal Health) per a preplanned treatment protocol. If a rectal temperature of $\geq 40^{\circ}\text{C}$ persisted past first antibiotic post-treatment interval of 2 d; cattle were re-treated with enrofloxacin (Baytril; Bayer Animal Health, Shawnee Mission, Kansas). Finally, if the calf had a rectal temperature $\geq 40^{\circ}\text{C}$ after the post-treatment interval for enrofloxacin of 2 d, ceftiofur (Excenel; Zoetis, Florham Park, New Jersey) was given as a final antibiotic. Ceftiofur was administered over 3 consecutive days, and the calves were rechecked 48 h after the final treatment. Calves were considered chronic if they were treated with all 3 treatment antibiotics and gained less than 0.23 kg per day. After calves were treated with any antibiotic, they were penned by treatment into sick pens, until their rectal temperature was $< 40^{\circ}\text{C}$.

Statistical Analyses

Growth performance, economic costs, and rectal temperatures were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Percentage morbidity was analyzed using the GENMOD and FREQ procedures of SAS to determine Chi-square probability. The experimental unit utilized was pen while individual calf served as the observational unit. Calf arrival date (block) and gender were the random effects while treatment was the lone fixed effect. Significance was declared at $P \leq 0.05$, with tendencies between 0.05 and 0.10.

RESULTS AND DISCUSSION

Body weights (Table 2) were not different ($P \geq 0.22$) across treatment throughout the duration of the study. Calves treated with Zn had a lesser ($P < 0.01$), ADG from d 7 to 28 and d 14 to 28 when compared to control. This result is comparable to Guernsey et al. (2009), who found that calves treated with intranasal Zn also had a lesser ADG when compared to controls

and oral Zn groups. Overall ADG throughout the entirety of the study did not differ ($P = 0.98$) between treatment groups, (1.01 kg/d).

Rectal temperatures were not different ($P = 0.10$) for cattle across both treatments for the duration of the study (Figure 1). Guernsey et al., (2009) found calves treated with Zn nasal spray had greater rectal temperatures initially, but no other differences in rectal temperatures were observed throughout the remainder of the study. Administrations of first and second antibiotics (Table 3) were not different ($P \geq 0.22$) in both treatments. Control calves tended ($P = 0.06$) to be treated with 3 antibiotics more often than Zn treated calves. Therapeutic trials using intranasal ZnAc in humans have shown reductions in overall duration of symptoms and severity (Wintergerst et al., 2006), which could explain the control calves tendency to being treated more frequently. Overall antibiotic costs did not differ across treatments ($P = 0.64$). Although costs between treatments were not statistically different, there was a numerical \$2.09 difference between the treatments (Table 3). Similarly, number of antibiotics given/calf was not affected ($P = 0.14$), but was numerically lower for the control calves. A calf in each treatment group died during the study. Eight calves throughout the duration of the study were deemed chronic ($P = 0.72$), of which 5% were of the control group and 3% were treated with Zn. Relapse rate (cattle treated with a second antibiotic) were not different ($P = 0.40$) across both treatments.

The nasal atomizer used to deliver the spray to the calves, was a little uncomfortable for the calves as well as awkward for the handler as also seen by Guernsey et al. (2009). Therefore, calves may not have received Zn at the desired level. Zinc treated calves were restrained longer in the chute than the control calves for the administration of the intranasal solution. It has been determined in humans that an intranasal Zn spray leaves the individual with a burning sensation in their nasal passage (Hirt et al., 2000). The greater chute time and potential burning sensation

associated with the application of the Zn could have applied more stress, therefore negatively affecting their immune systems. Previous research has reported that calves under greater stress are more likely to have decreased feed intake (Galyean and Hubbert, 1995; Cole, 1996), which coincides with the decrease in ADG for Zn treated calves. Zinc supplemented in the diet, regardless of the Zn source, has been shown to increase ADG (Spears and Kegley, 2002). In the present study, calves were treated one time with a Zn solution in comparison to being treated with Zn in the diet at a daily rate; however, if treated more frequently with the intranasal Zn solution it is possible the outcome of ADG in the Zn treated calves could differ from our current results.

The use of a Zn nasal spray in humans can result in anosmia, or a loss of sense of smell (Cohen, 2006) and a decrease in smell can lead to taste disturbance, which leads to a loss of pleasure from eating resulting in changes in weight (Gaines, 2010). It is possible, that the Zn treated calves began to display anosmia, explaining the decrease in ADG from d 7 to 28 and 14 to 28. Zinc ions have been reported to be toxic to the olfactory epithelium derived from ion concentration and individual sensitivity, but it is not affected by the source of the ion (Jafek et al., 2004).

Implications

Intranasal Zn did not appear to have a positive effect on ADG or to decrease morbidity and mortality in calves susceptible to BRD. Blindly treating calves appears to have a negative effect on performance, however, targeting treatment to calves with BRD could be more efficacious. Anosmia derived from the treatment of Zn may have had a negative effect on feed intake.

Table 1: Ingredient composition of grain supplement on an as-fed basis

Ingredient	%
Cracked corn	68.36
Dried distillers' grain	26
Salt, white	1
Limestone	2
Molasses	2
Vitamin A, D, E premix ^a	0.1
Vitamin E, premix ^b	0.05
NB-8675 Ruminant trace mineral, premix ^c	0.085
Corn/Rumensin, premix ^d	0.4

^a Contained 8,800,000 IU/kg Vitamin A, 1,760,000 IU/kg Vitamin D, and 1,100 IU/kg Vitamin E

^b Contained 44,000 IU/kg

^c Contained 12% Zn, 8% Mn, 4% Cu, 1% Fe, 500 mg Co, 2,000 mg I, and 600 mg Se/kg

^d Provided 160 mg/d monensin (Elanco Animal Health)

Table 2: Effect of intranasal Zn on growth performance of high risk cattle

Item	Treatment ¹		SEM ²	<i>P</i> - value
	Control	Zn		
Body weight, kg				
Initial	275.8	276.8	1.3	0.60
Day 7	278.3	278.8	1.5	0.83
Day 14	283.6	285.1	1.7	0.55
Final	315.1	312.4	2.1	0.38
Average daily gain, kg				
Day 0 to 7	1.4	1.5	0.19	0.65
Day 0 to 14	1.0	1.2	0.11	0.34
Day 0 to 28	0.9	0.8	0.05	0.67
Day 7 to 14	0.8	0.9	0.09	0.23
Day 7 to 28	0.9	0.7	0.06	0.04
Day 14 to 28	1.0	0.7	0.08	0.01
Day 28 to 45	1.0	1.2	0.08	0.21
Overall	1.01	1.01	0.05	0.98

¹Control- receiving no intranasal treatment; Zn- receiving 3ml per nostril of a 36.24 mg intranasal Zn solution

²SEM- Standard error of the mean

Table 3: Effect of intranasal Zn on the morbidity of high risk cattle

Item	Treatment		<i>P</i> - value
	Control	Zinc	
Morbidity, %			
Treated once	71.7	67.2	0.40
Treated twice	36.7	29.4	0.22
Treated thrice	17.5	9.2	0.06
Chronic ^a	4.2	3.4	0.72
Dead	0.83	0.84	0.98
Relapse ^b	51.2	43.8	0.40
Antibiotic cost, \$/calf ¹	24.75	22.66	0.64
Antibiotics used/calf ²	1.26	1.05	0.14

^a Chronic was declared if calf was treated with an antibiotic 3 times and gained less than 0.23 kg/d

