# A FORTY-NINE DAY EVALUATION OF BIO-MOS® REPLACEMENT OF ROXARSONE IN A COMMERICALLY BASED BROILER FEEDING PROGRAM

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# A FORTY-NINE DAY EVALUATION OF BIO-MOS® REPLACEMENT OF ROXARSONE IN A COMMERICALLY BASED BROILER FEEDING PROGRAM

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## ABSTRACT

A study was conducted to investigate the effects of roxarsone and Bio-Mos® on broiler production, gut morphology and bone strength. Three thousand and ten broilers were randomly assigned to 1 of 5 dietary corn-soybean meal based treatments: 1) negative control (NEG), basal diet; 2) positive control (POS; NEG + 27 ppm Bacitracin MD); 3) roxarsone (ROX; POS + 50 ppm of roxarsone); 4) Bio-Mos® (BIO; POS + 0.15 and 0.5% Bio-Mos® added during the starter and grower periods, respectively); 5) Bio-Mos®+All-Lac XCL (BIO+LAC; POS + 0.2, 0.1, and 0.05% Bio-Mos® during the starter, grower and finishing periods, respectively and 0.25g All-Lac XCL/bird sprayed at hatchery). On day 14, 7 of the 14 replicate pens/treatment were challenged with *Eimeria maxima* (3 x $10^4$ oocysts/bird). Tibias were collected on day 28 and 49 to determine bone-breaking strength. Non-challenged birds had higher body weight gains (BWG) and lower feed conversion (FCR) from day 0 to 49 than challenged birds ($P < 0.05$). Jejunal crypt depth was increased in challenged broilers compared to non-challenged broilers at 28 days-of-age ($P < 0.05$). From day 0 to 35, ROX birds had lower BWG and FI than BIO and BIO+LAC birds ($P < 0.05$), while FCR was similar. Supplementing roxarsone resulted in reduced feed intake and BWG, but no significant differences were noted in FCR compared to feeding Bio-Mos®. ROX fed broilers had decreased ileal crypt depth compared to all other dietary treatments ($P < 0.05$). Muscle As concentration was lower than FDA allowable limits in broilers fed ROX without a withdrawal period at 28 days-of-age. Including roxarsone or Bio-Mos® did not generally improve production compared to broilers fed the negative diet.

Keywords: Bio-Mos, roxarsone, coccidia, broiler, performance

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## ATTRIBUTION

Several colleagues assisted in the design, research and writing of chapter 2 of this thesis. A brief description of their background and contribution follows.

**Curtis Novak**-Ph.D. (Department of Animal and Poultry Science, Virginia Tech) is the primary Advisor and Committee Chair. Dr. Novak provided project design and was active in the development of laboratory protocols and interpreting the data.

**Ted Sefton**-Ph.D. (Alltech®) collaborated and supported the research project.

**Audrey McElroy**-Ph.D. (Dpeartment of Animal and Poultry Science, Virginia Tech) is a collaborator and member of the Committee. Dr. McElroy was instrumental in the coccidia challenge design and implementation. Dr. McElroy provided histology protocols.

## TABLE OF CONTENTS

## LIST OF TABLES

### Chapter 2

## LIST OF FIGURES

**Chapter 2**

# CHAPTER ONE: REVIEW OF LITERATURE

## Introduction

Today's broiler production systems employee various dietary growth promoters to maintain production rates in coccidia challenged housing units. Growth promoters are dietary supplements that enhance production rates such as weight gain, feed intake and feed conversion. Common U.S. production practices include removal of caked litter, leaving a house empty for 2 to 3 weeks and top dressing with fresh litter before replacing a new flock to limit transferring disease to subsequent flocks (Allen and Fetterer, 2002b). Time between flocks may be limited due to pressure to increase the number of flocks per year. Although research has shown oocysts are no longer viable after two weeks in poultry litter, coccidiosis remains within the U.S. poultry flock (Reyna *et al.*, 1983). Coccidiosis is spread from flock to flock if down time is less than 2 to 3 weeks or litter has arthropod pests (such as flies and darkling beetles) which are able to carry oocysts (Reyna *et al.*, 1983). Coccidiosis costs the American broiler industry about $450 million annually (Allen and Fetterer, 2002b). Williams (1999) reported 46% of the cost of coccidiosis in the United Kingdom is due to reduced weight gain and 34% of the cost was due to poor feed conversion (**FCR**). Broiler nutrition programs utilize different anticoccidials and antibiotics during growth and finishing periods to reduce growth loses associated with diseases. Shuttle programs utilize different combinations of growth promoters during various stages, instead of using a single or combination of growth promoters throughout the entire lifespan of the animal. These feed additives improve weight gain and FCR during a disease challenges. Although antibiotics and anticoccidials are still used in the U.S., the use of antibiotic growth promoters and anticoccidials in poultry feed has been banned in many countries due to concern of bacterial resistance and residues in meat. As concern over the use of anticoccidials and antibiotics

continues, research must focus on determining alternative broiler nutrition programs for broilers encountering coccidia and other health challenges. This review outlines common broiler feed additives and the interactions of combining additives on broiler production and gastrointestinal health.

## Gastrointestinal Anatomy and Function

### *Gastrointestinal anatomy*

Maintaining broiler gastrointestinal tract health contributes to optimal performance. As both the central location for nutrient absorption and first line of defense against pathogens, the intestinal tract plays an important role in maintaining an animal's nutritional and immunological health. Indicators of gastrointestinal health include: villi length, crypt depth, lamina propria area and goblet cells. The three layers of the intestinal mucosa are first epithelial cells, the lamina propria consisting of sub epithelial connective tissue and lymph nodes, and a thin layer of smooth muscle called the muscularis mucosa (Pearson and Brownlee, 2005). Lamina propria is rich in both B and T lymphocytes. B cells present in the lamina propria are positive for Immunoglobulin M (IgM) and IgA, while the major T cells are $CD4^+$ (Schat and Myers, 1991). The intestinal epithelial consists of villi which are finger-like projections. The absorptive cells on the villi contain microvilli on their apical surface creating a brush boarder (Pearson and Brownlee, 2005). Between the absorptive cells on the villi are mucin secreting cells called goblet cells which create a mucus bilayer protecting the underlying epithelial cells (Pearson and Brownlee, 2005). At the base of each villus are the crypts of Lieberkuhn, which contain stem cells that give rise to the basic epithelial cell types associated with the small intestine—absorptive enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (Laux et. al., 2005).

Epithelial cells are continuously produced by the stem cells located within the crypts, migrate up from the basal portion of the villus to the apical tip, and are shed through processes involving exfoliation and apoptosis (Laux et. al., 2005).

*Mucin production and function*

Goblet cells are responsible for mucin production. Mucins are high-molecular-weight glycoproteins that are part of the secreted mucus gel in the airway and gastrointestinal tract (Pearson and Brownlee, 2005). Carbohydrates make up to 80% of the mucin molecule (Pearson and Brownlee, 2005). A central protein core attached with carbohydrate side chains via O-glycosidic links between serine and/or threonine and *N*-acetylgalactosamine make up the mucin structure (Pearson and Brownlee, 2005). N-acetylglucosamine, N-acetylgalactosamine, fructose, and galactose are the four primary mucin oligosaccharides (Deplancke and Gaskins, 2001). Mucin enables the flow of nutrients and creates a protective barrier between the epithelial layer of the mucosa and luminal contents (Pearson and Brownlee, 2005).

Mucins are categorized as neutral or acidic, which are further categorizes as sulfated (sulfomucins) or non-sulfated (sialomucins) (Deplancke and Gaskins, 2001). During late stages of embryonic development, goblet cells only contain acid mucin, while neutral mucin expression begins post-hatch (Uni *et al.*, 2003). A 48 hour post-hatch fast results in increased neutral mucin goblet cell density in the duodenum and jejunum compared to non-fasted chicks ($P < 0.05$). Acidic mucin density increased in the duodenum, jejunum and ileum in fasted chicks by 2-days old ($P < 0.05$). Mucin production tends to increase during starvation and may effect gut bacterial populations (Uni *et al.*, 2003). Conventional raised pigs have increased mucin in the small intestine, cecum and colon compared to germ-free pigs, indicating bacteria within the gut may

change mucin production (Meslin *et al.*, 1999). It is hypothesized that similar changes in mucin profiles would occur in other stressful growth period, such as a disease state and fasting.

The importance of different mucin subtypes is not well understood, but neutral and acid mucins may be expressed in response to disease state and diet. Fernandez and colleagues (2000) fed a wheat-based, wheat-based supplemented with 0.1% xylanase or a maize-based diet to chicks to 33 days of age. After inoculating the chicks with *Campylobacter jejuni* (at 5-days of age), chicks fed the wheat-based diet had higher acid mucin concentrations in the large intestine than chicks fed the xylanase-supplemented diet and decreased neutral mucin concentration compared to the xylanase supplemented and maize fed chicks (Fernandez *et al.*, 2000). Wheat-based diets contain non-starch polysaccharides (NSP) that are considered anti-nutritional factors. Digesta viscosity increased in broilers fed wheat NSP compared to broilers fed sorghum ($P < 0.01$) (Choct and Annison, 1992). It has been hypothesized the increased acidic mucin concentration during disease states is due to the less efficient degradation of these mucins by bacterial glycosidases and host proteases (Deplancke and Gaskins, 2001). These findings suggest that the broilers increase acid mucin while decrease neutral mucin production during sub-optimal management, such as feeding a diet containing anti-nutritional factors.

*Bacterial interactions with the gastrointestinal tract*

The normal broiler gastrointestinal flora is complex and has not been completely characterized. The mucosal layer acts as a physical barrier that bacteria must enter or pass through to reach the epithelial surface (Laux et. al., 2005). Bacterial attachment to the mucosal layer can have two outcomes: colonization or removal. Mucin can act as an attachment site for both bacterial colonization and to facilitate the removal of bacterial by trapping the bacterial in

the mucin and normal mucin turn over (Laux *et al.*, 2005). Vilma *et al.* (2000) reported *Salmonella typhrurium* bound to purified rat mucus. To define the structure of the binding site, lipids were extracted from the mucus. No significant differences in binding affinity were noted between delipidified and crude mucus determining that lipid did not act as the binding site. Bound mucus was subjected to SDS-polyacrylamide gel electrophoresis and then treated with a periodic acid glacial acetic acid stain to identify glycoproteins. The technique verified that glycoprotein act as the binding site. To confirm that glycoproteins in mucin act as the attachment site for bacteria in the gut, bound mucus was treated with proteolytic enzymes (Vilma *et al.*, 2000). Bacterial binding was reduced with the addition of proteolytic enzymes ($P < 0.001$). Pre-incubation of *S. typhimurium* with mannose resulted in a reduction in bacterial binding to mucus by 77.79% (Vilmal *et al.*, 2000). Oyofo *et al.* (1989) reported that *S. typhimurium* binding to chicken small intestine was also inhibited by 90% *in vitro* with the addition of D-mannose. Work by Oyofo *et al.* (1989) and Vilmal *et al.* (2000) demonstrate that bacteria attach to the mucosal layer at a mannose site.

## Coccidiosis

### *Life cycle of coccidiosis*

Coccidiosis costs the American broiler industry $450 million annually (Allen and Fetterer, 2002b). Coccidiosis is caused by the protozoa *Eimeria spp.* The life cycle of the *Eimeria* species has been well documented in a review by Allen and Fetterer (2002b). Oocysts shed in feces undergo sporulation within the environment or litter. The sporolated oocysts contain four sporocysts, which each contain two sporozoites. Broilers ingest oocysts in the feed and litter and during preening. Ingested oocysts excyst within the intestinal lumen and penetrate

the villous epithelial cells. Once in the host cell, sporozoites undergo asexual reproduction, resulting in the formation of merozoites that break free of the host cell causing cellular damage. In the lumen, merozoites can undergo sexual reproduction. Merozoites then enter the host cells and form into male or female forms. The male form seeks the female form for fertilization, then develop into oocysts that then exit the animal in the feces. Maximum oocysts output ranges from 6 to 9 days post infection (Allen and Fetterer, 2002b; Reyna *et al.*, 1983). Oocysts can survive in poultry litter for two weeks after a poultry house is depopulated (Reyna *et al.*, 1983). Clinical signs of coccidiosis include: diarrhea, morbidity, reduction in weight gain and poor feed conversion (Williams, 2005).

As sporozoites enter the epithelial cells of the villi, damage to the gut occurs. Two-week-old coccidia-free chicks were infected with a single dose of *E. acervulina* (80,000 oocysts) (Pout, 1967). Villus height:total mucosal thickness gradually decreased until the forth day post-infection. At day four, a decrease of 0.2 was noted in villus height:total mucosal thickness. After day four, the gut began to gradually recover from the infection and was totally recovered by 21-days post-infection. Broilers were housed in wire batteries, so re-exposure could not occur. In the industry, broilers will be constantly exposed to new cycles of coccidia, so recovery may not occur within 21-days (Pout, 1967). Although broilers will be constantly re-exposed to coccidia, Chapman and Saleh (1999) observed large number of oocysts 7 days post-challenge in poult litter. Oocysts output was decreased 10-fold 14 days post-challenge. Four weeks post-challenge, no oocysts were detected in the litter (Chapman and Saleh, 1999). These poults were raised on litter, constantly being re-exposed to oocysts. Seven days post-challenge is hypothesized to be the time period of greatest morbidity to coccidia. Anticoccidials and antibiotics will have the greatest impact on performance from 0 to 7 days post-infection.

*Eimeria maxima*

*Eimeria maxima* often parasitizis the mid-section of the small intestine from below the duodenal loop to beyond meckel's diverticulum. *Emeria maxima* is the most immunogenic of all *Eimeria* spp. that parasitize poultry (Rose and Long, 1962). This species is moderately pathogenic, with around 200,000 oocysts needed to sufficiently cause morbidity in the form of reduced weight gain and diarrhea (Rose and Long, 1962). Depressions in performance are observed in broilers infected with *E. maxima*. Allen and Fetterer (2002a) studied the effects of *E. maxima* infections in male broilers. Male Ross broiler chicks were orally inoculated with *E. maxima* at 14 days of age. Weight gain was depressed by 6 days post challenge compared to uninfected control birds ($P < 0.05$). Feed conversion was not effected by *E. maxima* inoculation (Allen and Fetterer, 2002a). *Eimeria maxima* is able to depress production in broilers.

*Necrotic enteritis: A secondary bacterial infection associated with Coccidiosis*

Necrotic enteritis is a disease caused by an overgrowth of the commensal bacteria, *Clostridium perfringens* type A and C. *Clostridium perfringens* is a gram-positive, spore-forming anaerobe found in the environment. *Clostridium perfringens* colonizes the gastrointestinal tracts of 75% to 95% of all birds, but only a small portion of these birds ever show symptoms of necrotic enteritis (McDevitt *et al.*, 2006). Worldwide, necrotic enteritis is considered the 4$^{th}$ most important bacteria found when examining diseased poultry (Kohler, 2000). The believed cost of necrotic enteritis in the U.S. is $0.05/bird (van der Sluis, 200b). The clinical signs of necrotic enteritis include: gastrointestinal edema, loose feces that is watery and light colored, ruffled feathers, anorexia, huddling, increased water:feed ratio, reduced daily

growth and a reduction in feed intake (van der Sluis, 2000a; Wilson *et al.*, 2005). Elanco Animal Health conducted a world wide survey among poultry experts and reported 88.9% of poultry experts in the U.S. and Canada had positively diagnosed necrotic enteritis (van der Sluis, 2000b). Disease prevention and control continues to be important in decreasing the incidence of necrotic enteritis.

