

Effects of probiotics and application methods on performance and response of broiler chickens to an *Eimeria* challenge

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ABSTRACT Coccidiosis is an inherent risk in the commercial broiler industry and inflicts devastating economic losses to poultry operations. Probiotics may provide a potential alternative to the prophylactic use of anticoccidials in commercial production. This study evaluated the effects of probiotic applications (feed and water) on bird performance and resistance to a mixed *Eimeria* infection in commercial broilers. On day of hatch, 1,008 commercial male broilers (Cobb 500) were assigned to 1 of 6 treatments (8 replicate floor pens; 21 birds/pen), including noninfected negative control (NEG), *Eimeria*-infected positive control (POS), anticoccidial control (0.01% salinomycin, SAL), intermittent high-dose water-applied probiotic (WPI), continuous low-dose water-applied probiotic (WPC), and feed-supplemented probiotic (FSP). On d 15, all birds except those in NEG were challenged with a mixed inoculum of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*. Measurements were taken on d 7, 15, 21, 28, 35, and 42. Fecal samples were collected from d

20 to 24 for oocyst counts, and lesion scores were evaluated on d 21. Data were analyzed using the Fit Model platform in JMP Pro 10.0 (SAS Institute Inc.). Differences in experimental treatments were tested using Tukey's honestly significant difference following ANOVA with significance reported at $P \leq 0.05$. Overall, NEG birds outperformed all other groups. For performance, the probiotic groups were comparable with the SAL-treated birds, except during the 6 d immediately following the *Eimeria* species challenge, where the SAL birds exhibited better performance. The WPC birds had lower duodenal and jejunal lesion scores, indicating a healthier intestine and enhanced resistance to *Eimeria* species compared with POS. Birds in the WPI treatment shed fewer oocysts in the feces, although this was not a trend for all of the probiotic treatment groups. The results of this study suggest probiotic supplementation without anticoccidials can enhance performance and help alleviate the negative effects of a mixed *Eimeria* infection.

Key words: probiotic, coccidiosis, broiler, performance, *Eimeria*

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INTRODUCTION

The decades-old practice of supplying food animals with subtherapeutic doses of antibiotics to protect against infections and improve general health has recently been under scrutiny. These practices are perceived to lead to microbial resistance to the drugs in use, resulting in consumer concerns regarding residues in food products. The relatively recent ban of subtherapeutic doses of certain antibiotics as feed additives in the European Union led to a general decline in animal health (Castanon, 2007). This outcome, as well as the

threat of a domestic ban, has led researchers to explore the next promising alternatives.

Probiotics present a potential alternative to the prophylactic use of antibiotics in feed animals. Also known as direct-fed microbials, probiotics are classified as live nonpathogenic microorganisms that are capable of maintaining a normal gastrointestinal microbiota (Patterson and Burkholder, 2003; Ohimain and Ofongo, 2012). Probiotic, meaning “for life” in Greek, has been defined as “a live microbial feed supplement, which beneficially affects the host animal by improving intestinal balance” (Fuller, 1989). Probiotics can be composed of one or many strains of microbial species, with the more common ones belonging to the genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus*, and *Pediococcus* (Gaggia et al., 2010).

Although the primary function of the gastrointestinal tract is to digest and absorb nutrients, a well-balanced

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gastrointestinal microbiota is crucial for optimal animal health and performance. The gastrointestinal tract also serves as a vital barrier preventing the entry of potentially harmful pathogens and other environmental antigens (Kogut and Swaggerty, 2012). Because the gastrointestinal tract begins to be colonized within hours after the chick hatches, the earlier the introduction of nonpathogenic microorganisms, the more effective their establishment in the digestive tract (Timmerman et al., 2006; Torok et al., 2007). Probiotics help maintain a healthy balance of microorganisms within the intestine, which is accomplished through multiple modes of action. Those mechanisms include competitive exclusion, pathogen antagonism, and stimulation of the immune system (Ohimain and Ofongo, 2012). The presence of probiotics reduces colonization of the gastrointestinal tract by pathogenic bacteria and attenuates enteric diseases, which ultimately result in enhanced performance of poultry (Kabir et al., 2004).

Coccidiosis is an inherent risk in the commercial broiler industry and inflicts devastating economic losses to poultry operations. Caused by development and reproduction of multiple species of the *Eimeria* protozoa, coccidiosis is estimated to result in a loss of US \$3 billion annually to the industry worldwide (Dalloul and Lillehoj, 2006). *Eimeria* species are unlike other protozoan parasites in that the primary target tissue is the intestinal epithelium, which results in considerable impairment of growth and feed utilization in poultry. Probiotics may provide a potential alternative to the prophylactic use of drugs in food animals due to their studied abilities to reduce enteric diseases in poultry (Patterson and Burkholder, 2003; Eckert et al., 2010). This study aimed to evaluate the effects of a multi-species, host-specific probiotic product containing *Enterococcus*, *Bifidobacterium*, and *Lactobacillus* species in the feed and water on performance parameters and resistance to coccidiosis (*Eimeria* infection) in commercial broiler chickens.