^b Relapse denotes animals treated with a second antibiotic

¹ Standard error of the mean = 3.12

² Standard error of the mean = 0.09

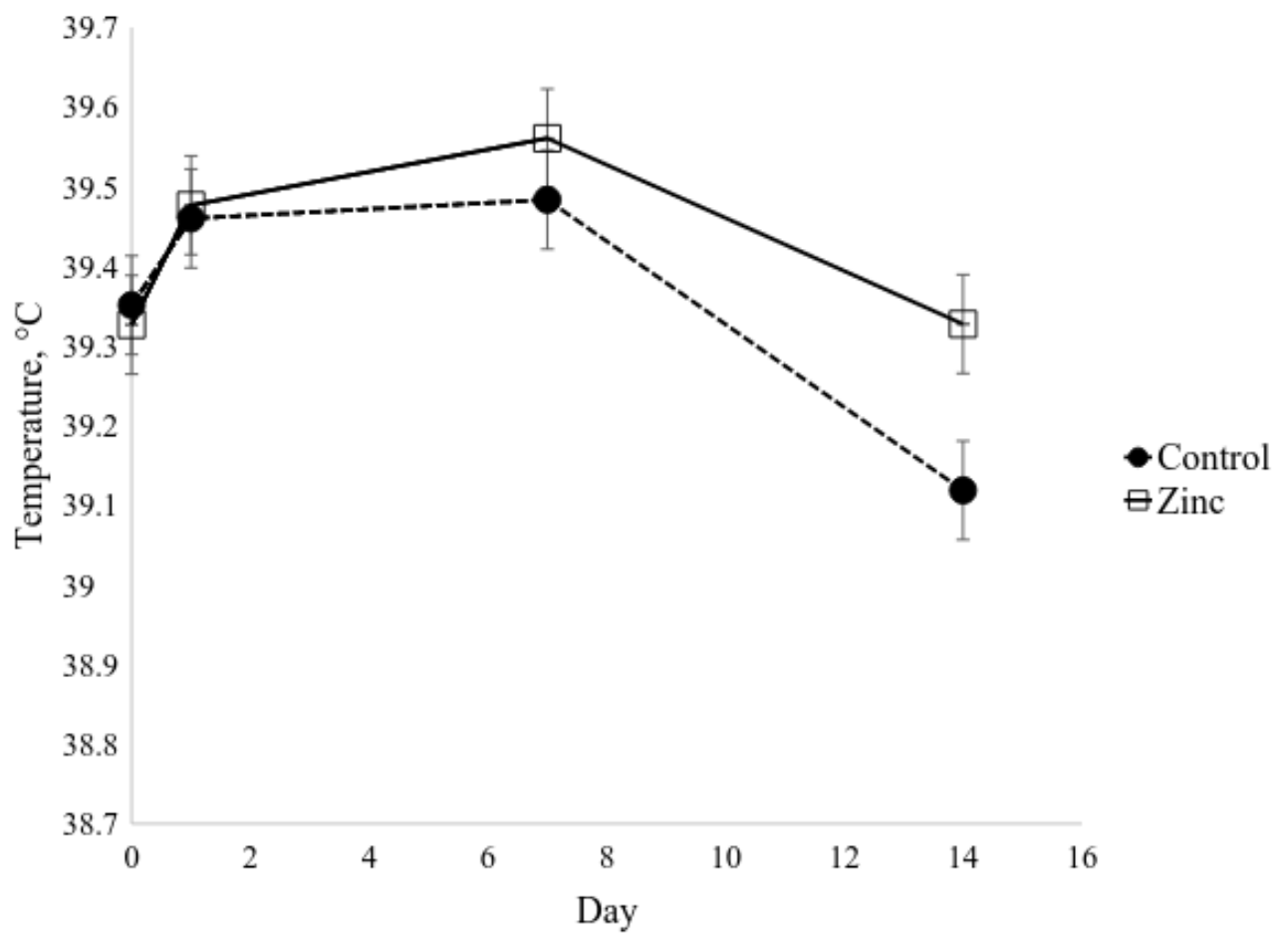


Figure 1. Effect of intranasal Zn on rectal temperatures in high-risk newly received beef cattle. Treatment x day, $P = 0.17$; day, $P < .0001$; treatment, $P = 0.10$.

CHAPTER IV

Efficacy of a novel intranasal zinc solution on bacterial populations in the nasal membranes of
high-risk, newly received stocker cattle

Abstract:

The objective of this study was to determine if using an intranasal zinc (**Zn**) solution would impact bacterial populations associated with bovine respiratory disease (**BRD**). Male beef calves (n = 76; initial BW = 277 ± 2.2 kg) were stratified by arrival gender and BW into 2 treatments: 1) treated with 3 ml of a Zn solution containing 36.24 mg of Zn administered intranasally, or 2) control, in which calves were not treated. In addition, nasal swabs were collected from 24 calves (12/treatment) and cultured for bacterial pathogens on d 0, 1, 2, and 7. Calves were observed daily for signs of morbidity and a clinical illness score (1 [normal] to 5 [morbid]) was recorded. Cattle that scored > 1 on the illness score and had a rectal temperature greater $\geq 40^{\circ}\text{C}$ were treated with an antibiotic. If rectal temperature $\geq 40^{\circ}\text{C}$ persisted past first antibiotic post-treatment interval, cattle were re-treated according to pre-planned protocol. If cattle were treated with an antibiotic for BRD there were classified as sick. With the exception of *Bacillus* spp. and *Pseudomonas aeruginosa* the prevalence of bacterial pathogens were not different ($P \geq 0.14$) between treatments. The presence of *Pseudomonas aeruginosa* was greater ($P \leq 0.04$) in the control compared to the Zn treated calves and *Bacillus* spp. tended ($P = 0.09$) to be greater in control calves. Swabs from d 7 had significant operational taxonomic units (**OTUs**) when comparing healthy vs. sick calves through LEfSe, however there were no differences between control and Zn treatment. Differences were found when comparing treatments in cattle that got sick (Zn and control), but there were no differences between treatments in cattle that were healthy. From the results of this study, calves treated intranasally with Zn showed minimal differences in bacterial populations in the nasal membranes compared to the control.

Keywords: Bovine Respiratory Disease, Zinc, Pathogens

INTRODUCTION

Bovine respiratory disease (**BRD**) can decrease total economic output due to increased medical costs and labor, decreases in production, and death loss (Guzel et al., 2010). Newly received cattle are at a high risk for contracting BRD due to stress associated with weaning, marketing, and travel (Duff and Galyean, 2007). Stress negatively affects the immune system (Blecha et al., 1984) at a time when calves are exposed to new bacterial and viral pathogens associated with BRD.

The study of microbial effects on human health has demonstrated evidence for species-dependent health consequences (Penner et al., 2014). Many of the pathogens associated with BRD are considered to be opportunistic as many reside in the upper respiratory tract of healthy and sick cattle (Allen et al., 1991; Timsit et al., 2016a). Understanding the nasopharyngeal microbiota as it changes upon feedlot arrival is vital to the health of calves susceptible to contracting BRD (Timsit et al., 2016a). Previous research correlates disrupting the microbiome could lead to an increase in susceptibility of disease and with continued research more will be discovered (Timsit et al., 2016a).

Zinc is an essential dietary mineral necessary for overall growth and health (Todd et al., 1934). Zinc holds antiviral properties and evidence suggests adequate intake of vitamin C and Zn can ameliorate symptoms shortening respiratory tract infections (Wintergerst et al., 2006). Zinc deficiencies can lead to impaired immune response resulting in an altered resistance to infection (Wintergerst et al., 2006), negatively affecting growth performance.

Bovine respiratory disease in the upper respiratory tract is similar to the common cold in humans with symptoms such as coughing, difficulty breathing, ocular discharge, fever, and decreased appetite (Bagley, 1997). For a rhinovirus to enter into the nasal epithelium, the virus

must bind to an intracellular adhesion molecule-1 (ICAM-1), which is a cellular receptor (Cohen, 2006). Zinc acts as a competitive inhibitor of ICAM-1, which disrupts the virus's ability to penetrate the cell wall and replicate (Cohen, 2006). The objective of this study was to evaluate whether a mucosal application of Zn would impact health and growth performance in high-risk, newly received stocker calves.

MATERIALS AND METHODS

The University of Arkansas Animal Care and Use Committee approved protocols and procedures used in this study, approval # 15030. A total of 76 male beef calves (initial BW = 277 \pm 2.2 kg) were obtained from regional auction markets. One calf was removed from the study due to refusing to drink from the waterer. Upon arrival, cattle were identified with individual ear tags, weighed (unshrunk), arrival castrate status recorded, rectal temperature taken, and an ear was notched for detection of persistent infection of bovine viral diarrhea (PI-BVDV), with ear notches sent to Cattle Stats (Oklahoma City, OK) for analysis. All cattle were negative for PI-BVDV. Cattle were vaccinated with a clostridial (Covexin 8, Merck Animal Health, Madison, New Jersey) and 5-way modified live against respiratory viruses including: infectious bovine rhinotracheitis (IBR), parainfluenza virus 3 (PI3) and bovine viral diarrhea (BVD) (Titanium 5+ PH-M, Elanco, Greenfield, Indiana). An anthelmintic was administered to control internal and external parasites (Dectomax Pour-on, Zoetis, Florham Park, New Jersey). Bull calves were castrated via banding (California bander, InoSol Co. LLC, El Centro, CA).

Calves were stratified by arrival castrate status and initial BW for allotment to 1 of 8 0.4-ha grass paddocks with 4 pen replications per treatment. Administration of treatments occurred on d 0. Zinc treated calves were given 3 ml per nostril of a solution containing 36.24 mg of Zn administered intranasally by a single use nasal atomizer (Mucosal Atomization Device, Wolfe

Tory Medical Inc., Salt Lake City, UT), and a control with calves not given anything intranasally. The Zn solution consisted of 3.62% zinc acetate (**ZnAc**) and 0.9% sodium chloride (**NaCl**) in deionized water solution. The nasal atomizer device helped to provide exact dosing and volume to the area with the optimal particle size (30-100 microns) for proper application to the mucosal membrane. Pens were arranged such that no fence-line contact occurred between treatments.

