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Performance Of Neonatal Holstein Heifers Fed Yeast Derivatives In Milk Replacer

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PERFORMANCE OF NEONATAL HOLSTEIN HEIFERS FED YEAST DERIVATIVES IN MILK REPLACER

By

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Objectives of the present study were to evaluate neonatal Holstein heifer performance while supplemented with coccidiostat (CX), yeast (YST), β-glucan or some combination of these products in non-medicated milk replacer. Efficacy of these products on E. coli and coccidia shedding was also observed. No differences ($P > 0.05$) were observed among treatment groups with respect to performance data collected which included growth, feed intake and efficiency, fecal and respiratory and blood parameters. Heifers fed YST or YST + β-glucan had higher ($P = 0.02$ and $P < 0.01$, respectively) fecal E. coli concentrations compared to heifers fed CX, CX + YST, or β-glucan. No heifers showed clinical signs of coccidiosis and appeared to be healthy. These results suggest neonatal heifers may be supplemented with yeast derivatives without negative effects on growth or health performance.

DEDICATION

I would like to dedicate this research to my family, who has helped keep me motivated and supported my decision to pursue a Master's degree

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. First, I would like to extend great thanks to my family who has supported me every step of the way throughout my college career. I am very blessed to have such loving people in my life. Along my educational journey, I met my husband, Kenneth, who has shown me more love and support than I could ask for and "thank you" doesn't say enough. Many thanks is due to my advisor and friend, Dr. Stephanie Hill Ward, who has guided me through my graduate student career. I appreciate her willingness and patience. To my committee members, Dr. Brian Rude, Dr. Jim Brett, and Dr. Yvonne Vizzier-Thaxton, I extend great thanks to all of you for your time. Each of you have influenced my life in a positive way. I owe a special thanks to Mr. John McReyonlds for taking a risk and letting a "girl" do farm work for him. We shared many good times together, along with a few stressful ones, from traveling around the southeast attending cow sales to driving cattle up the road! To Ali Anderson, thanks for being my partner in crime. I am very grateful for the fun times we've had over the past several years and hope there are many more ahead. Finally, I would like to thank all of my fellow office mates for new friendships and endless hours of entertainment and "educational sessions". I wish you all the best of luck with your endeavors.

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INTRODUCTION

Finding alternatives for sub-clinical antibiotic use in production animals is being encouraged not only by those in the medical community, but also by consumers. From the medical standpoint, it is believed that human pathogens are becoming resistant to certain antibiotics due to the sub-clinical use of antibiotics in animals that are reared for consumption and the potential for translation of these antibiotics into the food product. The administration of an antimicrobial, usually as a feed additive, over a period of time, to growing animals that results in improved physiological performance, is the definition of growth promotion given by, Phillips et al. (2004). In the 1940's, chickens were fed by-products of tetracycline (an antibiotic) and were found to grow faster than those not fed the by-products. This is when growth promoting effects of antibiotics were first discovered (Stokestad et al., 1949). Since this discovery, poultry, swine and cattle producers have utilized some antibiotics or derivatives, as growth promoters and gut health enhancers. Phillips et al. (2004) states that antimicrobials are an integral part of efficient and humane livestock production, as growing the best animal in the shortest length of time with the least amount of disease interference is a main objective in livestock production. Swine producers use therapeutic antibiotics at weaning to treat gastrointestinal disorders and then later in life to treat pneumonia, whereas cattle producers use antibiotics mostly to treat respiratory infections in calves and mastitis in cows. It is believed that the problem with human resistance to certain antibiotics may also

be caused by the over-use of antibiotics by humans and not just from the use in animals (Phillips et al., 2004). Consumers today are more health conscious than in years past and for this reason, producers aim to raise production animals to the standard in which the consumers feel comfortable buying and consuming their products. In March of 2009, Representative Louise Slaughter proposed to amend the Federal Food, Drug, and Cosmetic Act. The proposed bill, Preservation of Antibiotics for Medical Treatment Act of 2009, would eliminate the use of Critical Antimicrobial Animal Drugs, which are defined as drugs used for treatement in both humans and animals: Penicillin, tetracycline, macrolide, lincosamide, steptogramin, aminoglycoside and sulfonamide. Upon approval of this bill, these drugs will not be usable as a non-therapeutic drug (Library of Congress, 2009). In 2002, the National Animal Montoring System (NAHMS) conducted a study that represented 83% of the U.S. dairies and 85.7% of the total dairy cow population in the United States. Data for the study was collected from February 2002 to January 2003. The purpose of the study was to provide information about on farm antibiotic use to participants and industry. Data was collected on cows, unweaned heifers, weaned heifers and medicated milk replacer. The most common problem that required antibiotic treatment for cows was mastitis (16.3%) and of those infected, 91.9% were treated with an antimicrobial. Of the participants in the present only 14.7% used antimicrobials in weaned heifer rations compared to 82.6% who did not. The majority of antimicrobial use in unweaned heifers is due to diarrhea and/or digestive problems (15.3%) rather than respiratory disease (9.0%), while respiratory diseases are treated more often (95.6%) with antimicrobials compared to diarrhea/digestive problems (85.7%). Medicated milk replacers were utilized by 50.8% of the participants in this study, while the greatest percentage of those were operations with 100 to 499 cows. The most commonly used

drug in medicated milk replacers was oxytetracycline (23.5%). With the potential restriction of certain antibiotics, researchers are motivated to investigate the possibility of using live yeast cultures, yeast cell wall products, and β-glucans to promote gut health and reduce use of antibiotics in feed.

Advancements in biotechnology in recent years have allowed the use of microbial cultures as feed additives (Görgülü et al., 2003). Among these additives, probiotics, which has been defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance", are included (Fuller, 1989). In the late 1800s and into the early 1900s, the dietary use of probiotics and prebiotics bloomed as people became more interested in intestinal microbiology (Patterson and Burkholder, 2003). Along with probiotics, the use of prebiotics are also being evaluated as a supplement for both humans and animals. The basic definition of a prebiotic is, "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). Fructooligosaccharide products are the most commonly used prebiotics at this time, however, there are many other oligosaccharides being investigated (Patterson and Burkholder, 2003). Among these oligosaccharides used for both human and animals, mannanoilgosaccharides are used as a prebiotic, but they do not selectively enrich beneficial bacterial populations. Instead, they are thought to bind and remove pathogens from the intestinal tract, which results in immune system stimulation (Spring et al., 2000). Immune stimulation is enhanced due to the reduction of pathogen colonization in the intestinal tissue that helps fight infection (Patterson and Burkholder, 2003). Three of the possible alternatives being studied currently with and for dairy calves are live yeast culture (*Saccharomyces cerevisae*), yeast cell wall products

(oligosaccharides), and β-Glucans (derivative of yeast). To date, the research conducted thus far involving these three yeast derivatives has been very positive. Görgülü et al. (2003) conducted a study using 24 male Holstein calves assigned to either the control or the probiotic treatment group in two week intervals (used to eliminate time and age effect) until there were 12 calves per treatment group. The probiotic treatment which consisted of: *Lactobacillus plantarum, L. bulgaricus, L. acidphilus, L. rhamnosus, Bifidobacterium bifidum, Aspergillus orysa and Candida* pintolopesii, was added to milk during the morning feeding throughout the experimental period at a rate of 2 g per head per day. Calves were fed whole milk, twice daily until weaned at 60 days of age. Growth and health measures were recorded throughout the trial and calculated for 0-30, 30-60 and 0-60 d. Results from this study are based on 10 control calves and 12 probiotic fed calves, due to death of calves from scours and/or bloat. The present study concluded that calves supplemented with probiotics were superior in overall health compared to control, however, there were no differences in growth performance. Average daily gain and feed to gain ratio for the total experimental period for control and probiotic treatments were, 349.29 ± 53.36 g/d and 366.26 ± 29.68 g/d and 1.25 ± 0.24 and 1.44 ± 0.14, respectfully. The total number of diarrhea and/or bloat cases were lower (3 cases) for the probiotic treatment group compared to control (8 cases) group. Based on this information, Görgülü et al. (2003) saw reduced treatment and medication cost for calves fed probiotics.