MATERIALS AND METHODS

Birds and Experimental Treatments

The study performed was a 42-d trial with broilers housed on floor pens, with 8 replicate pens per treatment and 21 birds per pen. On day of hatch (DOH), 1,008 commercial male broilers (Cobb 500) were assigned to 1 of 6 treatments, including noninfected negative control (NEG), *Eimeria*-infected positive control (POS), anticoccidial control [salinomycin as Sacox-60 (Huvepharma, Peachtree City, GA) at the rate of 0.01%, SAL], continuous low-dose water-applied probiotic (WPC), intermittent high-dose water-applied probiotic (WPI), and feed-supplemented probiotic (FSP). Birds in the low-dose WPC group were given probiotics at the rate of 2 mg/bird per day. Birds in the high-dose WPI treatment were given probiotics at the rate of 20 mg/bird per day intermittently. The WPI birds received probi-

otics on the first 3 d of life, once a week, during the week of *Eimeria* species challenge starting 1 d before inoculation, and 1 d before, the day of, and 1 d after feed changes. The diet for birds receiving probiotics in the feed was mixed and pelleted weekly. Probiotics for the feed application were microencapsulated to protect the probiotic bacteria from high pelleting temperature. All animal protocols were approved and conducted under the guidelines of the Virginia Tech Institutional Animal Care and Use Committee.

Probiotic Mixture Preparation

The probiotic product used in the experiment was a multi-species, host-specific probiotic (PoultryStar, BIOMIN GmbH, Herzogenburg, Austria) containing *Bifidobacterium animalis* subspecies *animalis* DSM 16284, *Lactobacillus salivarius* subspecies *salivarius* DSM 16351, and *Enterococcus faecium* DSM 21913. For the WPC and WPI groups, the cfu content of the product was 5×10^{12} per kg. For the FSP group, the cfu content of the product was 10^{11} cfu per kg and was mixed into the finished feed at an inclusion rate of 1 g per kg of feed, giving a final concentration of 10^8 cfu per kg of feed. This dosage was determined based on recovery rate of probiotic bacterial counts in pelleted feed samples from previous studies.

Eimeria Challenge

On d 15 of age, all birds except those in the NEG group were challenged via oral gavage with a mixed inoculum of *Eimeria acervulina* (USDA isolate #12), *Eimeria maxima* (Tysons isolate), and *Eimeria tenella* (Wampler isolate). The dose was 1 mL per bird containing 50,000 *E. acervulina* oocysts, 10,000 *E. maxima* oocysts, and 2,500 *E. tenella* oocysts. The inoculation rates of salinomycin-sensitive *Eimeria* species were based on previous studies in our laboratories. On d 21 (6 d postinfection), 24 birds per treatment (3 birds from each replicate pen) were randomly selected and euthanized for scoring of intestinal lesions caused by *Eimeria* infection. Lesions in the duodenum, jejunum, and ceca were scored according to the method of Johnson and Reid (1970) by personnel blinded to treatment based on scores ranging from 0 (no gross lesion) to 4 (most severe lesion). Fecal samples were collected from each pen on d 20 to 24 (d 5–9 postinfection) and kept in separate airtight plastic bags. After homogenization, samples were stored at 4°C until assessed for oocyst counts, which were determined by dilution and counts via microscope using a McMaster counting chamber (JA Whitlock & Co., Eastwood, NSW, Australia) and expressed as oocysts per gram of excreta.

Performance Measurements

Pen and feed weights were taken on DOH, d 15, d 21, d 35, and d 42. From these data, BW, BW gain

(**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined on a pen basis, and then averaged by treatment.

Statistical Analysis

Data were analyzed using the Fit Model platform in JMP Pro 10.0 (SAS Institute Inc., Cary, NC). Values were considered statistically different at $P \leq 0.05$. Results are reported as least squares means (least squares means) with SEM.

RESULTS

Eimeria Challenge

Lesion Scores. On d 21, lesion scores in the duodenum caused by *E. acervulina* infection showed a significant effect of treatment ($P < 0.0001$), as presented in Figure 1A. The NEG treatment group had significantly lower lesion scores than all challenged treatment groups. The POS treatment group had higher lesion scores than the SAL group and WPC group. The WPC had lower lesion scores than the FSP treatment. Similar to the duodenum, lesion scores caused by *E. maxima* in the jejunum on d 21 (Figure 1B) showed a significant effect of treatment ($P < 0.0001$). The NEG treatment group had lower lesion scores compared with all challenged treatments. The POS treatment group had higher lesion scores than WPC and WPI, the 2 water-administered probiotics. No significant lesions were observed in the ceca (Figure 1C), which is the site of *E. tenella* infection ($P = 0.0473$).