Calves were observed at 0800 each morning to assess morbidity and a clinical illness score (1 [normal] to 5 [morbid]; Elanco Animal Health) was recorded. Calves were observed for the following symptoms: coughing, depression, ocular and nasal discharge. If a calf displayed 2 symptoms they were pulled from their pens and their rectal temperatures were taken. If calves scored > 1 on the illness score and had a rectal temperature greater than $\geq 40^{\circ}\text{C}$ calves were treated with florfenicol (Nuflor, Merck Animal Health, Madison, New Jersey) per a preplanned treatment protocol. If a rectal temperature of $\geq 40^{\circ}\text{C}$ persisted past first antibiotic post-treatment interval of 2 d; cattle were re-treated with enrofloxacin (Baytril; Bayer Animal Health, Shawnee Mission, Kansas). Finally, if the calf had a rectal temperature $\geq 40^{\circ}\text{C}$ after the post-treatment interval for enrofloxacin of 2 d, ceftiofur (Excenel; Zoetis, Florham Park, New Jersey) was given as a final antibiotic. Ceftiofur was administered over 3 consecutive days, and the calves were rechecked 48 h after the final treatment. Calves were considered chronic if they were treated with all 3 treatment antibiotics and gained less than 0.23 kg per day. After calves were treated with any antibiotic, they were penned by treatment into sick pens, until their rectal temperature was $< 40^{\circ}\text{C}$. After calves were treated with any antibiotic, they were penned by treatment into sick pens, until their rectal temperature was $< 40^{\circ}\text{C}$. If cattle were treated at least once for BRD they

were considered sick, and calves that were never treated with antibiotics were considered healthy.

To monitor bacterial loads, 3 calves per treatment were selected randomly for culture-based bacterial swabs (BBL CultureSwab; Becton, Dickinson and company, Sparks, MD) and 2 of the 3 calves were again selected randomly to have a second set of swabs taken for microbiota analysis (BD Universal Viral Transport; Becton, Dickinson, and company, Sparks, MD). Sick calves were administered an antibiotic, 4 calves being treated on d 2, 2 on d 4, 1 on d 6 and 2 calves on the d 7 of collection. Bacterial swabs were taken on d 0 prior to administration of treatment and again on d 1, 2, and 7 of the trial. Immediately after, the second swabs were taken to the University of Arkansas and stored in an -80° C freezer until analysis. The culture-based bacterial swabs were taken to the University of Arkansas Division of Agriculture Veterinary Diagnostic Laboratory (Fayetteville, Arkansas) and were cultured on 5 different agar plates. Each swab was plated on a chocolate agar, Columbia CNA agar of 5% sheep blood, MacConkey agar, blood agar of TSA with 5% sheep blood, and a hektoen enteric agar (Remel Microbiology Products, Lenexa, KS). Laboratory personnel monitored the plates and qualitative scores were given 24 and 48 h after incubation. The presence or absence of culturable pathogens was noted and a qualitative score was assigned from 0 to 4 with 0 being not present, 1 being 1 to 4 colonies, 2 being 5 to 19 colonies, 3 being 20 to 99 colonies and 4 having more than 100 colonies.

The DNA was extracted from the second swabs taken on d 7 of the trial (n = 12; 6 control: 3 healthy and 3 BRD, 6 zinc: 3 healthy and 3 BRD) using PowerLyzer® UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories, INC., Carlsbad, CA) according to the manufacturer's protocol. The DNA concentration was measured using Nanodrop (Thermo Scientific). The DNA next-generation sequencing was performed on an Illumina MiSeq

sequencer. The V4 hypervariable region of the bacterial 16SrRNA gene was then amplified from the extracted DNA using bar-coded primers (forward: GTGCCAGCMGCCGCGGTAA, reverse: GGACTACHVGGGTWTCTAAT). Each 50 µl PCR reaction contained 50 ng DNA, 41 µl molecular biology grade water, 5 µl 10 x FastStart High Fidelity Reaction Buffer with 18 mM MgCl₂, 1 µl dNTPs (10 mM each), 1 µl Fusion Primer A (10 µM), 1 µl Fusion Primer B (10 µM), and 1 µl FastStart High Fidelity Enzyme Blend (5 U/µl). The PCR was performed at 95° C for 2 min; followed by 30 cycles of 95° C for 20 s, 50° C for 30 s, and 72° C for 5 min; followed by a final extension at 72° C for 10 min.

Sequences were processed and analyzed using mothur v1.36 (Schloss et al., 2011) following the Miseq SOP on the mothur wiki and Schloss et al. (2011). The make.contigs command was used to combine the data from all of the samples. The chimeric sequences were then removed using the Uchime algorithm (Edgar et al., 2011) and a preclustering methodology (Huse et al., 2010) was used to reduce sequencing noise. The sequences that had a length ≥ 275 bp, passed the sequencing error reducing, chimera detection, and the removal steps were then considered high quality sequences. The high quality sequences were then assigned to operational taxonomic units (**OTUs**) by using an average neighbor algorithm that has a 97% similarity cut off. The OTUs were then classified at the genus level by using the Bayesian method (Cole et al., 2009).

Statistical Analyses

Culture-based bacteria data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) to analyze both fixed and random factors. Day was used in the model as a repeated measure analysis. Pen and sex were the 2 random factors while treatment was the lone fixed effect. Individual calf served as the experimental and observational units. When treatment

x day interactions were not significant, main effects for treatment and day were reported. Percentage of bacteria was analyzed using the GENMOD and FREQ procedures of SAS to determine Chi-square probability. Significance was declared at $P < 0.05$, with tendencies between 0.05 and 0.10. For the swabs from d 7, specific OTUs that were differentially distributed across treatments were identified using a linear discriminant analysis effect size (LEfSe; Segata et al., 2010), which focuses on statistical significance as well as biological relevance.

RESULTS AND DISCUSSION

Culture-based method

Numerous culturable pathogens were isolated from the nasal passage of calves (Table 1). There were no treatment x day interactions for the prevalence of bacteria ($P \geq 0.15$). With the exception of *Bacillus* spp. and *Pseudomonas aeruginosa* the prevalence of bacterial pathogens were not different ($P \geq 0.14$) between treatments (Table 1). The presence of *Pseudomonas aeruginosa* was greater ($P \leq 0.04$) in the control compared to the Zn treated calves and *Bacillus* spp. tended ($P = 0.09$) to be greater in control calves. Two of the common BRD bacterial pathogens, *Pasteurella multocida* and *Mannheimia haemolytica*, were isolated from both treatments, but did not differ ($P = 1.0$). The presence of *Pasteurella multocida* was substantial in both treatments, however they were not different ($P = 1.0$). Guernsey et al. (2009) reported the application of intranasal Zn initially reduced the presence of the pathogen and then increased by d 7 to levels that were not different then the control group. The presence of *Mannheimia haemolytica* was also isolated from 10.4% of control calves and 14.6% of Zn treated calves; however, its presence was not different ($P = 1.0$) across treatments (Table 1). *Rhizopus* spp. was isolated from 6.3% of control calves and 2.1% of Zn treated calves; however, the presence

between treatments were not different ($P = 0.28$). Guernsey et al. (2009) found intranasally Zn treated calves possessed fewer colonies of *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp.; however, in the current study, there were no differences ($P \leq 0.63$) between treatments.

Prevalence of *Pasteurella multocida*, *Bacillus* spp., *Escherichia coli*, and *α -streptococcus* sp. decreased ($P \leq 0.005$) over the 4 culture dates (Table 2). *Mannheimia haemolytica* tended ($P = 0.08$) to increase over time. The prevalence of *Staphylococcus* spp. increased ($P < 0.0001$) over the duration of the 4 swab dates. Prevalence of fungus increased ($P < 0.0001$) during the 7 d experimental period; while prevalence of *Rhizopus* tended ($P < 0.07$) to be increased on d 1 and 2.

Bacterial scores for the different pathogens did not differ ($P \geq 0.20$) across treatment (Table 3). There were no treatment x day interactions for bacterial scores ($P \geq 0.31$). When bacterial scores were analyzed with day as a main effect, 8 different pathogens were affected ($P \leq 0.05$; Table 4). Scores of *Pasteurella multocida* decreased ($P < 0.0001$) on d 7 compared to earlier dates; however, scores for *Mannheimia haemolytica* did not ($P = 0.14$). Both are known primary bacterial pathogens associated with BRD. Scores for Lactose gram-negative rods tended to be affected by day ($P = 0.08$).

