EFFECTS OF DIRECT-FED MICROBIALS AND VACCINE ROUTE OF ADMINISTRATION ON HEALTH AND PERFORMANCE OF NEWLY-ARRIVED CALVES TO FEEDLOTS

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Abstract

Management of newly-arrived beef calves into feedlots is of utmost importance to the long-term health and profitability of those animals. Two separate studies conducted individually examined the efficacy of particular commercially available products in the beef industry and collectively evaluated the viability of those products in commercial application. In the presented data, the use of direct-fed microbials (DFM) (Bovamine Defend®; Nutrition Physiology Co.; Guymon, OK) in recently-weaned, newly-received beef calves to feedlots did not improve average daily gain ($P = 0.98$), dry matter intake ($P = 0.33$), morbidity ($P = 0.33$), or mortality ($P = 0.34$).

In the second study, the efficacy of three different, commercially available multivalent modified-live viral respiratory vaccines was evaluated. No differences in final body weight, average daily gain, dry matter intake, or Gain:Feed were observed during the duration of the experiment. Calves receiving the INFORCE treatment required significantly fewer ($P = 0.01$) second treatments for Bovine Respiratory Disease (BRD) than did the calves receiving the VISTA treatment. Calves in the INFORCE and PYRAMID treatments required significantly fewer ($P = 0.03$) third treatments for BRD than did calves in the VISTA treatment. INFORCE treatment tended ($P = 0.09$) to have a lower percent mortality than VISTA. These data demonstrate that the use of products comprising the INFORCE treatment resulted in fewer calves requiring treatment for BRD or expiring from the disease.

It is the conclusion from the data reported in these studies that the inclusion of a DFM product into the ration of newly-arrived beef calves to feedlots was not beneficial to their growth and performance in the current situation. Moreover, the use of the products in the INFORCE treatment were able to prevent more losses associated with morbidity and mortality than the products used in the PYRAMID and VISTA treatments.

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CHAPTER I

INTRODUCTION

The production of beef in the United States is diverse and encompasses multiple, well established sectors and a wide array of production strategies. The first stage of production is often referred to as the "cow-calf sector". In this sector, producers own cows with the goal of selling a weaned calf at 6 to 8 months of age as the final end product. The majority of beef cow/calf operations in the United States have less than 49 head and comprise 27.7% of the nation's cow inventory. Moreover, 83.3% of the nation's cow herd is in operations containing less than 499 head (USDA NASS, 2012).

Due to the spatial distribution and large number of small, independent producers in the cow-calf sector, a need for an intermediate phase has been established to coordinate the assembly of calves from various locations and backgrounds into a more homogenous group for entry into the finishing phase of production. This sector is often referred to as the stocker, backgrounding, or growing phase of production, and its focus is to assemble smaller groups of cattle from various sources into larger groups of like individuals to market to large feedyards. The majority of cattle intended for slaughter and subsequent human consumption spend the last 3 to 9 months in concentrated animal feeding operation (CAFO). Most cattle entering this phase of production are recently-weaned calves weighing between 400 and 600 pounds. These cattle are generally

sold by the cow-calf operator at local sale barns or through regional auction markets and commingled with other groups of calves to form larger cohorts for management in the next phase of production. During this growing phase, cattle will be grown to a common end weight of approximately 750 pounds and usually marketed in larger groups to finish feedyards as "feeder cattle".

Within the industry, cattle being marketed into the growing phase of production are given a risk classification based on expected likelihood of developing bovine respiratory disease and whether they have been exposed to various unique respiratory pathogens. Bovine Respiratory Disease (BRD) is the most prevalent and costly health issue in beef cattle production in North America, accounting for 70 to 80% of morbidity and 40 to 50% of mortality in feedlots (Edwards, 2010). "Low Risk" cattle are considered to have a lower potential to develop BRD and an elevated potential to gain weight quickly and efficiently. The market generally places a premium on such cattle due to their low risk of investment and high likelihood of potential profitable return. Cattle in this classification often come from one common source and are not commingled with other sources prior to arrival in the growing phase, unless they come from a niche marketing sale of like-managed cattle. "High Risk" cattle are deemed to have an increased risk of developing BRD, which often leads to increased cost of therapeutic treatments, higher morbidity and mortality rates, decreased efficiency, and a more volatile profit potential. Most animals in this category have naïve immune systems that are not prepared to handle the various pathogens that commonly result in BRD. The industry consistently discounts cattle in this classification due to the extreme variability of health and performance results.

Many calves entering the growing phase will spend the first 30 to 60 days in a dry lot or in small grass paddocks so that individual health status can be closely monitored. Upon arrival, most calves will be vaccinated for common viral and bacterial pathogens, dewormed, horns will be tipped, intact bull calves will be castrated, and in high risk cattle antimicrobials are often administered metaphylatically to prevent BRD. This processing of newly-arrived cattle is done to

prepare them for the rest of their journey through the cattle production system and to ensure that cattle are immunologically prepared to handle a variety of health challenges.

In these intensively stocked systems, calves are generally fed high quality forages or total mixed rations to ensure that nutrient uptake is adequate even if intake is low. Often after cattle have spent 45 days in these intensive systems they are moved to less intensive grazing systems were the cost of weight gain is less expensive (i.e., wheat pasture or summer forage), because their risk of succumbing to BRD is much lower at this point. Once these cattle have achieved the desired weight gain they are marketed to finish feedyards.

In recent years, there has been much focus on improving health and performance in this sector of the industry due to inflated calf prices and volatile feed prices. The growth of housing these calves in feedlot pens due to increasing land costs coupled with drought conditions limiting forage resources has increased significantly. Within these situations many producers are investigating the utility of commercially available tools that are intended to increase performance or decrease morbidity and mortality. There are many products that promise significant biological and economic profit if utilized; however, it is important to have reliable information before committing to the use of such products.

In this thesis, the purpose of the first experiment was to evaluate the usefulness of Direct-Fed Microbial (DFM) application in growing cattle rations in a confined feeding situation. Previous research indicated that DFM have the potential to increase daily gains in cattle by 2.5% to 5% and increase efficiency by 2% (Krehbiel et al., 2003). The purpose of the second experiment was to investigate the efficacy of commercially available modified-live viral and bacterial combination vaccines. In tandem, the projects should provide enhanced information with regards to improved ways to manage health and nutrition of newly-received calves into confined feeding operations.

CHAPTER II

REVIEW OF LITERATURE

Direct-Fed Microbials

The United States Food and Drug Administration defines direct-fed microbials (DFM) as a "source of live, naturally occurring microorganisms" (Yoon and Stern, 1995) and has required feed manufacturers to use this terminology for bacteria, fungi, or enzymes fed to animals (Miles and Bootwalla, 1991; Krehbiel et al., 2003). There are many different commercial DFM products available on the market, but the most commonly used products in ruminant animal production often contain a lactate-producing and a lactate-utilizing bacterial species (Krehbiel et al., 2003; Wilson and Krehbiel, 2012). Interest in the inclusion of these products into ruminant diets has increased in recent years as the public concern over antibiotic use in livestock production has increased, with DFM being seen as a possible method to increase animal health and performance while decreasing reliance upon antibiotics and other growth promoting technologies (Krehbiel et al., 2003).

History and Use of DFM in Livestock Production and Nutrition

The use of microbial species to benefit the mammalian gastrointestinal tract has been a topic of interest for centuries. Often seen as the father of probiotics, Metchnikoff, in his book *The Prolongation of Life* (1908), theorized that consuming live lactobacilli cultures could be beneficial to human health. His evidence for this was his observation of Bulgarian peoples

consuming fermented milk products, and their subsequent resistance to enteropathogens. After World War II, the use of antibiotic therapy increased drastically due to innovation in this field of medicine. However, some of these antibiotics being used were so efficient they would eliminate all enteric bacteria in the specimen which they were applied. This elimination of all enteric bacteria often led to severe "antibiotic diarrhea" in human patients, so the interest in *Lactobacillus acidophilus* therapy to replenish gut microflora increased (Mannheim, 1951). In more recent years, there has been ever increasing interest from the livestock production industry to adapt this technology to their various biological models. Specifically, there has been much effort in the cattle feeding industry to evaluate DFM as a production tool, and successfully define their biological benefits in the ruminant animal consuming large amounts of concentrate rich diets. Although the original intent of feeding DFM was to improve intestinal health, much attention has been turned to research results quantifying DFM potential positive effects on daily gains, feed efficiency, immune function, and rumen health (Krehbiel et al., 2003). Some early work from Gill et al. (1987) demonstrated that feeding a bacterial DFM to calves during a 28-d receiving period resulted in a 9.3% increase in ADG, improvement in feed efficiency by 9.5%, and it reduced mortality rate by 10.9% compared to calves not fed the DFM. In 2004, VetLife conducted a survey evaluating the use of a DFM product by 267 feedyards with 118 responding. Closeouts from these responding facilities accounted for 10.9 million head of cattle during the period from January 1, 2003 to May 31, 2004. In this data, set steers and heifers fed a DFM product experienced an improvement in daily gains by 1.9% and 1.4% respectively, and advantages for feed efficiency were 1.9% and 3.9% for steers and heifers, respectively (VetLife, 2004). However, there are numerous published controlled studies which have not detected any differences in performance, feed efficiency, or health in cattle fed DFM versus cattle not fed DFM (Trenkle et al., 2001; Greenquist et al., 2004; Raeth-Knight et al., 2007; Neuhold et al., 2012; Dick et al., 2013).

The interest in feeding cattle DFM has increased over the past few decades as the need to explore new ways to produce food animals more efficiently has escalated. In turn, the mission to validate the claims associated with individual DFM has risen as well. The present research available gives evidence to the fact that response to DFM treatment is often variable with positive responses showing modest improvements in performance measurements.

Bovamine Defend®

Bovamine Defend® is a product manufactured by Nutrition Physiology Company, LLC. The product contains 1 x 10^9 *Lactobacillus acidophilus* strain LA51 and 1 x 10^9 *Propionibacterium freudenreichii* strain PF24 (a lactate-producing and a lactate-utilizing bacteria, respectively). The claim associated with this product is the lowering of pathogenic bacterial organisms in the gastrointestinal tract (GIT) of animals, mainly *Escherichia coli* O157:H7, and is generally used 20 to 60 days prior to slaughter to prevent *E. coli* shedding in slaughter facilities (Ware and Anderson, 2011).

Bacterial DFM Mode of Action

Bacterial DFM containing *Lactobacillus* were originally fed to animals with the intent of promoting health of the lower gastrointestinal tract. Much information has been collected on the effectiveness of these bacteria to reduce the pathogenic bacterial load in the intestine of ruminant animals intended for harvest through means of competitive attachment, production of antimicrobial substances, and through altering the environment of the GIT (Elam et al., 2003; Krehbiel et al., 2003; Loneragan and Brashears, 2005; Brown and Nagaraja, 2009; Wilson and Krehbiel, 2012). Elam et al. (2003) theorized that some of the potential reduction in pathogenic organisms found in the GIT of cattle fed DFM in their study was due to a reduction in thickness of the lamina propria of the intestine. This reduction could result in improved nutrient absorption in the small intestine by the animal, a possible reduction in GIT microflora turnover, and therefore a more stable environment in the animals GIT. All these factors have the potential to lead to better absorption and utilization of consumed nutrients by the host animal, as well as

lowered maintenance energy requirements which could have an ancillary improvement on performance. Moreover, *Lactobacilli* have been shown to produce hydrogen peroxide, a compound that is antagonistic to other bacterial species such as *E. coli* O157:H7 (Krehbiel et al., 2003). In summary, lactate-producing bacteria such as *Lactobacilli* confer improvements in intestinal health and function to the host animal being fed these substrates, which in-turn has potential to lead to fewer dietary losses and improvement in performance and nutrient utilization.

DFM in the Rumen

Although the original focus of DFM treatment was to improve the health and function of the lower GIT, there is evidence that certain species of bacteria in DFM have the potential to influence rumen microflora and health (Krehbiel et al., 2003). Yoon and Stern (1995) reported that their summary of data suggested lactate-producing bacterial species when introduced into the rumen had the propensity to reduce the effects and severity of subacute acidosis. It is believed that *Lactobacilli* producing lactate as an end product of fermentation in the rumen help to condition the rumen microbiome to lower pH levels. In doing this, when low pH levels are experienced due to the rapid fermentation of starch particles, the rumen microflora are acclimated to this decreased pH. Therefore, the rapid lysis of bacterial and protozoal cells does not occur, preventing an acidotic situation. Others have investigated the inclusion of lactate-utilizing species, such as *Megasphaera elsdenii* or *Propionibacterium freudenreichii*, in concurrence with a lactate producer (Krehbiel et al., 2003). With lactate being a precursor of propionate, the introduction of bacteria that can make this conversion could increase the efficiency of ruminal fermentation for energy capture in cattle fed high-concentrate diets. Although *M. elsdenii* is a major utilizer of ruminal lactate, there is much interest in the use of *Propionibacterium* because they more readily convert the lactate to propionate (Krehbiel et al., 2003).