Necrotic enteritis is a potentially fatal disease that can occur following coccidiosis infection. Hermans and Morgan (2003) reported a strong association between necrotic enteritis and coccidiosis ($R^2 = 2.56$). Hume *et al.* (2006) observed dramatic population shifts in microbial communities of the duodenum, ileum and cecum 7-days after broilers were infected with *E. maxima, E. acervulina* and *E. tenella.* Although *C. perfringens* are part of the bacterial community of a healthy broiler gut, damage to the gut induced coccidiosis, allows *C. perfringens* proliferation and produce an alpha-toxin, which further damages the gut. Baba *et al.* (1997) compared male White Leghorn chicks that were either uninfected controls, infected with *E. necatrix* ($2 \times 10^4$ oocysts), infected with *C. perfringens* ($2 \times 10^8$ CFU) or infected with a combination of *E. necatrix* ($2 \times 10^4$ oocysts) and *C. perfringens* ($2 \times 10^8$ CFU). Mean *C. perfringens* counts increased in birds infected with *E. necatrix* or *E. necatrix* and *C. perfringens* on day 7 ($P < 0.05$) compared to uninfected birds, but the concurrent infection did not increase the clostridia populations compared to the *E. necatrix* infection. Edemas in the duodenum through the jejunum increased in birds infected with both *E. necatrix* and *C. perfringens* compared to birds solely infected with *E. necatrix* indicating a concurrent infection caused more gastrointestinal damage than coccidia infection alone (Baba *et al.*, 1997).

Necrotic enteritis decreases bird performance. Hofacre *et al.* (1998) orally inoculated 12-day old male broiler chicks with *E. acervulina* followed by serial oral inoculations of *C.*

*perfringens* and compared production parameters to uninfected chicks. Mortality and feed consumption increased in *C. perfringens*-infected birds ($P < 0.05$) 15-days post-infection. Body weight gains were depressed in *C. perfringens*-infected birds ($P < 0.05$), although feed conversion was not significantly different. Necrotic enteritis decreases bird performance, which decreases producer net gains.

Necrotic enteritis is controlled by antibiotic growth promoters (AGP) like virginiamycin and bacitracin (Williams, 2005). In a retrospective study, affects of a necrotic enteritis outbreak in Norway following a withdrawal of AGP from July 1995 to December 1997 was analyzed (Lovland and Kaldhusdal, 2001). Flocks with high levels of *C. perfringens*-associated hepatitis were compared to flocks with low levels. At 35-days of age, body weight, feed conversion, total mortality, downgrades and condemnations were poorer ($P < 0.05$) in flocks with high levels of *C. perfringens*. These factors negatively effected producer margins by 33% ($P < 0.001$). Increased feed conversion caused the most significant effect on producer margins contributing 44% to the decrease (Lovland and Kaldhusdal, 2001). Research needs to be conducted on alterative feed ingredients to antibiotics to control necrotic enteritis.

Enteritic bacterial species increase in the gut following a coccidiosis challenge. Baba *et al.* (1992) infected cecums from conventionally hatched White Leghorn and germ-free chickens exposed to *E. tenella* with *Salmonella typhimurium* L-55 and *Clostridium perfringens* KGW-1 *in vitro*. Germ-free chickens were housed in a sterile environment and conventionally hatched chicks were raised on wired-floor battery cages. Half of the chickens were inoculated orally with 5,000 *E. tenella* oocysts at 4 days-of-age, while the other half were unexposed. All birds were killed by cervical dislocation 7-days post-infection and cecums were collected. Bacterial *in vitro* adhesion tests using *S. typhimurium* and *C. perfringens* were than conducted. The number of *S.*

*typhimurium* and *C. perfringens* adhering to *E. tenella*-infected ceca was higher ($P < 0.05$) than that adhering to uninfected ceca for both the conventionally raised and germ-free chickens. The change of bacterial adhesion resulted only due to the coccidia infection, regardless of the microflora already present in the cecum (Baba *et al.*, 1992). Coccidia increases salmonella attachment to the gut mucosal surface.

## Antibiotic and Anticoccidial Growth Promoters

### *Roxarsone*

Roxarsone or 4-hydroxy-3-nitrophenyl arsenic acid ($C_3H_6As_4NO_6$) is an organic form of arsenic fed to broilers to improve performance. Approximately 70% of the U.S. broiler industry utilizes roxarsone (Chapman and Johnson, 2002), yet its metabolism is not well understood. The reduction of roxarsone to an inorganic form of As has been established as part of the utlilization of roxarsone (Czarnecki *et al.*, 1984; Lowry and Baker, 1989). Czarnecki *et al.* (1984) conducted a series of experiments to determine the interactions of sulfur amino acids and roxarsone in order to understand roxarsone metabolism. Cysteine and roxarsone interacted to depress growth at toxic roxarsone levels (300 mg/kg diet) ($P < 0.05$). Further studies were conducted to examine why cysteine would exacerbate the toxic effects of roxarsone, when often cysteine is used as a detoxifying agent. When comparing cysteine with a trivalent and pentavalent form of organic arsenic, Czarnecki *et al.* (1984) observed that the pentavalent form of As (phyenylarsonic) interacted with cysteine ($P < 0.025$). Because of this interaction with phyenylarsonic and not the trivalent organic arsenic (phenylarsine oxide), it was hypothesized that cysteine excalibrates the effects of arsenic toxicity in roxarsone by reducing roxarsone from its pentavalent form into a trivalent form. The trivalent form is considered more toxic than the

pentavalent form. Lowry and Baker (1989) studied the ameliorating Selenium toxicity properties of different forms of arsenic. Positive control chicks were fed 15 mg Se/kg diet. Birds fed both roxarsone and L-cysteine increased weight gain and gain:feed ($P < 0.05$) compared to birds fed roxarsone or L-cysteine separately. Roxarsone and L-cysteine acted synergistically to ameliorate Se toxicity, implicating cysteine as a reducer in roxarsone metabolism in birds (Lowry and Baker, 1989). The exact mechanism of roxarsone metabolism is still unknown, but dietary cysteine has been observed to reduce roxarsone to an inorganic form of As.

*Roxarsone as an anticoccidial*

When roxarsone is reduced to its inorganic form, the reduction may protect the gastrointestinal tract from coccidial gut infections. Czarnecki *et al.* (1984) suggested roxarsone would be most successful at reducing effects of *E. tenella*, an *Eimeria* species that infects the cecum, because of the reducing effects of the anaerobic cecal conditions. Izquirdo *et al.* (1987) found that roxarsone supplementation (50mg/kg diet) reduced cecal lesion scores ($P < 0.05$) in chicks infected with *E. tenella* and *E. acervulina*, but did not reduce duodenal lesion scores supporting the hypothesis of Czarnecki *et al.* (1984). The reduction of roxarsone may be able to trigger apoptosis of coccidia in the distal small intestine (Florea *et al.*, 2005).

*Roxarsone as a growth promoter*

During the late 1940s, researchers discovered that feeding roxarsone and its salts to poultry accelerated growth rates when fed below toxic levels (Morehouse, 1948). Thus, roxarsone may be effective as a growth promoter and anticoccidial. Commercial poultry producers continue to fed roxarsone because of its effectiveness and low cost (Chapman and

Johnson, 2002). In the U.S., 69.8 and 73.9% of poultry production units utilized roxarsone in starter and grower feeds, respectively (Chapman and Johnson, 2002). Due to federal regulations, roxarsone is not used in withdrawal feed. A combination of ionophore, antibiotic and roxarsone was the most common drug program used for the starter and grower periods in 2000, but use of this feeding program is declining in favor of an ionophore and roxarsone based-program (Chapman and Johnson, 2002). While the use of antibiotics declined from 1995 to 2000, roxarsone use remained steady (Chapman and Johnson, 2002).

Roxarsone is most effective as a growth promoter during stressful periods of growth, including the first week of growth, feeding diets low in nutritional value, cold stress and immune challenges. Fernandez *et al.* (1973) fed White Rock male chicks a corn or rye based diet supplemented with various levels of roxarsone (0, 25, 37 and 50 ppm) for 3 weeks. Body weight gains were positively affected by roxarsone supplementation at levels of supplementation ($P < 0.05$) for rye based diets, while improvements were not as significant in chicks fed corn based diets. Rye based diets contain anti-nutritional factors that increase digesta viscosity, decrease body weight gains and increase small intestine weights compared to corn based diets (Làzaro *et al.*, 2004). Roxarsone was observed to improve body weights when birds were fed rye-based diets due to an ability to compensate for the lower nutritional value of this diet compared to the corn-based diet (Fernandez *et al.*, 1973).

Roxarsone is also effective at simultaneously limiting coccidia infections and maintaining performance. Mitrovic *et al.* (1977) fed day old broilers a diet supplemented with roxarsone (50 ppm) or unmedicated fed. Birds were infected with *E. tenella* at two-weeks of age. A positive (unmedicated and uninfected) control was also maintained during the 9-day infection period. The roxarsone treated birds had 88% of the weight gain of the unmedicated and

uninfected birds, while the negative control birds (infected, unmedicated) had only 69% of the weight gain as the control (Mitrovic *et al.*, 1977). Lesion scores of the roxarsone treated birds were reduced to 1.5 compared to the scores of 3.0 from the negative control birds (Mitrovic *et al.*, 1977). Roxarsone effectively limits the effects of the primary infection associated with coccidiosis.

Although roxarsone is able to limit coccidial infections, it is less effective at controlling necrotic enteritis. Stutz and Lawton (1984) found more than 100 ppm of roxarsone is need to control *C. perfringens in vitro*. Hofacre *et al.* (2007) observed bacitracin methylene disalicylate (**BMD**) to be more effective at improving broiler performance following a *C. perfringens* challenge than roxarsone. Male, Cobb x Cobb broilers were fed a basal diet with or without supplementation of roxarsone (45.4 g/ton) or BMD (50 g/ton) for 42 days. On day 15, the necrotic enteritis challenge model was used by administering a 25x dose of a coccidia vaccine, Cocciva-B. A group of chicks on the non-supplemented feed was not infected to serve as a positive control. On days 19, 20 and 21, broilers were fed *C. perfringens*. Body weights, mortality and feed conversion were different between the BMD, roxarsone and negative control animals ($P < 0.05$). Body weights were most improved by BMD supplementation and then roxarsone supplementation compared to the control broilers ($P < 0.05$). Feed conversion and mortality was more positively affected by BMD treatment than roxarsone supplementation ( $P < 0.05$). Although BMD treated birds greatly improved performance compared to roxarsone, *Salmonella* spread between birds was reduced more by roxarsone. On day 1, half of the birds/pen were orally inoculated with *Salmonella* Heidelberg. On day 22, the total number of broilers positive for *Salmonella* Heidelberg was decreased in roxarsone treated birds compared to BMD supplemented broilers. The discrepancy of roxarsone effectiveness may be due to the

tendency of *Salmonella* to growth in areas of the gut where roxarsone is most effective (cecum and distal small intestine) during *E. tenella* infections.

*Negative impacts associated with feeding roxarsone*

Resistance to arsenic has been shown in some bacterial species. Susceptibilities to arsenite (As III), arsenate (As IV) and roxarsone were measured in *Campylobacter* isolates from retail poultry products (Sapkota *et al.*, 2006). All isolates expressed some phenotypic resistance to roxarsone, arsenite and arsentate. Isolates from conventional poultry products were more roxarsone resistant ($P < 0.0001$) than isolates from producers that claim not to use antimicrobials or roxarsone (Sapkota *et al.*, 2006).

Feeding roxarsone results in the excretion of As in the feces. Poultry manure is commonly applied to land resulting in a build-up of As in the environment. After feeding a diet supplemented with 45.5 g/ton of roxarsone for 7 weeks, the average fecal arsenic concentration was 13.8 ppm (Anderson and Chamblee, 2001). In a study of 40 various poultry litter samples from Alabama, Georgia and South Carolina, Jackson and colleagues (2003) observed As levels ranging from 1 to 39 mg/kg. Because poultry litter contains As from feeding roxarsone, management of litter presents some difficulties. Research has found that land applied with poultry litter contains As levels above that of land that has not previously been spread with poultry litter (Gupta and Charles, 1999). Fields from the Delmarva area (known for long time application of litter from birds feed roxarsone) has 2.6 times higher total As than fields that had been spread for one season (Rutherford *et al.*, 2003). Roxarsone supplementation can cause bacterial resistance and As build-up in the environment.

Roxarsone requires a five-day withdrawal period prior to slaughter to limit human exposure to arsenic. The FDA has an allowable limit of 500 ppb of As in poultry muscle tissue. Little research has been done studying the accumulation of arsenic in muscle tissue due to the feeding of roxarsone. Currently, As accumulation in skeletal muscle is based on liver-to-muscle ratios (Lasky *et al.*, 2004). Liver-to-muscle ratios for arsenic levels vary from 11, 4.2, and 2.9 for calculating muscle As levels in poultry (Lasky *et al.*, 2004). With these ratios, it is found that consuming one meal of poultry a day (60g of meat) will not cause an adult to exceed the daily tolerable level of 2 μg/kg of body weight/day of inorganic arsenic set by the World Heath Organization (Lasky *et al.*, 2004). VanderKop and MacNeil (1989) studied As residues in broiler tissues after feeding arsanilic acid (another organic arsenic compound). From 10 to 31-days of age, male Hubbard broilers were fed a corn, wheat and soybean meal based diet containing various levels of arsanilic acid (0, 100 and 500 ppm). Arsenic concentrations in skeletal muscle of birds fed 0, 100 or 500 ppm in skeletal muscle were as follows: 20, 70 and 130 ppm, respectively. This study did not include a withdrawal period. Liver residues were reported as 340 (0), 230 (100) and 162 ppm (500 ppm) of arsenic. Although roxarsone requires a withdrawal period of 5 days, in this study arsanilic acid was fed continuously until slaughter. Even with no withdrawal period, muscle As levels were below FDA requirements (VanderKop and MacNeil, 1989). Arsenic was observed in skeletal muscle of birds not fed an arsenical, indicating environmental As can accumulate in muscle tissue (Vanderkip and MacNeil, 1989). Further investigation of muscle As contamination due to roxarsone supplementation needs to be completed.

*Bacitracin Metheylene Disalicylate*

Bacitracin metheylene disalicylate, an antibiotic produced by *Bacillus licheniformis*, is used as a growth promoter for production animals and as a topical preparation in human and veterinary medicine. Active against most gram-positive bacteria, bacitracin interferes with the dephosphorylation of the $C_{55}$-isoprenyl pyrophosphate, a molecule which carries the building blocks of the bacterial cell wall outside of the inner membrane (Stone and Strominger, 1971). The FDA limits the amount of BMD residues in uncooked chicken muscle tissue to 500 ppb, but no withdrawal period is required when BMD is fed at levels to improve growth rate from broilers.

Bacitracin is effective at manipulating bacterial profiles by decreasing lactic acid producing bacteria and reducing clostridia. Stutz and Lawton (1984) found BMD (3.1 ppm) inhibited *Clostridium perfringens* growth *in vitro*. Sims *et al.* (2004) observed *in vivo* reductions ($P < 0.05$) of clostridia in turkey digesta when BMD was supplemented compared to turkeys fed unmedicated fed. Feeding BMD for 42 days increased *Lactobacillus salivarius*/gram of broiler ileal digesta compared to non-medicated birds (Guban *et al.*, 2006). Increases in digesta bifidobacteria were observed in turkeys fed BMD compared to non-medicated fed (Sims *et al.*, 2004). With changes in microbial populations, BMD also changes gut morphology. Decreased intestinal length and weight was observed in broilers fed BMD (50 g/ton in the starter and 25 g/ton in the grower and finisher diets) compared to birds fed unmedicated fed for 49 days ($P < 0.05$) (Miles *et al.*, 2006).

*Bacitracin metheylene disalicylate as a growth promoter*

The affects of bacitracin supplementation on broiler performance is contingent on health status. Some research has shown no change in growth performance with the supplementation of bacitracin (Henry *et al.*, 1987; Leeson *et al.*, 2005; Yang *et al.*, 2007). Male Ross x Ross broilers were fed non-medicated or BMD (50 ppm) supplemented feed for 42 days (Leeson *et al.*, 2005). Duodenal samples were collected on day 21 to determine changes in gut morphology. Weight gain, feed intake, feed efficiency and carcass weight did not differ significantly between treatments. Crypt depth was reduced in BMD fed birds ($P < 0.05$) indicating less cell turnover within the gut, but this reduction in gut cell turnover did not relate to an increase in feed efficiency or weight gain (Leeson *et al.*, 2005). Yang *et al.* (2007) observed similar findings when male Cobb broilers were fed bacitracin Zn (50 ppm and 30 ppm in the first and last 3 weeks, respectively) for 42 days. Feed intake, feed efficiency and body weight gain did not differ significantly from control animals (Yang *et al.*, 2007). Guban *et al.* (2006) also observed no differences in body weight at 42 days when BMD was supplemented in feed. Body weight gain was increased from 36 to 42 days of age with BMD supplementation compared to non-medicated fed broilers (Guban *et al.*, 2006).