Bacterial phyla and genera differentially represented

Eleven different identifiable phyla were isolated and the distribution of the bacteria at the phylum level is illustrated in Figure 1a. The calves treated for BRD across treatments varied in bacterial communities. *Proteobacteria* was present in each of the nasal membranes of the sick calves across treatments. In the Zn treated BRD calves, *Proteobacteria* was as high as 64% and in the control BRD calves it was as high as 29% (Figure 1a). The sick calves displayed less

Proteobacteria in comparison to the healthy calves, which had a high of 89% in the Zn calves, and 93% in the control calves. It is possible the application of intranasal Zn, could have depleted the population of *Proteobacteria*. The phyla *Firmicutes*, *Bacteroidetes*, and *Tenericutes* were present in each sick calf, with *Tenericutes* more heavily distributed in the control calves treated for BRD in comparison to Zn treated (Figure 1 a). *Firmicutes*, *Tenericutes*, and *Bacteroidetes* were also present in the healthy calves; however, the distribution of each phylum was less in abundance in comparison to the sick calves. *Proteobacteria* is the phylum that houses multiple genera associated with BRD such as *Pasteurella* spp. and *Mannheimia* spp. (Holman et al., 2015b), which is more present in the healthy calves on d 7 in comparison to the sick calves. Both pathogens are considered to be opportunistic meaning they are present in both healthy and sick animals (Timsit et al., 2016a). In previous research, Holman et al. (2015b) found at feedlot entry a majority of sequences were of the *Proteobacteria* phylum and *Firmicutes* were present across all sequences while at d 60 of the trial, *Actinobacteria*, *Tenericutres*, and *Bacteroidetes* were isolated at a relative abundance of greater than 1% of sequences. The current study focuses on the nasal passages of stocker calves; however, it is interesting the phyla that are similar between treatments to the feedlot calves (Holman et al., 2015b) at both entry and d 60. This supports the variation and changes of the microbial community as the calves go through stocker as well as the feedlot phases of production.

The genus level analysis followed similar patterns, however 55 identifiable genera were isolated and their distribution is illustrated in Figure 1b. Calves treated for BRD displayed a greater population of *Escherichia/Shigella* as high as 38% in comparison to healthy calves with a high of 15%. The presence of *Escherichia/Shigella* was present in each of the control BRD treated calves in comparison to the Zn treated BRD calves and it was minimally present in

healthy calves. *Mycoplasma* was a dominating genus in 2 of the control calves treated for BRD at 68 and 52%. *Mycoplasma bovis* is also a pathogen that is frequently isolated in calves with BRD (Timsit et al., 2016a). Zinc treated sick calves displayed less *Mycoplasma* than the control calves, which could be contributed to the intranasal application. However, other pathogens or potentially viruses could have contributed to the onset of BRD in the sick Zn treated calves.

Healthy calves between treatments varied in abundance for the genera *Pasteurella*, *Pasteurellaceae*, *Gammaproteobacteria*, *Moraxella*, and *Bacteroidetes*. *Pasteurella* was greater in the control calves at a relative abundance of 53% in one calf in comparison to the Zn treated calves with one calf having a high of 14%. It is possible the intranasal Zn application could have depleted a healthy population of *Pasteurella*. *Moraxella* was greater in the healthy Zn treated calves at a relative abundance as high as 44% in comparison to the control calves. At d 60 in the feedlot, Holman et al. (2015b) reported *Staphylococcus*, *Mycoplasma*, *Mannheimia*, and *Moraxella* were the most prevalent genera. Two of the 4 genera Holman reported are pathogens associated with the onset of BRD. In the current study, *Mycoplasma* dominated the control calves at d 7 treated with BRD, but was of minimal presence in the healthy calves between treatments. *Pasteurella* spp. were greater in the healthy calves in comparison to the sick calves; it is possible the treatment of intranasal Zn could have depleted a beneficial population of *Pasteurella* spp. in the nasal membrane.

LEfSe Analysis

Healthy and sick groupings were analyzed at both the phylum and genus levels through LEfSe analysis. Sick calves were considered calves treated at least 1 time for BRD. Operational taxonomic units belonging to phylum *Plantomycetes* and “other” were significantly overrepresented in sick calves while OTUs associated with *Proteobacteria* were more abundant

in the healthy calves across treatments (Figure 2a to d). The phylum *Proteobacteria* includes 2 bacterial pathogens of interest to BRD; however, it was not a phylum with significant presences for the sick calves when analyzed.

At the genus level for the healthy vs. sick comparison, 22 OTUs were identified from the nasal passages of both healthy and sick calves. A total of 18 OTUs were increased in sick calves and 4 OTUs were increased in the healthy calves (Figure 3a). To show the distribution of an individual genus considered significant, 2 different genera were selected: *Escherichia/Shigella* for the sick calves and *Pasteurella* for the healthy calves. Sick calves displayed a greater amount of *Escherichia/Shigella* in their nasal microbiome in comparison with healthy calves. The individual distribution of the relative abundance for *Escherichia/Shigella* is illustrated in Figure 3b. In contrast, healthy calves had a greater amount of *Pasteurella* in comparison to the sick calves (Figure 3c).

Experimental treatments (Zn vs. control) were analyzed through LEfSe, but there were no differences. To further break down the treatments, subgroups were formed under the main groups: healthy and sick. The groups analyzed were healthy and sick with subgroups of Zn treated and control calves. Bacteria in healthy control and Zn treated calves were not different when analyzed, but differences were observed at the genus level between the sick control and Zn treated calves (Figure 4a). A total of 10 identifiable OTUs were of significance for the sick Zn treated and control calves. Eight OTUs were increased for the sick Zn treated calves at the genus level, one of being *Faecalibacterium* (Figure 4b), a bacteria known to reside in the gut. The presence of this bacterium in the nasal membrane could be caused by regurgitation of contents being broken down in the rumen. Two OTUs were increased in the sick control calves, 1 being *Mycoplasma* (Figure 4c).

A number of calves were treated for BRD 1 to 2 d prior to collecting the sample from their nasal passage, and 1 calf was treated the day of the collection. The treatment with an antibiotic could be a factor in the variation among the phyla and genera observed in the sick calves in comparison to the healthy. As previously reported, control calves tended ($P = 0.06$) to be treated with 3 antibiotics more often than Zn treated calves. Therapeutic trials using intranasal ZnAc in humans have shown reductions in overall duration of symptoms and severity (Wintergerst et al., 2006), which could explain the control calves tendency to being treated more frequently. Also, it is possible the application of the Zn to the nasal microbiome could have had a positive role in preventing relapse (animals treated with a second antibiotic). However, overall antibiotic costs did not differ across treatments ($P = 0.64$). Holman et al. (2015b) reported significant changes of the structure of the nasopharyngeal microbiota occurred overtime from d 0 to d 60 in the feedlot. The current swabs reported for the microbiota of stocker calves only represent d 7 of the trial. To collect swabs later than d 7 of the study could represent more pathogens associated with BRD than what were observed currently. In addition, the microbial diversity could be different between the sick Zn treated and control calves later in the study in comparison to the healthy treatment groups. Results that were not different for the microbiota data could be correlated with the small sample size and could vary drastically when all samples are analyzed.

Implications

Intranasal Zn did not appear to have a positive effect on the percent of bacteria isolated within the nasal passages or to decrease morbidity and mortality in calves susceptible to BRD. The culture based isolation method identified a smaller number of bacteria but analyzing the microbiota showed a wider variety of both phyla and genera providing researchers with more

bacterial taxa associated to BRD. To accurately identify and determine how the microbiota changes in association with the onset of BRD, culture-based are not sufficient as molecular methods. Treatment of intranasal Zn could be more efficacious if not treated blindly to calves, but treated upon the display of symptoms.

Table 1: Prevalence of bacteria cultured from the nasal membrane of cattle treated with an intranasal zinc solution compared to no intranasal application

Item	Treatment		<i>P</i> - value *
	Control	Zinc	
Bacteria, %			
<i>Pasteurella multocida</i>	83.3	87.5	1.0
<i>Bacillus</i> sp.	66.7	58.3	0.09
<i>Escherichia coli</i>	35.4	29.2	0.63
<i>α-streptococcus</i> sp.	85.4	85.4	0.14
<i>Mannheimia haemolytica</i>	10.4	14.6	1.0
<i>Staphylococcus</i> sp.	68.8	79.1	0.17
Lac gram-negative rods	60.4	64.6	0.67
<i>Psuedomonas aeruginosa</i>	6.3	0.0	0.04
<i>Enterococcus</i> sp.	0.0	2.1	0.24
<i>Klebsiella</i> sp.	0.0	2.1	0.24
Fungus, %			
Rhizopus	6.3	2.1	0.28
Fungus	20.8	18.8	0.77

^{*}Chi square probability from GENMOD procedure

Table 2: Main effect of day for the prevalence of bacteria cultured from the nasal membrane of cattle treated with an intranasal zinc solution compared to no application