Effect of DFM on Receiving Cattle Performance

Some initial interest in DFM use for cattle was for the benefit of newly-received, stressed cattle with the purpose being the reestablishment of GIT microflora (Birkelo, 2003). This

response was observed by Gill et al. (1987) when they fed a bacterial DFM to newly-arrived calves for 28 days. They reported a 9.3% increase in ADG, a 9.5% improvement in feed conversion, and a 10.9% reduction in BRD morbidity for cattle treated with the DFM versus non-treated control cattle (Gill et al., 1987). Siverson et al. (2012) conducted two experiments to evaluate the effects of DFM inclusion into diets of newly-received, lightweight stocker calves. In the first experiment, heifers (n = 279, BW = 226 kg) were fed for 44 days. The DFM was included in the diet via liquid suspension. In the second experiment, heifers (n = 287, BW = 226 kg) were fed for 44 days with the DFM being administered via dry suspension in the diet. In both experiments, there was no difference in DMI, ADG, or morbidity of calves fed the DFM product versus those on the control diet. Morbidity for Exp. 1 and Exp. 2 were 38.9% and 14.3%, respectively, when averaged across treatments with no difference due to experimental treatment. Morbidity rates in these two studies were low (0 to 20%) to moderate (20 to 40%) in magnitude. These data suggest that cattle classified as having a low to moderate risk of developing BRD may not respond with increased performance to DFM treatment. This difference could be attributed to the overall good performance of the group masking any treatment differentiation or to some other unknown factor.

In 2001, Krehbiel and colleagues conducted a study with 466 newly-received male calves (mixed bulls and steers) at the Willard Sparks Beef Research Center in Stillwater, OK. Over the 42-d trial, a probiotic gel was administered to calves (15 mL/calf) during their first pull for clinical signs of BRD. At the end of 42 d, there were no differences in DMI or daily gains for cattle receiving the probiotic gel (n = 85) as opposed to those not receiving the probiotic treatments (n = 212); however, the cattle receiving the gel tended to have fewer retreatments for BRD than those not treated and less of those were treated within 96 hours of first BRD antimicrobial administration. In their experiment, overall morbidity was 100% since only cattle treated for BRD were eligible to receive probiotic treatment. Second treatment morbidity was 20.8% and 12.9% for calves not receiving and receiving probiotic gel, respectively. These data

show that DFM have the potential to improve the health of newly-received calves in a feedyard. Gill et al. (1987) postulated that extremely healthy or extremely sick cattle may not respond to DFM treatment due to the overwhelming poor or positive nature of their health status masking the response to treatment; however, these data suggest that extremely sick cattle (100% morbidity) will respond in a favorable manner to DFM treatment in terms of decreased longevity of clinical BRD signs and reduced need for continued antimicrobial therapy.

These data demonstrate that DFM treatment in newly-received cattle can be advantageous in certain situations; however, responses to treatment can be variable and are often of low magnitude. Route of administration is also important to keep in mind when assessing DFM protocol effectiveness. When delivered through the feed, correct dosage rates are dependent on individual feed intake. It is possible that the calves that need the DFM the most get the least of it due to low feed intake. Limit feeding situations could also influence the response to DFM treatment when delivered via the feed. In contrast, when pulse doses of DFM are delivered to targeted individuals based on some qualifying criteria (e.g., during first antimicrobial treatment for BRD, during processing or revaccination, etc.) the effectiveness of this dose may have greater potential to exert beneficial effects in the animal. In these situations, it can be certain the dose delivered is appropriate for each animal (based on size, age, health/nutritional status), therefore ensuring that the animal has optimal chance to respond.

Effects of DFM on Finishing Cattle Performance

Much more information has been produced regarding the action and efficacy of DFM use in cattle on high grain finishing diets. Due to the volume of published data and the diversity of diet, environment, management, and composition of the DFM used in each experiment, the results are variable and at times non-congruent. This review will focus on studies that will expose the differences seen in published data in order to more fully understand the complex environment associated with DFM use.

In 2005, McDonald et al. evaluated survey results from a 2004 survey of 267 feedyards in North America who contributed to the VetLife Benchmark Database. Of these feedyards, 118 responded as using a DFM product from 1 January 2003 to 31 May 2004 and the other 149 responded as not using a DFM during this time. Closeouts from these yards representing 73,870 lots comprised of 10,900,504 head of cattle were evaluated to determine the efficacy of DFM use in commercial feedyards. The results of analyzing these data showed an improvement in ADG for heifers and steers of 1.4% and 1.9%, respectively, when fed a DFM product. Furthermore, steers experienced a 1.9% improvement in feed efficiency when fed a DFM with heifers exhibiting a 3.9% improvement. Analysis also showed that lighter weight cattle on arrival (less than 700 lbs) that incurred over $20 of processing and treatment costs experienced a greater response to DFM administration than cattle of similar weight groups with less than $20/animal processing and treatment costs. Furthermore, cattle entering the feedyard at greater than 700 lbs experienced decrease loss associated with mortality when fed a DFM product. Although these data were not the product of a designed, controlled experiment and should be evaluated with caution, it does provide insight into the potential value of DFM inclusion in the commercial cattle feeding industry. If the improvements in gain and efficiency of nutrient conversion, coupled with the reduction of morbidity and mortality are repeatable outcomes of DFM application to cattle in the finishing phase, the biologic and economic implications to the industry would be great and far reaching.

A study was conducted utilizing 240 steers (BW = 370 ± 6 kg) at Texas Tech University in 2007 (Vasconcelos et al., 2008). The diet was 92% concentrate consisting of approximately 76% steam-flaked corn. In this study, three levels of DFM inclusion((1) $1x10^9$ cfu *Propionibacterium freudenreichii* NP24 + $1x10^7$ cfu *Lactobacillus acidophilus* NP51; (2) $1x10^9$ cfu *Propionibacterium freudenreichii* NP24 + $1x10^8$ cfu *Lactobacillus acidophilus* NP51; and (3) $1x10^9$ cfu *Propionibacterium freudenreichii* NP24 + $1x10^9$ cfu *Lactobacillus acidophilus* NP51) were evaluated against a negative control. From d 0 to the end of the feeding period, the average

of the three DFM treatments had an improved body weight gain to feed dry matter intake (G:F) ratio compared to the control diet. Also, there was a quadratic effect of increasing *L. acidophilus* NP51 dose on G:F. Carcass data were collected and analyzed, but no differences were identified as a result of DFM treatment.

Ponce et al. (2011) used 96 steers (BW = 321 kg) to determine if differences existed when cattle were fed BeefPro® (a DFM product containing various lactate-producing bacteria as well as a variety of digestive enzymes) at 200 mg/steer/d. Cattle were fed for 175 days with the final diet being 90% concentrate consisting of 72.6% steam-flaked corn. Results showed that steers fed BeefPro® for the duration of the study tended to have increased ADG on a live weight basis and had a significantly greater carcass adjusted ADG. Dry matter intake was significantly increased by BeefPro® treatment over the 175 DOF. Carcass adjusted final BW tended to be greater for BeefPro® steers, and no differences were detected in feed efficiency due to treatment. Carcass data were collected and no differences were observed as a result of BeefPro® treatment.

A recent experiment was conducted to examine the effects of feeding bacterial DFM to neonatal Holstein bull calves on digestive tract morphology, and also on the effects of long-term bacterial DFM supplementation on performance of Holstein steers (Dick et al., 2013). In the first experiment, 43 Holstein bull calves (BW = 42 kg) were placed on one of two treatments. Treatment 1 was a negative control and Treatment 2 had a proprietary mixture of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (Bovamine®; Nutrition Physiology Co.; Guymon, OK) added to the control diet. Calves were fed a commercial milk replacer (Calvita Supreme; Milk Specialties Co; Dundee, IL) for 50 d at which time one-half of each treatment group was randomly selected for harvest with the other half being moved to concrete floor pens and fed a high-grain diet for 14 d at which time they were harvested. For both harvest groups there were no differences in DMI, ADG, or feed conversion between treatments. At harvest, portions of the rumen and ileum were removed for further examination of intestinal morphology.

It was determined that calves on the DFM treatment had greater ileal height (villus + crypt) when slaughtered at weaning, and greater rumen papillae width when slaughtered 14 d post weaning.

In the second experiment, 264 Holstein bull calves (initial BW = 37 kg) were fed for approximately 406 days on a final diet containing 85% concentrate (72% steam-flaked corn). Cattle were assigned to one of three treatments: (1) control; (2) $1x10^5$ cfu *Lactobacillus acidophilus* + $1x10^9$ cfu *Propionibacterium freudenreichii;* or (3) $1x10^6$ cfu *Lactobacillus acidophilus* + $1x10^9$ cfu *Propionibacterium freudenreichii* (Bovamine®; Nutrition Physiology Co; Guymon, OK). Average daily gain for treatment (2) tended to be greater than treatments (1) or (3). There were no differences in DMI, G:F, BW, or carcass quality due to treatment.

These studies demonstrate that DFM added to finishing diets have the potential to positively influence ADG, final BW, and feed conversion. It also appears that there are optimum levels of dosage for various DFM combinations which are most likely influenced by diet, animal type, level of production, and other unspecified management factors (Vasconcelos et al., 2008; Dick et al., 2013). One possible explanation for the improvement of feed conversion and subsequent animal performance is morphological changes to the rumen and small intestine, which act to increase surface area and nutrient absorptive capacity (Dick et al., 2013). Moreover, bacterial DFM might elicit greater effect if fed in the presence of digestive enzymes (Ponce et al., 2011). Although feed conversion, DMI, and ADG have the propensity to be influenced by DFM addition to the diet, it seems from the present body of work that DFM do not cause any changes in carcass quality (Vasconelos et al., 2008; Ponce et al., 2011; Dick et al., 2013).

Although there is published data that show the efficacy of DFM products in high concentrate finishing diets for beef cattle, there are also many sets of published data that failed to see any expected benefits of DFM inclusion. A brief review of these studies follows.

In 2003, Trenkle fed varying concentrations of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* to steers for 174 days (n = 161; BW = 284 kg). The final diet contained 92% concentrate (59.6% steam-flaked corn, 30% wet corn gluten feed (WCGF)). The

four treatments consisted of a (1) control; (2) $5x10^6$ cfu *L. acidophilus* NP45 X $5x10^6$ cfu *L. acidophilus* NP51 X $1x10^9$ cfu *P. freudenreichii* NP24; (3) $2x10^9$ cfu *L. acidophilus* NP51 X $1x10^9$ cfu *P. freudenreichii* NP24; and (4) $2x10^9$ cfu *L. acidophilus* M35 X $1x10^9$ cfu *P.* freudenreichii NP24. No differences were observed in DMI, ADG, feed efficiency, or carcass quality due to treatment.

In 2004, a larger experiment was conducted in a commercial feedyard environment utilizing steers and heifers (n = 3,539; BW = 362 kg; Greenquist et al., 2004). The control diet was 93% concentrate (66% steam-flaked corn, 15.4% wet distiller's grains plus solubles (WDGS)) with the treatment diet having $1x10^9$ cfu *P. freudenreichii* NP24 X $1x10^6$ cfu *L. acidophilus* NP45 X $1x10^9$ cfu *L. acidophilus* NP51 applied to the control diet via a micro ingredient machine, which would be common industry practice for DFM application. Cattle were on feed for 122 d, at the end of which no differences in DMI, ADG, feed efficiency, and carcass merit were detected.

In another experiment, the effects of 10-G Brand® (a DFM containing a proprietary blend of *L. acidophilus, E. faecium, P. acidilactia, L. brevis, L. plantarum* administered to supply $5x10^6$ cfu to the diet) were evaluated using 144 steers (BW = 335.5 ± 12.2 kg) on feed for 173 days. The diet, similar to the Greenquist et al. (2004) diet, contained 91% concentrate (69% steam-flaked corn, 15% WDGS). At the conclusion of the study, there were no differences observed due to DFM treatment in BW, DMI, ADG, feed efficiency, and calculated NE_m or NE_g. The only observed difference was an increase in %KPH by the 10-G® treated cattle (Neuhold et al., 2012).