Positive improvements in growth with BMD supplementation have been reported when broilers are stressed due to common industry problems: disease and stocking density. Male broilers supplemented with bacitracin (50 g/ton) and housed on previously used pine shavings containing coccidia (*E. tenella, E. maxima* and *E. acervulina)* increased chick body weights at 42 days-of-age compared to unsupplemented broilers ($P < 0.05$) (Stanley *et al.*, 2004). Hooge *et al.* (2003) fed Ross x Ross broiler chicks a corn and soybean based diet with or without BMD (55 (0-21d), 55 (21-42d) and 27.5 (42-49d) ppm). Body weight was improved and feed efficiency

decreased by 20 points at 49-days in broilers fed BMD supplemented feed ($P < 0.05$). Though chicks were housed on new litter, the stocking density was high (0.667 $ft^2$/bird), which may have added enough stress to the animals to accent the positive contributions of BMD to growth (Hooge *et al.*, 2006).

Although bacitracin is fed to limit necrotic enteritis, some research has not observed improvements in performance in necrotic enteritis challenged broilers. Hofacre *et al.* (1998) fed BMD ( 50 g/ton) supplemented feed to male broilers orally inoculated with *E. acervulina* followed by *C. perfringens* for 15 days-of-age. Intestinal lesion scores, feed consumption and body weight gain were not different from broilers infected with *C. perfringens* challenge and fed unsupplemented fed compared to birds fed BMD supplemented feed. Mortality was decreased when BMD was fed ($P < 0.05$). Although BMD is effective at limiting clostridium growth *in vitro* (Stutz and Lawton, 1984), BMD is not effective at improving body weight gain during a necrotic enteritis infection (Hofarce *et al.*, 1998).

*Bacitracin MD interactions with other growth promoters*

Bacitracin is used as the only feed antibiotic in 13.7% of all U.S. poultry production units (Chapman and Johnson, 2002). More commonly, a combination of BMD with other AGP is used. Bacitracin in combination with other growth promoters is more popular than feeding it alone and utilized by 73.6% of all U.S. poultry production units (Chapman and Johnson, 2002). Bacitracin MD in combination with roxarsone has not been shown to improve body weight or feed efficiency when broilers were fed a corn based diet for 3 weeks (Fernandez *et al.*, 1973). However, improvements in body weight and feed efficiency were observed when broilers were fed a rye based diet supplemented with roxarsone and BMD (Fernandez *et al.*, 1973).

Improvements in performance may be more detectable when supplementation is done in a less nutritive diet, such as a rye-based diet, compared to a corn-soybean diet. In a study that compiled data from 5 different trials, feeding a combination of BMD (55 mg/kg) and roxarsone (50 mg/kg) improved body weight ($P < 0.05$) and feed efficiency ($P < 0.05$) in broilers grown to 49 days (Waldroup *et al.*, 1986). All diets were supplemented with salinomycin (66 mg/kg) (Waldroup *et al.*, 1986). Bacitracin metheylene disalicylate is a popular and effective growth promoter feed in combination with other AGP.

*Virginiamycin*

Virginiamycin is an antibiotic used in poultry diets to prevent necrotic enteritis. Continuous feeding of virginiamycin is allowed because virginiamycin does not lead to high levels of residues in meat. Proudfoot *et al.* (1990) found only 0.5µg of virginiamycin per gram muscle and liver tissue in broilers fed 11 mg of virginiamycin /kg of diet continuously for 42 days. Virginiamycin is a mixture of virginiamycin M and virginiamycin S, which are considered virginiamycin type A and B, respectively (Thai and Zervos, 1999). Mixtures of type A and B improve the potency of this bacteriocide (Cocito, 1979). In a review of his work, Cocito (1973) describes virginiamycin blocking the formation of ribosomal subunits within gram-positive bacteria. Inhibiting the formation of ribosomal subunits will cause protein synthesis to cease, eventually causing cell death.

Virginiamycin is effective at manipulating bacterial profiles *in vitro* and *in vivo*. Stutz and Lawton (1984) observed virginiamycin (0.02 ppm) added to bacterial media inhibited *Clostridium perfringens*. Dumonceaux and colleagues (2006) compared bacterial libraries in virginiamycin (20 g/ton) treated male Cobb 500 broilers to control broilers using chaperon 60

gene sequencing and quantitative PCR. Dietary virginiamycin tended to increase the abundance of all of the bacterial targets that were analyzed in the proximal gastrointestinal tract (duodenal loop, mid-jejunum and proximal ileum). The libraries included the gram-positive bacteria: *Lactobacillus spp., Pediococcus parvulus, Enterococcus cecorum, Globicatella sanguinis, Staphylococcus spp., Clostridium polysaccharolyticum* and *Macrococcus spp.* There were no effects on bacterial libraries in the distal intestine. Virginiamycin may alter the abundance of certain species, which may have led to an increase in gram-positive bacteria even though virginiamycin is considered a bacteriocide against these bacteria. Changes in bacterial populations occurred in the proximal small intestine, leading the authors to conclude that virginiamycin is most effective in that region of the intestine (Dumonceaux *et al.*, 2006). Although virginiamycin inhibits gram-positive bacteria, research has observed increases in certain gram-positive bacteria in broilers treated with virginiamycin.

Recent research shows an alteration in gut morphology of broilers fed virginiamycin. Intestinal length and weight were decreased in birds fed virginiamycin (15 g/ton during the starter period and 10 g/ton during the grower and finisher periods) at 49 days compared to birds fed drug free diets or birds fed BMD (50 g/ton in the starter and 25 g/ton in the grower and finisher diets) (Miles *et al.*, 2006). Duodenal villi:unit area were increased with virginiamycin supplementation compared to BMD supplementation or drug free diets (Miles *et al.*, 2006), further supporting Dumonceaux *et al.* (2006) findings that virginiamycin is most effective in the proximal small intestine. Ileal villus height and crypt depths were shorter in the ileum of birds fed virginiamycin compared to drug free or BMD supplemented birds (Miles *et al.*, 2006). Broiler villi length is decreased with virginiamycin. This decreased villi length in birds fed

virginiamycin indicates a decreased absorptive area within the gut, however the reported increase in villi number may have compensated for the decrease in length (Miles *et al.*, 2006).

*Virginiamycin as a growth promoter*

Virginiamycin supplementation is hypothesized to positively affect broiler performance in "unclean" conditions, while having no effect in "clean" conditions. Male Arbor Acre broilers housed on clean litter were fed virginiamycin supplemented feed (11 mg/kg) for 42 days (Proudfoot *et al.*, 1990). No differences were found in mortality, feed conversion or live body weight at 42 days of age in supplemented birds compared to unsupplemented birds (Proudfott *et al.*, 1990). Miles *et al.* (1984) reported contradicting results. Cobb broilers housed on clean litter for 51 days had improvements in body weight gain, feed efficiency and decreased mortality when supplemented with virginiamycin (10 mg/kg) compared to control fed birds (Miles *et al.*, 1984). The differences in these studies may be due to the inclusion of female birds in the Miles *et al.* (1984) study. A study of female Large White Hybrid turkey poults observed increased overall body weights by 8.6% and decreased feed conversion from 0 to 14 weeks of age when poults were supplemented with virginiamycin (22 mg/kg diet) compared to birds fed a non-supplemented control (Parks *et al.*, 2005). Poults were housed on top-dressed litter from a previous flock, but overall the cumulative mortality (0.6%) and cull (3.6%) rate were low, indicating a relatively "clean" environment ($P < 0.05$). Parks *et al.* (2005) observations of changes in body weights and feed conversion in female turkey poults further support Miles *et al.* (1984) hypothesis that female birds are more affected by virginiamycin supplementation.

Supplementation of virginiamycin in broiler diets maintains performance while broilers are raised under stress-inducting conditions. George and colleagues (1982) challenged male

broilers with *C. perfringens* and supplemented their diets with 20 or 40 g of virginiamycin/ton of feed. No differences were observed in body weight gain, feed efficiency, mortality or lesions in broilers fed diets supplemented with virginiamycin compared to unchallenged drug-free fed broilers (George *et al.*, 1982). Hofacre *et al.* (1998) observed similar results in male broiler chicks fed virginiamycin (50 g/ton) and infected with *C. perfringens*. Fifteen days post-infection, intestinal gross lesion scores and feed efficiency were reduced compared to challenged birds fed non-medicated feed ($P < 0.05$). Although improvements in some production parameters were observed, body weight gain was depressed in virginiamycin fed broilers compared to unchallenged non-medicated fed broilers ($P < 0.05$). Female poults were inadvertently subjected to cold stress (29$^{o}$C) during the first 2 days post hatch (Parks *et al.*, 2005). Poults feed virginiamycin (22mg/kg diet) improved overall body weight gains by 8.6% ($P < 0.05$) (Parks et a., 2005).

Although under stressful conditions virginiamycin improves performance, virginiamycin does not improve performance following coccidiosis vaccination. Leeson *et al.* (2005) fed male Ross x Ross broilers nonmedicated feed or virginiamycin (11 ppm) supplemented feed for 42 d following a gel-spray coccidiosis vaccine. Body weight gain, mortality and carcass weight were not different between treatments. Feed intake was reduced in birds fed virginiamycin causing a reduction in feed efficiency ($P < 0.05$). Male birds, which are not as sensitive to virginiamycin, were used in this study which could have affected results (Leeson *et al.*, 2005). Virginiamycin is not effective at controlling coccidiosis.

In a current study, virginiamycin depressed growth (Baurhoo *et al.*, 2007). Male Cobb 500 broilers were fed a corn-soybean meal diet for 42 days with or without virginiamycin (11 mg/kg). At 7 until 42 days-of-age, broilers supplemented with virginiamycin had lower body

weights ($P < 0.05$). Feed intake was suppressed from 1 to 35 days ($P < 0.05$) and 1 to 42 days ($P < 0.05$) in broilers supplemented with virginiamycin. Depressed body weights were due to a depression in feed intake, but no similar effects of virginiamycin have been reported in clean conditions (Miles *et al.*, 1984; Proudfoot *et al.*, 1990; Parks *et al.*, 2005).

*Virginiamycin interactions with other growth promoters*

Virginiamycin is often added to broiler finish diets following BMD used fed during the starter and grower periods. Hooge and colleagues (2003) fed BMD during the starter and grower periods (27.5 and 55 ppm) and virginiamycin during the finisher period (11 ppm) to straight run Ross x Ross broilers. Compared to broilers fed a drug-free control diet, broilers fed BMD/virginiamycin increased body weight gain by 3.8% ($P < 0.05$). Feed:gain and mortality were not different between the treatment groups. Overall, the net income per bird increased by $0.022 when BMD and virginiamycin were supplemented in the diet ($P < 0.05$) (Hooge *et al.*, 2003). Feeding virginiamycin and BMD in a shuttle program improves body weight gain and net income/bird.

*Monensin*

Monensin, an ionophore antibiotic and classified as a monovalent carboxylic ionophorous polyether antibiotic, is the most popular drug for treating coccidiosis (Suls, 1999). *Streptomyces cinnamonensis* produces monensin (Butaye *et al.*, 2003). In a review of the effectiveness of monensin, Butayle *et al.* (2003) explained that monensin transports $Na^+$ more efficiently than $K^+$ interfering with the natural ion transport systems of both prokaryotic and eukaryotic cells. Energy barriers necessary for the transmembrane transport of ions are lowered. Neutral cation-

proton exchange across the barrier is slowed, abolishing $K^+$ and $Na^+$ gradients (Butaye *et al.*, 2003). The change in ion gradients lead to the cell death of gram-positive bacteria, while gram-negative bacteria are not effected because the cell wall cannot be penetrated by monensin.

Monensin affects the bacterial community within the gut of broilers by increasing gram-negative bacteria populations and decreasing gram-positive populations. Lu *et al.* (2006) fed monensin (90 g/ton of feed) for 49 days. Ileum contents were collected on days 3, 7, 14, 21, 28 and 49 days of age for bacterial phylotyping using terminal restriction fragment length polymorphism analysis combined with 16S rDNA clone libraries. *Clostridium lituseburense* and *C. irregularis* dominated the bacterial community of monensin fed birds. Lactobacilli were the primary community found in control animal digesta. Necrotic enteritis is caused by clostridium species and is often a secondary bacterial infection followed by a coccidia infection in broilers. Monensin supplementation may increase the amount of necrotic enteritis causing bacteria (Lu *et al.*, 2006), but improves production due to its anticoccidial effects. Feeding a wheat based diet supplemented with monensin (0.5g/kg), BMD (0.5 g/kg) or monensin (0.5g/kg) + BMD (0.5 g/kg) resulted in decreased *L. salivarius* ileal concentrations at 10 days of age in straight-run broilers compared to non-supplemented controls (Guban *et al.*, 2006). Birds fed monensin had increased lipid digestion ($P < 0.05$) and increased conjugated bile salts ($P < 0.05$) compared to control birds. Guban *et al.* (2006) hypothesized monensin supplementation decreases bacteria (*L. salivarius*) that deconjugate bile acids. By decreasing the amount of deconjugating bacteria, conjugated bile salt levels increased, increasing lipid digestion (Guban *et al.*, 2006). Monensin supplementation affects intestinal bacterial communities in broilers, but the outcome of this effect needs further study.

Monensin disrupts coccidia in a similar fashion as gram-positive bacteria. Wang *et al.* (2006) collected oocysts from chickens orally inoculated with *E. tenella* 7-days prior to collection. The sporozoites were treated with 1.0 μg/ml monensin for one hour and subsequently washed. Using atomic absorption spectrophotometry, Wang and colleagues (2006) analyzed sodium, potassium and $Na^+$-$K^+$-ATPase activities. Monensin treatment increased sodium, potassium and $Na^+$-$K^+$-ATPase activity levels compared to control sporozoites. The authors reported that these results were due to a lowered energy barrier caused by increased $Na^+$ entering the sporozoite. Monensin may cause sodium and potassium to be pumped into sporozoites at a rate that exceeds the pump's ability to remove sodium from the cell causing swelling and eventual cell death (Wang *et al.*, 2006).

Coccidia resistance to monensin has been documented. Wang and colleagues (2006) treated birds for 7 days with two different types of *E. tenella*, one that had no history of exposure to anticoccidials and one that had been passed through chickens medicated with monensin 35 times. Birds were treated with monensin. Lesion scores and oocyst outputs were increased in birds treated with the resistant oocysts. Sporozoites were studied *in vitro* to test for sensitivity to monensin. Resistant sporozoites had decreased sodium and $Na^+$-$K^+$-ATPase activity levels compared to the sporozoites that had never been exposed to anticoccidials (Wang *et al.*, 2006). Coccidia resistance to monensin may be the result of a decrease in the ability of monensin to cause sodium influx into the cell.

Past research indicates that monensin does not leave residues in poultry tissues. Ten-day old broilers were fed monensin supplemented feed at different levels (0, 50 and 70 ppm) until 31-days of age (VanderKop and MacNeil, 1989). Monensin was not detected in cardiac or skeletal muscle, nor in the tissue of the liver or kidney. The detection limit of the assay was 250

ug/kg and tissue monensin recoveries were 93 – 97% (VanderKop and MacNeil, 1989). Monensin does not leave detectable residues in poultry meat.

*Monensin as a growth promoter*

Past research on performance effects of monensin is inconsistent due to variation in disease state and incorporation of non-nutrient dietary additives. The addition of monensin (5g/kg diet) to a wheat-based diet had no effect on body weight gain or feed conversion in floor raised straight-run broilers (Guban *et al.*, 2006). Welch *et al.* (1986) found that monensin (121 mg/kg and 140 mg/kg) depressed growth rates in chicks fed a corn soybean meal basal diet (20% crude protein) by decreasing feed intake. Harms *et al.* (1989) also observed a reduction in weight gain and feed intake in chicks reared in batteries when fed a corn-soybean meal basal diet supplemented with monensin at 101, 121, 141 and 161 ppm compared to chicks feed unmedicated feed. The reduction in body weights increased feed:gain ratios in the birds supplemented with 121, 141 and 161 ppm of monensin (Harms *et al.*, 1989). Monensin suppresses performance in broilers fed nutrient rich diets.