CHAPTER II

REVIEW OF LITERATURE

Coccidiostat Supplementation

In the United States, there are currently at least 13 different coccidial species known with the ability to infect cattle, however, not all of them are pathogenic. The two species that pose the greatest concern are: *Eimeria bovis* and *Eimeria zuernii*. Coccidia typically infect the epithelial cells of the gut mucosa, but there are exceptions , Exception number one is: the first generation schizonts (sporozoites that have undergone nuclear division, of *E. bovis* are located in the endothelial cell of the small intestine, while the second exception is the first generation schizonts of *E. zuernii* are located in the connective tissue cells of the lamina propria (a loose layer of connective tissue located just below the epithelium). If not treated properly, calves with coccidiosis normally die from diarrhea or dehydration (Ernst and Benz, 1986). The lifecycle of coccidia take approximately 21 d to complete. From d 1 to 15, the coccidia mature within the small intestine, around d 16, the coccidia enter the large intestine and then is excreted between days 17 and 21. The coccidia may stay in the environment until the oocyst are ingested at which the lifecycle starts over (Quigley, 2001). Due to the persistence of coccidia in the environment, the most effective treatment for coccidiosis is prevention. New calvesshould be housed in a clean environment where feeding and water buckets should be placed so there is limited fecal contamination. If contamination occurs, the chances of calves ingesting coccidia oocyts increases, which may ultimately lead to infection

(coccidiosis). The prevalence of coccidiosis may be controlled or reduced by the use of preventative drugs such as ionophores (lasalocid or monensin) and quinolones (decoquinate), as used by in studies by Waggoner et al. (1994) and Heinrichs et al. (1991). According to Ernst and Gerald (1986), coccidosis was first effectively treated in chickens by flowers of sulfur in 1936. While this pioneer drug was too toxic to be used for general purposes, it did encourage the discovery of a variety of sulfonamides which were able to be used in chickens and other animals. Sulfonamides are still effective drugs for treating coccidiosis, they are also used in human medicine, therefore, if the proposed bill "Preservation of Antibiotics for Medical Treatment Act of 2009" gets passed, this would result in the elimination of this particular class of drug. Another drug currently being used to treat coccidiosis is Amprolium (Corid), according to Ernst and Gerald (1986).

Research in Cattle

In 1990, Heinrichs et al. conducted a study to evaluate the effectiveness of coccidiostat (decoquinate) on growth and health parameters in calves weaned early (4 wk) or conventionally (7wk). Forty-four Holstein calves were assigned to one of four treatment groups; Early weaning/control grain, early weaning/ decoquinate grain, conventional weaning/control grain, or conventional weaning/decoquinate grain. The experimental design for the present study was a 2 x 2 factorial. Calves were initially assigned to a respected weaning method and then to either control calf starter or starter containing decoquinate. Calves fed grain with decoquinate were later fed control starter after consuming their initial treated grain. This was to ensure total consumption of decoquinate before being offered more grain with no additive. Decoquinate (Deccox,

Rhone-Poulenc, Atlanta, GA) was fed at a rate of 100mg/kg grain until weaning. Once calves were weaned, those supplemented with decoquinate continued to be fed at a rate of 0.5mg/kg BW/d. All calves were fed whole milk at 10% of initial body weight twice daily until 1 wk before weaning, at which 5% of initial body weight of milk was fed one time daily. Grain was offered daily, while refusals were collected and weighed weekly. Samples were collected weekly for feed analysis. After weaning, calves were moved outdoors and placed in groups of 6 to 8 (by treatment) and remained on trial until 24 wk of age. During this period, grain was fed at a rate of 3 kg/d along with free choice alfalfa hay. Calves were weighed weekly until 8 wk of age and then biweekly through the duration of the trial, while wither height, body length and heart girth were recorded biweekly until 8 wk of age. Fecal scores were recorded and fecal samples were collected from each calf one time per week until calves were 7 wk of age. Group-housed animals had fecal samples collected weekly through the duration of the trial. Early weaned calves had greater ($P < 0.01$) intakes of grain between weeks 5 and 7. There were no apparent differences observed between weeks 8 and 24, meaning there were no long-term effects to early weaning. Feed intake was not different, overall, for treated calves. Coccidia counts were significantly lower in calves treated with decoquinate ($P < 0.01$), however, the age of weaning had no effect. Actual coccidian counts for weaning treatment and decoquinate treatment were: Early weaning (377.9), conventional weaning (196.1), no decoquinate (535.4) and decoquinate (38.7). Conclusions from this study suggest treating neonatal calves with decoquinate may reduce the number of coccidia oocysts shed in feces. Also, early weaning had no negative long-term effects when compared to conventional weaning. Waggoner et al. (1994) conducted a 56 d study to evaluate the growth differences of calves fed lasalocid and decoquinate or a combination of the two

additives. Lasalocid is the drug name for an antibacterial and coccidiostat, which is also the active drug in the commonly used feed additive, Bovatec®. Sixty-four Holstein calves between 15 and 17 wk of age with a mean BW of 185 ± 3 kg, were used in this study; they were randomly assigned to one of four treatments. Treatments were: control (unmedicated), lasalocid (Bovatec®,1.0 mg/kg of BW), decoquinate (Deccox®, 0.5 mg/kg of BW) or combination of lasalocid and decoquinate (1.0 mg/kg + 0.5 mg/kg of BW, Bovatec® and Deccox®, respectfully). The combination group was fed the additives at the same feeding rate, however, calves were fed the same recommended feeding rate of decoquinate for the first 28 d on trial and then fed the recommended feeding rate of lasalocid for the last 28 d on trail. Deccox® was fed to calves during the first 28 d of trial because it is an anticoccidial treatment used to prevent calves from being infected with coccidia before weaning, while Bovatec® was fed once calves were weaned and placed into a group environment, the time at which coccidia oocyts are shed in feces. Bovatec®, an ionophore, is a growth promoter, which helps calves during stressful situations, such as weaning. Calves were housed in a single barn with four calves per pen. One week before the study, qualitative fecal exams were conducted to determine the presence of coccidial oosyts: *E. zuernii* and *E. bovis*. Oocyst numbers were graded subjectively (rare, few, moderate, or many). Upon arrival at the Purdue University Animal Science Research Farms, calves were weighed for 2 consecutive days and then weekly for 56 d. Calves were also weighed consecutively on the final 2 d of the study. Fecal samples were collected from each calf at the beginning of the trial and then weekly for 8 wk. Daily body weight, DMI, and feed efficiency were calculated per pen. Results for overall feed intake showed calves fed decoquinate had lower (6.76 kg/d, $P < 0.05$) intakes when compared to calves fed lasalocid (7.00 kg/d, $P < 0.05$) or lasalocid plus

decoquinate (7.07 kg/d, $P < 0.05$). Body weight gain for all calves was similar ($P = 0.75$) with an average of 1.29 ± 0.03 kg/d over the duration of the 56 d trial. Waggoner et al. (1994) noted, but did not show results for: lower oocyst shedding rates at wk 2 for all treatment groups which caused a slight increase in body weight gain. There was an increase shedding of oocyst at wk 6 among all treatment groups. During this time, all calves exhibited a decreased body weight gain. Calves did not show signs of coccidial infection, suggesting that coccidial infection may not be a major factor in rate of gain. Feed efficiency was similar ($P = 0.79$) and averaged 0.189 ± 0.004 kg of gain/kg of DMI for all treatment groups. Conclusions for this study suggest that under natural exposure to coccidiosis, there was little advantage for medicating calves with lasalocid or decoquinate at recommended dosages when compared to nonmedicated calves with respect to increased gain and calf performance.