Direct-fed microbial products have also received much interest in high-producing dairy cow rations. An experiment consisting of 53 dairy cows with three treatment groups (Control; DFM1: $1x10^9$ cfu *L. acidophilus* NP747 + $2x10^9$ cfu *P. freudenreichii* NP24; and DFM2: $1x10^9$ cfu *L. acidophilus* NP747 + $5x10^8$ cfu *L. acidophilus* NP45 + $2x10^9$ cfu *P. freudenreichii* NP24) was carried out at the University of Minnesota (Raeth-Knight et al., 2007). The diet contained

less concentrate and more roughage than a beef feedlot diet (48% corn silage, 13.5% corn grain). DFM treatment in this experiment resulted in no difference in DMI, milk volume produced, or milk quality as compared to the control.

Conclusions can be made based on these studies that DFM do not exhibit biological efficacy every time they are included in a diet. Presented here are examples of similar and different bacterial DFM in rations with varying levels of concentrate:roughage with a common outcome of no difference in measured variables due to treatment. There has been speculation that inclusion of corn co-product feedstuffs into beef finishing diets (especially WCGF) can limit the effectiveness of DFM (Trenkle, 2003). It is thought that in these diets or diets containing increased levels of roughage (>15%) that the mode of action to acclimate the rumen to high levels of lactate in order to reduce acidosis potential is limited; therefore, the ability of the product to display its biological efficacy is impaired (Birkelo, 2003; Trenkle, 2003; Brown and Nagaraja, 2009; Wilson and Krehbiel, 2012). It should not be assumed that DFM will produce the positive effects on DMI, ADG, and feed conversion as seen by some studies, and that factors such as diet composition, route of administration, bacterial species and strain, animal type and stage of production, along with management factors may influence the efficacy of these products.

Escherichia coli O157:H7 Shedding

Escherichia coli O157:H7 exhibits multiple different virulence factors and is the most prominent serotype associated with the enterohaemorrhagic (EHEC) group (Phillips et al., 2000). Virulence factors of *E. coli* O157:H7 include the production of Shiga toxin (Stx), an attaching and effacing phenotype, and the possession of plasmid p0157 (Phillips et al., 2000; LeBlanc, 2003; Hussein, 2007). The attachment and colonization of *E. coli* O157:H7 to the lumen of the intestine in humans and bovine has demonstrated the ability to damage the epithelial surface and remove existing symbiotic bacterial species (Phillips et al., 2000). This loss of intestinal homeostasis can result in severe diarrhea in the host. Moreover, the production of Shiga toxin has been described as the preeminent virulence factor associated with *E. coli* O157:H7 (Su and

Brandt, 1995). This toxin inhibits protein synthesis with biological ramifications being the ability to elicit conditions in the host such as hemorrhagic colitis and hemolytic-uremia (Su and Brandt, 1995; Hussein, 2007). *E. coli* O157:H7 infection resulting in food borne illnesses in humans has been associated with the consumption of animal products, mainly processed beef (LeBlanc, 2003). The specific virulence factors exhibited by *E. coli* O157:H7 make it a particularly potent pathogen, and its abilities to cause detrimental intestinal effects in a variety of host animal species demands diligence in management and prevention of its prevalence in the food chain.

For many years, the occurrence of *Escherichia coli* O157:H7 infection within the human food supply chain has been linked to bovine fecal material (Lejeune and Wetzel, 2007). Much work has been done by the packing industry to reduce the prevalence of *E. coli* O157:H7 contamination within meat products. Implementation of hide washing, carcass washing, organic acid rinses, steam chambers, and thorough kill floor sanitation has done much to reduce the incidence of *E. coli* outbreaks in humans. However, the greatest improvement in this effort will have to come from the reduction of pathogenic bacteria within the host animal while it is alive (LeJeune and Wetzel, 2007). Implementation of on-farm technologies to reduce the carriage of *Escherichia coli* O157:H7 in the animal pre-shipment are imperative to complete or near-complete elimination of the threat (LeJeune and Wetzel, 2007). The only current on-farm intervention that has been investigated in-depth is the inclusion of *Lactobacillus acidophilus* strain NP51 into finishing feedlot diets (Loneragan and Brashears, 2005). Although there are other techniques to effectively reduce *E. coli* shedding prior to harvest (vaccination, sodium chlorate, neomycin sulfate), these technologies are either not licensed for this application or have not been repeatedly tested in controlled experiments (Loneragan and Brashears, 2005). *Lactobacillus acidophilus* strain NP51 (LANP51) has been shown to reduce *E. coli* O157:H7 by 58% as compared to cohorts not fed the DFM. Moreover, it has also been shown that the effectiveness of this treatment increases as the dose concentration of LANP51 increases (tested

10^7, 10^8, 10^9 cfu) (Loneragan and Brashears, 2005 citing Younts et al., 2004 and Younts-Dahl et al., 2005).

Elam et al. (2003) examined the effects of differing dose and *L. acidophilus* strain combinations accompanied by *Propionibacterium freudenreichii* NP24 on intestinal morphology and occurrence of *E. coli* O157:H7 shedding. Two hundred and forty steers were used (initial BW = 332.8 kg). Treatments consisted of (1) control; (2) $1x10^9$ cfu *L. acidophilus* NP51 X $1x10^6$ cfu *L. acidophilus* NP45 X $1x10^9$ cfu *P. freudenreichii* NP24; (3) $1x10^9$ cfu *L. acidophilus* NP51 X $1x10^9$ cfu *P. freudenreichii* NP24; and (4) $1x10^6$ cfu *L. acidophilus* NP51 X $1x10^6$ cfu *L. acidophilus* NP45 X $1x10^9$ cfu *P. freudenreichii* NP24. The results showed treatments (2) and (3) reduced the thickness of the lamina propria and also reduced the incidence of *E. coli* O157:H7 shedding when measured at shipping and 7d prior as compared to treatments (1) and (4). The authors proposed that the *L. acidophilus* treatment was able reduce the thickness of the lamina propria by means of reducing inflammation of the lumen associated with the attaching/effacing phenotype characteristic of *E. coli* O157:H7 as a result of *L. acidophilus'* ability to outcompete *E. coli* O157:H7 for intestinal attachment sites (Phillips et al., 2000; Elam et al., 2003).

A recent experiment evaluated the efficacy of a commercial vaccine for *E. coli* O157:H7 (SRP vaccine; Zoetis; Florham Park, NJ), a commercial DFM (Bovamine®, Nutrition Physiology Co.; Guymon, OK), or the combination of the two treatments in the presence of a negative control group. The experiment used 17,148 animals contained in 40 pens (initial BW = 393.7 kg, weighted average DOF = 165). In this experiment, the vaccine was effective in reducing *E. coli* O157:H7 prevalence on hides and shedding, but the DFM and the combination DFM/vaccine treatment were not effective (Cernicchiaro et al., 2014). One possible explanation offered by the author for the lack of reduction by the DFM or DFM/vaccine treatment was the use of a "low-dose" product ($1x10^6$ cfu *L. acidophilus* NP51 X $1x10^9$ cfu *P. freudenreichii* NP24). Based on results from Elam et al. (2003) this explanation seems plausible.

Bacterial DFM including lactate-producing strains, especially those with *L. acidophilus* NP51, have been shown to be efficacious in reducing pathogenic bacterial loads in the hindgut of cattle. Although other effective technologies exist for combating *E. coli* O157:H7 in the live, pre-harvest animal, either a lack of published information, industry acceptance, or legal roadblocks impair their effective use. As it is, *L. acidophilus* NP51 is the best, most effective technology available to the industry to reduce carriage of pathogenic strains of *E. coli*.

Vaccination and Receiving Cattle Management

History of Vaccines and Immune System Overview

Vaccination, or the inoculation of healthy individuals or populations with modified or weakened strains of a pathogen, has been a large focus of human and animal health since the late 1700s (Janeway's Immunobiology, 7th Edition). In the late 18th century, Edward Jenner observed that milk-maids who contracted cowpox were much less susceptible to suffering ill effects associated with smallpox. By the 19th century, Robert Koch had determined infectious diseases were spread by pathogenic microorganisms and classified four potential disease causing agents: viruses, bacteria, fungi, parasites (Janeway's Immunobiology, 7th Edition). As time has progressed, our understanding of biological immune function has continued to increase.

The immune system is often broken down into two separate units that work in unison. Animals are born with functional innate immunity. The innate immune system has many components such as: mucosal epithelium in body openings to prevent bacterial colonization, structural design to inhibit passage of pathogens to internal tissues, a variety of cells whose function is to recognized potential foreign pathogens and trigger a large scale immune response, and chemical messengers and pathways that alert the body to the site of infection and create a proinflammatory response (Janeway's Immunobiology, 7th Edition). The innate immune response is rapid, yet it is relatively non-specific and retains no memory to previous pathogen invasion

(Banse et al., 2014). Innate immunity is effective in healthy, non-stressed, or immunocompromised individuals in preventing disease pathogenesis.

The adaptive immune response is much slower to respond to foreign antigens (7 to 10 days from initial infection), but it carries with it immunological memory of previous infections and is extremely effective in combating a plethora of specific pathogens (Janeway's Immunobiology, 7th Edition; Banse et al, 2014). The adaptive immune response is often broken down into two subunits: cell-mediated immunity and humoral immunity. Cell-mediated immunity (CMI) is carried out by T-cells and is primarily responsible for protecting the body against intracellular pathogens such as viruses (Banse et al., 2014). Humoral immunity is comprised of B cells which produce antibodies to extracellular antigens specific to foreign infectious agents. When animals are born, their adaptive immune system is referred to as naïve, and newborn livestock mammals must consume colostrum in order to begin establishing a competent immunity. As animals encounter specific pathogens throughout their lifetime, the innate and adaptive immune responses work together to protect the body from infection and disease, and the adaptive immune response stores immunological memory in regards to the specific invading pathogen. The memory response allows the animal to be less susceptible to reinfection from the same pathogenic organism. Vaccines stimulate mainly the adaptive immune response to build antigen recognition of specific pathogens seen as most harmful to a species.

Bovine Respiratory Disease Complex

Bovine Respiratory Disease (BRD) is the single most costly loss to the cattle production industry to date, accounting for 1,055,000 hd loss in 2010 valued at $643 million (NASS, 2010). BRD is a disease complex consisting of multiple viral and bacterial components (Table 1) that combine to create severe respiratory distress for the animal (Griffin, 1996). BRD disproportionally affects light weight, recently-weaned, stressed calves that enter a feedyard or stocker program. Many of these calves are weaned and immediately transported from the farm of origin to local or regional auction markets where they are purchased and combined with other

calves of like nature to form larger, more marketable cohorts. During these events, calves experience greater levels of stress which has been shown to down-regulate immune function, increased vocalization due to disruption of social order which irritates the mechanical defenses of the upper respiratory tract, and are often exposed to novel infectious pathogens (Mackenzie et al., 1997; Galyean et al., 1999; Loerch and Fluharty, 1999; Duff and Galyean, 2007). Often times, these calves are at a higher risk of succumbing to BRD infection because their naïve adaptive immune systems, increased level of stress due to transportation and a new social environment, accompanied by a probable decline in their level of nutrition leaves them with an impaired ability to mount an effective immune response.

Vaccine Mode of Action

A comprehensive review of immunology and vaccine function is provided in Janeway's Immunobiology, 7th Edition. The purpose of this review is to highlight the functional components behind an effective response to vaccination. There are two main requirements that must be met for vaccination to be effective. First, an efficacious vaccine that stimulates the appropriate immune response and confers long-lasting immunological memory must be administered. Second, the efficacious vaccine must be administered to an immunocompotent animal, as administration of an effective vaccine to an immunosuppressed animal will not result in the appropriate immune response or long-lasting immunity (Perino, 1996; Janeway's Immunobiology 7th Edition). The most effective vaccines perfectly imitate the pathogen of concern within the body while being unable to reproduce and cause full-scale infection (Perino, 1996). There are vaccines for both viral and bacterial pathogens. It is important for viral vaccines to stimulate an effective T cell response and memory, as T cells are the lymphocyte associated with viral immunity. Bacterial vaccines (bacterins) need to ensure a strong B cell response and memory, so that antibodies will be made to their extracellular defining antigens (Janeway's Immunobiology 7th Edition). When vaccines are administered, the innate immune response is immediately triggered because of the non-self material administered to the animal. Antigen-presenting cells

(APCs) of the innate immune system present the vaccine antigens to lymphocytes which undergo selection and proliferation. During this process, the body creates various chemical messengers that produce varying effects on immune function. Interferon gamma (IFNγ) secreted by dendritic cells (an APC) and natural killer cells (NK cells) are important chemical mediator in the stimulus of T cell activation in viral infections (Woolums et al., 2002).