Although negative effects are observed in unchallenged birds supplemented with monensin, chicks infected with *E. acervulina* did not show decreased performance when supplemented with monensin compared to control animals (Welch *et al.*, 1986). Welch *et al.*, 1986 fed 50,000 *E. tenella* oocysts to broilers and lesion scored the birds 7-days post-inoculation. Birds fed monensin (125 ppm) had decreased lesion scores and oocysts output compared to control animals. Performance depressions associated with monensin supplementation in non-infected birds may be due to a reduction in feed intakes. Despite these negative effects, monensin is able to decrease the damaging effects of coccidiosis.

*Monensin interactions with other growth promoters*

The effect of monensin and roxarsone supplementation on broiler performance is still unclear. In the presences of an *E. tenella* infection, monensin and roxarsone did not improve weight gain or feed conversion ($P < 0.05$) compared to feeding monensin alone (Izquierdo *et al.*, 1987). Unchallenged chicks experienced depressed body weight gains when supplemented with monensin and roxarsone. Izquierdo *et al.* (1987) concluded that following an infection with *E. tenella* and *E. acervulina*, roxarsone was efficacious in the absence of monensin. In turn, monensin was more efficacious in the absence of roxarsone. This interpretation was challenged by McDougald and colleagues (1996) when birds orally inoculated with *E. acervulina, E. maxima* and *E. tenella* were fed a diet supplemented with monensin, roxarsone and BMD. Birds fed the supplemented diet had reduced lesion scores in the cecum and middle gut, improved weight gain, feed conversion and shank pigmentation score compared to birds fed only roxarsone and BMD ($P < 0.05$) (McDouglad *et al.*, 1996). Feed intakes were 4.456 kg and 3.489 kg for the control fed birds and monensin fed birds, respectively. Monensin supplementation did not significantly impact feed intake in this study. The lack of treatment response could be due to the coccidia challenge as indicated by Welch *et al.* (1986) or the additional supplementation of roxarsone and BMD positively affected feed intakes. A study by Guban *et al.* (2006) supports the hypothesis that additional supplements of monensin does not improve performance in healthy animals. Monensin (5g/kg diet) and bacitracin (5g/kg diet) added to a wheat-based diet had no effect on body weight gain or feed conversion in floor raised straight-run broilers as compared to birds fed monensin (5g/kg diet) nor those fed no monensin or bacitracin (Guban *et al.*, 2006).

Further research needs to be conducted examining the effects of supplementing roxarsone, BMD and monensin in coccidia challenged and non-challenged broilers on feed intake.

Diets supplemented with virginiamycin and monensin do not depress performance. Salmon and Stevens (1990) fed turkeys diets supplemented with monensin (99mg/kg), virginiamycin (22mg/kg) or a combination of monensin (99mg/kg) and virginiamycin (22mg/kg) for 12 weeks. Birds fed the monensin + virginiamycin diet had improved feed efficiency compared to other diets, although final body weights were similar to the virginiamycin fed birds. Birds fed the monensin diet had depressed final body weights and gain:feed. Supplementation of monensin + virginiamycin increased gain:feed, while no differences were noted in final weights. This suggests that feed intake was depressed due to monensin supplementation but, the effects of virginiamycin on increasing body weight gains caused the improvement in gain:feed (Salmon and Stevens, 1990). Depressions in feed intake associated with monensin may be counterbalanced by virginiamycin. Miles and colleagues (1984) further support this claim, reporting that birds supplemented with monensin (0.011%) and virginiamycin (10 ppm) had increased body weight gains compared to birds supplemented with monensin only. There was no effect of treatment on feed efficiency. Virginiamycin appears to improve feed efficiency in diets supplemented with monensin.

Adding virginiamycin to a monensin supplemented diet has been hypothesized to improve weight gain by increasing feed intake while not changing the bacterial population of the gut. Elwinger and colleagues (1998) found that the addition of monensin to a diet with Avoparcin, a growth promoting antibiotic, did not effect mortality rates or the number of *Clostridium perfringen* positive cecal samples from broilers at 14, 21, 28, 35 or 46 days of age. Avoparcin is fed as an antibiotic growth promoter, similar to the feeding of virginiamycin.

Further research needs to be conducted to determine the effects of virginiamycin and monensin supplementation on bacterial populations in broilers. Although antibiotics and anticoccidials do not always produce significant improvements in production parameters, poultry producers continue to use these growth promoters.

**Bone Strength in Association with Feed Additives**

Most feed additives do not affect bone strength in broilers. Supplementation with roxarsone (45.4 g/ ton of feed) had no effect on leg issues in 3 week-old broilers compared to control animals not fed roxarsone (Rath *et al.*, 1998). Rath *et al.* (1998) also found no differences in the stress, strain or modulus of elasticity of digit flexoral tendons after roxarsone supplementation. No differences were observed in collagen, sulfated glycosaminoglycans or pyridinoline content of tendons with roxarsone supplementation (Rath *et al.*, 1998). Rath *et al.* (1998) also studied the effects on monensin (45.5 g / ton feed) on digit flexoral tendons and leg problems of 3 week old broilers. No differences were noted in the biomechanical and collagen content of the tendons (Rath *et al.*, 1998). Furthermore, no differences in leg problems were observed (Rath *et al.*, 1998). Veltmann and Jensens' (1981) work further supports Rath's work. Broilers were fed a corn soybean meal diet with either monensin (100 mg/kg), monensin (100 mg/kg) or roxarsone (11 mg/kg) or without any supplements for 4 weeks. At the end of the study no differences were noted in the incidence of twisted legs or tibia dyschondroplasia between broilers fed diets supplemented or unsupplemented (Veltmann and Jensens, 1981). Current research needs to be conducted on growth promoters and their affect on bone strength of today's broiler genetics.

## Alternatives to Antibiotics and Anticoccidials

### *Probiotics*

Probiotics are purposely ingested microorganisms that beneficially affect the host animal by improving its intestinal microbial balance and stimulating the immune system. Probiotic bacteria are generally lactic acid producing bacteria. Interaction between bacteria and the gut lining occurs at the epithelial cell lining. Bacteria must first colonize the mucosal surface before interacting with epithelial cells. The process of colonization begins with attraction of the bacteria to the mucosal surface and attachment to the mucosal layer, which may lead to colonization of the gut epithelial cells (Gusils *et al.*, 2003). Probiotic bacteria have shown a similar sequence of events leading to colonization, but not constantly. Not every species of *Lactobacillus* interacts with the gut in the same manner; therefore the exact mechanism of action for probiotics remains undefined (Jin *et al.*, 1998a; Jin *et al.*, 1998c; Koene *et al.*, 2004; Lan *et al.*, 2004; Smirnov *et al.*, 2005). Craven and Williams (1998) pretreated broiler cecal mucin *in vitro* with *Lactobacillus* strains and observed reduced *S. typhimurium* attachment to mucus compared to untreated mucin. Probiotics have been shown to shift bacteria populations in digesta, attach to epithelial cells *in vitro* and competitively exclude some bacterial species from attachment to the epithelial surface.

Changes in bacterial populations of the digesta and *in vitro* studies of probiotic supplementation have lead to the hypothesis probiotics colonize the gut. Ehrmann and colleagues (2002) were able to demonstrate that probiotic strains colonize within the gut. Twenty-eight days after two antibiotic resistant strains of *Lactobacillus* were fed to ducks these species were still detected in the crop and cecum. According to Tannok (2005), *Lactobacilli* shed from the crop inoculate the digesta and are therefore detected throughout the remainder of

the gut. The authors suggest that the presence of probiotic bacteria strains 28-days post-inoculation was due to their colonization of the gastrointestinal tract (Ehrmann *et al.*, 2002). Ehrmann *et al.* (2002) indicated that probiotics shift bacterial specie populations and are able to colonize the crop. Tierney and colleagues (2004) found that *Lactobacillus* species were able to inhibit *E. acervulina in vitro.* Using scanning electron microscopy, Chichlowski *et al.* (2007a) observed a greater number of rod-shaped bacteria (morphology similar to lactobacilli) on the surface of the ileal, cecal and colonic mucosal of broilers continuously fed a probiotic than broilers fed control fed. The bacteria were closely associated with goblet cells in probiotic fed broilers compared to control broilers, which could change the mucin profile and morphology of the gastrointestinal tract (Chichlowski *et al.*, 2007a). Probiotics are able to limit enteritic bacteria while increase beneficial bacterial within the gut.

Probiotics have been shown to change gut morphology in chickens (Smirnov *et al.*, 2005; Yurong *et al.*, 2005). Birds fed probiotics for 3 days had increased cecal microvilli density and length compared to control fed birds (Yurong *et al.*, 2005). In the duodenum, jejunum and ileum the goblet cell "cup" area was increased by 18%, 82%, and 40% respectively when chickens were fed probiotics, while feeding an antibiotic growth promoter (Avilamycin, 5 mg/kg) did not affect the goblet cell "cup" area (Smirnov *et al.*, 2005). Alterations in goblet cell area caused similar alterations in mucin glycoprotein concentration. Mucin glycoprotein concentration in the jejunum of birds fed probiotics was 110% compared with control birds ($P < 0.05$) (Smirnov *et al.*, 2005). The mucin glycoprotein storage in birds supplemented with Avilamycin was 53% lower in the duodenum than probiotic supplemented birds ($P < 0.05$). Increases in mucin mRNA expression suggest the possibility of increased mucin production in Avilamycin-fed animals compared to probiotic-fed animals. Feeding Avilamycin limited storage of mucin glycoproteins

in the goblet cell increasing mucosal turnover because the glycoproteins were unable to be stored. Increased mucin turnover is a protective mechanism of the mucosal layer (Laux *et al.*, 2005). By increasing turnover, mucin and the bacteria that have penetrated the layer are shed to avoid colonization of the epithelial cells. Meslin *et al.* (1999) reported increased goblet cell density and therefore mucin production with bacterial interaction in the gut. The antibiotic was less able to decrease bacteria interaction with the gut compared to probiotics (Smirnov *et al.*, 2005). Probiotics may reduce mucin turnover by competitively excluding pathogens from entering the mucosal layer and decreasing turnover.

Changes in mucosal morphology may decrease energy expenditures in broilers. Chichlowski *et al.* (2007a) observed increased villus height and perimeter in the jejunum ($P < 0.05$) in broilers fed probiotics compared to broilers fed diets supplemented with salinomycin and decreased crypt depth ($P < 0.05$). These observations indicate that the increase in villus height and parameter were not associated with actual increases in cell turnover because crypt depth, an indicator of cell turnover, was actually decreased in probiotic-fed broilers (Chichlowski *et al.*, 2007a). Probiotics may increase absorptive areas that relate to a decrease in body energy expenditures (Chichlowski *et al.*, 2007a; Chichlowski *et al.*, 2007b). Using indirect calorimetry, broilers continuously fed probiotics had a 6 to 16% decrease in whole-body energy expenditures and a 47% decrease in ileal energy expenditures ($P < 0.05$) compared to broilers fed non-medicated fed or feed supplemented with salinomycin (Chichlowski *et al.*, 2007b). Changes in mucosal morphology associated with feeding probiotics increase energy efficiency in broilers.

Recent research results of immune status alterations due to probiotic supplementation are inconsistent. Haung *et al.* (2004) fed a commercially available probiotic continuously at a high and low level. Broilers fed low levels of the probiotic had increased serum IgA concentration in

response to the antigen keyhole limpet hemocyanin ($P < 0.05$) compared to broilers not fed probiotics. Increased serum IgA levels indicate an activated gastrointestinal immune system. Serum IgG levels were not different among control broilers and probiotic fed birds (Haung *et al.*, 2004). Feeding probiotics enhanced the number of intestinal epithelial lymphatic cells in the cecal tonsils of chickens (Yurong *et al.*, 2005). These studies show that probiotics activate the immune system to protect the gut from coccidial invasion. The increase in immune function may relate the differences seen with production parameters. Although probiotics have been described to inhibit *E. acervulina*, O'Dea *et al.* (2006) observed a clinical outbreak of necrotic enteritis in broilers sprayed or feed probiotics and housed in isolated environmental chambers. Although probiotics may not limit necrotic enteritis infection, by limiting coccidial infections probiotics may be a useful addition to a broiler shuttle feeding scheme.

*Probiotics as growth promoters*

Improvements in broiler performance have been observed in broilers fed probiotics. Broilers had significantly higher body weights when continuously providing probiotics as compared to control birds at 49 days of age ($P < 0.05$), while feed intake and feed conversion was not affected (Cavazzoni *et al.*, 1998). Comparing probiotic effectiveness to an antibiotic growth promoter, Cavazzoni *et al.* (1998) also observed no differences in body weights of birds fed supplied probiotics or virginiamycin (Cavazzoni *et al.*, 1998). Jin and colleagues (1998a) found that supplementation with a *Lactobacillus* culture at 0.1% increased body weight and improved feed conversion at 42 days of age compared to birds fed non-supplemented feed ($P < 0.05$). Studies have shown that feeding probiotics can lower feed conversion ($P < 0.05$) compared to birds fed non-supplemented feed (Jin *et al.*, 1998b; Kalavathy *et al.*, 2003).

However, findings by Cavazzoni and colleagues (1998) contest these findings reporting no differences in feed conversion when supplementing virginiamycin or probiotics compared to birds fed non-medicated fed. Decreasing mortality is an important economical benefit of supplementing probiotics and antibiotics to broiler feed. Following heat stress, probiotic supplementation was able to decrease mortality from 57% to 22% compared to control chicks (Lan *et al.*, 2004). Probiotic supplementation may have important production implications related to body weight gain, feed conversion and decreased mortality in poultry.

*Bio-Mos®*

Bio-Mos®, a mannan oligosaccharide (**MOS**) derived from the cell wall of the yeast *Saccharomyces cerevisiae*, is a prebiotic that may be an appropriate replacement for antibiotics or roxarsone in broiler diets. Mannan oligosaccharides bind to bacteria with type-1 fimbriae *in vitro*. Many different species of Enterobacteriaceae (gram-negative, rod shaped bacteria) express type-1 fimbriae (Mulvey *et al.*, 2004). In an agglutination study, Spring and colleagues (2000) found that MOS aggregated with *Escherichia coli, Salmonella enteritidis, S. typhimurium, S. montevideo, S. give, S. kedougou,* and *S. dublin.* The mannan portion of MOS is thought to be the attachment point for bacteria. Mannose added to bacterial growth media prior to treatment with MOS inhibited aggregation in all those bacteria species that aggregated with MOS (Spring *et al.*, 2000). Mannan oligosaccharides, such as Bio-Mos®, act as a prebiotic by reducing competition of beneficial bacteria binding sites in the small intestine with that of Enterobacteriaceae species.