Table 1 Growth and intake performance of calves fed coccidostats, probiotics, prebiotics, or no additive

*	Animals	Week	Treatment	BW, kg	ADG, kg/d	P-value
1	Holstein & Swiss	1 - 4	Control	46.6	0.20	> 0.05
1	Holstein & Swiss	1 - 4	Decoquinate, 0.5 mg/kg of BW/d	46.6	0.19	> 0.05
1	Holstein & Swiss	1 - 4	Lasalocid,1 mg/kg of BW/d	45.3	0.15	> 0.05
1	Holstein & Swiss	5 - 8	Control	57.2[b]	0.41	< 0.05
1	Holstein & Swiss	5 - 8	Decoquinate, 0.5 mg/kg of BW/d	60.0[a]	0.52	< 0.05
1	Holstein & Swiss	5 - 8	Lasalocid,1 mg/kg of BW/d	57.2[b]	0.48	< 0.05
2	Holstein	1 - 4	Control	44.9	-	0.94
2	Holstein	1 - 4	Decoquinate, 0.5 mg/kg of BW/d	45.6	-	0.94
2	Holstein	5 - 8	Control	58.1	-	0.79
2	Holstein	5 - 8	Decoquinate, 0.5 mg/kg of BW/d	59.6	-	0.79
3	Holstein	1 - 8	Control	54.6±2.14	0.35	> 0.05
3	Holstein	1 - 8	Probiotic, *Lactobacillus* mixture	56.3±2.89	0.37	> 0.05
4	Holstein	1 - 9	Control	-	0.57	0.38

Table 1 (continued)

*	Animals	Week	Treatment	BW, kg	ADG, kg/d	P-value
4	Holstein	1 - 9	MOS, 3 g/d	-	0.53	0.38
4	Holstein	1 - 9	YST, 4 g/d	-	0.54	0.38
4	Jersey	1 - 9	Control	-	0.37	0.55
4	Jersey	1 - 9	MOS, 3 g/d	-	0.40	0.55
4	Jersey	1 - 9	YST, 4 g/d	-	0.41	0.55
5	Holstein	1 - 6	Control	60.2[b]	0.44[b]	<0.05
5	Holstein	1 - 6	YST, 1% yeast culture	61.4[ab]	0.44[ab]	<0.05
5	Holstein	1 - 6	YST1, 2% yeast culture	65.3[a]	0.51[a]	<0.05

* 1=Heinrichs et al., 1991 2=Heinrichs et al., 1990 3= Görgülü et al., 2003 4= Hill et al., 2009 5=Lesmiester et al., 2004.

[a,b] Means within the same row differ, $P < 0.05$.

Mannanoligosaccharide Supplementation

Research in Ruminants

Mannanoligosaccharide (MOS) is a carbohydrate fraction of the yeast cell wall that is not digestible, therefore as the bacteria attaches to MOS, it leaves the gut still attached. Mannanoligosaccharide attracts certain gut bacteria such as E. *coli*, which has an affinity for mannose fractions (Hill et al., 2009). In the past, oligosaccharides have been used most frequently in the poultry and swine industries. The popularity of MOS is becoming more familiar to the dairy industry as producers are encouraged to use other products to increase gut health without the use of sub-therapeutic antibiotics. In a study by Heinrichs et al. (2003), 72 Holstein calves were used to test the effects of MOS or antibiotics in milk replacer. After being fed colostrum for 2 d, calves were then switched to milk replacer (12.5% DM) containing 20% CP and 20% fat. Calves were assigned to one of three treatment groups: Control (no additive), Antibiotic (400 g/440 kg of neomycin + 200 g/440 kg of oxytetracycline) or MOS (4 g Bio-Mos per animal daily) after being removed from dam. Milk replacer was fed twice daily until 6 wks of age. Calves had *ad libitum* access to starter and water throughout the trial. Weekly samples included body measurements and blood sample, while fecal and respiratory scores were recorded daily. Heinrichs et al. (2003), reported, overall, calves fed antibiotics or MOS had a higher probability ($P < 0.01$) for normal feces for the duration of the 6 week trial. Regards to feed intake, there were no differences between treatment groups at the beginning of the trial, however, at week 6, calves fed MOS consumed more starter than calves supplemented with antibiotics ($P < 0.05$). No differences in growth or blood parameters were found in this study. This study suggests the addition of MOS in milk

replacer may benefit overall calf health. According to manufacturer's suggestion, the recommended feeding amount of MOS varies from 4 to 10 g/d.

Table 2 Means of intake and performance of dairy calves fed antibiotic, MOS, or no additives in milk replacer[1]

	Control	Antibiotic	MOS	SE
Body Weight, kg				
Initial	51.4	51.5	51.4	1.1
Final	66.5	67.2	65.7	1.3
ADG, kg/d	0.36	0.38	0.34	0.03
Grain Intake, kg/d				
Wk 1 to 5	0.130	0.117	0.137	0.012
Wk 6	0.850[ab]	0.793[b]	0.944[a]	0.047

[a,b] Means differ; $P < 0.05$.
[1] Heinrichs et al. (2003).

Hill et al. (2009) compared the supplementation of MOS (3 g/d) or live yeast (YST- 4 g/d) in a whole milk diet. All calves were fed 3.8 L colostrum once daily for 2 d, then fed whole milk according to body size; Holsteins were fed 3.8 L/d and Jerseys fed 2.8 L/d. Calves were weaned at 42 d and remained on trial until 63 d of age. Body measurements were taken weekly, while rectal temperatures, respiratory and fecal scores were recorded daily. Feed and orts samples were pooled by month and analyzed for DM, CP, NDF, and ADF according to AOAC protocols. No differences were observed by Hill et al. (2009) in Holstein body weights (BW) among treatments, however, Jersey calves supplemented with either MOS or YST had a greater BW at the end of the trial compared to those with no supplementation. On average, fecal scores were lower (indicating less scours) for Holstein calves fed MOS (1.35) or YST (1.26) compared to those with no supplementation (1.46), which is consistent with Heinrichs et al. (2003) who reported improved fecal scores in calves supplemented with MOS at a rate of 4 g/d. Because of its

immune boosting characteristic, MOS aids in the performance of animals when under stress (Spring et al.,1998). Franklin et al. (2005) conducted a study to determine if feeding MOS to close-up dry period dairy cows had an influence on cow immune status or on the transfer of passive immunity to their calves. At 4 weeks pre-partum, immune system parameters were monitored, blood samples were collected, and cows were vaccinated against rotavirus, coronavirus, and *E. coli* toxoid. Fifty cows (30 Holstein, 20 Jersey) were randomly assigned to treatment approximately 3 weeks pre-partum to one of two treatments: Control (18% CP, 3.1% fat) or control diet with MOS (10 g/hd/d). At this time, cows were also weighed and moved to the close-up maternity pens. Two weeks pre-partum, cows were vaccinated again and then one week before and at parturition, blood samples were collected. Blood samples were analyzed for serum protein concentrations (SPC), serum Ig concentrations, and rotavirus neutralization titers. Cows were given an intramuscular injection of oxytocin and milked soon after calving to ensure the complete removal of colostrum. Weight, temperature and quality of colostrum was recorded; samples were frozen for later IgG analysis. At the second milking, cows were weighed. Serum rotavirus titers were greater ($P < 0.01$) for Holsteins cows compared to Jersey cows. Serum rotavirus titers were also greater ($P = 0.04$) for MOS-fed cows compared to cows fed the control diet, indicating the potential for MOS-fed cows to have greater passive transfer of immunity against rotavirus to calves. Calves were removed from dams before suckling, they were weighed, bled via jugular venipuncture and fed maternal colostrum (1.9 L Holstein and 1.2 L Jersey). The second feeding was 12 h after the first and an additional blood sample was collected 24 h after the first bleeding. The quantity of colostrum produced was greater ($P = 0.04$) for Holsteins compared to Jerseys, as was expected, however, lactation number or MOS supplementation had no affect on the

amount of colostrum produced. For calves born from MOS-fed cows, there was a tendency (P = 0.08) for a greater increase in SPC from birth to 24 h of age. Franklin et al. (2005) concluded that MOS supplementation to close-up dry cows may enhance transfer of immunity to offspring, which may decrease the need for therapeutic antibiotics in calves, leading to a decrease in morbidity and medical cost.