Vaccines come in two main forms, either attenuated/modified-live (MLV) or killed. In general, MLV vaccines elicit a more "real to life" infection producing a stronger, longer-lasting immune response, yet they are less stable in storage, require prompt use, and possess the potential to revert to pathogenic strains inducing clinical infection (Stokka and Perino, 2000). Killed vaccines are usually composed of purified components of the virus or bacteria and adjuvants (chemical carriers that bind the killed antigen and target specific immune functions). Killed vaccines are generally more stable in storage and much less likely to cause clinical infection; however, they also are not near as effective at stimulating a strong, long-lasting immune response (Stokka and Perino, 2000). Vaccination is the most effective and successful method currently available to prevent loss associated with communicable disease in cattle; however, it is important to remember that vaccines do not prevent infection and do little to modulate innate immune response to pathogens. For vaccines to express their effect the pathogen must overcome the mechanical defenses and innate immune response, enter the body, and begin to replicate (Bowersock and Martin, 1999; Stokka and Perino, 2000).

Vaccine Route of Administration and Efficacy

Most commercial vaccines for viral and bacterial pathogens related to BRD are intended for parenteral administration (either subcutaneous or intramuscular). Vaccination via this route should induce a systemic immune response resulting in increasing levels of circulating antibodies; however, less response is seen in mucosal tissues when this route of administration is used (Shewen et al., 2009). This presents possible immunological issues since approximately 90% of all infectious pathogens enter through mucosal surfaces (Potter et al., 2008). Vaccination of

mucosal surfaces could provide local and systemic immune protection, and could prove useful in the presence of maternal antibodies in young calves (Kimman et al., 1989). Immunization of mucosal surfaces has the potential to produce immune protection with the ability to prevent pathogen invasion, while still conferring some level of systemic immunity (Bowersock and Martin, 1999). Intranasally administered MLV IBR (infectious bovine rhinotracheitis or bovine herpes virus 1) and PI3 (parainfluenza 3) confer protective immunity to upper respiratory tract infection within 60-72 h after inoculation (Bowersock and Martin, 1999). Shewen et al. (2009) reported that immunoglobulin production and effector T cells differ between mucosal and systemic immunity. They acknowledged the benefits of stimulating mucosal immunity through vaccination procedures, but cautioned that intranasal vaccines must be delivered in dosages capable of overcoming the innate clearance mechanisms of the upper respiratory tract (Shewen et al., 2009).

Concern over maternal antibody interference, proper priming of the immune system, and antigen interference have all been topics of concern over the recent years in regards to vaccination (Harland et al., 1992; Ellis et al., 2010; Cortese et al., 2011; Stoltenow et al., 2011; Kavanagh et al., 2013; Woolums et al., 2013).

Antigen Interference and Vaccine Efficacy

Immunodominance (often referred to as antigen interference) is the immunological phenomenon in which the presence of one epitope or antigen interferes with the recognition and response to a second distinct epitope or antigen (Cortese et al., 2011). Essentially, it describes the body's preferential response to one pathogen at the expense of a response to another (Cortese et al., 2011). Little is known or understood about how the viral and bacterial antigens present in most commercial MLV vaccines affect the immune response of one another when administered concurrently. Currently, much attention is being devoted to the understanding of exactly how these vaccine antigens interact with the immune system once administered, and if potential

detrimental interactions can be avoided through diversifying routes of administration of certain pathogens.

In 2005, Ellis et al. used 4 to 6 month old beef calves (n = 60) to investigate the longevity of immunity provided by a MLV viral combination vaccine administered parenterally (Reliant 4; MLV IBR/PI3/BVD with killed BRSV; Merial Ltd; Athens, GA). Six treatment groups (including a negative control) were used to determine the duration of efficacy after two vaccinations separated by approximately 20 d. Initial vaccination times for groups ranged from 146 d to 42 d prior to challenge, with revaccination occurring in each group approximately 20 d after initial vaccination. The results revealed all animals had adequate immunological memory conferred with vaccinations out to 126 d (longest duration from revaccination to challenge with Cooper strain BHV-1) as evidenced by the decreased severity and extent of clinical signs associated with BRD, magnitude and duration of viral shedding, and degree of change in rectal temperature post BHV-1 challenge as compared to the unvaccinated control (Ellis et al., 2005). The results in this study indicated no detrimental interactions among the four viral components of the vaccine administered.

Another potential route of vaccine inactivation is the presence of maternal antibodies. Some speculation has been raised in regards to vaccination of young calves with moderate to high levels of circulating maternal antibodies as to the effectiveness of vaccine methods designed to elicit a systemic antibody response (Kimman et al., 1989). In 2013, Kavanagh et al. used 65 Holstein/Friesian calves (18 d old) to determine the effects of intranasal vaccination for BRSV in the presence of maternal antibodies. They reported an increase in mucosal immunity (secretory IgA) but no apparent increase in systemic immune capabilities (serum IgG or serum IFNγ) as a result of intranasal inoculation (Kavanagh et al., 2013). These results support the notion that mucosal immunization may not confer a strong systemic response, but may be an effective route of administration if maternal antibody interference is likely.

One of the more severe antigen interactions is thought to occur between BHV-1 and coadministered *Mannheimia haemolytica* (formerly *Pasturella* haemolytica) bacterin. This interaction was first reported by Harland et al. in 1992. Their experiment utilized 7 to 10 month old calves (n = 2,324; avg BW = 250 to 350 kg) administered a MLV (BHV-1/PI3) or BHV-1 subunit (gIV) in a 2x2 factorial design with the addition of *Pasteurella haemolytica* (PhV) to each treatment (Harland et al., 1992). The gIV + PhV treatment significantly reduced BRD associated morbidity and mortality along with incidence of fibrinous pneumonia mortality as compared to all other treatments. The inclusion of PhV vaccine with the MLV (BHV-1/PI3) vaccine was not different from the two treatments that did not include PhV for BRD related morbidity, mortality, and fibrinous pneumonia mortality (Harland et al., 1992). Similarly, Cortese et al. (2011) used 642 calves seronegative for BHV-1 to evaluate the effects of commercially available vaccines combining MLV viral preparations (IBR/BRSV/PI3/BVD) with *M. haemolytica* toxoid. Their results express similar findings to the results reported by Harland et al. (1992). Calves receiving a *M. haemolytica* toxoid coadministered with the MLV viral vaccines had a depressed response to *M. haemolytica* leukotoxin as compared to those who received these two vaccinations at separate times. Their conclusion was that the MLV BHV-1 had a significant negative influence on the response to *M. haemolytica* inoculation (Cortese et al., 2011). These two experiments reveal the potential for antigen interaction between MLV BHV-1 and *M. haemolytica* when coadministered, consequently leaving the calf immunologically susceptible to colonization and infection by *M. haemolytica*. The recommendation of Cortese et al. (2011) is to vaccinate for these two pathogens at separate times or at least through separate routes of administration if administered simultaneously.

In a separate experiment, 154 spring-born calves were vaccinated with either a *M. haemolytica* (MH) commercial preparation (OneShot®; Zoetis; Florham Park, NJ), the MH preparation and a commercial intranasal vaccine containing BHV-1/PI3 (TSV-2®; Zoetis; Florham Park, NJ), or the MH preparation and a commercial intranasal vaccine containing BHV-

1/PI3/BRSV (Inforce3®; Zoetis; Florham Park, NJ). All animals were revaccinated on d 91 with a pentavalent MLV vaccine containing BHV-1/PI3/BRSV/BVD type 1 & 2 (BoviShield Gold 5®; Zoetis; Florham Park, NJ) and the same MH preparation previously administered. From blood samples collected at multiple time points during the 112 d trial, no difference among treatments in MH leukotoxin neutralizing antibody were demonstrated (Stoltenow et al., 2011). These results confirm the theory that intranasally applied MLV BHV-1 vaccines will not interfere with parenterally administered MH.

These experiments outline the potential antigen interactions experienced when MLV preparations containing BHV-1 are coadministered with *M. haemolytica* toxoid parenterally. However, there is potential to reduce or eliminate this antigen interference through delaying administration of a MH toxoid or by utilizing separate routes of administration concurrently. If ignored, this interaction could result in significant increases in losses associated with BRD morbidity and mortality in feedlots (Harland et al., 1992).

Route of Administration: Mucosal vs. Parenteral

As previously discussed, vaccination of mucosal tissues presents a possible alternative to conventional vaccination parenterally and provides a potential means by which to avoid detrimental maternal antibody suppression and antigen interference. Controlled studies comparing the utility of mucosal vs parenteral vaccine delivery are not as ubiquitous as needed to make a definite claim as to which is most effective in all circumstances, but some work has been completed that allows for linear comparison.

A 2010 experiment conducted by Ellis et al., used 66 Holstein bulls calves (3 to 8 days old; mixture of seropositive and seronegative animals for BRSV) to compare the administration of a commercial pentavalent MLV viral vaccine (VistaOnce® SQ; Merck Animal Health; Millsboro, DE) given via subcutaneous or intranasal routes (Ellis et al., 2010). It should be noted that VistaOnce® SQ is not labeled for intranasal administration. Calves were challenged with BRSV 4.5 months post-vaccination. Seropositive calves receiving intranasal application of the

vaccine experienced greater maximum change in rectal temperature than seronegative intranasally vaccinated calves. Calves seronegative for BRSV prior to vaccination responded similarly to BRSV challenge regardless of route of administration (Ellis et al., 2010). These data affirm that in calves with similar immunological backgrounds both intranasal and subcutaneous administration of vaccines have similar efficacy. It is important to remember all vaccines should be administered per label directions, and the lack of doing so could explain the increased change in rectal temperature observed in seropositive calves administered intranasal vaccine.

Another possible application of interest for intranasal vaccines is the use in young calves to mitigate the effects of maternal antibody interference. A recent experiment was conducted in young beef calves (n = 184) to evaluate the effect of a priming dose of vaccine at 2 or 70 d of age using either intranasal (IN) or subcutaneous (SQ) routes of administration (Woolums et al., 2013). All calves were revaccinated again at weaning. Although calves vaccinated SQ at d 70 showed a higher serum antibody titer for BVDV1, all other serum antibody responses were similar across treatment at weaning and 45 d post-weaning (Woolums et al., 2013).

Although some practical administration difficulties have limited the utilization and effectiveness of intranasally delivered vaccines, research shows their comparable efficacy to vaccines administered subcutaneously or in the muscle. In some scenarios, intranasal administration has proven more effective due to limiting the effects of antigen interaction specifically between BHV-1 and *M. haemolytica* vaccines. With continued education on potential benefits, training of personnel responsible for vaccine administration, and more data produced from controlled experiments; the potential to improve the health of cattle entering the feedlot could be significantly enhanced with the use of intranasal vaccines in correct application.

Receiving Cattle: Timing of Vaccination

It is well understood and accepted that the optimal time to vaccinate animals is prior to their encountering infectious agents; however, the duration from vaccination to exposure and frequency of vaccinations has been a topic of debate and much research attention. Due to the

current market structure of the commercial beef cattle production industry, many calves are never vaccinated until their arrival at a feedlot or stocker program. By this point, they have generally been commingled with calves of different origins and often times exposed to BRD contributing pathogens. Most cattle are vaccinated on arrival as part of a comprehensive health management program aimed at increasing the level of herd immunity and reducing the likelihood of disease epidemics (Edwards, 2010). Unfortunately, this time period is also associated with stress related to transportation, marketing, and acclimatization to new surroundings and is recognized as the single most stressful event in the life of a typical feeder calf (Loerch and Fluharty, 1999). The stress of this event acts in an immunosuppressive manner, hindering the ability of cattle to respond to vaccines (Duff and Galyean, 2007). It is generally accepted that two to three weeks are required from time of inoculation to build an acceptable level of immunity (Edwards, 2010). With these things in mind, some have stressed the critical importance of length of time vaccinated prior to arrival and/or revaccination in the feedlot phase. It becomes very important to understand the true biological effects of these management practices in order to best advocate how they should be applied to the current market structures and demand.