Although MOS is hypothesized to reduce the number of gram-negative bacteria within the gut by binding to the bacteria's type-1 fimbriae enabling bacterial attachment to the gut wall,

*in vivo* experiments have not consistently found reductions in gram-negative bacteria when MOS is supplemented. One-day-old male Cobb broiler chicks were fed a corn, wheat and soybean meal basal diet with monensin (1 g/kg) for 42 days (Yang *et al.*, 2007). Chicks were fed a negative control (basal diet), positive control (basal + bacitracin Zn (50 ppm and 30 ppm for first and last 3 weeks) or a MOS-supplemented diet (basal + MOS (2 g/kg)). The MOS fed animals had significantly higher coliform-forming bacteria in the duodenum and ileum compared to the positive and negative control fed animals ($P < 0.05$). The amount of *C. perfringens* was not different among dietary treatments. Gut morphology was also examined on day 14 and 35 in this study. Villus height and crypt depth did not change in the jejunum or ileum. Alkaline phosphatase, an indicator of intestinal maturation, was decreased in birds fed MOS on day 35 compared to birds fed the positive control. Monensin was added to all diets, which may have effected how bacitracin Zn or MOS interacted with the gut (Yang *et al.*, 2007). Spring *et al.* (2000) observed decreases in gram-negative bacteria *in vivo* when feeding Bio-Mos®. Broiler chicks were fed a control diet or diet containing Bio-Mos® (4,000 ppm) for 10 days and housed in bacterial isolation chambers in three separate trials. Chicks were inoculated with a standard inoculum from batching debris on day of hatch. Trials involved an additional inoculation of the chicks with *S. typhimurium* or *S. dublin*. Overall, total cecal coliforms were not different among dietary treatments, in contrast to Yang *et al.* (2007) who observed increases in coliform bacterial with dietary Bio-Mos®. Lactobacilli, enterococci and anaerobe bacteria within the cecas were also not different between dietary treatments. Salmonella was reduced in Bio-Mos® fed birds compared to control fed birds ($P < 0.05$). The prevalence of salmonella was also reduced in Bio-Mos® fed broilers ($P < 0.05$) inoculated with *S. dublin* (Spring *et al.*, 2000). Baurhoo *et al.* (2007) observed decreased *E. coli* in litter of broiler chicks fed Bio-Mos® compared to control

broilers after 28 and 42 days ($P < 0.05$). After 42 days of feeding Bio-Mos®, the amount of bifidobacteria and lactobacilli bacteria in the cecum broilers fed Bio-Mos was increased ($P < 0.05$) compared to broilers fed virginiamycin (11 mg/kg) (Baurhoo *et al.*, 2007). The amount of *E. coli* in the cecum of turkeys fed Bio-Mos® for 16 weeks has also been observed to reduce compared to non-medicated control animals (Zdunczyk *et al.*, 2005). The reduction of gram-negative bacterial within the gut of broilers fed Bio-Mos® may result in improvements in production parameters.

*Bio-Mos® as a growth promoter*

Results using MOS as a growth promoter in poultry diets have been inconsistent. Parks and colleagues (2001) fed Hybrid Large White male poults a corn-soybean meal diet unsupplemented (control) or supplemented with MOS (1 kg/ton to 6 weeks then 0.5 kg/ton), virginiamycin (20 g/ton), or a combination of MOS and virginiamycin at the same levels as other diets. Body weights of birds fed MOS were similar to controls except on week 20, with body weights improved by 0.46 kg/poult ($P < 0.05$) compared to control fed poults. Poults supplemented with only virginiamycin had higher body weights on weeks 12, 15 and 20 compared to control poults. Feed:gain was improved by 6 points in poults fed MOS from week 0 to 3 ($P < 0.05$), but no other cumulative feed:gain was different. Cumulative feed:gain, mortality and cull rates were not different among treatments (Parks *et al.*, 2001). Mannan oligosaccharides may improve early body weights by improving feed:gains. These positive improvements in production are not observed with Bio-Mos® supplementation in situations of cold stress. Turkey poults that were moderately exposed to cold stress (29°C) and fed Bio-Mos® (500 mg/kg diet) for 6 weeks did not have significantly different body weights, feed intake nor feed conversion (Parks

*et al.*, 2005). Bio-Mos® is a prebiotic that has been observed to improve body weight gain and feed conversion and is a possible replacement for antibiotic growth promoters.

*Bio-Mos® supplementation with antibiotics and anticoccidials*

Resent research indicates broilers fed medicated diets supplemented with Bio-Mos® improved performance. Supplementation of Bio-Mos® and BMD limit beneficial bacterial activities of both feed additives, but improvements in growth parameters have been observed. Sim *et al.* (2004) fed Hybrid turkey toms a basal diet supplemented with BMD, Bio-Mos® or a combination of BMD and Bio-Mos® for 18 weeks. Feeding diets supplemented with either BMD or Bio-Mos® resulted in an increase of the amount of Bifidobacteria ($P < 0.05$) and decrease Clostridia ($P < 0.05$) in digesta compared to control toms. No differences in bacteria were reported between combination and unsupplemented feed. However, at 18 weeks toms fed the combination were heavier than all other birds ($P < 0.05$) and had improved feed efficiency ($P < 0.05$). Growth parameters are improved when BMD is fed in combination with Bio-Mos® (Sim *et al.*, 2004). A shuttle program utilizing BMD, virginiamycin and Bio-Mos® improved growth parameters and net income per bird (Hooge *et al.*, 2003). A corn and soybean meal basal diet supplemented with nicarbazin (0 to 3 week) and monensin (110 mg/kg, 3 to 6 week) was not further supplemented or supplemented with BMD (27.5 ppm) and Bio-Mos® (500 ppm) from 0 to 42-days of age and then virginiamycin (11 ppm) and Bio-Mos® (500 ppm) from 42 to 49 days of age. The shuttle program improved 49-day body weight ($P < 0.05$) and feed efficiency ($P < 0.05$). Net income was increased by $ 0.039/bird ($P < 0.05$) utilizing the shuttle program (Hooge *et al.*, 2003). Bio-Mos® supplementation has been observed to increase production performance in a shuttle nutrition program utilizing antibiotics.

Additions of virginiamycin in a Bio-Mos® feed program improves production in commercial broiler diets under non-stressful conditions. A 49-day broiler feeding program including Bio-Mos®, BMD during the grower phase and virginiamycin during the finishing period improved body weight gain and feed efficiency compared to birds fed a drug free program (Hooge *et al.*, 2003). The feeding program also resulted in increased body weight gains compared to animals fed programs with Bio-Mos® and BMD or only Bio-Mos® (Hooge *et al.*, 2003). A study involving female turkey poults fed a MOS (500mg/kg diet) from 0 to 6 weeks and then virginiamycin (22mg/kg diet) from 6 to 14 weeks (Parks *et al.*, 2005). The shuttle program improved feed conversion 2.37 to 2.34 compared to poults not supplemented with growth promoters ($P < 0.05$), but did not improve body weights at 14 weeks. Although the shuttle program improved feed conversion compared to controls, poults fed solely virginiamycin (22mg/kg diet) did not differ in body weights and feed conversion compared to poults fed the shuttle program (Parks *et al.*, 2005). Feeding programs shuttling Bio-Mos® and virginiamycin improve feed efficiency and body weight gain.

*Probiotic and Bio-Mos® Interactions as Growth Promoters*

A feeding program including probiotics and Bio-Mos® has shown promise as an alternative for antibiotic and anticoccidial use. Broilers treated with probiotics on day of hatch and fed Bio-Mos® have been observed to increase performance. Sun *et al.* (2005) fed Cobb 500 broiler chicks a corn and soybean meal based diet unsupplemented, supplemented with Lincomycin (2 g/ton starter, 4 g/ton grower and removed after 29 days) or supplemented with Bio-Mos® (1.81 kg/ton starter, 0.91 kg/ton grower, 0.45 kg/ton finisher and withdrawal) to 49-days of age. Broilers fed Bio-Mos® supplemented fed were also sprayed with All-Lac XCL at

the hatchery (5 g/2,000 birds). All-Lac XCL is a spray probiotic that contains *Lactobacillus,* *Enterococcus,* and *Pediococcus*. For the first 14 days, there were no differences in mortality, feed conversion, body weight gain or feed intake reported. At day 14, birds were challenged with coccidia (*E. acervulina, E. maxima,* and *E. tenella*). Mortality was decreased by 5.24% for broilers fed Bio-Mos® compared to unsupplemented diet fed broilers ($P < 0.05$). During days 36 to 49, broilers fed supplemented diet feed lowered fed conversion by an average of 3.7 points ($P < 0.05$). Although feed conversion was improved by supplementing the feed with Bio-Mos®, cumulative body weight gains and feed intakes were not different. Improvements in feed conversion were not related to an improvement gastrointestinal morphology. Duodenum and ileum villus lengths and crypt depths were not significantly different, nor were cecal lamina propria depth measurements (Sun *et al.*, 2005). A nutritional program including Bio-Mos® with All-Lac XCL resulted in similar feed conversion as feeding a medicated feed during a coccidiosis challenge. This nutrition program shows promise as a replacement for traditional medicated feeding programs.

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## Chapter 2: A Forty-Nine Day Evaluation of Bio-Mos® Replacement of Roxarsone in a Commercially Based Broiler Feeding Program

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### ABSTRACT

A study was conducted to investigate the effects of roxarsone and Bio-Mos® on broiler production, gut morphology and bone strength. Three thousand and ten broilers were randomly assigned to 1 of 5 dietary corn-soybean meal based treatments: 1) negative control (NEG), basal diet; 2) positive control (POS; NEG + 27 ppm Bacitracin MD); 3) roxarsone (ROX; POS + 50 ppm of roxarsone); 4) Bio-Mos® (BIO; POS + 0.15 and 0.5% Bio-Mos® added during the starter and grower periods, respectively); 5) Bio-Mos®+All-Lac XCL (BIO+LAC; POS + 0.2, 0.1, and 0.05% Bio-Mos® during the starter, grower and finishing periods, respectively and 0.25g All-Lac XCL/bird sprayed at hatchery). On day 14, 7 of the 14 replicate pens/treatment were challenged with *Eimeria maxima* (3 x $10^4$ oocysts/bird). Tibias were collected on day 28 and 49 to determine bone-breaking strength. Non-challenged birds had higher body weight gains (BWG) and lower feed conversion (FCR) from day 0 to 49 than challenged birds ($P < 0.05$). Jejunal crypt depth was increased in challenged broilers compared to non-challenged broilers at 28 days-of-age ($P < 0.05$). From day 0 to 35, ROX birds had lower BWG and FI than BIO and BIO+LAC birds ($P < 0.05$), while FCR was similar. Supplementing roxarsone resulted in reduced feed intake and BWG, but no significant differences were noted in FCR compared to feeding Bio-Mos®. ROX fed broilers had decreased ileal crypt depth compared to all other dietary treatments ($P < 0.05$). Muscle As concentration was lower than FDA allowable limits in

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broilers fed ROX without a withdrawal period at 28 days-of-age. Including roxarsone or Bio-Mos® did not generally improve production compared to broilers fed the negative diet.

(*Key words:* Bio-Mos, roxarsone, coccidia, broiler, performance)

## INTRODUCTION

Coccidiosis costs the U.S. broiler industry about $450 million annually (Allen and Fetterer, 2002b). Williams (1999) reported 46% of the cost of coccidiosis in the United Kingdom is due to reduced weight gain and 34% of the cost is due to poor feed conversion. Broiler nutrition programs rely on shuttling different anticoccidials and antibiotics during growth and finishing periods to reduce growth loses associated with diseases. Shuttle programs utilize different combinations of growth promoters during various stages, instead of using a single or combination of growth promoters throughout the entire lifespan of the animal. These feed additives improve weight gain and feed efficiency during disease challenges. Although antibiotics and anticoccidials are still used in the U.S., the use of antibiotic growth promoters and anticoccidials in poultry feed has been banned in many countries due to concern of bacterial resistance and antibiotic residues in meat. As concern over the use of anticoccidials and antibiotics continues, research must focus on determining alternative dietary programs for broilers facing coccidia and other health challenges.

Enteric diseases, such as coccidiosis, reduce performance by causing damage to the gastrointestinal tract. Coccidia negatively impacts the gastrointesintal tract by embedding themselves within the villious epithelial layer (Allen and Fetterer, 2002b). The resulting destruction of the epithelial cells results in impaired nutrient utilization and growth. As both the central location for nutrient absorption and the first line of defense against pathogens, the

intestinal tract plays an important role in maintaining an animal's nutritional and immunological health. The mucosal layer enables the flow of nutrients and creates a protective barrier between the epithelial layer of the mucosa and luminal contents (Pearson and Brownlee, 2005). Goblet cells produce the mucins which form the mucosal layer. Mucins maybe categorized as neutral or acidic and further categorized as sulfated (sulfomucins) or non-sulfated (sialomucins) (Deplancke and Gaskins, 2001). The importance of different mucin subtypes is not well understood, but neutral and acid mucins may be expressed in response to disease state and diet. It has been hypothesized that the increase in acid mucin frequency during disease states is due to the less efficient degradation of these mucins by bacterial glycosidases and host proteases (Deplancke and Gaskins, 2001). The affects of coccidia and diets containing various growth promoters on goblet cell mucin subtype production has not been previously published.

In 2000, the most common drug program for growing broilers included a combination of ionophore, antibiotics and roxarsone (Chapman and Johnson, 2002). Roxarsone is an organic arsenical used to improve production and reduce coccidial infection in broilers. Although roxarsone use remains steady, concerns of arsenic (As) contamination in the environment and human food supply may limit its use in the future. Roxarsone is reduced to an inorganic trivalent As form during metabolism and is thought to accumulate in muscle tissue (Czarnecki *et al.*, 1984). Lasky *et al.* (2004) reported daily consumption of muscle (60 g of meat) from broilers fed roxarsone, calculated based on liver-to-muscle ratios, would not cause an adult to exceed the World Heath Organization's (WHO) daily tolerable level of 2 μg/kg of body weight/day of inorganic As. If the required 5-day withdrawal period of roxarsone prior to slaughter is maintained, the average person will consume 5.24 μg As/day (Lasky *et al.*, 2004). Research is limited on actual muscle As concentration after feeding roxarsone to broilers.

Mannan oligosaccharides, such as Bio-Mos®, are being considered a viable replacement for roxarsone in poultry diets. Mannan oligiosaccharides have been reported to significantly improve body weight gain and feed efficiency of 49-day old broilers compared to control fed birds (Hooge *et al.*, 2003). Bio-Mos® has the ability to act as a decoy within the GI tract, attaching to certain bacteria possessing Type-1 fimbriae, thus limiting bacterial interaction with the gut mucosa (Spring *et al.*, 2000). Supplementing a mannan oligosaccharide decreased the percentage of *Salmonella* positive birds from 89.8 to 55.7% after broilers were challenged with *S. dublin* (Spring *et al.*, 2000). A feeding program including probiotics and Bio-Mos® has shown promise as an alternative for antibiotic and anticoccidial use. Broilers treated with probiotics on day of hatch and fed Bio-Mos® had improved feed conversion after a coccidia challenge (Sun *et al.*, 2005). A nutritional program including Bio-Mos® with a spray probiotic resulted in similar feed conversion as feeding a medicated feed during a coccidiosis challenge. This dietary program showed promise as a replacement for traditional medicated feeding programs.

The objectives of the present trial were to explore the effects of spraying broiler chicks with a probiotic at hatch and feeding a commercially formulated broiler diet supplemented with Bio-Mos®. The effects of feeding Bio-Mos® or roxarsone on body weight gain, feed intake, feed efficiency, mortality, intestinal morphology, muscle tissue As accumulation, and bone breaking strength were determined.

## MATERIALS AND METHODS

### *Broiler Husbandry and Sampling Periods*

Three-thousand and ten straight-run Cobb x Cobb broiler chicks were obtained from a commercial hatchery in Virginia. Chicks were randomly assigned by body weight to one of five

dietary treatments, and each treatment was replicated by 14 pens for the 49 day trial. Forty-three chicks were randomly assigned to each pen (0.76 $ft^2$/chick). Pens were randomly blocked by location to minimize the influence of ventilation on performance differences. Each pen contained fresh pine shavings top dressed with 30 lbs of previously used litter to simulate an industry built-up litter management program. The used litter was obtained from a trial which chickens were challenged with *Eimeria tenella*. Pens were prepared in October 2005 and birds were placed in January 2006. Birds were reared on 24 hours/day light for the first 8 days and 20 light :4 dark thereafter. Feed and water were provided *ad libitum* via tube feeders and nipple drinker lines, respectively.

Feed intake was determined at 14, 28, 35 and 49 d. Pen BWG were measured at 0, 14, 28, 35 and 49 d. Feed conversion and BWG were calculated for both individual feeding phases and cumulative periods during the trial. Mortality was recorded daily. Feed conversion was adjusted for mortality.

***Dietary Treatments***

A corn and soybean meal basal diet was formulated to meet commercially recommended nutrient specifications and obtained from a local integrator. Additional ingredients were added to constitute dietary treatment groups. A four phase feeding program consisted of starter (0-14 d), grower (15-28 d), finisher (29-35 d) and withdrawal (35-49 d) phases. The basal diet included 0.075% of monensin as Coban[2] during the starter and grower phases. The finisher and withdrawal diets contained 16 ppm of virginiamycin as Stafac[3]. After final mixing at Virginia

[2]Elanco Animal Health, Greenfield, IN
[3]Phibro Animal Health, Ridgefield Park, NJ

Tech, diets were crumbled (starter diets) or pelleted at Augusta Cooperative Farm Bureau Feed Mill[4]. Basal diet ingredients are listed in Table 1.