Item	Day				<i>P</i> – value [*]
	0	1	2	7	
Bacteria, %					
<i>Pasteurella multocida</i>	91.7	95.8	95.8	58.3	<0.0001
<i>Bacillus</i> sp.	75.0	54.2	87.5	33.3	<0.0001
<i>Escherichia coli</i>	54.2	8.3	29.2	37.5	0.005
<i>α-streptococcus</i> sp.	95.8	87.5	95.8	62.5	0.003
<i>Mannheimia haemolytica</i>	4.2	8.3	25.0	12.5	0.08
<i>Staphylococcus</i> sp.	37.5	70.8	91.7	95.8	<0.0001
Lactose gram-negative rods	70.8	66.7	41.7	70.8	0.11
<i>Psuedomonas aeruginosa</i>	4.2	0	4.2	4.2	0.62
<i>Enterococcus</i> sp. ^a	0	0	0	4.2	-
<i>Klebsiella</i> sp. ^a	0	0	4.2	0	-
Rhizopus	0	12.5	4.2	0	0.07
Fungus	0	41.7	33.3	4.2	<0.0001

^{*}Chi square probability from GENMOD procedure

^aDay effects did not converge for these culturable pathogens

Table 3: Scores for cultured bacteria from the nasal membrane of cattle treated with an intranasal zinc solution compared to no intranasal application

Item	Treatment		SEM	P - value
	CON	Zn		
Qualitative Score ^a				
<i>Pasteurella multocida</i>	2.6	2.9	0.19	0.44
<i>Bacillus</i> sp.	0.9	0.8	0.11	0.37
<i>Escherichia coli</i>	0.5	0.3	0.11	0.37
<i>α-Streptococcus</i> sp.	1.9	2.2	0.19	0.20
<i>Mannheimia haemolytica</i>	0.3	0.4	0.16	0.58
<i>Staphylococcus</i> sp.	1.3	1.4	0.13	0.66
Lactose gram-negative rods	0.9	0.8	0.11	0.79
<i>Psuedomonas aeruginosa</i>	0.1	0	0.08	0.27
<i>Enterococcus</i> sp.	0	0.02	0.01	0.33
<i>Klebsiella</i> sp.	0	0.02	0.01	0.32
Total Bacteria	4.2	4.2	0.19	0.81
Rhizopus	0.06	0.02	0.03	0.30
Fungus	0.3	0.2	0.07	0.82
Total Fungi	0.3	0.2	0.05	0.41

^aScores were 0 to 4: 0 having no bacteria present, 1 being 1 to 4 colonies, 2 being 5 to 19, 3 being 20 to 99, and 4 having more than 100 colonies present

Table 4: Main effect of day on for scores for bacteria cultured from the nasal membrane of cattle treated with an intranasal zinc solution compared to no application

Item	Day [*]				SEM	P - value
	0	1	2	7		
Qualitative Score ^a						
<i>Pasteurella multocida</i>	3.5 ^a	3.0 ^a	3.0 ^a	1.5 ^b	0.22	<0.0001
<i>Bacillus</i> sp.	1.0 ^{ab}	0.6 ^b	1.2 ^a	0.3 ^b	0.15	0.0006
<i>Escherichia coli</i>	0.6 ^a	0.1 ^b	0.3 ^{ab}	0.4 ^{ab}	0.13	0.05
<i>α-Streptococcus</i> sp.	2.7 ^a	2.0 ^b	2.3 ^{ab}	1.1 ^c	0.24	0.0002
<i>Mannheimia haemolytica</i>	0.1	0.2	0.7	0.3	0.20	0.14
<i>Staphylococcus</i> sp.	0.5 ^b	1.3 ^a	1.6 ^a	1.7 ^a	0.18	0.0001
Lactose gram-negative rods	1.0	0.7	0.5	0.9	0.15	0.08
<i>Psuedomonas aeruginosa</i>	0.1	0	0.08	0.04	0.07	0.42
<i>Enterococcus</i> sp.	0	0	0	0.04	0.02	0.39
<i>Klebsiella</i> sp.	0	0	0.04	0	0.02	0.41
Total Bacteria	4.3 ^{ab}	3.9 ^b	4.7 ^a	3.7 ^b	0.23	0.0098
Rhizopus	0	0.1	0.04	0	0.04	0.11
Fungus	0 ^b	0.5 ^a	0.3 ^a	0.04 ^b	0.09	0.0002
Total Fungi	0 ^b	0.5 ^a	0.3 ^a	0.04 ^b	0.08	<0.0001

^{*}Numbers lacking common superscripts within row differ ($P < 0.05$)

^aScores were 0 to 4: 0 having no bacteria present, 1 being 1 to 4 colonies, 2 being 5 to 19, 3 being 20 to 99, and 4 having more than 100 colonies present

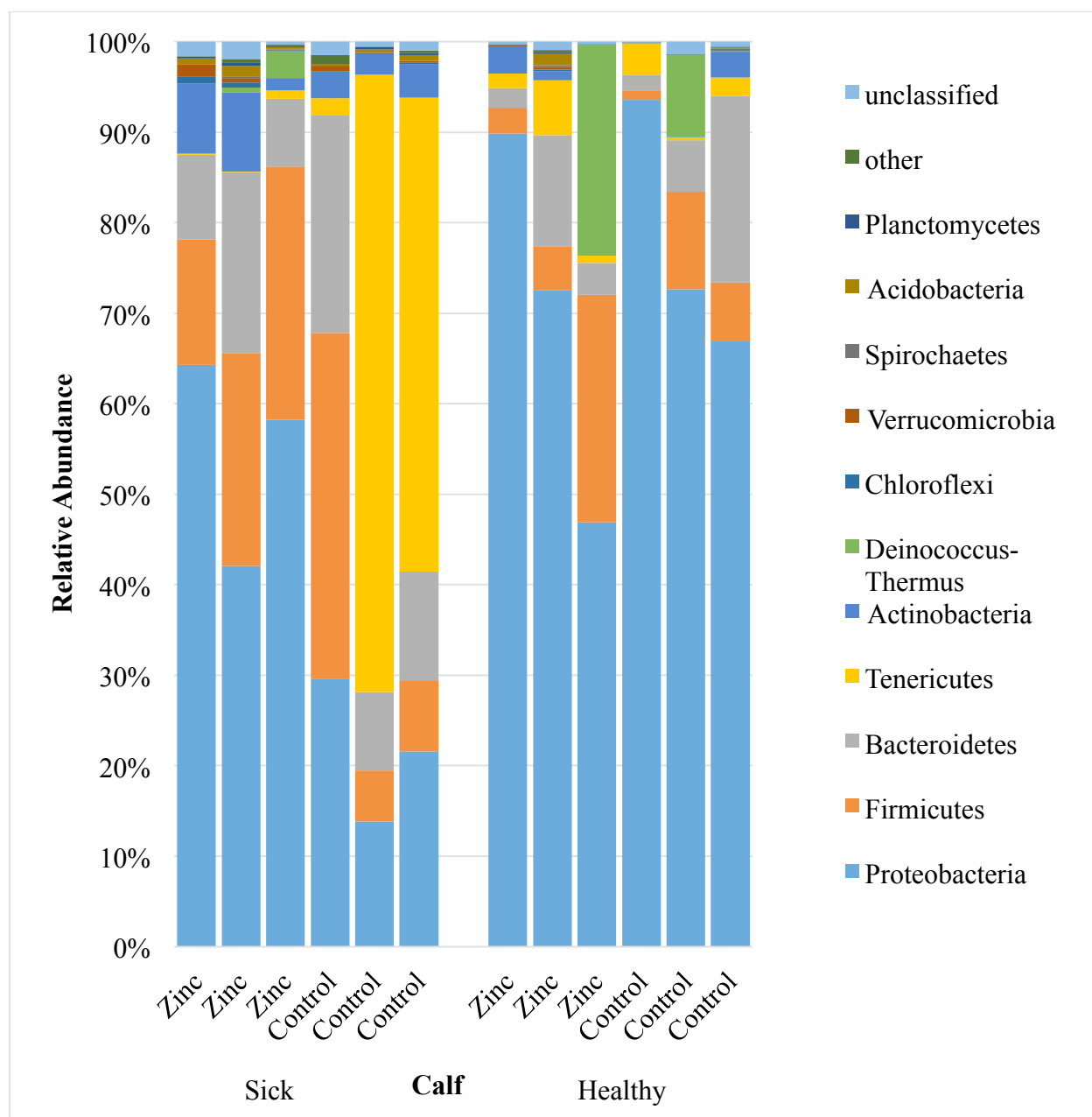


Figure 1a. Relative abundance at the phylum level in the nasal membranes of calves across treatments