In 2008, data were reported by White et al. from a volunteer enrollment study regulated by stringent vaccination and husbandry guidelines. Producers had to provide proof that all calves were weaned 30 d prior to shipment and vaccinated at least twice with a MLV vaccine containing IBR/PI3/BRSV/BVD, for clostridial diseases, and against *M. haemolytica* (White et al., 2008). Information was recorded on time in days between first vaccination and booster, days from booster to shipment, days from weaning to shipment, and subsequent morbidity and death-loss during a backgrounding phase. Cattle receiving a booster less than 14 d after initial vaccination experienced 18.4% higher morbidity rates during the backgrounding phase than calves boostered after 14 days (White et al., 2008). Days from booster to arrival along with days from time of weaning to arrival displayed no effect on subsequent morbidity during the backgrounding phase in this study (White et al., 2008).

A separate experiment evaluated the effect of time in hours from vaccination with BHV-1 to BHV-1 challenge on morbidity and performance (Fogarty-Fairbanks et al., 2004). Calves (n = 43) either received a commercial MLV vaccine containing BHV-1 or no vaccine (non-vaccinated control (NVC)). Animals were challenged intranasally with the Cooper strain BHV-1 at 48, 72, or 96 h after vaccination. Calves challenged 72 h or 96 h after vaccination exhibited fewer days of clinical depression associated with BRD and had 40 to 75% more weight gain over the 29 d trial than did the calves in the NVC and 48 h challenge groups (Fogarty-Fairbanks et al., 2004). The data from these experiments reveal that commercial vaccines can confer adequate levels of protective immunity to viral pathogens within 72 to 96 h of administration. Moreover, when cattle are vaccinated twice prior to arrival in a feedlot there seems to be no significant correlation between time of second vaccine application in relation to shipment and subsequent morbidity; however, there does appear to be some interaction in regards to the time between first vaccination and booster. It appears best to allow at least 14 d from initial vaccine application to revaccination.

Likewise, there is limited data produced via controlled experiments regarding the delayed application of vaccines after arrival to the feedlot or need to revaccinate during the feeding phase. Richeson et al. (2009) used a mixture of bulls and steers (n = 264, BW = 239 ± 1.2 kg, bulls castrated on arrival) from multiple auction markets located in western Arkansas and eastern Oklahoma to investigate any potential effects with delaying clostridial (CLOS) or respiratory (RESP) vaccines. Calves had been commingled prior to arrival at the experiment station and were considered to be at a high risk for developing clinical BRD (Richeson et al., 2009). A 2x2 factorial design was used to segregate treatments into: arrival CLOS/arrivalRESP, arrival CLOS/delayed RESP, delayed CLOS/arrival RESP, and delayed CLOS/delayed RESP. Arrival treatments were administered on d 0 and delayed treatments were administered on d 11. All calves received two doses of RESP either on d 0 and 11 for the arrival RESP treatments or d 11 and 28 for the delayed RESP treatments. No differences in morbidity, mortality, or BW gain

were observed during the 56 d trial due to treatment. The arrival CLOS/delayed RESP treatment did result in a significantly higher percentage of chronics (Richeson et al., 2009). Another experiment conducted at Oklahoma State University examined the utility of revaccination in high-risk, commingled auction market calves (n = 612, BW = 219.5 ± 23.6 kg) after initial vaccination on arrival to the backgrounding facility (Step et al., 2009). All cattle received a commercially available pentavalent MLV viral vaccine (Vista® 5 SQ; Merck Animal Health; Millsboro, DE) at processing (d 0), and the revaccination group (RVAC) received the same vaccine on d 11. Cattle in the RVAC group experienced significantly greater morbidity rates during the backgrounding phase than did cattle in the SVAC group; however, the authors noted this outcome was most likely due to a randomization effect because the DOF to first antimicrobial treatment was not different between SVAC and RVAC and was less than the number of DOF to revaccination (SVAC = 7.62 d, RVAC = 7.21 d, revaccination occurred on d 11; Step et al., 2009). Mortaliy rates did not differ significantly due to revaccination treatment (SVAC = 2.36%, RVAC = 1.08%). Cattle in the RVAC group expressed slightly better feed conversion during a subsequent finishing period than SVAC cattle (Step et al., 2009). Revaccination in backgrounding facilities or feedyards is often used as a tool to improve herd immunity by ensuring animals not responding to the first vaccination treatment, due to some complicating factor, are exposed to "safe" form of pathogens of concern (Step et al., 2009). These studies reveal that a single vaccination on arrival for respiratory and clostridial pathogens is effective in conveying adequate immunity to groups of light-weight incoming cattle. Although revaccination or delaying vaccination may not be overly detrimental to health and performance, these practices would most likely increase labor costs associated with production and would not be necessary in every situation.

Receiving Cattle Studies: Pathogen Selection

Since many different vaccine combinations are commercially available, it is important to vaccinate for all potential offending pathogens. Multiple studies have evaluated vaccination

against only specific pathogens or with different variants of vaccines. In 1982, Martin et al. vaccinated calves (n = 849) three weeks prior to shipment with two different IBR/PI3 vaccines. No differences were observed due to treatment. Ellis et al. (2009) used three treatment groups (unvaccinated control, MLV without BHV-1, MLV with BHV-1) to evaluate the health outcome of calves (n = 63) challenged 30 d or 97 d post vaccination with BHV-1. In both challenge groups, the calves vaccinated with the MLV containing BHV-1 had reduced rectal temperatures, fewer observed clinical signs of respiratory disease, less shedding of BHV-1 virus, and increased antibody titers to BHV-1 as compared to the unvaccinated controls and MLV without BHV-1 (Ellis et al., 2009). Conversely, cattle in a large scale study (n = 5,163; BW = 253 to 274 kg) were vaccinated at processing and 70 d later with either a univalent MLV BHV-1 vaccine or a quadravalent MLV vaccines (BHV-1/PI3/BRSV/BVD) (MLV4) (Schunicht et al., 2003). This experiment reported cattle receiving the MLV4 treatment had greater ADG and lower morbidity rates. DMI, DOF, feed efficiency, mortality, and carcass merit were not affected by vaccine treatment. Altogether, these experiments confirm that commercial vaccines are efficacious in preventing disease onset from the pathogens they are specific for. Therefore, management strategies intended at targeting specific pathogens believed to cause the greatest losses can be very effective, but caution must be applied in large scale commercial settings not to leave cattle unvaccinated and immunologically naïve to potential pathogens.

Receiving Cattle: Management

It is important to have an established protocol for disease prevention and treatment when managing newly-received cattle into feedyards (Apley, 2006). Generally, upon arrival, cattle are processed which may involve: castration of intact males, tipping/removal of horns, deworming, vaccination, and metaphylactic treatment (Duff and Galyean, 2007). Multiple methods of castration exist and should be chosen base on preference, experience, and ability of those attending the cattle, but it is important to understand the effects of each method on subsequent health and performance (Hicks, 2014). A definition of a clinically morbid animal should be

determined and antimicrobial treatment protocols established and followed to allow for unbiased decisions to be made in regards to program efficacy (Apley, 2006). Diets should be palatable; familiar to the animal in texture, taste, and smell; and nutrient dense with special attention paid to protein and energy levels (Galyean et al., 1999; Loerch and Fluharty, 1999; Duff and Galyean, 2007). In all things, animal husbandry and handling must be closely monitored, as these factors have the largest impact on animal stress and have the potential to exhibit the greatest influence on the innate immune system (Edwards, 2010).

CHAPTER III

EFFECTS OF DIRECT-FED MICROBIALS ON PERFORMANCE AND HEALTH OF GROWING CATTLE

Abstract

With the cost of beef production increasing and profit margins becoming narrower, the need to utilize tools and employ techniques that increase cattle performance and efficiency becomes imperative. Direct-fed microbials (DFM) have received increasing attention in recent years in regards to the potential these products have displayed to boost performance and improve the efficiency of nutrient utilization of beef cattle in feedlots. Wide spread drought conditions in recent years have ushered in an increase in the percentage of recently-weaned calves going directly to feedlots due to the shortage of available pasture, and the need to validate the effects of DFM treatment in this class of cattle has increased. In the present experiment, twenty-four 18.2 m x 45.5 m open-air, dirt floor pens (12 pens per treatment) were utilized to feed steers (n = 562; BW = 238.2 ± 9.0 kg) a 75% concentrate, 25% roughage ration for 90 d with Bovamine Defend® (DFM) applied to the ration in 12 pens using randomized complete block design. The results showed no difference ($P > 0.05$) in BW, ADG, or DMI between the control and treatment groups.

Feed efficiency (G:F) was decreased in the DFM treatment ($P = 0.03$) by 5.9% during the first 28 days on feed compared to the control treatment. Likewise, DFM treatment had a tendency ($P = 0.06$) to decrease G:F from d 0 to 90 by 2.4% compared to the control treatment. No effects due to treatment ($P > 0.05$) were observed in morbidity or mortality. These data suggest that inclusion of Bovamine Defend® into diets of recently-weaned, newly-received calves to feedlots does not impact performance during the receiving and growing phase.

Introduction

The feeding of beneficial microbial products including bacteria, fungi, and enzymes to cattle has received increasing interest over the past few decades as a result of increased pressure to enhance cattle performance amidst increasing scrutiny over antibiotic and hormone use (Krehbiel et al., 2003). The FDA has required feed manufacturers to label such products fed to animals as Direct-Fed Microbials (DFM) and defines such products as a "source of live, naturally occurring microorganisms" (Yoon and Stern, 1995; Krehbiel et al., 2003). The most widely utilized DFM products in ruminant nutrition often contain species of lactate-producing and lactate-utilizing bacteria which are believed to have the propensity to reduce the effects of subclinical acidosis in feedlot cattle, improve daily gains and feed conversion, and improve the health of young, newly-received cattle to feedlots (Krehbiel et al., 2003; Wilson and Krehbiel, 2012). A 2004 survey of the VetLife Benchmark Database participants representing 267 feedyards and 10.9 million head of cattle revealed DFM increased daily gains by 1.9% and 1.4% in steers and heifers, respectively, and improvements in feed conversion were 1.9% and 3.9% for steers and heifers, respectively (McDonald et al., 2005). Furthermore, results showed that light-weight cattle on arrival (< 318 kg) that accrued over $20 in processing and treatment costs experienced a greater degree of response to DFM treatment than cohorts of the same weight class receiving less than $20 in processing and treatment (McDonald et al., 2005). Recently more attention has been devoted to elucidating the performance and intestinal health effects of DFM

inclusion into high grain finishing diets. However, some work has been completed to evaluate the effects of DFM inclusion on health and performance of light-weight calves recently received into feedlots (Gill et al., 1987; Krehbiel et al., 2001; Siverson et al., 2012). DFM products and routes of administration used differed among these experiments, and the results demonstrated considerable variation in efficacy as well. In the past few years, there has been an influx of weaned calves going directly to feedlots as a result of a decrease in cattle numbers and lack of forage resources due to drought conditions. Therefore, the need to validate the effects of DFM in this class of cattle on moderate energy, high roughage feedlot diets is of increasing importance.

Bovamine Defend® (Nutrition Physiology Co., Guymon, OK) is a product that contains 2 x 10^9 cfu of a combination of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24. The current experiment evaluated the effects of Bovamine Defend® on the performance and health of recently weaned, newly-received calves to a feedlot when included in the diet at 1 g/animal/d.

Materials and Methods

Steers (n = 562; initial BW = 238.2 ± 9.0 kg; Table 4) were shipped to the Willard Sparks Beef Research Center at Oklahoma State University in Stillwater, OK from October 20, 2013 to November 14, 2013. A total of 6 different sources were used to procure the cattle for this experiment (one load from Arkansas, one load from Louisiana, one load from North Dakota, and three loads from Texas). All calves were transported directly from ranch of origin to the Willard Sparks Beef Research Center (WSBRC) without previous commingling with other cohorts. Upon arrival to the WSBRC, calves were unloaded, a group weight obtained on a pen scale, and were allowed to rest for approximately one hour with no access to water or feed. After the resting period, all calves were individually weighed, checked for presence of testicles, and given a unique numbered identification tag in the left ear. After this process, cattle were kept in an open

dirt floor holding pen with ad libitum access to clean water and prairie hay (9.3% Crude Protein and 52.5% TDN on DM basis). Calves received initial processing 12 to 72 h after arrival which included: an individual weight, 5-way MLV viral respiratory vaccine (VistaOnce SQ®; Intervet/Merck Animal Health; Omaha, NE), a 7-way clostridial bacterin (Vision7® with Spur®; Intervet/Merck Animal Health; Omaha, NE), injectable dewormer (Ivermax® Plus; Aspen Veterinary Resources, Ltd; Liberty, MO), oral dewormer (SafeGuard®, Intervet/Merck Animal Health; Omaha, NE), a long duration, low dose estrogenic implant (Compudose®, Elanco; Greenfield, IN), metaphylaxis with tilmicosin phosphate at 2.0 mL/45.45 kg BW via subcutaneous injection (Micotil 300 USP; Elanco; Greenfield, IN), and a colored tag corresponding to treatment in the right ear.