The five dietary treatments included 1) basal diet (NEG), 2) positive control (POS): NEG + 27 ppm bacitracin methylene disalicylate (BMD) added during the starter and grower phase, 3) roxarsone[5] treatment (ROX): POS + 50 ppm of roxarsone in the starter and grower phase (11.06 and 14.33 ppm of As analyzed for the starter and grower diet, respectively), 4) low Bio-Mos®[6] treatment (BIO): POS + 0.15% and 0.5% Bio-Mos® added during the starter and grower phases, respectively, and 5) high Bio-Mos® + All-Lac XCL[5] treatment (BIO+LAC): POS + 0.2, 0.1, and 0.05% Bio-Mos during the starter, grower and finisher phases, respectively (Table 2). BIO+LAC chicks were sprayed with 0.25g All-Lac XCL/bird on day of hatch. All-Lac XCL is a probiotic consisting of *Lactobacillus, Enterococcus,* and *Pediococcus spp*. Feed samples were collected at the beginning of each feeding phase. Samples were ground with a Tecator and analyzed for: dry matter, crude protein, gross energy and Arsenic.

[4]Augusta Cooperative Farm Bureau Feed Mill, Fairfield, VA
[5]3-Nitro 20, Alpharma Animal Health Division, Fort Lee, NJ
[6] Alltech, Lexington, KY

**Table 1. Ingredient list of basal diets per feeding phase.**

| Feeding Phase | Starter 0 to 14 d | Grower 14 to 28 d | Finisher 28 to 35 d | Withdrawal 35 to 49 d |
|---|---|---|---|---|
| | % of Basal Diet | | | |
| Corn | 52.74 | 56.91 | 67.99 | 69.80 |
| Soybean Meal | 36.12 | 31.30 | 23.13 | 20.95 |
| Bakery RD Doswel[1] | 5.00 | 5.00 | 3.25 | 4.63 |
| Poultry Fat | 1.91 | 2.77 | 2.05 | 1.25 |
| Phosphate | 1.56 | 1.31 | 1.11 | 0.97 |
| Meat and bone meal blend[2] | 1.25 | 1.25 | 1.25 | 1.25 |
| Limestone | 0.60 | 0.60 | 0.60 | 0.59 |
| Alimet | 0.27 | 0.26 | 0.14 | 0.12 |
| Salt | 0.16 | 0.20 | 0.24 | 0.24 |
| Liquid Lysine, 50% | 0.09 | 0.12 | 0.03 | 0.05 |
| Copper Sulfate | 0.07 | 0.07 | 0.07 | 0.07 |
| Choline Chloride-70 | 0.07 | 0.06 | 0.04 | 0 |
| Trace Minerals[3] | 0.06 | 0.05 | 0.03 | 0.02 |
| Vitamins[4] | 0.02 | 0.02 | 0.01 | 0.01 |
| Coban | 0.08 | 0.08 | 0 | 0 |
| Stafac | 0 | 0 | 0.04 | 0.04 |
| Calculated Nutrient Content | | | | |
| ME, kcal/kg | 1365.00 | 1410.00 | 1430.00 | 1430.00 |
| GE, kcal/kg *analyzed* | 22.97 | 20.99 | 17.32 | 16.56 |
| CP, % *analyzed* | 22.03 | 22.27 | 17.52 | 16.16 |
| As, ppm *analyzed* | 2.59 | 1.59 | 0.44 | 4.084 |

[1]Bakery Feeds, a division of Griffin Enterprises, Doswell, VA.
[2]Blend of meat and bone meals and blood, Valley Protein, Winchester, VA.
[3]Suplied per kilogram of mix: iron ($FeSO_4 \cdot H_2O$), 33.5 g; zinc (ZnO), 214 g; manganese (MnO), 300 g; copper ($CuSO_4 \cdot H_2O$), 3.4 g; iodine (Ca Iodate), 2.1g; selenium ($Na_2SeO_3$), 500 µg.
[4]Supplied per kilogram of mix: vitamin A (retinyl acetate), 30,870,000 IU; vitamin $D_3$, 13,230,000 ICU; vitamin E (DL-α-tocopheryl acetate), 66,150 IU; vitamin $K_3$ (menadione dimethypyrimidinol bisulfite), 6,006 mg; thiamin 6,174 mg; riboflavin, 26,460 mg; pyridoxine, 11,025 mg; pantothenic acid (D-calcium pantothenate), 39,690 mg; niacin, 154,350 mg; folic acid, 3,528 mg; biotin, 264 mg; vitamin $B_{12}$ (cyanocobalamin), 53 mg.

**Table 2. Dietary treatment list for each feeding phase.**

| Dietary Treatment | POS | NEG | ROX | BIO | BIO+LAC |
|---|---|---|---|---|---|
| | | | % of Basal Diet | | |
| Feeding Phase | | | Starter Phase (0 to 14 d) | | |
| BMD | 0.025 | 0 | 0.025 | 0.025 | 0.025 |
| Roxarsone | 0 | 0 | 0.025 | 0 | 0 |
| Bio-Mos | 0 | 0 | 0 | 0.05 | 0.2 |
| Feeding Phase | | | Grower Phase (14 to 28 d) | | |
| BMD | 0.025 | 0 | 0.025 | 0.025 | 0.025 |
| Roxarsone | 0 | 0 | 0.025 | 0 | 0 |
| Bio-Mos | 0 | 0 | 0 | 0.05 | 0.1 |
| Feeding Phase | | | Finisher Phase (28 to 35 d) | | |
| BMD | 0 | 0 | 0 | 0 | 0 |
| Roxarsone | 0 | 0 | 0 | 0 | 0 |
| Bio-Mos | 0 | 0 | 0 | 0 | 0.05 |

***Coccidia challenge***

Coccidia challenge began at d 0 with used litter being placed in all pens from previously *E. tenella* challenged broilers. The building was blocked into two sections separating the coccidia challenged birds from the unchallenged birds to limit cross-contamination. On d 14, seven pens from each dietary treatment were infected with *Eimeria maxima* prepared to provide $3 \times 10^4$ oocysts per bird administered via feed. Feed sprayed with oocysts was placed in chick feed trays and removed when all feed had been consumed. To prevent cross-contamination from the coccidia challenged area workers were required to change plastic boots before entering the non-challenged area and non-challenged birds were weighed and sampled prior to challenge birds.

***Intestinal Morphology***

On day 28, one bird/pen (n = 7/treatment) was randomly selected for intestinal morphology sampling. Each bird was weighed and euthanized by cervical dislocation. Tissue

samples (1 cm in length) were collected every seven centimeters from the duodenum (located from the top of the duodenal loop to the pancreatic duct), jejunum (past the pancreatic duct posterior to the Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileo-ceco-junction). One-centimeter cecal tissue sub samples of both tonsils were taken every 3 cm. Samples were placed into neutral buffered formalin and stored until further processing. To process each 1 cm sub sample, sections were then further cut into 5 mm sections and placed into Tissue Tek Casettes™. Tissue sections were processed, embedded in paraffin, cut at 7 μm thickness, and placed onto slides at Histo-Scientific Research Laboratories, Inc.[7]. Two slides were made from each sampled animal and five different sub sampled sections were placed on each slide. One slide was stained with hemotoxlyin and eosin to identify changes in gut morphology. The second slide was stained with alcian blue-periodic acid-Schiff stain to identify neutral and acid mucin producing goblet cells. After staining with hemotoxlyin and eosin, pictures were taken of tissues to determine villus length, crypt depth and laminia propria thickness. Pictures were obtained using an Olympus DP 70™ camera mounted to a BX50™ photomicroscope[8]. Ileal and jejunal villus length and crypt depth, and cecal lamina propria width were measured using SigmaScan Pro 5[9]. From procedures reported by Sun *et al.* (2005) villi length was measured from the tip of the villus to the top of the crypt. Crypt depth was measured from the top of the crypt to the bottom of the crypt. Lamina propria width was measured from the bottom of the crypt to the muscularis mucosae. Four villus, crypt and lamina propria measurements were made from each gut section and three gut sections from each tissue were measured.

[7] Histo-Scientific Research Laboratories, Inc., Woodstock, VA
[8] Olympus America Inc., Melville, NY
[9] SPSS Inc., Chicago, IL

The slide stained with alcian blue-periodic acid-Schiff stain was used to count goblet cell types. Following procedures reported by Brown *et al.* (2006) the number of acid (blue), acid/neutral (purple) and neutral (pink) goblet cells were counted on 2 villi from each of the 5 gut sections on each slide for a total of 10 counts per sampled animal. The proportion of goblet cell types was compared to determine the effects of disease challenge and diet on the mucin profile of broilers.

***Determining Muscle and Feed As Concentration***

On days 28 and 49, one bird/pen (n = 7/treatment) was randomly selected for determining As muscle concentration. Each bird was weighed and euthanized by cervical dislocation. Pectoralis minor muscles were collected, frozen and stored for further processing. The right tibia was collected, frozen and stored until further analysis of bone strength. Muscle tissue samples were pulverized using a Sunbeam Oscar Jr. Chopper Plus for five sec. After mixing, a sub-sample (1 to 2 g) was collected for digestion. Concurrently, milled diet samples (0.5 g) were collected for digestion. Samples were examined for As in duplicate following the procedures of the Virginia Tech Veterinary Medical Experiment Station Toxicology Diagnostics for arsenic determination using induced coupled plasma spectrometry. Muscle and diet samples were digested in an 80% nitric acid and 20% perchloric acid solution at 265$^{o}$C until a dense white smoke formed. Samples were then evaporated until 1.5 mL of liquid remained at 265$^{o}$C. Samples were cooled for 5 minutes and then brought to 15mL using distilled, deionized water. Samples were analyzed using Inductively Coupled Plasma-Dynamic Reaction Cell-Mass Spectrometry (ICP-DRC-MS) at Applied Speciation and Consulting, LLC[10].

[10] Applied Speciation and Consulting, LLC, Tukwila, WA

***Bone breaking strength***

Right tibias were thawed and cleared of adhering soft tissue. Tibias were measured for bone length and bone diameter at the mid-diaphysis using a dial caliper and then refrozen. Maximum force required to cause shear failure at the mid-diaphysis was determined after a second thaw using an MTS System Corporation Sintech 5/G™ machine[11]. Force was applied at a speed of 5 mm/min. Following shear testing, tibias were sawed in half at the point where force had been applied. Tibia wall thickness was measured at the breaking point using a dial caliper. Cross-sectional area of the bone was calculated as a quarter circle following the procedures of Combs *et al.* (1991). Tibia breaking strength was determined as yield force divided by the cross-sectional area of the bone (Combs *et al.*, 1991).

***Statistical Analysis***

Data was analyzed as a RCBD using the MIXED procedure of SAS[12]. Pens were defined as the experimental unit and blocked by row to minimize the effect of ventilation on performance. The statistical model was $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$; where $y_{ijk}$ = observed dependent variable, $\mu$ = grand mean, $\alpha_i$ = *i*th infection status (*A*), $\beta_j$ = *j*ith dietary treatment effect (*B*), $\alpha\beta_{ij}$ = random effect due to the *i*th level of factor *A* and the *j*th level of factor *B*, $\varepsilon_{ijk}$ = error for the *k*th replicate of factors *A* and *B*. When the main effect was significant ($P < 0.05$), means were compared with a Tukey's adjustment. Alpha was determined to be less than 0.05. Mortality and goblet mucin cell types were transformed with arcsine (square root of percent) prior to analysis. Significance was assigned at $P < 0.05$.

[11] MTS Headquarters, Eden Prairie, MN
[12] SAS User's Guide, 1999, SAS Institute, Cary, NC

# RESULTS

## *Effect of Coccidia Challenge on Bird Performance*

Cumulative (d 0 to 49) BWG and FI were decreased and FCR and mortality were increased in broilers challenged with coccidia compared to non-challenged broilers ($P < 0.05$) suggesting the presence of a coccidia infection (Table 3). Although no challenge occurred from 0 to 14 d, BWG and FI were decreased in "challenged" broilers. Block location did not significantly effect BWG or FI from 0 to 14 d. Simple effects during the finisher phase (28 to 35 days) and 0 to 35 days for mortality and FCR are not listed because a treatment by challenge interaction occurred. During the grower phase (14 to 28 days), body weight gain, feed intake and FCR were depressed due to the coccidia challenge ($P < 0.05$). No differences were noted between challenged and non-challenged birds during the finisher phase (28 to 35 days), which may relate to a treatment by challenge interaction or possible recovery from the coccidia challenge.

**Table 3. The affect of *E. maxima* challenge on performance of broilers at various ages.**

| | BWG (kg)[1] | FI (kg) | FCR | Mortality (%) |
|---|---|---|---|---|
| | Starter Phase 0 to 14 d | | | |
| Non-Challenge | 0.43[a] | 0.45[a] | 1.03 | 2.27 |
| *E. maxima* Challenge | 0.42[b] | 0.43[b] | 1.03 | 1.73 |
| SEM[2] | 0.0026 | 0.0039 | 0.0091 | 0.3868 |
| | Grower Phase 14 to 28 d | | | |
| Non- Challenge | 1.19[a] | 1.75[a] | 1.48[b] | 3.25 |
| *E. maxima* Challenge | 1.05[b] | 1.66[b] | 1.58[a] | 2.85 |
| SEM[2] | 0.0109 | 0.0089 | 0.0114 | 0.3893 |
| | Finisher Phase 28 to 35 d | | | |
| Non- Challenge | 0.43 | 0.96 | 2.24 | -----[3] |
| *E. maxima* Challenge | 0.42 | 0.95 | 2.34 | ----- |
| SEM[2] | 0.0100 | 0.0064 | 0.0664 | ----- |
| | Withdrawal Phase 35 to 49 d | | | |
| Non- Challenge | 1.06 | 2.75 | 2.61 | 4.10[b] |
| *E. maxima* Challenge | 1.08 | 2.74 | 2.64 | 6.25[a] |
| SEM[2] | 0.0241 | 0.0173 | 0.0791 | 0.724 |
| | 0 to 28 d | | | |
| Non- Challenge | 1.65[a] | 2.23[a] | ----- | ----- |
| *E. maxima* Challenge | 1.50[b] | 2.12[b] | ----- | ----- |
| SEM[2] | 0.0114 | 0.011 | ----- | ----- |
| | 0 to 35 d | | | |
| Non- Challenge | 2.22[a] | 3.38[a] | ----- | ----- |
| *E. maxima* Challenge | 2.05[b] | 3.27[b] | ----- | ----- |
| SEM[2] | 0.0118 | 0.0150 | ----- | ----- |
| | 0 to 49 d | | | |
| Non- Challenge | 3.48[a] | 6.41[a] | 1.85[b] | 10.78[b] |
| *E. maxima* Challenge | 3.32[b] | 6.31[b] | 1.89[a] | 11.50[a] |
| SEM[2] | 0.0205 | 0.0311 | 0.0109 | 0.8595 |

[a-b]Means within the observational unit and time period without a common superscript differ significantly ($P < 0.05$).

[1]BWG are reported per bird.

[2]SEM are reported as the largest SEM.

[3]Dashed lines represent treatment by challenge interactions.

***Effect of Dietary Treatment on Bird Performance***

No differences were observed between broilers fed the NEG and POS diets. Because differences were not observed between control diets, it is difficult to compare dietary treatments with control diets. Body weight gain, FCR and mortality were not different between dietary treatments from 0 to 49 days (Table 3). Differences were noted from 0 to 35 days. Broilers fed the ROX diet had decreased BWG and FI from 0 to 35 days compared to broilers fed the NEG, BIO and BIO+LAC diets. During this period, a treatment by challenge interaction occurred for mortality, which likely affected other production parameters. No differences were noted for cumulative (0 to 49 day) mortality.