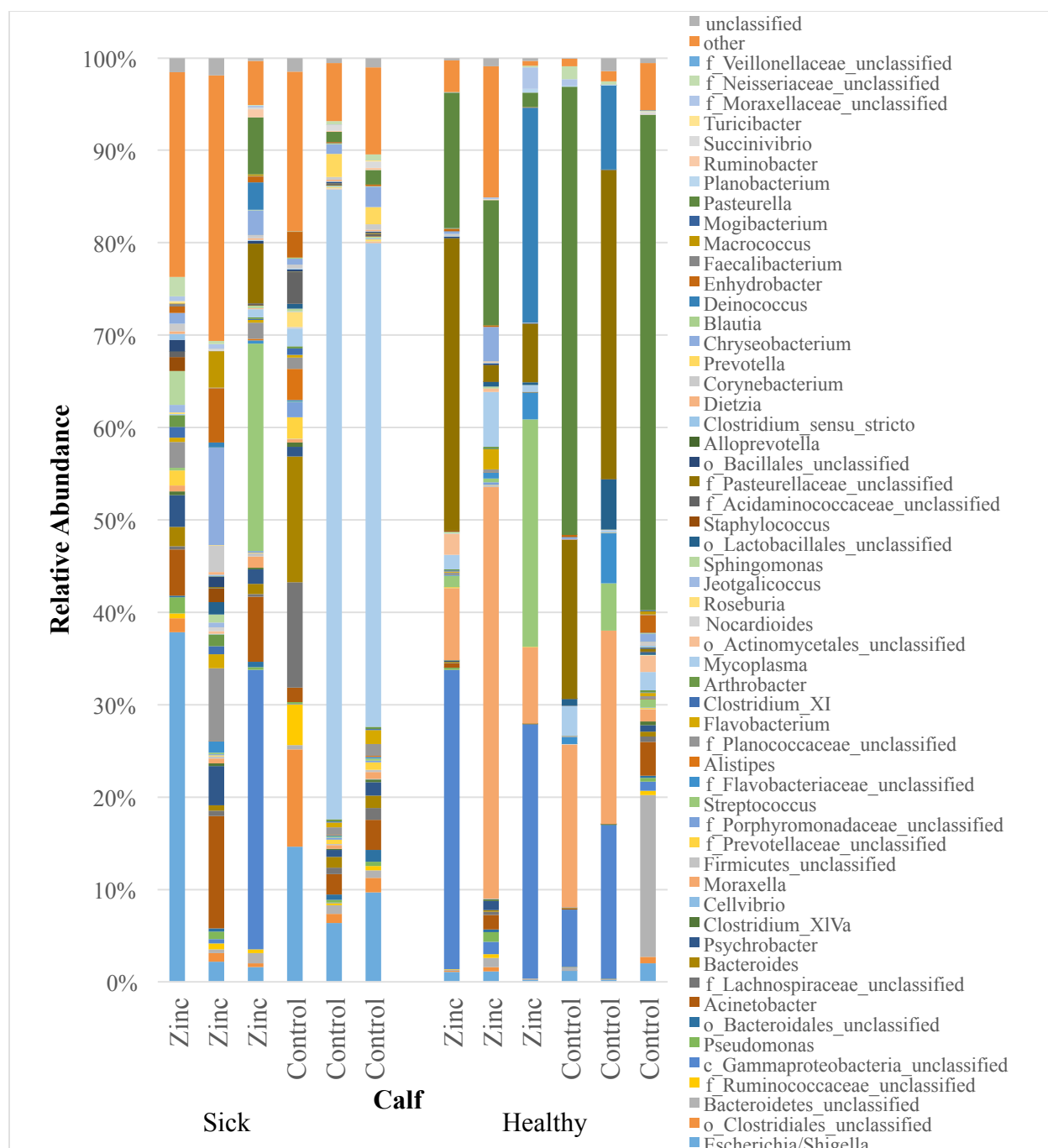


Figure 1b. Relative abundance at the genus level in the nasal membrane of calves across treatments

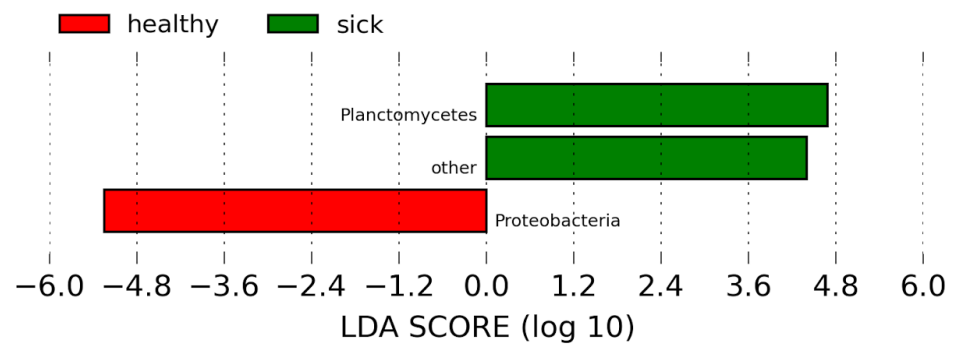


Figure 2a. Histogram displaying OTUs more abundant in the sick calves (green) or control calves (red) ranked by the effect size at the phylum level across both treatments

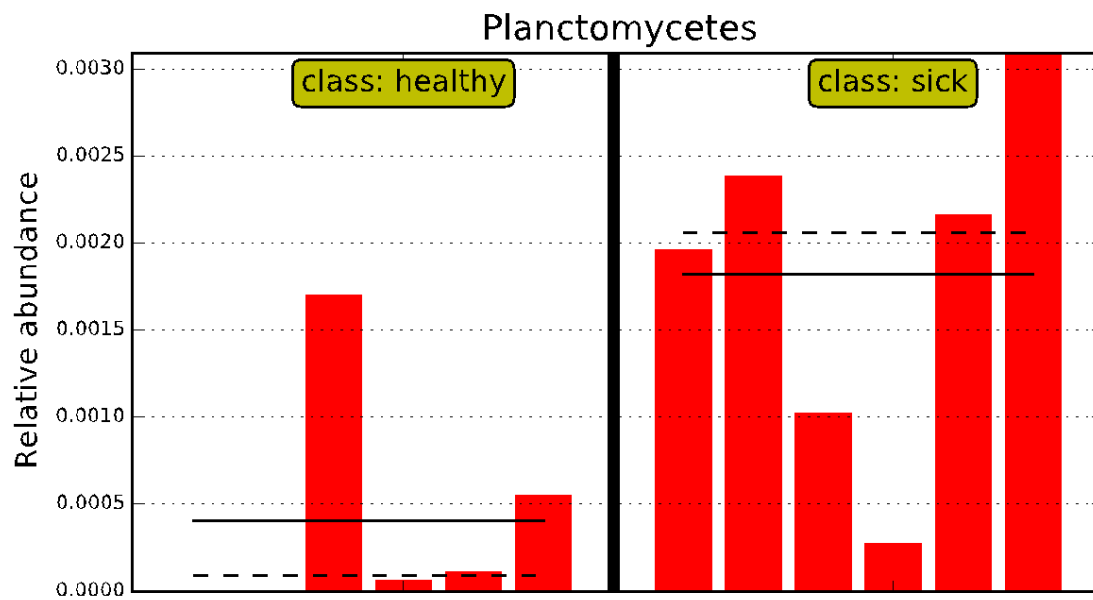


Figure 2b. The distribution of the significant OTUs in the healthy and sick calves at the phylum level across treatments

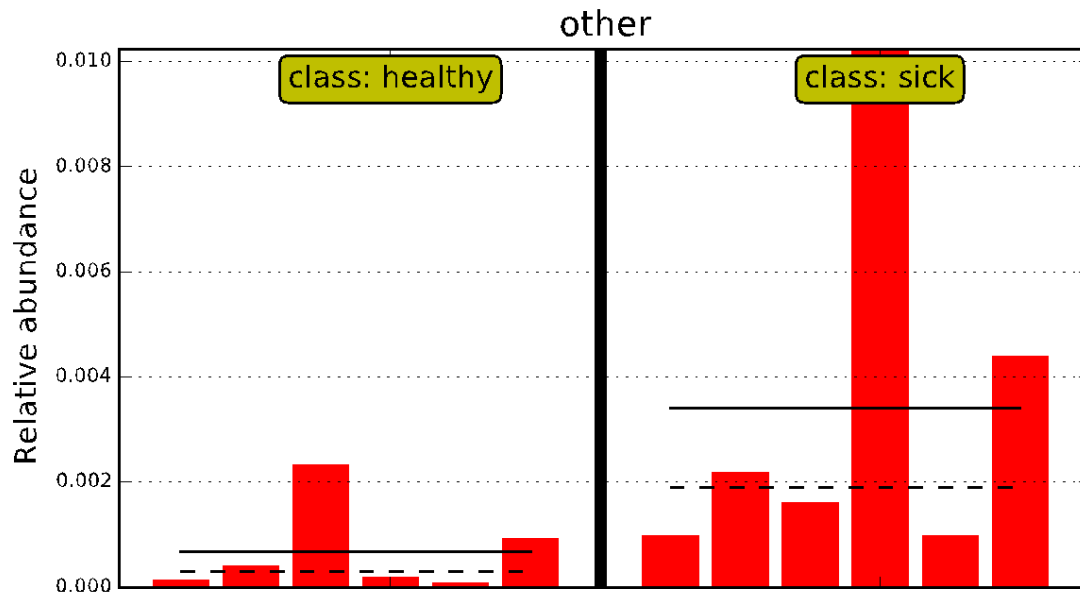


Figure 2c. The distribution of the significant OTUs in the healthy and sick calves at the phylum level across treatments