Calves were blocked by source and arrival body weight within source and randomized to treatment. Twenty four 18.2 m X 45.5 m pens were used with 18.2 m of bunk space in each pen. Pens contained between 20 and 26 head depending on quantity of cattle supplied by each source. Pens were randomly assigned to treatment prior to initiation of the study, and calves were allocated to pen based on treatment in a sequential order.

Pen and individual BW were collected on arrival, d 0, 28, 56, and 90. Calves were weighed prior to morning feeding on each of these days and were weighed in the same pen order each day.

The diet consisted of 15% dry-rolled corn, 10% dried distiller's grains, 44.8% wet corn gluten feed (Sweet Bran®; Cargill; Dalhart, TX), 5.2% dry supplement (formulated to deliver 30 g/ton monensin and 8.25 g/ton tylosin phosphate to the final ration), and 25% chopped prairie hay (Tables 2 and 3). Bunks were observed multiple times daily and managed so that no feed remained at the 0530 observation. Feed was scheduled daily after bunks were read. Pens were fed twice daily at 0700 and 1300, and 50% of the days feed call was delivered at each feeding. Two feed mixing wagons were used in this experiment, a Kuhn Knight 3100 series horizontal feed mixer and a Rotomix 274-12B horizontal feed mixer. Prior to initiation of the study both

mixers were validated for consistency, and no differences in kilograms of feed delivered, ration consistency, or ration dry matter were detected between them. Daily pen feed intake was recorded on MicroBeef's Read-N-Feed (MWI/MicroBeef, Amarillo, TX) bunk management system. Individual morbidity, antimicrobial treatments, and mortality were recorded daily using Excel (Microsoft Corp., Redmond, WA).

On days that calves were weighed, any feed remaining in the bunk was removed, weighed as-is, and a dry matter sample was taken. Representative samples of both rations (CONTROL and DFM) were taken once weekly, and DM analysis was performed on each at that time. At trial termination, all dried feed samples were ground to 2 mm particle size, composited according to treatment, and nutrient analysis performed (ServiTech Laboratories; Dodge City, KS).

Bovamine Defend® was prepared daily for delivery to pens. Bovamine Defend® was administered to cattle at a rate of 1g/animal/d split equally between the two feedings and delivered in the mixed ration via dry suspension. Daily a new packet of Bovamine Defend® was opened and the appropriate amount for each batch (grams per batch adjusted for number of cattle fed on each batch) was mixed with 2.27 kg of ground corn for 5 minutes in a KitchenAid® mixer. The ground corn Bovamine mixture was stored in resealable buckets in a freezer at approximately -17^{o}C until application to the appropriate ration. The ground corn Bovamine mixture was added into the ration in substitution of 2.27 kg of dry-rolled corn at the time that each batch of feed was manufactured. All feed batches were mixed for 4 minutes upon the completion of loading the last ingredient prior to delivery to the pen.

Cattle were evaluated once daily at 0700 for clinical signs of respiratory illness by professionally trained evaluators. Cattle were evaluated following standard Willard Sparks Beef Research Center feedlot protocol for depression, appetite, respiratory signs, and temperature (DART™). The subjective evaluation was assigned a severity score (1=mild, 2=moderate, 3=severe, 4=moribund; Step et al., 2009). Cattle evaluated as having a clinical score of 1 or 2

were required to have a rectal temperature greater than or equal to 40°C to receive an antimicrobial treatment for clinical BRD. Cattle exhibiting a clinical score of 3 or 4 were administered antimicrobial treatment regardless of rectal temperature for humane reasons. All cattle were returned to their original pen unless it was determined that their ability to thrive in their home pen had been compromised due to severe respiratory distress, lameness, or other medical condition.

After metaphylaxis, a 5 d post-metaphylactic interval was established in which animals were not eligible for additional antimicrobial therapy for clinical signs of BRD. Calves eligible for first pull antimicrobial treatment were administered Resflor® Gold (Merck Animal Health; Omaha, NE) at 6.0 mL/45.45 kg of body weight with a post-treatment interval of 5 days. After the second post-treatment interval, calves which met the criteria for treatment were administered Excede® (Zoetis, Florham Park, NJ) at 1.5 mL/45.45 kg of body weight via subcutaneous injection at the base of the ear. After treatment with Excede®, cattle were no longer eligible for antimicrobial treatment for clinical respiratory disease. For humane reasons, post-treatment interval was decreased by 50% if an animal received a clinical score of 3 or 4. All calves with non-respiratory health issues requiring antimicrobial treatment were treated with oxytetratcycline (Biomycin® 200, Boehringer-Ingelheim Vetmedica, St. Joseph, MO; 200 mg/mL at 4.5 mL/45.45 kg of BW via subcutaneous injection). For all animals that died during the experiment, a gross field necropsy was conducted to determine the cause of death.

Data were analyzed using a mixed model with either the GLIMMIX or MIXED procedure in SAS with pen serving as the experimental unit (SAS Institute, Cary, NC). Categorical variables (e.g., mortality, morbidity) were analyzed with the GLIMMIX procedure and continuous variables (e.g., average daily gain, daily feed intake) were analyzed with the MIXED procedure. Each BW block within source block served as a replication, and replication was used as a random variable within the model. Deads-in analysis was used to calculate ADG, DMI, and G:F.

Results

There were no differences in BW or ADG as a result of DFM treatment ($P > 0.05$; Table 4). Dry matter intake per steer did not differ between treatments ($P > 0.05$; Table 5). From d 0 to 28 G:F was significantly greater for the calves receiving CONTROL treatment than for cattle on DFM treatment ($P = 0.03$). There was no difference in G:F between treatments from d 28 to 56 or d 56 to 90, but there tended ($P = 0.06$) to be an improvement in feed efficiency for cattle in the CONTROL treatment from d 0 to 90. No treatment effects were observed in morbidity or mortality ($P > 0.05$; Table 6).

Discussion

The inclusion of Bovamine Defend® into the diet at a rate of 1 g/animal/d had no significant effect on the BW, rate of gain, or intake of feed DM in this experiment. Some have proposed that DFM fail to exhibit much effect in newly-received cattle to feedlots when those cattle are either extremely healthy or extremely morbid (Gill et al., 1987). In the current experiment, calves were metaphylatically treated in order to prevent them from becoming extremely ill due to the belief that excessive morbidity would have potential to mask any treatment effects. Morbidity and mortality rates in these calves were low, and these cattle could be classified as healthy and high performing. The overall health and performance experienced by these steers could have been a contributing factor to why no intake or gain response was detected. Siverson et al. (2012) used heifers (n = 287; BW = 226 kg) to evaluate the effect of a DFM product delivered in the diet via dry suspension on health and performance of newly-arrived, stressed calves to feedlots. Similar to the present experiment, heifers in that experiment experienced high levels of DMI (7.45 kg/hd/d) and ADG (1.83 kg/hd/d) with low levels of morbidity (14.25%). They reported no effect on growth performance or morbidity due to DFM treatment. These reported findings by Siverson et al. (2012) coupled with the findings from the present experiment, support the theory of Gill et al. (1987) that extremely healthy, high performing cohorts may not respond to DFM treatment.

Furthermore, it is generally accepted that a partial mode of action of DFM in ruminants is the conditioning of the rumen microorganism to elevated levels of lactate. This conditioning results in tempering the detrimental effects of subclinical acidosis in these animals allowing them to maintain superior levels of performance at high levels of dietary concentrate intake (Krehbiel et al., 2003; Brown and Nagaraja, 2009; Wilson and Krehbiel, 2012). Wet corn gluten feed (WCGF) contains high levels of lactate from the addition of steep to corn bran in the milling process, which results in WCGF exhibiting a low pH (approximately 2.0). Much effort has been devoted to validating the effects WCGF has on ruminal fermentation, VFA concentration, pH, and diet digestibility when used as a substitute for corn. Krehbiel et al. (1995) reported more rapid declines in ruminal pH when WCGF was substituted for dry-rolled corn (DRC) at 50 or 100% of the DRC fraction of the diet. However, the inclusion of WGCF resulted in an increased minimum pH as compared to the DRC treatment, as well as less total area under the curve (Krehbiel et al., 1995). Data reported in other experiments support these results concerning WCGF inclusion into corn based diets (Montgomery et al., 2004; Siverson et al., 2014). Moreover, it has been reported that cattle fed diets containing high levels of wet corn gluten feed (>30% of DM) have not displayed a response to DFM treatment (Trenkle et al., 2003). A possible result of this effect was elevated lactate levels and reduced pH conferred from the intake of WCGF mitigated the effects supplemented lactate producing bacteria could exert on ruminal VFA profile and overall ruminal pH. The diet fed in our present experiment contained 44.8% WCGF and 25% chopped prairie hay. The elevated diet lactate levels in combination with the moderate rate of forage inclusion may have been critical factors in limiting the beneficial ruminal effects often associated with DFM.

In the current experiment, calves receiving the CONTROL treatment exhibited increased rates of feed efficiency during the first 28 d after arrival ($P = 0.03$) and tended to have increased feed conversion during the duration of the experiment ($P = 0.06$). Bovamine Defend® contains a mixture of *L. acidophilus* and *P. freudenreichii* at a level of 2×10^9 cfu. This product has been

marketed more on the basis of controlling the shedding of *Escherichia coli* O157:H7 in feedlot cattle intended for harvest, and is considered a "high-dose" DFM product. A separate "low-dose" product has also been marketed containing 1 x 10^7 cfu *L. acidophilus* NP51 and 1 x 10^9 cfu *P. freudenreichii* NP24 with label claims to improve DMI, ADG, and feed efficiency in feedlot cattle (Ware and Anderson, 2011). There has been speculation that inclusion of the "high-dose" product for long durations could actually have a detrimental impact on cattle performance. This is based on the assumption that the continuous inoculation of the rumen with a foreign microbial species artificially forces rumen microbial turnover to an extent that rumen microbial fermentation is decreased. If this hypothesis were true, it could be a potential factor in the decrease in feed efficiency associated with the Bovamine Defend® treatment in this experiment from arrival to d 28.

Implications

More data need to be generated in controlled research environments in regards to the effects of long-term supplementation with DFM products. Due to the wide array of DFM products available on the market and the inherent diversity of beef cattle production systems, the available research displays variable results to DFM treatments. Although the current experiment reported no beneficial outcomes resulting from DFM inclusion, the positive impact reported by other studies warrants an increase in the volume of data to validate the repeatability of such results.

CHAPTER IV

EVALUATION OF COMMERCIALLY AVAILABLE MULTIVALENT MODIFIED-LIVE VACCINES ON CATTLE HEALTH AND PERFORMANCE IN FEEDLOTS

Abstract

Successful prevention of Bovine Respiratory Disease (BRD) in newly-arrived cattle results in improved animal well-being and economic returns. Improvement of vaccination protocols upon arrival to feedlots will be essential to combating BRD prevalence in cattle with unknown vaccination histories. In this experiment, auction market derived, crossbred calves (n = 1,442; average arrival BW = 216 ± 20 kg, 64.77% bulls and 35.23% steers) with unknown health histories were obtained from February to May 2014. Cattle were transported an average of 600 km to the Willard Sparks Beef Research Center in Stillwater, OK. Upon arrival, calves were vaccinated with one of three treatments: INFORCE (Inforce™ 3 & OneShot® BVD; Zoetis; Florham Park, NJ), PYRAMID (Pyramid® 5 + Presponse; Boehringer-Ingleheim; St. Joseph, MO), or VISTA (Vista® Once SQ; Merck/Intervet; Omaha, NE) per label instructions (2 mL per animal). On d 14, calves were revaccinated according to their respective treatments INFORCE (BoviShield® 5; Zoetis; Florham Park, NJ), PYRAMID (Pyramid® 5; Boehringer-Ingleheim; St. Joseph, MO), and VISTA (Vista® 5; Merck/Intervet; Omaha, NE). No differences as a result of vaccine treatment were detected for BW, ADG, DMI, or G:F from d 0 to 60 ($P > 0.05$). Calves in the INFROCE treatment required 5.6 percentage units fewer ($P = 0.01$) second treatments for clinical signs of BRD than did calves receiving the VISTA treatment. INFORCE

and PYRAMID required three treatments for clinical signs of BRD 3.0 percentage units less (P = 0.03) than VISTA. Calves in the INFORCE treatment group tended to experience a lower percentage death loss (P = 0.09) than calves in the PYRAMID or VISTA treatment groups. Results suggest that vaccination with INFORCE decreased the number of animals requiring second and third antimicrobial treatments for BRD, and also reduced the number of calves lost as a result of BRD infection.