Differences in production parameters were noted during various feeding phases (Table 4 and Table 5). During the starter phase, which was prior to the coccidia challenge, broilers fed the BIO diet had increased BWG compared to the broilers fed the POS diet ($P < 0.05$). No differences in BWG were observed during the grower phase, which was during the coccidia challenge. Feed intake was depressed for broilers fed the ROX and BIO+LAC diets compared to broilers fed the POS diet ($P < 0.05$) during the starter phase. The depression in FI related to a decrease in FCR for broilers fed the ROX and BIO+LAC diet compared to broilers fed the POS diet ($P < 0.05$) during the starter phase. Broilers fed the BIO+LAC diet also improved FCR by 10 points during the grower phase compared to broilers fed the BIO diet. Feed intake was also depressed for broilers fed the ROX diet compared to broilers fed the NEG, BIO and BIO+LAC diets ($P < 0.05$) and BIO diet during the finisher and withdrawal phases, respectively. The ROX feeding program resulted in decreased FI which related to a decrease in BWG. Broilers fed the ROX diet had decreased BWG compared to the broilers fed the BIO diet ($P < 0.05$) during the finishing phase (29 to 35 days).

Treatment by challenge interactions occurred during the finisher phase and 0 to 35 day cumulative period for mortality and FCR. No differences were noted between non-challenged broilers fed various dietary treatments or broilers of different infection status for mortality during the finisher phase (Figure 1). Differences in morality were noted for broilers challenged with coccidia and fed various diets. Broilers fed the ROX diet and infected with coccidia had higher mortality than broilers fed the NEG diet and infected with coccidia ($P < 0.05$). The treatment by challenge interaction for 0 to 35 day cumulative mortality data was significant ($P = 0.0326$), but differences were not noted when pair-wise comparisons were done (Figure 2) although broilers fed the same diet reacted differently based on disease challenge.

Feed conversion ratio during the cumulative 0 to 35 day period had a significant treatment by challenge interaction (Figure 3). Broilers fed POS, BIO and BIO+LAC diets had increased FCR during coccidia challenge compared to non-challenged broilers fed the same diet ($P < 0.05$). Coccidia challenge did not effect FCR for broilers fed NEG and ROX diets. Unchallenged broilers fed BIO+LAC had lowered FCR compared to all diets challenged with coccidia ($P < 0.05$). Broilers fed the POS and BIO diets and challenged with coccidia had the higher FCR compared to non-challenged broilers ($P < 0.05$). Broilers feed ROX had similar FCR levels in both challenged and non-challenged broilers, but broilers fed the BIO and BIO+LAC diets were affected by coccidia challenge as evidenced by increasing FCR rates.

**Table 4. The affect of dietary treatment on cumulative bird performance.**

| | BWG (kg)[1] | | | FI (kg)[2] | | | FCR[3] | Mortality, % |
|---|---|---|---|---|---|---|---|---|
| Age (day) | 0-28 | 0-35 | 0-49 | 0-28 | 0-35 | 0-49 | 0-49 | 0-49 |
| POS | 1.56 | 2.11$^{ab}$ | 1.84 | 2.19$^{a}$ | 3.30$^{bc}$ | 6.27$^{b}$ | 1.84 | 8.80 |
| NEG | 1.60 | 2.17$^{a}$ | 1.88 | 2.19$^{a}$ | 3.36$^{ab}$ | 6.38$^{ab}$ | 1.88 | 11.46 |
| ROX | 1.54 | 2.07$^{b}$ | 1.88 | 2.12$^{b}$ | 3.23$^{c}$ | 6.29$^{ab}$ | 1.88 | 12.14 |
| BIO | 1.56 | 2.17$^{a}$ | 1.89 | 2.20$^{a}$ | 3.41$^{a}$ | 6.48$^{a}$ | 1.89 | 11.81 |
| BIO+LAC | 1.60 | 2.16$^{a}$ | 1.85 | 2.17$^{ab}$ | 3.33$^{ab}$ | 6.38$^{ab}$ | 1.85 | 11.50 |
| SEM[4] | 0.0180 | 0.0186 | 0.0177 | 0.0171 | 0.0242 | 0.0504 | 0.0177 | 1.3589 |

$^{a-c}$Means within the observational unit and time period without a common superscript differ significantly ($P < 0.01$).

[1]BWG is expressed per animal and adjusted for mortality.

[2]FI is expressed per animal and adjusted for mortality.

[3]FCR is expressed per animal and adjusted for mortality.

[4]SEM is the largest SEM for each period.

**Table 5. The affect of dietary treatment on body weight gain and feed intake for various feeding phases.**

| | BWG (kg) [1] | | | | FI (kg)[2] | | | |
|---|---|---|---|---|---|---|---|---|
| Age (d) | 0-14 | 14-29 | 28-35 | 35-49 | 0-14 | 14-28 | 28-35 | 35-49 |
| Phase | Starter | Grower | Finisher | W/draw | Starter | Grower | Finisher | W/draw |
| POS | 0.421[b] | 1.11 | 0.43[ab] | 1.15 | 0.454[a] | 1.71 | 0.95[bc] | 2.73[ab] |
| NEG | 0.429[ab] | 1.14 | 0.44[ab] | 1.07 | 0.441[ab] | 1.72 | 0.98[ab] | 2.76[ab] |
| ROX | 0.429[ab] | 1.10 | 0.39[b] | 1.02 | 0.429[b] | 1.67 | 0.91[c] | 2.68[b] |
| BIO | 0.439[a] | 1.09 | 0.46[a] | 1.09 | 0.450[ab] | 1.73 | 0.99[a] | 2.82[a] |
| BIO+LAC | 0.424[ab] | 1.15 | 0.41[ab] | 1.02 | 0.428[b] | 1.71 | 0.96[ab] | 2.73[ab] |
| SEM[3] | 0.0042 | 0.0172 | 0.0158 | 0.0381 | 0.0063 | 0.0144 | 0.0102 | 0.0274 |

[a-c]Means within the observational unit and time period without a common superscript differ significantly ($P < 0.05$).

[1]BWG is expressed per animal and adjusted for mortality.

[2]FI is expressed per animal and adjusted for mortality.

[3]SEM is the largest SEM for each period.

**Table 6. The affect of dietary treatment on feed conversion and mortality for various feeding phases.**

| | FCR[2] | | | | % Mortality | | |
|---|---|---|---|---|---|---|---|
| Age (day) | 0-14 | 14-28 | 28-35 | 35-49 | 0-14 | 14-28 | 35-49 |
| Phase | Starter | Grower | Finisher | W/draw | Starter | Grower | W/draw |
| POS | 1.08[a] | 1.54[ab] | 2.20 | 2.39 | 1.33 | 2.95 | 3.47 |
| NEG | 1.03[ab] | 1.52[ab] | 2.36 | 2.64 | 2.99 | 3.30 | 5.14 |
| ROX | 1.00[b] | 1.53[ab] | 2.38 | 2.67 | 1.17 | 2.61 | 5.81 |
| BIO | 1.03[ab] | 1.59[a] | 2.17 | 2.63 | 2.50 | 3.70 | 5.60 |
| BIO+LAC | 1.01[b] | 1.49[b] | 2.34 | 2.80 | 2.00 | 2.70 | 5.85 |
| SEM[3] | 0.0147 | 0.0185 | 0.1050 | 0.1250 | 0.6116 | 0.6156 | 1.1451 |

[a-b]Means within the observational unit and time period without a common superscript differ significantly ($P < 0.05$).
[2]FCR is expressed per animal and adjusted for mortality.
[3]SEM is the largest SEM for each period.

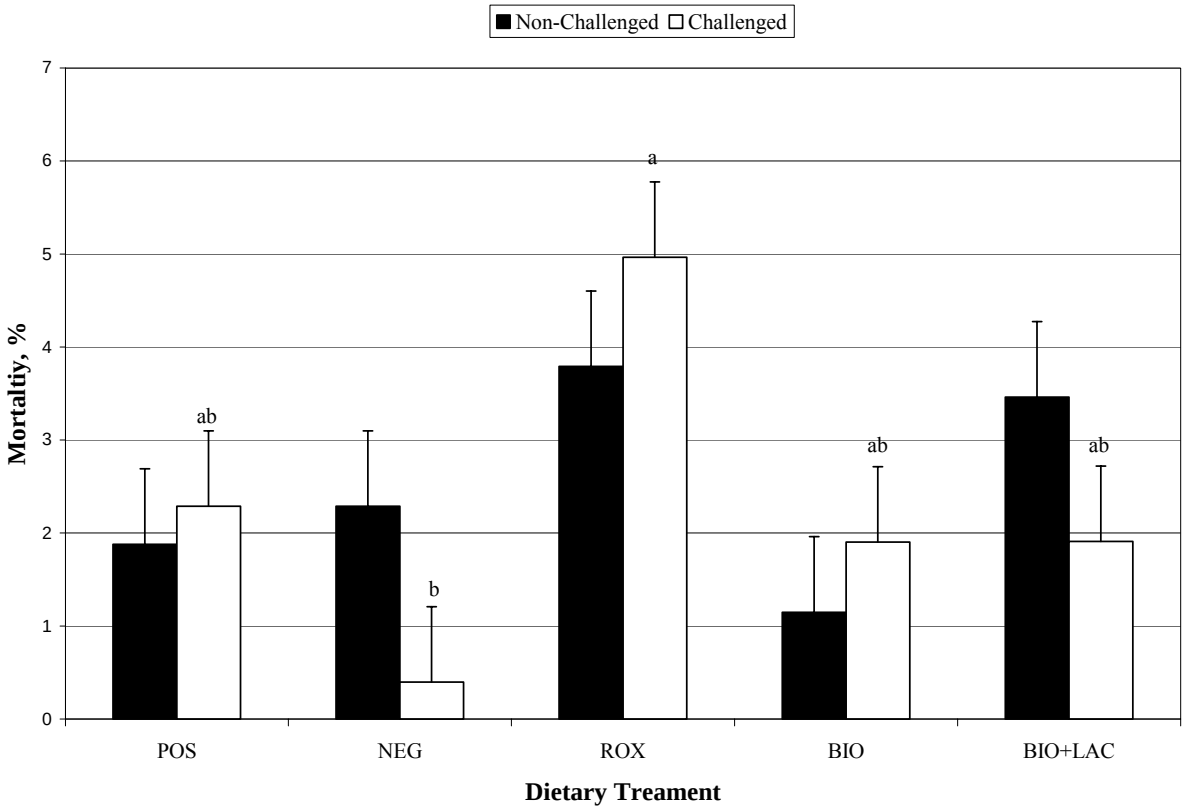


**Figure 1. Interaction of treatment by challenge on broiler mortality from day 28 to 35 (finisher phase).** Treatment by challenge interaction overall *P*-value = 0.0257. Error bars represent the largest standard error of the mean.
[a-b]Means within the infection status without a common superscript differ significantly ($P < 0.05$).

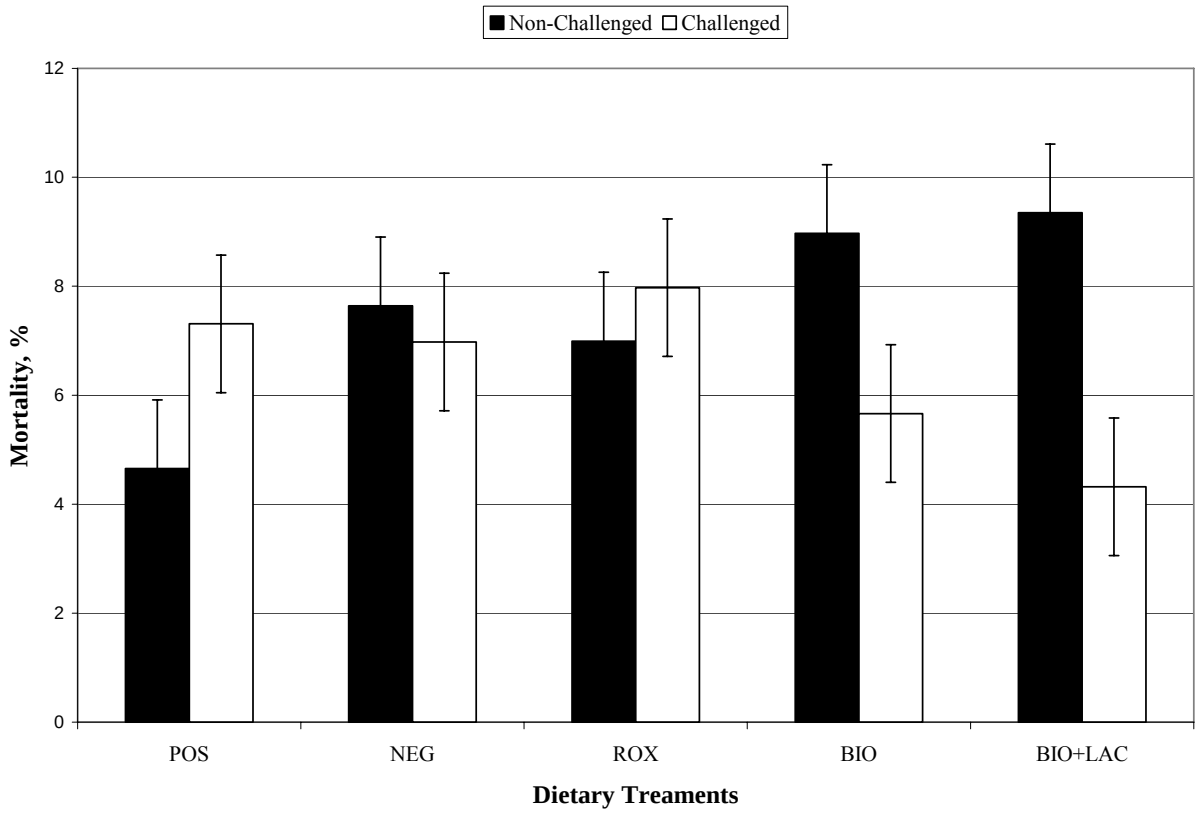


**Figure 2. Interaction of treatment by challenge on broiler mortality from day 0 to 35.** Treatment by challenge interaction overall *P*-value = 0.0326. Error bars represent the largest standard error of the mean.

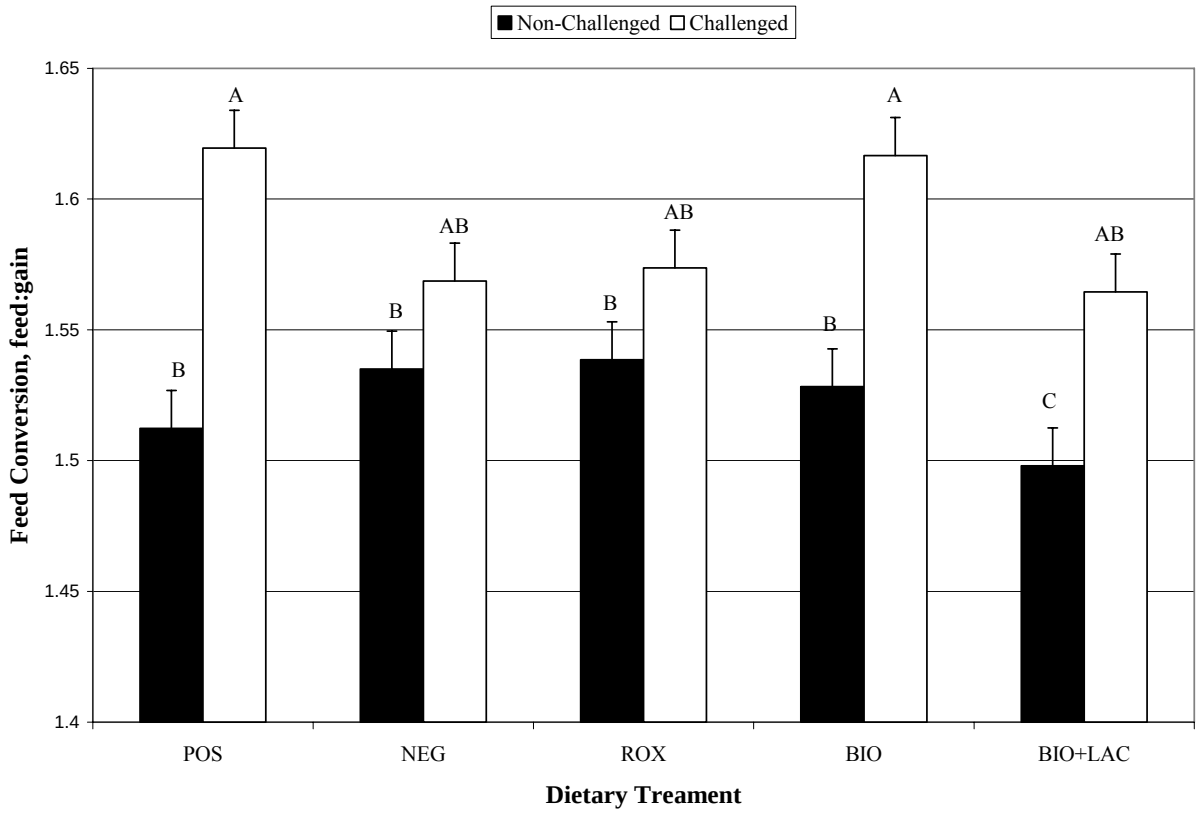


**Figure 3. Interaction of treatment by challenge on broiler feed conversion from day 0 to 35.** Treatment by challenge interaction overall *P*-value = 0.0295. Error bars represent the largest standard error of the mean.
[A-B]Means from different infection status without a common superscript differ significantly ($P < 0.05$).