Research in Poultry and Swine

Saccharomyes cerevisiae (yeast) is the most common source for MOS however, dried brewers yeast needs to be researched further as a source of MOS, which lead to the following study. White et al. (2002) conducted 2 experiments, using brewers dried yeast as a source of mannan oligosaccharides. The yeast contained 5.2% MOS. Agglutination test were performed to confirm that brewers dried yeast would agglutinate gram-negative bacteria having Type-1 fimbriae. The first experiment (28 d), 140 crossbred barrows and gilts were used from the University of Kentucky Swine Research herd. These weanling pigs were assigned to one of four treatment groups: Treatment 1- nonmedicated basal diet, Treatment 2- basal diet plus 55 mg carbadox/kg, Treatment 3- basal diet with 3% brewers dried yeast and Treatment 4- basal diet with 3% yeast and 2% citric acid. At the end of weeks 2 and 4, fecal swabs were collected to evaluate microbial enumerations. At the end of the experiment, one pig per pen was harvested for collection of intestinal samples. In Experiment 2, a total of 24 cross bred pigs were used in a 39 day isolation trial and assigned to one of three treatment groups. Treatment 1- basal diet, Treatment 2- basal diet with 55 mg carbadox (antimicrobial agent)/kg or Treatment 3-basal diet with 3% yeast. Pigs were inoculated with *E. coli* K88 after a 29-d preliminary period. Fecal samples were collected daily and intestinal samples were collected and analyzed

postmortem from one pig per pen as in Experiment 1. Results from Experiment 1 over the 28 d test period showed reduced feed intake (633g and 636g, yeast and yeast + acid, respectfully; $P < 0.05$) in pigs fed diets containing yeast leading to reduced growth rates and weight gains (393g and 391g, yeast and yeast + acid, respectfully; $P < 0.05$). The addition of yeast did not enhance growth performance. While the addition of yeast to pig diets tended to decrease the total amount of coliforms (*E. coli* K88 and Carbadox-resistant coliforms) present at 14 and 28 d, it was not significant ($P > 0.10$). Pigs in Experiment 2 fed brewers yeast shed fewer ($P < 0.05$) total coliforms than pigs fed basal diet. The addition of yeast and carbadox to pig diets resulted in reduced colonization of total coliforms in the jejunum ($P < 0.01$) and cecum ($P < 0.05$). Figure 1 shows total coliform counts in the jejunum were significantly different ($P < 0.01$) between pigs fed the basal diet (2.86 ± 0.06) and those supplemented with carbadox (2.00 ± 0.06) or yeast (2.22 ± 0.06).

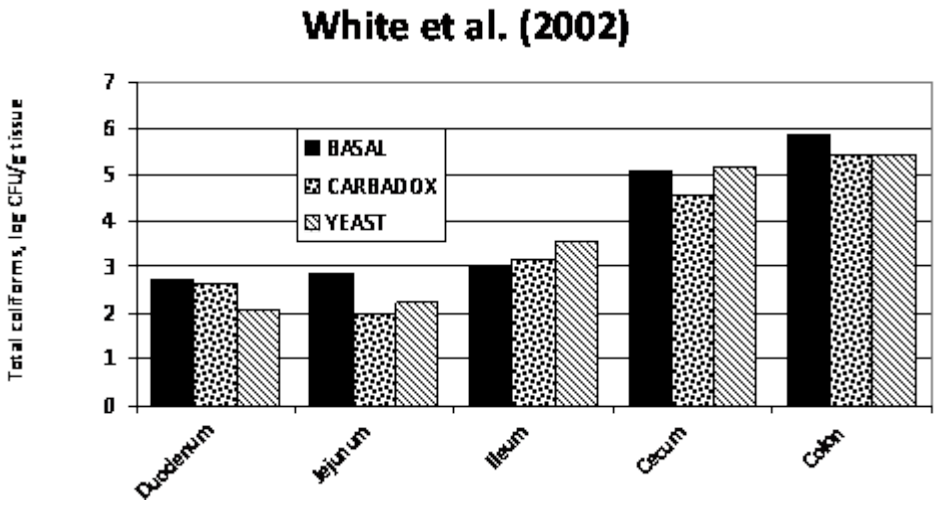

Figure 1 Total Intestinal Coliform Counts 10 d after *E.coli* inoculation in weanling pigs supplemented with brewers dried yeast- Exp. 2

There was significant differences between pigs fed the basal diet (5.70 ± 0.12) and those that received either carbadox (4.55 ±0.12) or yeast (5.16 ± 0.12) with respect to total cecal coliform counts. It should be noted that greater differences in were observed between basal and carbadox groups ($P < 0.01$) compared to basal and yeast groups ($P < 0.05$). According to the two experiments by White et al. (2002), the addition of 3% brewers yeast to pig diets may not improve growth response as found in other studies. Results from this study suggest that MOS may have a positive effect on immunoglobulins leading to enhanced immune function as pigs fed brewers yeast tended to have higher ($P < 0.10$) IgG and IgA levels. Davis et al. (2004) conducted a study to evaluate the effects of phosphorylated mannans on growth and immune response of 32 weanling pigs. Pigs were weaned on average at 19 d of age with an initial body weight of 5.7 ± 0.2 kg and assigned randomly within 16 pens, with two pigs per pen. Pigs were fed one of two diets both of which contained growth-promoting antibiotics (0.15 g of neomycin as neomycin sulfate and 0.11 g of oxytetracyline per kg/feed) with one of the diets containing phosphorylated mannans (0.3% of diet) substituted at the expense of corn in the basal diet. Pigs were bled via vena cava puncture at the beginning of the trial, 14 d after weaning and again just before euthanasia on d 19, 21, 24, and 26, respectively, via vena cava puncture. Differential blood samples showed an increased lymphocyte percentage, while the neutrophil percentage tended to decrease from pigs fed mannan diet. Neutrophils are often associated as the first line of defense in sub-clinical and clinical infection. Alleviation of inflammatory responses due to stress of weaning and improvements in gain and efficiency may all be supported by the trend of decreased ($P < 0.08$) neutrophil percentages in mannan-fed pigs, thus, phosphorylated mannans may improve growth and immune response in weanling pigs. Pigs were slaughtered in order

to collect 2 jejunal samples, which were used to isolate intraepithelial lymphocytes. On d 14 and 21 after weaning, BW and feed intake were measured to calculate ADG, ADFI and gain:feed (G:F) ratio. Results from this study indicated greater ($P < 0.05$) ADG and G:F, 0 to 14 d post weaning in pigs fed diets supplemented with mannans compared to pigs fed basal diets. Overall (0 to 21 d, postweaning) pigs supplemented with phosphorylated mannans performed better than pigs fed the basal diet with respect to ADG ($P = 0.03$) and G:F ratio ($P = 0.02$). Implications from the current study suggest the addition of phosphorylated mannans to newly weaned pigs may serve as a growth-enhancer.

β-Glucan Supplementation

The pre-weaned calf's digestive system functions similarly as those of some monogastrics, specifically pigs. Therefore, research and the data collected using pigs as the experimental unit can be very helpful in understanding the pre-ruminant calves and their digestive systems. The study mentioned above by Hahn et al. (2006), is an example of some data that may be reviewed and modified to fit a study using pre-ruminant calves.

β-Glucans are currently being studied by both human and animal scientist with hopes to finding new products that improve gut health and overall gut function. Improved weight gain, growth efficiency, disease resistance, and reduction in stress due to environment and/or immune function, are all examples of the improvements that may be made by supplementing β-glucans. β-glucans are immunomodulators derived from yeast cell walls, much like MOS, and are linked polysaccharides. β-glucans may be found in a variety of yeast and grains: yeast culture, yeast cell wall products, barley, oats, distillers grains (wet or dry), and brewers grains. It is recommended that β-glucans be fed in