Introduction

Bovine Respiratory Disease Complex (BRD) continues to be the single most costly loss associated with commercial beef production in the United States, accounting for 1,0555,000 animals lost in 2010 valued at $643 million (NASS, 2010). Bovine respiratory disease is the result of a combination of pathogenic microorganisms infecting the host animal. Generally, the most severe cases begin with stress and an initial viral infection leading to a compromised immune system that allows for bacterial colonization in the upper and lower respiratory tract from pathogens such as *Mannheimia haemolytica* or *Pasturella multocida* (Griffin, 1996). Cattle that experience the greatest risk of developing BRD are light-weight calves (< 250 kg) that have been recently weaned and are commingled with other calves from different origins before arrival at a feedlot. The weaning, assembly, marketing, and transportation process is one of the most stressful experiences in a calf's life, and the subsequent disruption of feed and water intake, coupled with increased vocalization due to the disturbance of known social order, leave the calf physiologically susceptible to infection in the respiratory tract (Loerch and Fluharty, 1999).

Vaccination against viral and bacterial pathogens is the most successful management tool currently available to prevent losses associated with infectious diseases (Bowersock and Martin, 1999). It is well understood that for an efficacious vaccine to have potential to display true efficacy it must be administered to an animal that is immunocompetent and prior to exposure with infectious agents (Perino, 1996). However, vaccines are generally delivered on arrival to feedlots when cattle may be immunocompromised due to the stress of assembly and transportation, and

often times have already been exposed to disease causing pathogenic agents (Mackenzie et al., 1997). Increasing our understanding of how to effectively confer immunological protection to such animals through vaccination procedures is imperative to decreasing losses associated with BRD.

Most vaccines are parenteral in delivery route, but the understanding and application of vaccinating mucosal membranes to confer protective immunity has received increasing amounts of attention and interest. Kimman et al. (1989) reported that mucosal vaccination of calves against BRSV was an effective method of conferring mucosal and some systemic antibody protection. Moreover, it has been determined that some degree of protective immunity can be imparted to intranasally vaccinated animals within 60 to 72 h of vaccination (Bowersock and Martin, 1999).

Concern over antigen interference has also been placed on commercially available multivalent MLV products, especially those containing toxoids for *M. haemolytica* (formerly known as *Pasturella haemolytica*). Harland et al. (1992) demonstrated this effect when they vaccinated incoming calves (n = 2,324) to a feedlot with a MLV BHV-1 product or a BHV-1 glycoprotein subunit (gIV) both in the presence or absence of a *P. haemolytica* vaccine. The results demonstrated the MLV BHV-1 inhibited the efficacy of the *P. haemolytica* vaccination (Harland et al., 1992). Reported outcomes from a 2011 experiment evaluating the efficacy of concurrent administration of multivalent MLV viral vaccines in the presence of *M. haemolytica* bacterin-toxoid agree with the results of Harland et al. (1992) (Cortese et al., 2011). In contrast, intranasal administration of multivalent MLV viral vaccines in concurrence with subcutaneous delivery of *M. haemolytica* bacterin-toxoid has been reported to confer effective immunity to calves against all vaccinated pathogens (Stoltenow et al., 2011). These results would suggest that administration of these vaccines by different routes has the potential to prevent antigen interference among common vaccines when administered concurrently.

The present experiment evaluated these effects among two common subcutaneously administered pentavalent MLV viral respiratory vaccines containing a *M. haemolytica* bacterin-toxoid and concurrent administration of an intranasal trivalent MLV viral vaccine with a parenteral BVDV types 1 and 2 with *M. haemolytica* bacterin-toxoid.

Materials and Methods

Crossbred bull and steer calves (n = 1,442; average arrival BW = 216 ± 20 kg, 64.77% bulls and 35.23% steers) with unknown health histories were obtained from multiple auction markets from February to May 2014. Calves were obtained from auction markets in Oklahoma, Kentucky, Louisiana, Mississippi, and Florida. All cattle were shipped to the Willard Sparks Beef Research Center (WSBRC) in Stillwater, OK where the experiment was conducted (average shipping distance 600 km). The experiment was completed in two separate replications with the first replication containing 724 bulls and steers (average arrival BW = 196 ± 8 kg), and the second replication containing 718 bulls and steers (average arrival BW = 236 ± 16 kg).

Upon arrival, calves were unloaded and allowed to rest for approximately one hour before being individually identified in the left ear with a unique identification number, weighed, and sex determined. After weighing and identifying, cattle were held in large receiving pens for 12 to 72 hours with ad libitum access to grass hay (CP = 9.3%; NDF = 71%) and fresh water. Any animals deemed clinically lame or morbid at this time were removed from the sample population, administered the appropriate treatment, and were not enrolled in the experiment. Within each arrival load, cattle were ranked based upon weight within sex (bull or steer) and randomly assigned to one of the three treatments.

Twenty four, 18.2 m × 45.5 m open air, dirt floor pens were used to house the cattle. Each pen contained 18.2 m of bunk space and shared an automatic water tank (J360 continuous flow 20 gallon capacity; Johnson Concrete Products, Hastings, NE) with an adjacent pen. Pens were assigned to treatment in 3 blocks of 8 pens per treatment, with a solid plywood barrier

separating pens assigned to differing treatments. Pens containing different treatments did not share a water tank. In each replication of the experiment, there were 30 steers/pen and 8 pens/treatment (n = 16 total pens per treatment for the experiment).

Calves were processed 12 to 72 h after arrival and placed in their home study pen. Processing involved surgical castration of bull calves (procedure involved the use of Newberry knife, White's Emasculator, and scalpel), tipping of horns, administration of a 7-way clostridial bacterin/toxoid injected subcutaneously in the neck per label directions (UltraChoice™ 7; Zoetis; Florham Park, NJ; 2 mL per animal), endectocide injected subcutaneously based on truck load average weight (Dectomax® injectable dewormer, Zoetis; Florham Park, NJ; 1 mL per 50 kg BW), and a multivalent viral modified live vaccine per assigned treatment administered per label instructions (Inforce™ 3 & OneShot® BVD; Zoetis; Florham Park, NJ; Pyramid® 5 + Presponse®; Boehringer-Ingleheim; St. Joseph, MO; or Vista® Once SQ; Merck/Intervet; Omaha, NE) per label instructions (2 mL per animal). On d 14, calves were weighed by pen and individual body weights were recorded on each animal. At the time of individual weighing, steers were revaccinated according to their respective treatments (BoviShield® Gold 5; Zoetis; Florham Park, NJ; Pyramid® 5; Boehringer-Ingleheim; St. Joseph, MO; or Vista® 5; Merck/Intervet; Omaha, NE), respectively.

The diet consisted of 10% dry-rolled corn, 54.8% wet corn gluten feed (Sweet Bran®; Cargill, Dalhart, TX), 5.2% dry supplement (formulated to deliver 30 g/ton monensin and 8.25 g/ton tylosin phosphate to the final ration), and 30% chopped prairie hay. Bunks were managed so that no feed remained at 0530 when they were read. Feed was scheduled daily after bunks were read. Pens were fed twice daily at 0700 and 1300, and 50% of the days feed call was delivered at each feeding. A Rotomix 274-12B horizontal mixer and delivery wagon was used to feed all pens. Representative samples of the ration were taken once weekly and DM analysis was performed on each at that time. At trial termination, all dried feed samples were ground to 2 mm particle size, composited, and nutrient analysis performed (ServiTech Laboratories; Dodge City,

KS). On days that cattle were weighed, orts were collected from the bunk, weighed, and a DM analysis was performed to determine feed removed.

On d 60, cattle were weighed by pen, and individual body weights were recorded for every animal. Daily pen feed intake was recorded in MicroBeef's Read-N-Feed (MWI/MicroBeef, Amarillo, TX) bunk management system. Individual morbidity, antimicrobial treatments, and mortality were recorded daily using Excel (Microsoft Corp., Redmond, WA).

Cattle were evaluated once daily at 0700 for clinical signs of respiratory disease by two evaluators that were blinded to treatments. Cattle were evaluated following standard Willard Sparks Beef Research Center feedlot protocol for depression, appetite, respiratory signs, and temperature (DART™). The subjective evaluation was also assigned a severity score (1=mild, 2=moderate, 3=severe, 4=moribund; Step et al., 2009). Cattle evaluated as having a clinical score of 1 or 2 were required to have a rectal temperature greater than or equal to 40°C to receive an antimicrobial treatment for clinical BRD. Cattle exhibiting a clinical score of 3 or 4 were administered antimicrobial treatment regardless of rectal temperature for humane reasons. All cattle were returned to their original pen unless it was determined that their ability to thrive in their home pen had been compromised due to severe respiratory distress, lameness, or other medical condition.

Calves eligible for antimicrobial treatment were administered Draxxin® (Zoetis, Florham Park, NJ) at 1.1 mL/45.45 kg of body weight with a post-treatment interval of 10 days. After the first post-treatment interval, calves which met the criteria for treatment were administered Excede® (Zoetis, Florham Park, NJ) at 1.5 mL/45.45 kg of body weight with a 7-day post-treatment interval. After the second post-treatment interval, if cattle were determined to be clinically ill Advocin™ (Zoetis, Florham Park, NJ) was given at 2.0 mL/45.45 kg of body weight. After treatment with Advocin, cattle were no longer eligible for antimicrobial treatment for clinical respiratory disease. For humane reasons, post-treatment interval was decreased by 50% if an animal received a clinical score of 3 or 4 (i.e., Draxxin® $\geq$ 5 days or Excede® $\geq$ 4 days). All

calves with non-respiratory health issues requiring antimicrobial treatment were treated according to standard disease treatment protocols for the WSBRC.

Cattle receiving three antimicrobial treatments for clinical bovine respiratory disease (BRD) were weighed 14 days after their third treatment. If the animal lost weight from the recorded weight during the third antimicrobial treatment, the animal was deemed to be a "chronic". For all animals that died during the experiment, a gross field necropsy was conducted to determine the cause of death.

Data were analyzed using a mixed model with either the GLIMMIX or MIXED procedure in SAS (SAS Institute, Cary, NC). Categorical variables (e.g., mortality, morbidity) were analyzed with the GLIMMIX procedure and continuous variables (e.g., average daily gain, daily feed intake) were analyzed with the MIXED procedure. The mixed model included a fixed effect of BRD treatment, and random effects of replication (1 or 2) and arrival lot within replicate. Pen was the experimental unit for all variables analyzed. Deads-in analysis was used to calculate ADG, DMI, and G:F.

Results

The criteria required for removing an animal from the data set in this experiment were as follows: severe respiratory distress, severe lameness, neurological abnormalities, or death (all deads from d 0 to d 60 were used in calculation of mortality rates). A total of 103 animals died during the course of the experiment. There were 22 "chronics" by study case definition as previously described (15 from Rep. 1 and 7 from Rep. 2). Of the 22 "chronics", 9 subsequently died from severe respiratory disease or were euthanized for humane reasons (5 from Rep. 1 and 4 from Rep. 2). An additional 10 animals were removed from the experiment for severe respiratory distress that did not fit the "chronic" definition with one eventually dying (6 from Rep. 1 and 4 from Rep. 2). A total of 16 animals were removed for severe lameness including one animal that received a claw amputation on its right hind limb due to extensive septic arthritis (8 from Rep. 1

and 8 from Rep. 2; claw amputation from Rep. 1; one died from Rep. 2 after removal). One animal was removed for neurological abnormalities (from Rep. 2).

Animal Performance

There was no difference among treatment for initial shrunk BW ($P = 0.84$). No difference in d 60 BW was measured ($P = 0.94$). ADG from d0 – 60 did not differ among the three treatments ($P = 0.69$). DMI from d 0 to 60 was similar among all treatments ($P = 0.83$), and subsequently feed conversion did not display any differences among treatments ($P = 0.27$). There were no differences among the three vaccine treatments for any performance variables measured.