### *Intestinal Morphology*

There was a significant increase in jejunal crypt depth ($P < 0.05$) for broilers challenged with *E. maxima* compared to non-challenged broilers (Table 7) suggesting that challenge induced an increase in cell turnover rate. No significant differences in ileal morphology between coccidia challenge and non-challenge were noted. Broilers fed the ROX diet had decreased ileal crypt depths ($P < 0.05$) compared to all other dietary treatments, suggesting a decrease in cell turnover for broilers fed the ROX diet. No significant differences were noted for cecal lamina propria measurements between dietary treatment or infection status.

No significant differences were observed in ileal or jejunal goblet cell mucin types between dietary treatments or coccidial challenge on day 28. From all sampled broilers, the acidic, neutral/acidic and neutral ileum mucin producing goblet cells represented 47.9%, 41.4% and 9.9% of total ileal goblet cell population, respectively and 41.94%, 43.99% and 5.8% of total jejunal goblet cell population, respectively (data not shown).

**Table 7. Effects of feeding programs and coccidia challenge on broiler intestinal morphology.**

| Treatment | Jejunum | | | Ileum | | | Cecum |
|---|---|---|---|---|---|---|---|
| | Villus Length (mm) | Crypt Depth (mm) | Villus/Crypt | Villus Length (mm) | Crypt Depth (mm) | Villus/Crypt | Lamina Propria (mm) |
| POS | 1.1756 | 0.1019 | 12.0105[b] | 0.6295 | 0.0937[a] | 6.9246[b] | 0.0119 |
| NEG | 1.2359 | 0.1066 | 11.7770[b] | 0.6428 | 0.1023[a] | 6.3408[b] | 0.0171 |
| ROX | 1.0624 | 0.0924 | 11.5160[b] | 0.6516 | 0.0758[b] | 8.6776[a] | 0.0134 |
| BIO | 1.0371 | 0.0961 | 14.2340[a] | 0.6695 | 0.0984[a] | 7.2752[ab] | 0.0148 |
| BIO+LAC | 1.2680 | 0.1099 | 11.7604[b] | 0.6056 | 0.0974[a] | 6.3842[b] | 0.0172 |
| SEM[1] | 0.0745 | 0.0057 | 0.7028 | 0.0373 | 0.0052 | 0.5360 | 0.0019 |
| Coccidial Infection | | | | | | | |
| Non-Challenged | 1.1936 | 0.0910[b] | 13.3256[a] | 0.6301 | 0.0900 | 7.2546 | 0.0150 |
| *E. maxima* Challenged | 1.2261 | 0.1118[a] | 11.1936[b] | 0.6495 | 0.0970 | 6.9864 | 0.0148 |
| SEM[1] | 0.0447 | 0.0034 | 0.4217 | 0.0236 | 0.0033 | 0.3390 | 0.0012 |

[a-b]Means within the observational unit and time period without a common superscript differ significantly ($P < 0.05$).
[1]SEM are reported as the largest SEM.

***Bone-Breaking Strength***

On day 28, no differences were observed for tibia length or breaking strength between broilers fed the various dietary treatments (data not shown). On day 49, there were no differences in tibia length or breaking strength between broilers fed the various dietary treatments and disease challenge. Birds infected with *E. maxima* had shorter tibia lengths (63.66 mm) than control birds (67.42 mm) ($P < 0.05$) on day 28, but tibia lengths were not significantly different on day 49. Increased bone length were observed in non-challenged broilers, which had greater body weight gains from 0 to 28 days compared to challenged broilers ($P < 0.05$). Increased bone length suggests increased frame size in non-challenged broilers, which is further evidenced by increase BWG of non-challenge broilers compared to challenged broilers.

***Muscle Tissue As Concentration***

Broilers fed the ROX diet had a greater concentration of As in the pectoralis minor at 28 d compared to broilers fed the POS diet ($P < 0.05$) (Table 8). Roxarsone was fed until 28 d, so this time period represents no withdrawal period. Even though there was a difference between treatments, the broilers fed the ROX diet did not exceed the FDA allowable As limit of 500 ppm. By day 49, broilers fed the different diets did not differ in As muscle concentration. Broilers fed the ROX diet had decreased As muscle concentration on day 49 compared to broilers sampled at 28-days.

**Table 8. The effect of diet and date on muscle tissue As concentration.**

| | Muscle Tissue As Concentration (ppb) | |
|---|---|---|
| Treatment | Day 28 | Day 49 |
| POS | 14.88[b] | 11.39 |
| ROX | 202.83[a,A] | 17.41[B] |
| SEM[1] | 9.07 | 9.44 |

[a-b]Means within the same column without a common superscript differ significantly ($P < 0.05$).
[A-B]Means within the same row without a common superscript differ significantly ($P < 0.05$).
[1]SEM are reported as the largest SEM.

## DISCUSSION

Growth and performance in terms of livability, FCR and BWG was negatively associated with *E. maxima* challenge ($P < 0.05$). Past research on the effect of *E. maxima* challenge on broilers housed in wire batteries has shown depression in BWG, but not FCR 6 days-post challenge in this trial (Allen and Fetterer, 2002a). In our trial, the effect of *E. maxima* challenge on broilers housed on litter had negative impacts on body weight gain, feed intake and FCR 14-days post-challenge. The coccidia challenge involved low amounts of oocysts ($3 \times 10^4$) compared to past research which determined the number of oocysts needed to cause morbidity to be around 200,000 oocysts (Rose and Long, 1962). However, each *Eimeria* isolate can react different passed on isolate strain and age of oocysts, which may account for the negative impacts on performance by *E. maxima* challenge observed during this trial. Broilers experienced stress induced by coccidia challenge which affected BWG, FI, FCR and mortality.

No data was collected to determine if necrotic enteritis had occurred in this trial, however necrotic enteritis may have occurred during the trial. After the coccidia challenge (d 14), mortality was not effected (14 to 28 d), but mortality was increased in broilers challenged with *E. maxima* from 0 to 49d and during the withdrawal phase (28 to 35 d). At d 28, the coccidia would

have time to cycle through the animals and cause sufficient damage to the gut to promote an overgrowth of *C. perfringens*. Also, necrotic enteritis has been prevalent on this farm in past flocks. Although necrotic enteritis was not confirmed through veterinary diagnosis, based on performance data this secondary bacterial infection likely occurred in this flock.

No differences were noted between broilers fed the POS and NEG diets. The POS diet contained BMD during the starter and grower phases and the NEG diet did not. Both diets contained monensin during the starter and grower phases and virginiamycin during the finisher and withdrawal phases. Monensin is also considered an anticoccidial. In the current trial, shuttling BMD in a basal diet containing monensin and virginiamycin had no effect on production. Virginiamycin, monensin and BMD are active against gram-positive bacteria (Butaye *et al.*, 2003; Cocito, 1973; Stone and Strominger, 1971). Guban *et al.* (2006) observed similar results when straight-run broilers fed a wheat-based diet supplemented with monensin (5g/kg diet) and bacitracin (5g/kg diet) did not have significantly different BWG or FCR compared to birds fed monensin (5g/kg diet) or those fed no monensin or bacitracin. Bacitracin supplementation has been observed to improve performance in chicks challenged with coccidia (Stanley *et al.*, 2004). Although BMD is feed to control *C. perfringens* outbreaks, Hofarce and colleagues (1998) observed no improvement in BWG during a clostridial challenge compared to control challenged animals. If necrotic enteritis had occurred in this flock, our results would mirror Hofarce and colleagues' (1998) observations.

Cumulative mortality was not affected by dietary treatment, but diet affected BWG and FI. Feeding roxarsone decreased FI and BWG. Roxarsone is most effective at maintaining performance during stress induced by disease, such as a coccidiosis infection (Mitrovic *et al.*, 1977). However, roxarsone is hypothesized to be most effective against coccidia infections in

the cecum or distal small intestine because roxarsone is able to be reduced to an inorganic form of arsenic under anaerobic conditions and cause sporozoite apoptosis (Czarnecki *et al.*, 1984). Prior to coccidial challenge, broilers fed ROX were not different in BWG from other dietary treatments, but FI was reduced compared to broilers fed the POS diet. Feed conversion was lowered compared to POS fed broilers. After the coccidia challenge, BWG and FI were lowered in broilers fed ROX, but FCR was not different from other dietary treatments. From day 0 to 35, no differences were noted between ROX fed broilers of different coccidia challenge status, while broilers fed diets BIO+LAC, POS and BIO were different, demonstrating the ability of dietary roxarsone to maintain performance during coccidial challenge (Mitrovic *et al.*, 1977). The decrease in FI associated with feeding roxarsone was not expected. Past research has not shown decreases in feed intake with the addition of roxarsone to a diet at 50 ppm (Fernandez *et al.*, 1973).

Diets including Bio-Mos® were effective at improving performance in broilers not challenged with coccidia. Prior to coccidial challenge, broilers fed the BIO diet had increased BWG compared to POS fed broilers. Feeding the BIO+LAC diet decreased broiler FI prior to the coccidial challenge, but BWG was not affected, while there was a decrease of 7 points in FCR during that period. After the coccidia challenge, feeding Bio-Mos® only affected performance during the finisher phase. Broilers fed the BIO diet had increased FI compared to POS fed broilers, while FCR was not affected. The treatment by challenge interaction on FCR during day 0 to 35 further supports the hypothesis that Bio-Mos® improves performance during non-challenged periods. Both Bio-Mos® treatments had decreased FCR of unchallenged broilers compared to broilers challenged with coccidia. Broilers not challenged with coccidia and fed the BIO+LAC diet had the lowest FCR of all treatment groups. Sun *et al.* (2005) also observed

improvements in FCR of broilers fed Bio-Mos® and sprayed with All-Lac XCL, but improvements were observed after a coccidia challenge.

Broilers challenged with *E. maxima* appeared to have increased jejunal cell turnover as indicated by increased crypt depth ($P < 0.05$). *E. maxima* parasitizes the mid-section of the small intestine from below the duodenal loop to beyond Meckel's diverticulum. The impact of this infection on the gut is ileal, an increase in cell turnover, but not an alteration in villus length. Broilers infected with *E. acervulina,* a species of coccidia that parasitizis the duodenum, have been shown to have a decrease in villus height:total mucosal thickness (Pout, 1967). In our coccidia model, villus were not damaged perhaps due to the increased cell turnover. As sporozoites came in contact with the mucosal lining, cell turnover increased to limit the migration of sporozoites into villus epithelial cells by sloughing of cells (Laux *et al.*, 2005).

Ileal crypt depth was decreased in broiler fed ROX diets suggesting a decrease in cell turnover. Differences were not observed in the jejunum. Izquirdo *et al.* (1987) also observed differences in distal, but not proximal gut morphology with roxarsone supplementation in broiler feed. Roxarsone supplementation (50mg/kg diet) reduced cecal lesion scores ($P < 0.05$), but not duodenal lesion scores in chicks infected with *E. tenella* and *E. acervulina* (Izquirdo *et al.*, 1987). Czarnecki *et al.* (1984) suggested roxarsone would be most successful at reducing effects of *E. tenella* (an *Eimeria* species that infects the cecum) because of the reducing effects of the anaerobic cecal conditions. The reduction of roxarsone in the ileum may be able to trigger apoptosis of *Eimeria* reducing its interaction with the gut mucosal layer, while roxarsone was not reduced in the jejunum. By causing apoptosis of coccidia in the ileum, cell turnover rate was decreased.

Bio-Mos® supplementation did not affect broiler gut morphology at 28 days. Sun *et al.* (2005) observed an interaction of age and diet on gut morphology of broilers fed drug-free diets, antibiotic growth promoters and Bio-Mos®. Yang *et al.* (2007) also observed no changes in gut morphology between broilers fed a diets containing monensin, monensin with BMD and monensin with Bio-Mos® at 14 and 35 days-of-age. Bio-Mos® does not directly improve gut morphology, but may maintain gut health during an enteritic bacteria challenge. At 28 days-of-age enteritic bacteria may not have been causing damage in control animals, so positive attributes of Bio-Mos® were not observed.

There were no differences in the goblet cell mucin profile in either both the ileum or jejunum. Past research has found differences in mucin profiles based on management practices (Meslin *et al.*, 1999; Uni *et al.*, 2003). Conventionally raised pigs have increased mucin in the small intestine, cecum and colon compared to germ-free pigs, indicating bacteria within the gut cause increased mucin production (Meslin *et al.*, 1999). Neutral and acidic mucin are all increased in the small intestine of conventationally raised swine, but sulphomucins are less affected by management (Meslin *et al.*, 1999). In poultry, neutral mucins do not begin to be expressed until post-hatch, but after 2-days post-hatch differences in management style can cause changes to the mucin profile (Uni *et al.*, 2003). Also, mucin production tends to increase during stress induced by starvation (Uni *et al.*, 2003). Our research observed the percentage of different mucin types did not differ but total number of goblet cells producing both neutral and acid mucin may have increased.

Most As accumulation data in chickens is reported as ratios based on liver As concentrations (Lasky *et al.*, 2004). Between 1994 to 2000, the mean As concentration in chicken meat was calculated to be 390 ppb based on liver-to-muscle ratios (Lasky *et al.*, 2004).

Roxarsone must be removed from the diet 5-days prior to slaughter. In our study, broilers without a withdrawal period (d 28) only had 202.83 ppb of As in muscle tissue, which is lower than what has been previously reported based on liver-to-muscle ratios (Lasky *et al.*, 2004). Roxarsone is typically fed during the starter and grower phase at 50 ppm, which is how roxarsone was fed in the current study (Chapman and Johnson, 2002). Our research has shown As concentrations in muscle tissue are lower than previously predicted by liver-to-muscle As ratios (Lasky *et al.*, 2004).

Overall, broilers fed the NEG diet performed similar to broilers fed other diets indicating a drug program with virginiamycin and monensin is helpful during coccidia challenges. Broilers fed diets containing roxarsone or Bio-Mos® performed differently. Bio-Mos® fed broilers had better FCR during non-challenge periods, while broilers fed roxarsone maintained similar performance during challenge compared to unchallenged broilers. Roxarsone likely resulted in a decrease in ileal cell turnover further supporting the hypothesis that roxarsone is effective against coccidia by reducing to an inorganic form in anaerobic conditions in the distal intestine (Czarnecki *et al.*, 1984). Feeding Bio-Mos® had no effect on gut morphology, but differences were observed in performance for unchallenged broilers. The improvement in performance may be related to an improved humoral immune response (Çetin et al, 2005). Turkeys fed a diet containing mannan oligosaccharides increased serum IgG and IgM, while inhibiting the peripheral T lymphocyte ratio (Çetin *et al.,* 2005). Improvements in production may be related to immune response. Although differences were observed between ROX and Bio-Mos® fed broilers, overall the addition of roxarsone or Bio-Mos® to broiler diets had limited effect on production and intestinal development compared to the NEG control fed broilers.

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