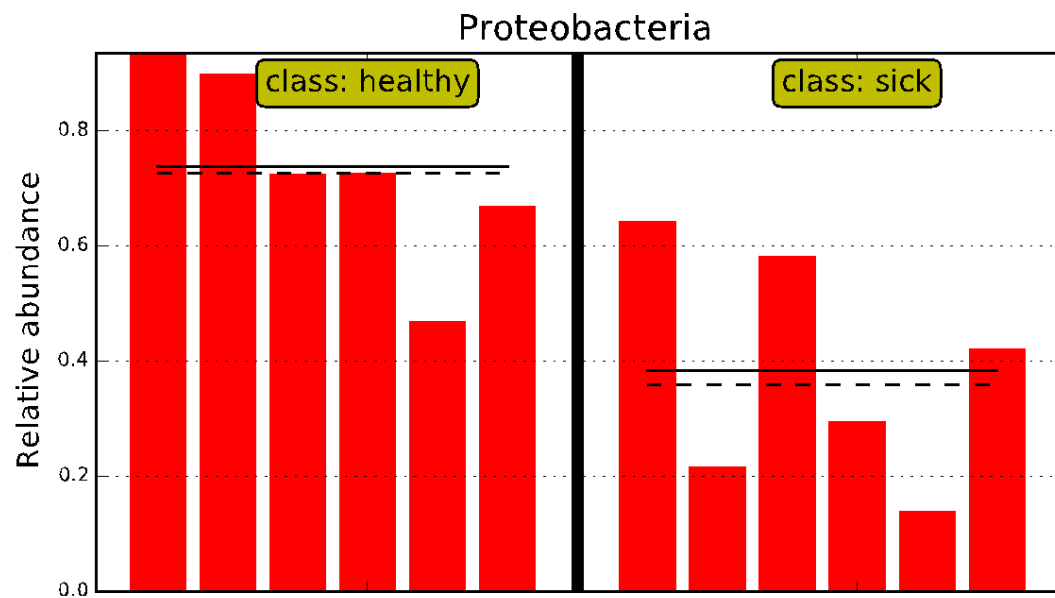


Figure 2d. The distribution of the significant OTUs in the healthy and sick calves at the phylum level across treatments

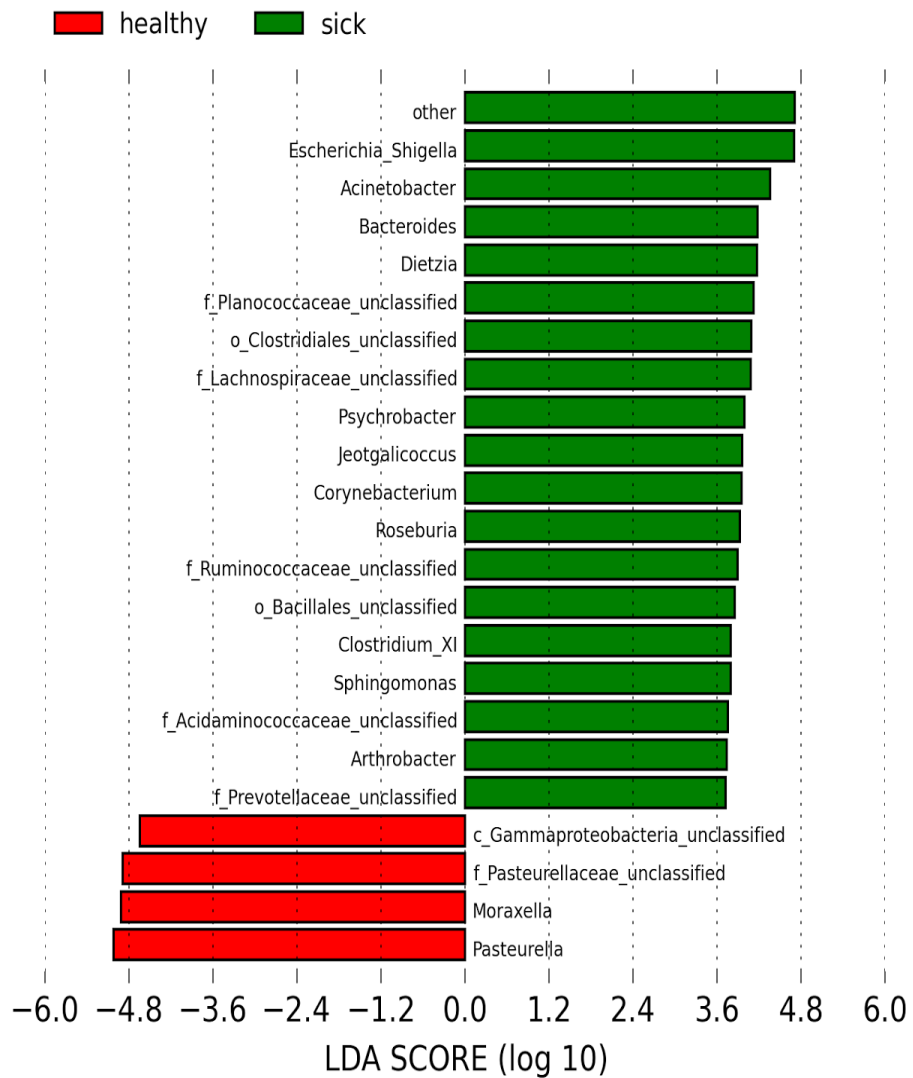


Figure 3a. Histogram displaying OTUs more abundant in the sick calves (green) or control calves (red) ranked by the effect size at the genus level across treatments

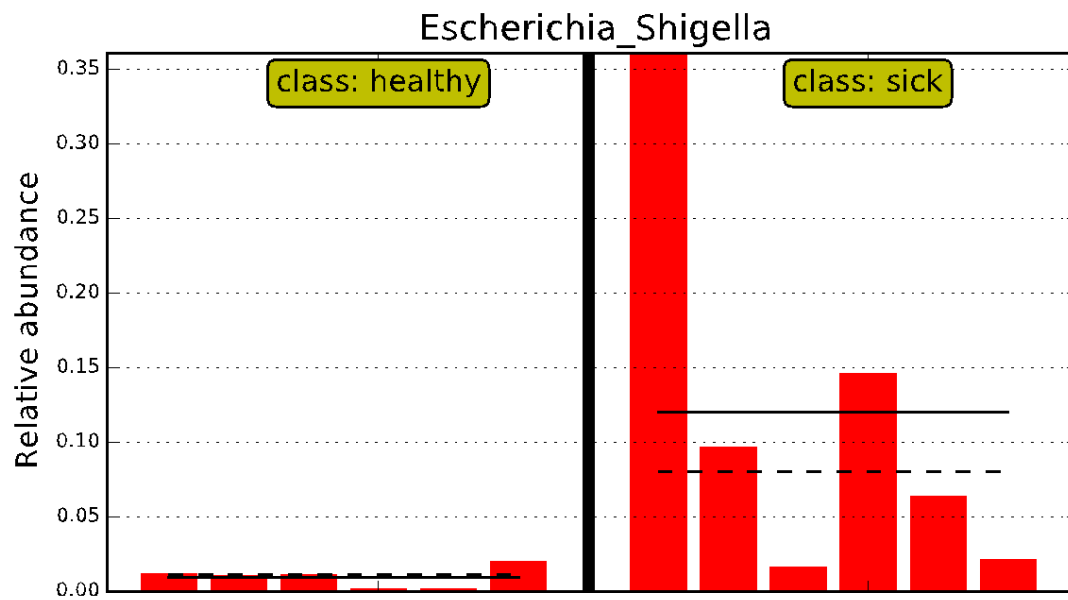


Figure 3b. The distribution of the significant OTUs in the healthy and sick calves at the genus level across treatments