Animal Health

Morbidity and mortality data are recorded in Table 10. There was no difference among treatments for percentage of animals receiving one antimicrobial treatment ($P = 0.49$). Days from processing to first treatment did not differ among vaccination treatments ($P = 0.13$). INFORCE had 8.0 ± 2.8% second treats as compared to 13.6 ± 4.3% second treats for VISTA ($P = 0.01$). INFORCE and PYRAMID not differ ($P > 0.05$) in the percentage second treats. PYRAMID was not different ($P > 0.05$) from VISTA for percentage second treats. Days to second antimicrobial treatment from processing were not different ($P = 0.78$) across all vaccine treatment groups. INFORCE (3.7 ± 1.4%) and PYRAMID (3.7 ± 1.4%) had lower ($P = 0.03$) third treats than VISTA (6.7 ± 2.3%), but INFORCE and PYRAMID were not different ($P > 0.05$) from each other. Days from processing to third antimicrobial treatment were similar ($P = 0.56$) for all vaccine treatments. The number of "chronics" in relation to the number of animals enrolled into a treatment did not differ ($P = 0.55$) across all treatment groups. Mortality percentage for INFORCE was 4.4 ± 1.9% which tended ($P = 0.09$) to be lower than PYRAMID (6.4 ± 2.7%) and VISTA (7.6 ± 3.1%), with PYRAMID expressing a tendency to be lower than VISTA.

Discussion

Due to the relatively short duration of this experiment, any potential long-term performance differences resulting from the different vaccination protocols were not able to be measured. All treatment groups exhibited similar first pull morbidity rates; therefore, it was concluded that all animals were equally likely to experience clinical signs associated with Bovine Respiratory Disease (BRD) regardless of vaccination protocol. However, due to the decrease in subsequent need of antimicrobial treatment for clinical BRD signs in cattle receiving the INFORCE treatment, these results demonstrate that INFORCE treated animals experienced greater protection from BRD causing pathogens.

Most vaccines are administered parenterally and designed to elicit a systemic immune response commonly measured by increased serum antibody titers. However, over 90% of all infectious pathogens enter the body through mucosal surfaces (Potter et al., 2008). In the case of BRD, these pathogens not only enter through a mucosal route (i.e. the respiratory tract), this is also where they colonize and elicit their detrimental effects to the host. Vaccination of mucosal surfaces presents a means to provide protective immunity at the source of infection, rather than relying on an adaptive immune response to be mounted after pathogen colonization, reproduction, and entrance into vital body tissues has occurred (Kimman et al., 1989; Bowersock and Martin, 1999; Stokka and Perino, 2000).

Furthermore, research has shown that MLV combination viral vaccines containing a *Mannheimia haemolytica* (MH) bacterin have the potential to experience antigen interference between Bovine Herpesvirus-1 (BHV-1/IBR) and the *M. haemolytica*. Harland et al. (1992) reported observing antigen interference in calves entering a feedlot (n = 2,324; BW = 250 to 350 kg) coadministered a MLV BHV-1/PI3 vaccine with a *Pasteurella haemolytica* bacterin. This phenomenon of antigen interference between coadministered BHV-1 and *M. haemolytica* was also observed by Cortese et al. (2011). It has been proposed that if these two antigens were administered via different routes of administration (i.e., subcutaneous and intranasal) this antigen

interaction would be mitigated (Cortese et al., 2011). This result was displayed in calves vaccinated for BHV-1 intranasally at the same time a *M. haemolytica* bacterin was administered subcutaneously exhibiting similar BHV-1 and *M. haemolytica* titers 112 d post vaccination to the treatment receiving only the MH bacterin (Stoltenow et al., 2011).

In the current study, cattle receiving the INFORCE treatment had BHV-1 administered intranasally with the MH bacterin administered subcutaneously; in contrast to the PYRAMID and VISTA groups receiving all pathogens coadministered at one subcutaneous injection site. This differentiation in site of administration could have led to the apparent increase in immunological capacity manifested in the INFORCE treatment.

Stimulation of mucosal immunity through vaccination procedures in cattle has the potential for great benefit; however, it is important to note that dosages administered into the respiratory tract must be given in concentrations capable of overcoming the innate clearance mechanisms present on mucosal surfaces (Shewen et al., 2009). Mucosal vaccination procedures have the ability to confer faster or more effective immune capability to the animals, in-turn reducing the need for antimicrobial therapy and lowering costs associated with animal losses. Ancillary benefits would include but not be limited to: improved animal performance, increased animal well-being, lower labor investment, and a possible reduction in injection site reactions causing losses in carcasses. Further investigation needs to be conducted so more accurate understanding of the complex nature of antigen interaction and the true long-term immunological protection conveyed by mucosal vaccination can be validated.

Implications

If results experienced in this experiment are repeatable in the field, the efficacy of concurrent administration of multivalent MLV viral respiratory vaccines in combination with *M. haemolytica* bacterin-toxoids could be significantly increased in newly-received cattle in feedlots. This approach could result in improved animal health, decreased use of therapeutic antimicrobials, and fewer losses associated with morbidity and mortality.

CHAPTER V

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TABLE AND FIGURES

Table 1. Common viral and bacterial pathogens associated with bovine respiratory disease

Viral Pathogens	Bacterial Pathogens
Bovine Herpesvirus-1 (IBR)[1]	*Mannheimia haemolytica*[1]
Bovine Herpesvirus-3	*Pasteurella multocida*[1]
Bovine Parainfluenza 3 virus (PI3)[1]	*Haemophilus somnus*
Bovine Viral Diarrhea Virus type 1a (BVDV1)[1]	*Mycoplasma spp.*
Bovine Viral Diarrhea Virus type 2a (BVDV2)[1]	*Chlamydia spp.*
Bovine Respiratory Syncytial virus (BRSV)[1]	
Bovine Adenovirus	
Bovine Rhinovirus	
Bovine Reovirus	
Bovine Enterovirus	
Bovine Coronavirus	

[1]Pathogens commonly vaccinated for

Griffin, D. 1996. Etiology, pathogenesis, and clinical signs of bovine respiratory disease. Bovine Respiratory Disease: Sourcebook for the Veterinary Professional. Veterinary Learning Systems Co.

Table 2. Ration composition fed to newly-received calves to dry-lot environment in a 90 d growing experiment (DM basis)

Ingredient	% Ration
Dry Rolled Corn	15.00%
Dried Distiller's Grains (DDGS)	10.00%
Wet Corn Gluten Feed (Sweet Bran[1])	44.80%
Dry Supplement[2]	5.20%
Chopped Prairie Hay	25.00%

[1]Cargill; Dalhart, TX

[2]Formulated to deliver 33 g/ton monensin and 9 g/ton tylosin phosphate to final diet

Table 3. Ration nutrient profiles of control and DFM inclusion rations in a 90 d receiving and growing experiment with newly-received calves to a dry-lot environment (DM basis)

Diets	CONTROL	DFM
% Ration Dry Matter[1]	73.10	73.02
NEm, Mcal/kg	0.34	0.34
NEg, Mcal/kg	0.21	0.22
% Crude Protein	19.25	19.70
% Crude Fat	3.00	3.10
% Crude Fiber	17.05	16.60
% NDF	46.05	45.25
% TDN	70.10	70.60
% Ca	0.66	0.68
% P	0.61	0.62
% Mg	0.305	0.317
% K	1.25	1.28

[1]Reported on AS-IS basis

Table 4. Body weights and average daily gains of newly-received calves to dry-lots supplemented with DFM in a 90 d receiving and growing experiment

	CONTROL	DFM	SEM	*P*-Value
BW (kg)				
d 0	238.20	238.06	9.01	0.60
d 28	281.03	279.52	9.36	0.24
d 56	327.48	327.99	9.70	0.73
d 90	381.33	381.27	10.49	0.98
ADG (kg)				
d 0-28	1.51	1.47	0.12	0.27
d 28-56	1.66	1.73	0.06	0.21
d 56-90	1.59	1.57	0.06	0.71
d 0-90	1.59	1.59	0.06	0.98

Table 5. Dry matter intake per head and feed conversion of newly-received calves to dry-lot environments supplemented with DFM in a 90 d receiving and growing experiment

	CONROL	DFM	SEM	*P*-Value
DMI/hd (kg)				
d 0-28	5.21	5.24	0.17	0.62
d 28-56	8.02	8.21	0.25	0.12
d 56-90	8.89	8.99	0.25	0.53
d 0-90	7.47	7.56	0.21	0.33
G:F (kg BW gain/kg DMI)				
d 0-28	0.286	0.269	0.016	0.03
d 28-56	0.207	0.208	0.006	0.83
d 56-90	0.178	0.174	0.006	0.41
d 0-90	0.212	0.207	0.005	0.06

Table 6. Morbidity and mortality of newly-received calves to a dry-lot environment when supplemented with DFM in a 90 d receiving and growing experiment

	CONTROL	DFM	SEM	*P*-Value
% 1st Treat, Metaphylaxis	100	100	-	-
% 2nd Treat Morbidity	15.36	19.00	4.83	0.33
% 3rd Treat Morbidity	3.73	4.25	1.69	0.69
% 3rd of 2nd Treats	12.00	14.49	5.83	0.74
% Mortality	0.65	1.05	5.00	0.34

Table 7. Ration composition fed to calves in a 60 d receiving period when administered various commercially available multivalent MLV viral respiratory vaccines on arrival (DM basis)

Ingredient	% Ration
Dry Rolled Corn	10.00%
Wet Corn Gluten Feed (Sweet Bran[1])	54.50%
Dry Supplement[2]	5.50%
Chopped Prairie Hay	30.00%

[1]Cargill; Dalhart, TX

[2]Formulated to deliver 33 g/ton monensin and 9 g/ton tylosin phosphate to final diet

Table 8. Nutrient analysis of ration fed to calves in a 60 d receiving period when administered various commercially available multivalent MLV viral respiratory vaccines on arrival (DM basis)

Diets	Replicate 1	Replicate 1	Combined Replicates
% Ration Dry Matter[1]	73.37	71.16	72.55
NEm, Mcal/kg	0.34	0.33	0.31
NEg, Mcal/kg	0.21	0.21	0.19
% Crude Protein	16.80	17.20	17.30
% Crude Fat	2.50	2.00	2.20
% Crude Fiber	17.90	18.10	20.30
% NDF	42.70	39.00	45.30
% TDN	69.70	69.40	66.70
% Ca	0.67	0.65	0.68
% P	0.65	0.60	0.60
% Mg	0.37	0.32	0.33
% K	1.09	1.34	1.19

[1]Reported on AS-IS basis

Table 9. Body weights, ADG, DMI/hd, and feed conversion of calves in a 60 d receiving period when administered various commercially available multivalent MLV viral respiratory vaccines on arrival

	INFORCE	PYRAMID	VISTA	SEM	*P*-Value
BW (kg)					
d 0	216.80	216.80	215.50	20.45	0.84
d 60	280.50	279.50	279.50	18.20	0.94
ADG (kg/d)					
d 0-60[1]	1.00	0.95	0.95	0.05	0.69
DMI/hd (kg)					
d 0-60[1]	5.82	5.91	5.91	0.41	0.83
G:F					
d 0-60[1]	0.175	0.167	0.171	0.006	0.44

[1]Deads in analysis

Table 10. Morbidity, mortality, and chronics observed in calves during a 60 d receiving period when administered various commercially available multivalent MLV viral respiratory vaccines on arrival

	INFORCE	PYRAMID	VISTA	SEM	*P*-Value
Number of Head	483	481	478	-	-
1st Treats, %	42.7	44.1	46.6	4.62	0.49
Days to 1st Treat	11.7	12.2	13.9	1.11	0.13
2nd Treats, %	8.0[a]	10.1[ab]	13.6[b]	4.32	0.01
Days to 2nd Treat	25.6	26.3	27.9	2.73	0.78
3rd Treats, %	3.7[a]	3.7[a]	6.7[b]	2.27	0.03
Days to 3rd Treat	31.1	31.5	35.0	2.54	0.56
Deads, %	4.4	6.4	7.6	3.10	0.09
Chronics, %	1.7	0.9	1.5	0.77	0.55

[a,b] Columns with differeing superscripts differ by less than ($P < 0.05$)

VITA

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Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF DIRECT-FED MICROBIALS AND VACCINE ROUTE OF ADMINISTRATION ON HEALTH AND PERFORMANCE OF NEWLY-ARRIVED CALVES TO FEEDLOTS

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