concert with ascorbic acid (Vitamin C) to obtain the most effective results. The preferred dosage of β-glucan supplementation is between 0.2 to 0.8 mg/ kg of BW, while the preferred amount of Vitamin C is between 250 to 500 mg/kg of feed per day. In a study conducted at the University of Missouri Swine unit by Eicher et al. (2006), 32 crossbred pigs were used to evaluate the effects of Vitamin C and β-glucan as growth enhancers in newborn pigs and as immunomodulators after an immune challenge in young pigs. Pigs were assigned to one of four treatments: control, *Saccharmyces cerevisae* with β-glucan (0.312 g/kg of BW, 2.5% of diet; Energy Plus, Natural Chem Industries, LTD, Houston, TX), vitamin C (75 ppm), or β-glucan plus vitamin C (0.312 g/kg and 75 ppm, respectively). Supplements were given via 10 mL of whole bovine milk within 36 h of birth (after adequate colostrum intake and absorption) then daily until weaned at 2 wk of age. Sows nursed pigs until weaning; then pigs were placed in individual pens and fed a starter containing no additive (control), *Saccharmyces cerevisae* with β-glucan, vitamin C, or β-glucan plus vitamin C. Body weights were recorded every third day until weaning and then weekly after weaning. Treatment dosages were adjusted as pigs' BW increased. Pigs were challenged with 150 μg of lipopolysaccharide/kg of BW (LPS; *Escherichia coli* serotype O111:B4) 14 d after weaning. Blood samples were collected every 0.5 h for 4 h via jugular catheter. ADG was greater ($P < 0.05$) in pigs supplemented with the combination (β-glucan + Vitamin C) than those that received control or Vitamin C, however, pigs supplemented with Vitamin C alone had lower ($P < 0.01$)) cortisol concentrations than control. The interaction between Vitamin C and β-glucan showed a trend ($P = 0.07$) for lower cortisol concentrations. It may be concluded from this study that β-glucan and Vitamin C do in fact have immunomodulating effects in young pigs.

Hahn et al. (2006) performed two experiments to evaluate the effects of β-glucans on growth performance, nutrient digestibility and immunity in weanling pigs. In Experiment 1, 210 weaned, males pigs were assigned to one of five treatments. β-glucans were fed in the basal diet at levels of: 0, 0.01, 0.02, 0.03, or 0.04% of diet, respectively. The purpose of Exp. 1 was to evaluate pig performance and conduct a digestibility trial. This experiment was broken into two phases; Phase I (2 wk after weaning) and Phase II (3 to 5 wk). During each phase, BW and ADFI were recorded. On d 28, pigs were fed assigned treatment diets mixed with 0.25% chromic oxide as an indicator and fecal samples were collected from all pigs between d 32 and 35. In Experiment 2, 144 weanling, male pigs were assigned to one of four treatments: T1 (no β-glucan or anitibiotics), T2 (0.02% β-glucan), T3 (antibiotic) or T4 (0.02% β-glucan and antibiotics). The antibiotics fed during this experiment were separated into two phases. Phase I, was between wk 0 to 2, was 0.15% apramycin and 0.10% carbadox. Phase II (wk 3 to 8) antibiotics were: 0.10% chlortetracycline and 0.10% carbadox. Pig performance and digestibility was evaluated the same as Exp. 1 and the immune response was studied until wk 8 (end of trial). The digestibility results showed a linear increase as dietary β-glucan was increased in DM, GE, CP, EE, and P. Overall ADG was greater ($P < 0.05$) for pigs fed T4 diet compared to T2 or T1, but was similar to pigs fed T3 diet. Final observations from this study suggest that antibiotics may be more effective in regards to nutrient digestibility and growth performance in young pigs compared to β-glucan.

Table 3 Overall growth performance of weanling pigs fed different concentrations of β-glucan[1,2]

	β-glucan, %					
Parameter	0	0.01	0.02	0.03	0.04	SEM[3]
ADG, g/d	363	380	401	403	402	28.0
Feed Intake, g/d	589	608	642	629	621	37.9

[1] Each mean represents 3 pens with 14 pigs each.
[2] Hahn et al. (2006).
[3] Pooled SEM.

Table 4 Overall growth performance of weanling pigs fed antibiotics, β-glucan, or no antibiotics[1]

	Treatment[2]				
Parameter	T1	T2	T3	T4	SEM[3]
ADG, g/d	355	376	386	403	23.6
Feed Intake, g/d	495	504	488	536	31.6

[a,b] Means within a row without a common superscript differ, $P < 0.10$.
[c-e] Means within a row without a common superscript differ, $P < 0.05$.
[1] Hahn et al. (2006).
[2] Four treatment diets: T1 (no β-glucan or antibiotics; control), T2 (0.02% β-glucan), T3 (antibiotic) and T4 (0.02% β-glucan + antibiotic).
[3] Pooled SEM.

The current study is an example of a trial that may be reviewed and modified to fit a study using pre-ruminant calves. In agreement with the Hahn et al. (2006) study, Dritz et al. (1995) found no positive influence on pig growth performance, immune response, or neutrophil and macrophage function, by adding 0.1% β-glucan to diets. However, pigs supplemented with 0.025% β-glucan had increased ($P < 0.02$) ADG, 28 d post-weaning. This evidence supports the idea of supplementing pigs with 0.025% β-glucan would increase growth performance.

Growth and Development

As a calf ages, it undergoes many physiological changes involving overall body development, rumen development, and the ability to cope with immune challenges. From birth until weaning, calf development is broken down into 3 phases: liquid-feeding phase (pre-ruminant), transition phase, and ruminant phase (NRC, 2001). During the liquid-feeding phase, calves rely solely on milk or milk replacers to meet nutrient requirements. The esophageal groove plays an important role during this phase, as it redirects milk or milk replacer into the abomasum, avoiding microbial breakdown in the reticulo-rumen (Orskov, 1972). Stimulation of the esophageal groove to close may be induced by visual and other stimuli such as: presence of caretaker or noises associated with feeding (Moran, 2002). As the calf ages, it begins to rely on both liquids and solids (starter grain) for nutrients, this is considered the transition phase. By the time the calf reaches the ruminant phase, it is completely reliant on solid feeds to meet nutrient requirements (NRC, 2001). For calves 2-3 weeks of age, receiving proper nutrition as their bodies transition into fully functional ruminants is crucial.

Pre-Ruminant Phase

Shortly after birth, a calf needs to receive adequate quality and quantity colostrum, which is the cornerstone to health and longevity. Colostrum contains numerous antibodies, including immunoglobulins, which help boost the immune system of the neonatal calf, which are absorbed by the small intestine. Calves are born with no immunity, therefore, it is vitally important to ensure the calf receives high quality colostrum. It is recommended that a newborn calf receive between 3 and 3.8 L of colostrum, depending on breed, containing at least 100 g of Immunoglobulin G (IgG) from multiparous cows within the first hours of birth (NRC, 2001). As time passes and

the calf gets older, the amount of immunoglobulins able to be absorbed decreases. Once the calf is 24 hours of age, no immunoglobulins may be absorbed. Hammon and Blum (1998) point out the necessity of colostrum due to the number of hormones and growth factors it contains, which are key for growth and development of the digestive tract and other organ systems.

As of 1995, 60% of U.S. dairy farms used milk replacers for some or all of the neonatal calf feeding (Heinrichs et al., 1995). In regards to quality, milk replacers are a very good source of liquid feed for neonatal calves (Heinrichs et al., 1995). Heinrichs et al. (1995) concluded from a study that focused on types of milk replacers used across dairies in the U.S. that producers in the southeast tended to feed less whole milk than those in other parts of the country. There are many influences that may sway producers either to use milk replacers or not, including: economics (price of milk/cwt), size of herd, and availability of waste milk. The quantity of milk replacer to be fed per calf is based on an equation for metabolizable energy (ME) by Toullec (1989):

$$\text{ME requirements (Mcal/d)} = 0.1\ LW^{0.75} + (0.84\ LW^{0.355})\ (LWG^{1.2}) \qquad (2\text{-}1)$$

where: LW (live weight) and LWG (live weight gain) are in kilograms. This formula is set in two different portions. The first sets the ME requirements at 100 kcal/$kg^{0.75}$ per day, while the second portion is used to derive ME required for LWG. Quality of milk replacer is important not only for overall health status, but also for the progression of rumen development. A calf's rumen is not fully functional until after weaning, therefore, it is crucial that milk replacers contain proteins able to be used by the calf. As mentioned previously, the esophageal groove redirects milk from the reticulo-rumen and into the abomasum. Milk or milk replacer clots when it reaches the abomasum and is then broken

down by enzymes rennin and pepsin. The clotting causes the rate of passage to slow, allowing the nutrients to be released at a steady pace (Moran, 2002). Protein sources and content may vary among milk replacer manufacturers. Examples of possible protein sources include: milk solids, skim milk, casein, whey protein concentrate, soy flour and animal (porcine and bovine) plasma protein (Davis and Drackley, 1998). According to Davis and Drackley (1998) there are two classifications of protein sources from which milk replacers are made of; milk or non milk proteins. Generally, milk protein sources (dried whey, skim milk and casein) are more desirable because they are highly digestible, have more desirable amino acid balance and tend to have less anti-nutritional factors than non milk sources. Major milk replacer manufacturers use whey proteins as the principle protein source as they allow for the manufacturers to produce a high quality product. Protein content will typically range from 18 to 24% crude protein, which is crucial to support adequate calf growth. Bartlett et al. (2006) discovered calves reached optimum efficiency when fed 22% CP compared to 14, 18 or 26% CP. Fat levels also vary in milk replacers from 10 to 24 percent with 15 to 20 percent being the most common. Because fats serve as an energy source of calves, quite often, a milk replacer with a higher fat level will be used in cold climates.