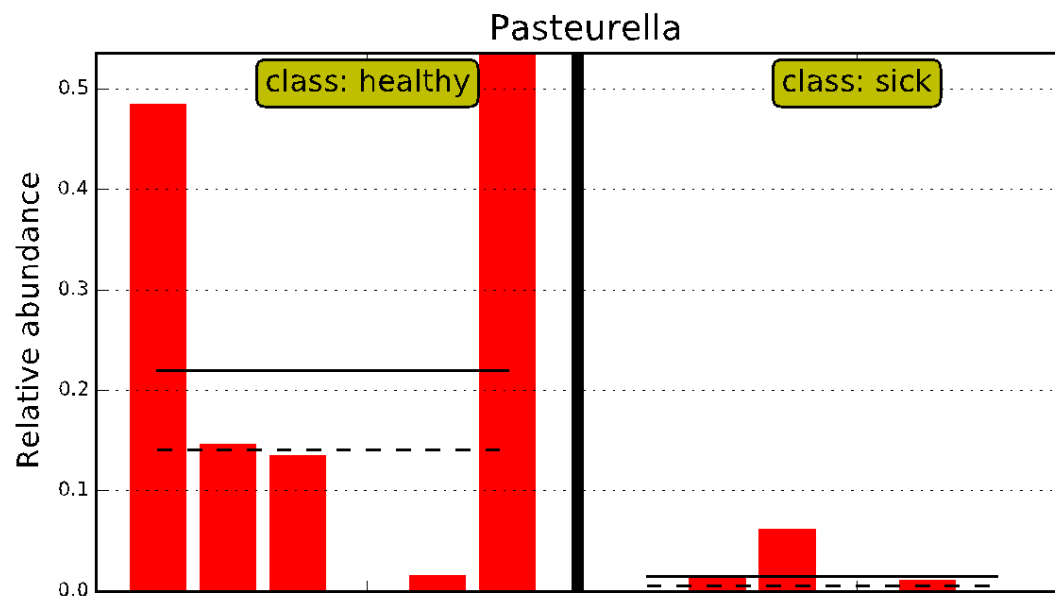


Figure 3c. The distribution of the significant OTUs in the healthy and sick calves at the genus level across treatments

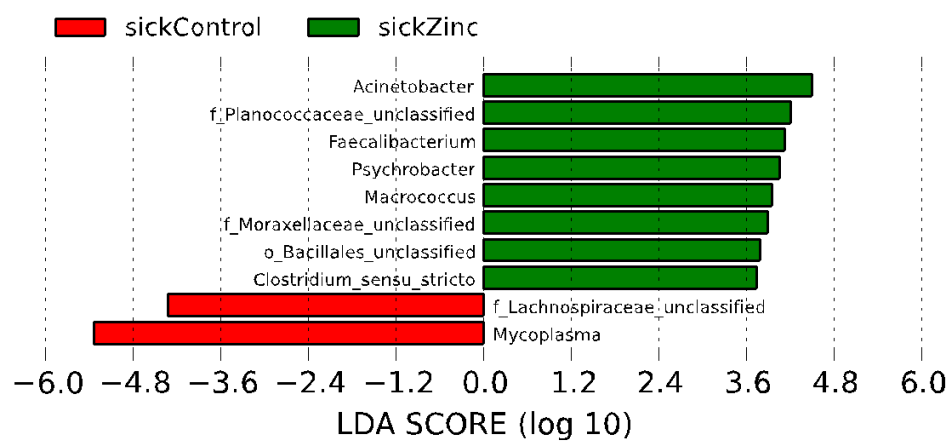


Figure 4a. Histogram displaying OTUs more abundant in the sick Zn treated calves (green) or control calves (red) ranked by the effect size at the genus level

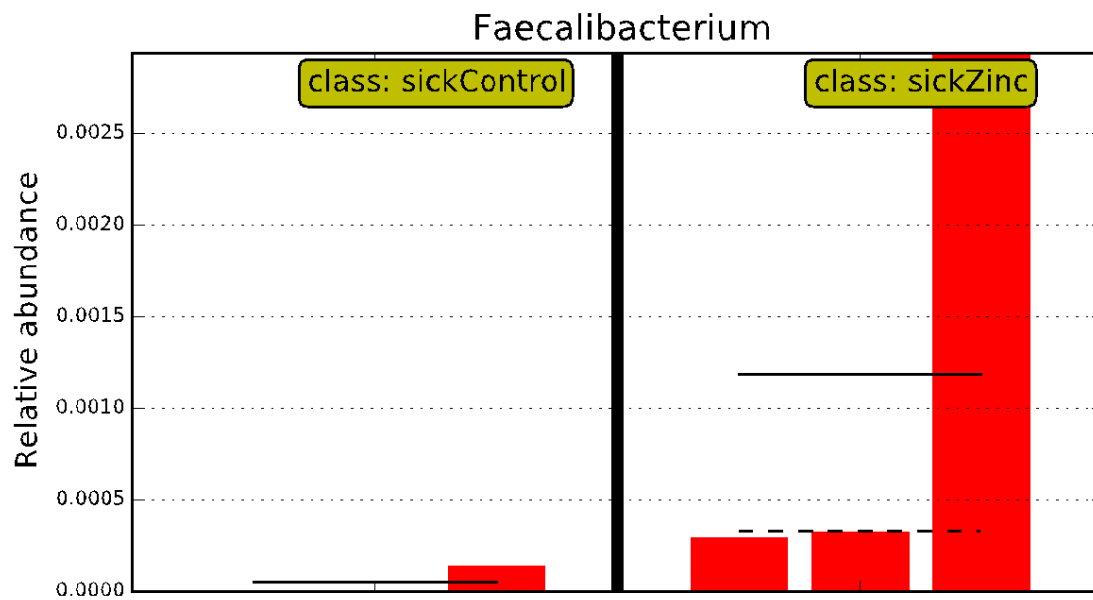


Figure 4b. The distribution of the significant OTUs in sick control and Zn treated calves at the genus level

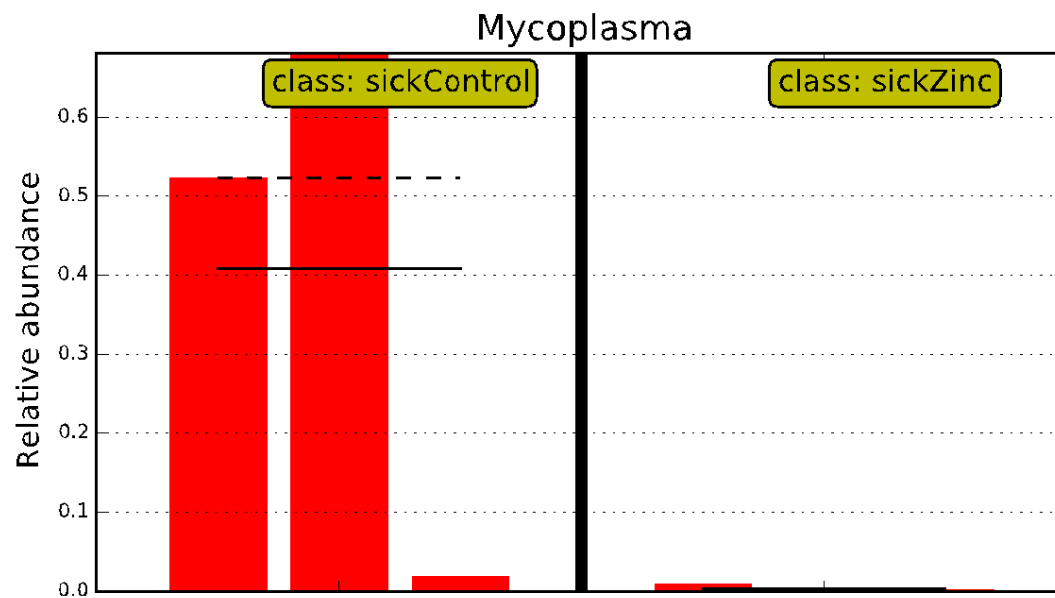


Figure 4c. The distribution of the significant OTUs in sick control and Zn treated calves at the genus level

CHAPTER V

CONCLUSION

The application of an intranasal Zn solution may not positively affect health and growth performance of stocker calves. Blindly treating calves may have a negative impact on performance. Control calves tended to be treated with 3 antibiotics more than the Zn treated calves. Intranasal Zn did not appear to have a positive effect on the percent of bacteria isolated within the nasal passages in calves susceptible to BRD. The culture based isolation method identified a smaller number of bacteria; however, analyzing the microbiota isolated a wider variety of both phyla and genera. Zinc may have depleted a healthy population of an opportunistic pathogen, *Pasteurella multocida*, which was present more in healthy calves than BRD treated calves. The phyla and genera isolated in the BRD calves could present additional pathogens associated with BRD outside of the 4 primary bacterial pathogens. To accurately identify and determine how the microbiota changes in association with the onset of BRD, culture based methods may not be as accurate. Further research is warranted in order to determine if the application of intranasal Zn may cause anosmia thereby resulting in reduced feed intake, and if intranasal administration to calves treated for BRD may be more efficacious. Therefore, the application of an intranasal Zn solution could potentially affect growth performance, health and the nasopharyngeal microbiome of newly received high-risk stocker calves.

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APPENDIX



UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Beth Kegley
FROM: Craig N. Coon, Chairman
DATE: Jan 23, 2015
SUBJECT: IACUC Approval
Expiration Date: Jan 22, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Protocol: 15030 "The Effectiveness of Zinc Given Intra-nasally Against Bovine Respiratory Disease"

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Jan 22, 2017 you must submit a modification or new protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

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