Transition Phase

Between weeks 2 and 3, the calf's digestive tract begins to undergo the most rapid changes with regard to digestible secretions and enzymatic activity (Toullec and Guilloteau, 1989; Davis and Drackley, 1998). Starter grain is generally offered to calves within the first two weeks of life and then continued through weaning. *Ad libitum* water is also provided to encourage starter consumption (NRC, 2001). Starter grains should be

highly palatable and meet the energy needs of each calf according to NRC Guidelines. Sander et al. (1959) concluded that the consumption of starter grain is critical in the development of an active, functioning rumen and also; solid feeds are responsible for the development of functional ruminal epithelial tissue. Reports show VFA concentrations are similar in calves consuming starter diets, as early as 3 weeks of age compared to those in adult cattle. Roth et al. (2009) conducted a weaning method trial using 26 Brown Swiss, 14 red Holstein, and seven Holstein-fresian calves. The objective of this trial was to analyze feeding behavior recorded via a feed intake-montoring computer as an appropriate method for predicting health status and rumen development. The calves were assigned to one of two treatment groups (conventional weaning or concentrate-dependant weaning). Both groups of calves were fed 6L of milk daily with ad libitum access to water. The conventionally weaned calves' milk was continuously reduced from weeks 8 to 12. Once they reached 12 weeks of age, calves were completely weaned from milk. These calves were only allowed grain according to their calculated nutritional requirements. For the concentrate-dependent weaned calves, the amount of concentrate was dependent upon each calf's individual feeding plan. Once calves consumed 700g of concentrate over 4 consecutive days, milk allowance was reduced. When calves consumed 2000g or more, 4 consecutive days, milk feeding was abruptly stopped. Calves in the concentrate-dependent weaning group were weaned at an average age of 76 d, while the conventionally weaned group had an average weaning age of 84 d. On average, concentrate-dependent weaned calves gained 0.88 kg/d, while conventionally weaned calves gain 0.87 kg/d. Roth et al. (2009) concluded faster physiological development without negative effects on rumen development, weight gain, or health status may be achieved by using the concentrate-dependent weaning method. Results

from this study showed that weight gain was a rather accurate measurement to determine rumen development. However, evaluation of rumen pH and VFA production are more common practices in determining rumen development. Calves fed grain diets with little to no roughage have in fact been shown to have increased VFA concentrations (Klein et al., 1987). Butyrate, in particular, is said to increase metabolic activity of the ruminal epithelium (Sutton et al., 1963).

Ruminant Phase

Once a calf is weaned (4 – 6 weeks of age) completely from its liquid diet, the rumen is beginning to be function. The rumen is not fully functional until the calf is approximately four months of age. As the calf ages and the rumen continues to develop and microbial populations increase. The rumen microbes must possess certain characteristics (anaerobic and fast growth in pH ranges from 5.0 to 7.0) in order to aid in a fully functional rumen. The esophageal groove no longer plays a role in the redirection of a liquid diet, allowing for solids to enter into the rumen to be broken down. Microbial fermentation now occurs in the reticulo-rumen (NRC, 2001). During this phase, the calf is relying on grain, forage, and other dry feeds to obtain nutrient requirements. Calves are sometimes offered a forage source (hay) with the intent rush rumen development, when in fact, the development of a fully functional rumen is not necessarily promoted by a forage source, but with a highly fermentable feed source. A good indicator of a well-functioning rumen, is the presence of VFA's (Volitile Fatty Acids: butyrate, proprionate, and acetate), which is yielded by forage sources in much lower concentrations than grain sources. With this said, it is not recommended to completely eliminate forage or fiber sources from the starter diet. It is important to provide the calf with a well-balanced diet

along with access to plenty of water to encourage dry matter intake to aid in the continuing of rumen development as well as overall body growth.

CHAPTER III

MATERIALS AND METHODS

Animals and Housing

Forty Holstein heifer calves (n=8) were used in the current trial at the Bearden Dairy Research Center at Mississippi State University. At birth, calves were randomly assigned to one of five different treatment groups. Treatments were as follows: CX (1 g/d Deccox), YST (10 g/d yeast), β-g (0.5 g/d β-glucan), CX + YST (1 g/d Deccox + 10 g/d yeast) and YST + β-g (10 g/d yeast + 0.5 g/d β-glucan). Treatments were noted as 1,2,3,4 and 5, respectively. The YST additive contained 50% mannanoligosaccharide and 25% β-glucan fractions. Treatment additives were mixed with 100 mL warm water and then added to milk replacer at feeding. Calves were individually housed in plastic hutches made by Calf Tel® which were bedded with wheat straw. In order to maintain a dry, insulated environment for calves, additional wheat straw was added as needed. Nose-to-nose contact between calves was eliminated by hutch arrangement. The trial was conducted over an eight week period, where calves were weaned at six weeks of age and remained in hutches until eight weeks of age. Calves were fed 3.8 L (710 g powder) of a non-medicated milk replacer (22% CP, 20% Fat; DM)from an open pail once daily at 0630 until d 35, at which daily milk allowance was then reduced to 1.4 L (355 g powder).

At d 42, calves were weaned. After milk feeding, buckets were rinsed and filled with water, allowing calves *ad libitum* access to water until next feeding. A non-medicated starter grain (18% CP) was offered in increments of 0.9 kg/d starting at 1 day

of age. When a calf had no feed refusals, grain allowance was increased by 0.9 kg/d. All 40 calves used for this study were born between September 2009 and February 2010.

Data Collection

Growth Performance

Calves were separated from dams by 12 h after birth. At that time, calves received 2.83 L of colostrum via esophageal feeding tube. A single blood sample was collected via jugular venipuncture between 2 and 7 d after birth to ensure adequate time for IgG absorption. Immunoglobulin G (IgG) concentrations were determined using a single radial immunodiffusion kit (VMRD Inc., Pullman, WA). Body weight (BW), hip height (HH), hip width (HW), wither height (WH) and body length (BL) was measured at birth, once weekly and the final day of trial. Rectal body temperatures were also recorded weekly. Orts were collected and weighed daily and then pooled by treatment for weekly feed analysis. Analyses of feed samples were completed in the Scales Nutrition Laboratory in the Animal and Dairy Sciences Department at Mississippi State University for dry matter, nitrogen, Fat, Neutral Detergent Fiber and Acid Detergent Fiber (according to AOAC methods).

Health status and blood sampling

Respiratory and fecal scores were evaluated and recorded daily at feeding according to Larson et al. (1977). Briefly, fecal scores were determined based on a 5 point scale where 1 represented normal (soft, solid, no fluid), 2 soft (semi-solid), 3 Runny (soft, mostly fluid), 4 Watery (fluid) and 5 bloody. Respiratory scores were as follows: 1-normal, 2- runny nose, 3-heavy breathing, 4-moist cough and 5-dry cough. Two blood samples were collected once weekly (Wednesdays), 4 hours after feeding; one 5 mL

vaccutainer containing EDTA for complete blood count (CBC) analysis with differential and one 10mL vaccutainer containing no anticoagulant. Samples were immediately transported (11 km) to the laboratory. The whole blood samples were analyzed for CBC in the Animal Pathophysiology Laboratory of the College of Veterinary Medicine at Mississippi State University. The blood samples containing no anticoagulant were processed in the Animal Physiology Lab at Mississippi State University; centrifuged at 3000 x g at 4 degrees C for 30 minutes and then serum was stored in 1.5 mL polypropylene tubes at -20 degrees C until further analysis of cortisol concentrations. Cortisol samples were analyzed via radioimmunoassay (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Los Angeles, CA).

Fecal Sampling

Fecal samples were collected weekly until calves reached 21 d of age and then twice weekly to analyze for the presence of coccidia. Analyses were conducted in the Animal Pathophysiology Laboratory of the College of Veterinary Medicine at Mississippi State University. Samples were graded subjectively based on the number of oocyst present: None (0), Rare (1 to 5), Few (10 to 25), Moderate or Many (>25). Twice weekly fecal samples were collected once calves reached 21 d of age due to the 21 d lifecycle of coccidia. Fecal E. *coli* shedding was recorded and analyzed by taking a fecal sample from each calf at weeks 2, 4 and 8. A sterile 10 µL loop of fecal material was placed into 990 µL of Luria-Bertani (LB) broth. A serial dilution was conducted to yield a 10^{-4} dilution and was then plated on MacConkey agar. Plates were incubated for 24 hrs at 37°C. After incubation time was complete, colony forming units (CFU's) were counted.

Feed Analysis

Grain refusals (orts) were collected and measured daily and then pooled by treatment for weekly feed analysis. Analyses of feed samples were completed in the H.W. Essig Nutrition Laboratory in the Animal and Dairy Sciences Department at Mississippi State University (according to AOAC methods). All samples were first dried for 48 hrs in a 64˚C drying oven to obtain an "Air Dry" basis. Samples were then ground in a Willy Mill through a 2- mm screen. Samples were analyzed in duplicate: DM, N, Fat, NDF and ADF.

Statistical Design and Analysis

A completely randomized design was used as the experimental design of this project. Class variables included Calf_ID, treatment, and week. All data were analyzed using the MIXED procedure of SAS (version 9.2). Orthogonal contrast statements were used to determine differences between treatments if present, the contrast statements were: YST vs. β-glucan, YST vs. YST + β-glucan, β-glucan vs. YST + β-glucan and CX vs. all. Data sets containing multiple measures per calf were analyzed by ANOVA for repeated measures. Treatment differences with $P \leq 0.05$ were considered significant, while $P \leq 0.10$ were considered a tendency.

CHAPTER IV

RESULTS AND DISCUSSION

Calf IgG concentrations were adequate for passive transfer of immunity in all calves in the present study. Nutrient intake was not different and as expected, growth measures among treatment groups were similar ($P \geq 0.05$; Table 1). Calves were fed the same basal diet, therefore, no differences were expected among treatments due to diet. Similar to findings of Lesmiester et al., (2004) and Morrill et al., (1995), and Galvão, et al. (2005) no differences were observed between treatment groups with respect to blood parameters ($P \geq 0.05$; Table 2). The effects of 1 or 2% live yeast culture (Lesmiester, et al., 2004 and Galvão, et al. (2005) or probiotics (Morrill et al., 1993) did not change total plasma protein concentrations when compared to control fed calves. Weekly body measurements were recorded and analyzed and no differences were found among treatments for BW, WH, HH, HW or BL ($P = 0.52$, 0.21, 0.19, 0.53 and 0.39, respectively). Hill et al. (2009), Hill et al. (2008), and Heinrichs et al. (2003) all supplemented Holstein calves with MOS and compared to calves with no supplementation of antibiotics, yeast, or coccidiostat, there were no differences in growth. Heinrichs et al. (2003) did however, report differences in grain intake during week 6 compared to calves fed antibiotics, while Hill et al. (2009) saw no change in starter intake due to supplementation of MOS in Holstein calves. Improved grain intakes ($P < 0.05$) were observed in calves supplemented yeast culture in starter grain compared to control, while a tendency ($P < 0.10$) for greater starter grain intake was shown by

calves supplemented with yeast culture in milk replacer compared to control (Galvão, et al., 2005). It can be noted that grain intake for calves fed yeast culture in both milk replacer and starter grain did not differ from other treatment groups. Lesmiester et al. (2004) found similar results when calves were supplemented 1 or 2% yeast culture in starter grain. Overall (wk 1 to 6) intakes were significantly higher ($P < 0.01$) for calves fed 2% yeast culture. No differences were found in fecal and respiratory scores or rectal temperatures ($P \geq 0.05$) among any treatment groups. These observations were similar to Galvão, et al. (2005), who found the addition of live yeast products in calf diets had no effect ($P > 0.10$) before or after weaning. In contrast, Heinrichs et al. (2003), Hill et al. (2009) and Magalhães et al. (2008), found calves fed MOS or yeast had a higher probability for more normal feces, lower average fecal scores, and/or fewer days scouring. Similar fecal coccidia scores ($P = 0.77$) were observed between treatment groups from weeks 0 to 8. Our findings are different from Heinrichs et al. (1991), Heinrichs et al. (1990) and Waggoner et al. (1994) who all found differences in coccidia shedding in calves treated with a anticoccidial, with shedding rates being greater for calves treated compared to control. Among all 40 calves in this study, cortisol concentrations ranged from 0.22 to 126.19 ng/mL over the course of the trial, while there were no differences between treatments ($P = 0.92$) the effect of week, was significantly different ($P < 0.01$) over the eight week course of the trial. These findings are similar to those of Cummings and Brunner (1990) who conducted a housing trial with colostrum-deprived Holstein calves. No differences ($P \geq 0.05$) were observed in blood nitrate or nitrite concentrations among treatment groups. Heifers fed YST or YST + β-glucan had higher ($P = 0.02$ and $P < 0.01$, respectively) fecal concentrations of E. *coli* compared to heifers fed CX, CX + YST, or β-glucan alone. Fecal E. *coli* concentrations were lower (P

< 0.05) in week 8 when compared to weeks 2 and 4. This decrease in concentrations may be a result of weaning, when additives were no longer included in the diet.

CHAPTER V

CONCLUSIONS AND IMPLICATIONS

The objectives of this study were to evaluate the efficacy of using antibiotic alternatives such as MOS and β-glucan on calf growth and health performance and feed intake/efficiency. The purpose of this study was also to evaluate the effects these products have on E. *coli* and coccidia shedding. Based on the results found, feeding these products at the given dosages, had no beneficial impact on growth and health performance, which includes feed intake and efficiency, fecal and respiratory health and blood parameters, however, these products had no negative effects on calf performance and are comparable to performance of calves supplemented with coccidiostats. All calves were healthy throughout the duration of the trial and no clinical signs of coccidiosis or respiratory infections were observed. Feeding these products may serve as a viable source of gut health enhancers, therefore reducing the need for non-therapeutic antibiotic use and the cost associated with them. The E. *coli* shedding results in the current study may be an indication of the gram (-) binding and non-digestible properties of YST, which increased E. *coli* shedding, serving as a suitable product to aid in balancing gut microflora, which ultimately leads to a healthy, productive calf. Further investigation is needed to determine the optimum dose and feeding method (example: milk/milk replacer or starter grain) of these products to maximize efficacy, while being an economical solution for producers to increase calf productivity.

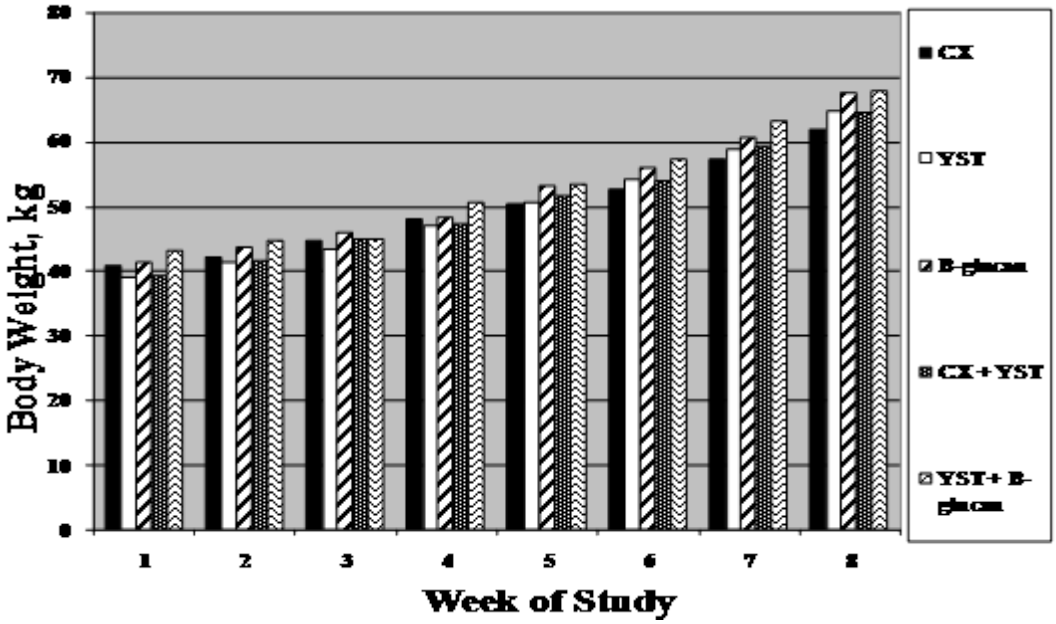

Figure 2 Growth performance of Holstein heifers fed CX, YST, β-glucan, or some combination

Table 5 Nutrient Intake and Growth Measures in Holstein heifers fed CX, YST, β-glucan, or some combination

	Treatment						P <	
	CX	**YST**	**β-glucan**	**CX + YST**	**YST + β-glucan**	**SEM**	**Trt**	**Period**
DMI: Starter grain, kg/d								
Period 1[1]	0.1	0.17	0.19	0.14	0.17	0.08	0.25	0.01
Period 2[2]	0.58	0.8	0.8	0.64	0.92	0.09		0.01
Period 3[3]	1.61	1.88	1.72	1.62	1.9	0.08		0.01
DMI: Milk, kg/d							-	
Period 1	0.71	0.71	0.71	0.71	0.71	-		-
Period 2	0.35	0.35	0.35	0.35	0.35	-		-
Period 3	0	0	0	0	0	-		-
DMI: Total[4], kg/d								
Period 1	0.81	0.88	0.91	0.85	0.88	0.08	0.25	0.001
Period 2	0.93	1.16	1.15	0.99	1.28	0.09		0.001
Period 3	1.61	1.88	1.73	1.63	1.9	0.08		0.001
CP Intake: Total, kg/d								
Period 1	0.16	0.17	0.18	0.17	0.17	0.02	0.29	0.001
Period 2	0.18	0.23	0.23	0.2	0.25	0.02		0.001
Period 3	0.31	0.36	0.32	0.31	0.38	0.02		0.001
NDF Intake: Starter, kg/d								
Period 1	0.02	0.04	0.03	0.03	0.03	0.02	0.26	0.001
Period 2	0.12	0.17	0.14	0.12	0.17	0.02		0.001
Period 3	0.34	0.4	0.32	0.31	0.32	0.02		0.001
ADF Intake: Starter, kg/d								
Period 1	0.01	0.02	0.02	0.01	0.01	0.01	0.28	0.001
Period 2	0.05	0.07	0.06	0.05	0.08	0.01		0.001
Period 3	0.13	0.16	0.14	0.12	0.13	0.01		0.001
Initial BW, kg	39.2	39.1	39.5	38.1	41.1	1.23	>0.05	-
Final BW, kg	61.9	64.9	67.7	64.4	67.3	2.67	>0.05	-
ADG, kg/d	0.41	0.46	0.5	0.47	0.48	0.04	>0.05	-
Feed Efficiency[5]	0.39	0.4	0.44	0.42	0.4	0.02	>0.05	-

[1] Period 1 = d 1 to 34, [2] Period 2 = d 35 to 41, [3]Period 3= d 42 to 55,
[4] Total DMI= Starter DMI + Milk DMI, [5] Feed Efficiency = Gain/Feed

Table 6 Mean Blood Parameters in Holstein heifers fed either CX, YST, β-glucan, or some combination from 0 to 8 weeks of age

	CX	MOS	β-GLUCAN	CX + MOS	MOS + β-GLUCAN	SEM	P-value
	Blood Parameters						
White Blood Cells, K/ul	8.39	7.92	7.73	8.29	8.80	0.65	≥0.05
Red Blood Cells, M/ul	8.40	8.44	8.27	8.48	20.2	5.42	≥0.05
Hemoglobin, g/dl	10.7	11.0	10.8	11.3	11.7	0.42	≥0.05
Hematocrit, %	29.1	30.2	28.2	31.0	31.4	1.17	≥0.05
Platelets, K/ul	1264	1118	1290.2	926	1229	125	≥0.05
Plasma Protein, g/dl	5.97	6.71	5.95	6.09	6.09	0.37	≥0.05
Neutrophil, #/ul	3455	3589	3332	3336	3643	401	≥0.05
Lymphocyte, #/ul	4829	3973	4073	4425	4806	351	≥0.05
Monocyte, #/ul	346	305	337	270.6	261.5	77	≥0.05
Eosinophil, #/ul	52.3	43.0	17.3	37.5	25.9	13.7	≥0.05
Basophil, #/ul	43.7	30.0	16.8	44.3	36.9	9.82	≥0.05

Table 7 Nitrate, Nitrite and Cortisol concentrations in Holstein heifers fed yeast derivatives in milk replacer from 0 to 8 weeks of age

	Treatment						
Item	**CX**	**YST**	**β-glucan**	**CX + YST**	**YST + β-glucan**	**SEM**	**P-value**
Nitrate, ppm	19.1	22.6	25.7	24.7	22.0	7.77	≥0.05
Nitrite, ppm	0.63	0.59	0.64	0.66	0.68	0.09	≥0.05
Cortisol, ng/mL	14.4	15.6	13.6	15.4	15.9	1.98	≥0.05

Table 8 Fecal Coccidia counts in Holstein heifers fed yeast derivatives in milk replacer from 0 to 8 weeks of age

	Treatment					
Item	**CX**	**YST**	**β-glucan**	**CX + YST**	**YST + β-glucan**	**Chi-Sq <**
None	72	79	71	75	74	0.65
Rare	15	12	20	16	11	
Few	7	8	8	7	9	
Moderate	6	2	3	5	6	
Many	0	2	0	0	2	

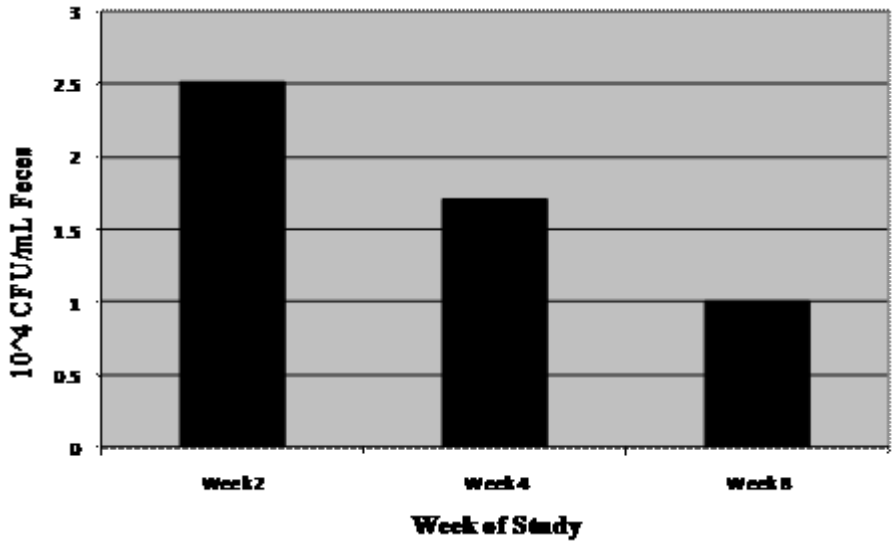

Figure 3 Fecal E. *coli* concentrations of Holstein heifers fed CX, YST, β-glucan, or some combination

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