Oocyst Shedding. Fecal samples were collected on d 20 to 24 from each pen for evaluating oocyst shedding in the feces as presented in Figure 2. There was a significant effect of treatment on number of oocysts shed ($P = 0.0014$). The NEG control birds shed significantly fewer oocysts than those in the POS and SAL treatments. The POS group shed significantly more oocysts than the NEG, WPI, and FSP treatments.

Performance

BW. Presented in Figure 3A, a significant effect of treatment on BW was noted on d 21 ($P < 0.0001$; d 6 after *Eimeria* infection). Birds in the NEG treatment had significantly higher BW than all other treatments, whereas the SAL birds exhibited higher BW than all 3 probiotic treatments and the POS group. A significant effect of treatment on BW was also noted on d 35 ($P < 0.0001$), shown in Figure 3B, and d 42 ($P < 0.0001$), presented in Figure 3C, with the NEG group showing heavier BW.

BWG. A significant effect of treatment was evident from d 15 to 21, with the NEG group showing a greater BWG compared with all other treatments ($P < 0.0001$), as presented in Figure 4A. The SAL group showed greater BWG compared with the POS

and all probiotic product treatments, though still less than the NEG birds. From d 21 to 35, a significant effect of treatment was observed ($P < 0.0001$) where the NEG treatment had greater BWG than all other treatment groups (Figure 4B). When comparing total BWG through the course of the study (DOH to d 42), there was also a significant effect of treatment (Figure 4C). The NEG birds gained significantly more weight compared with all of the challenged treatment groups,

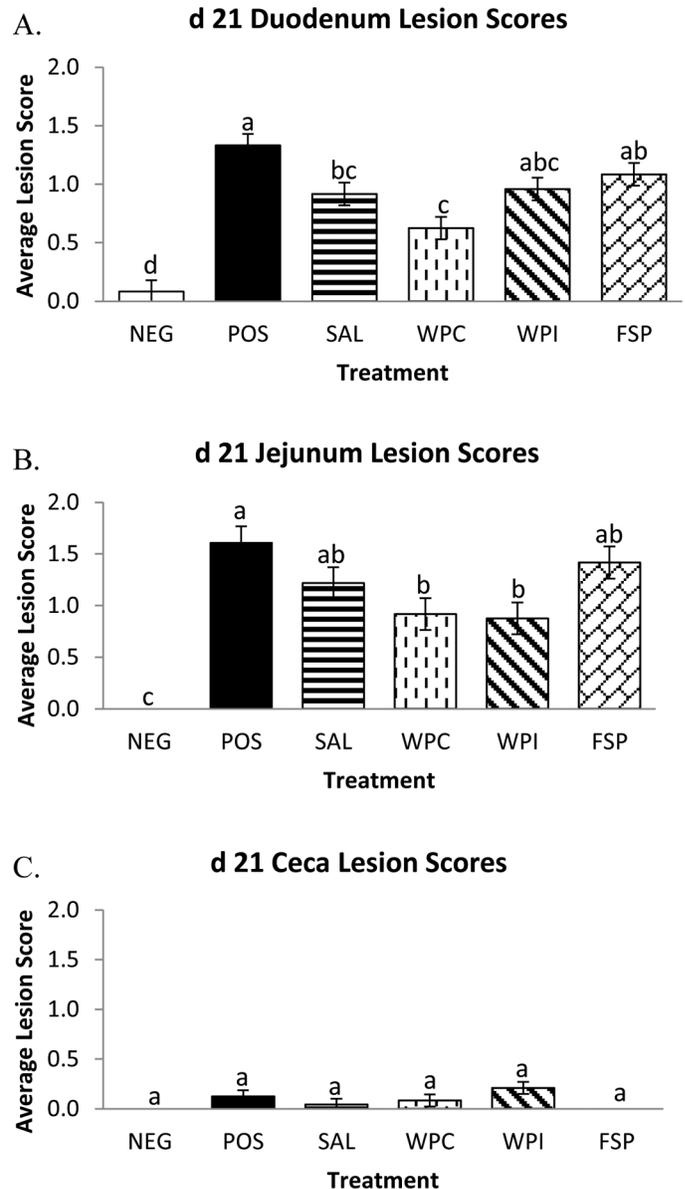


Figure 1. Effect of administration of probiotics (PoultryStar, BIO-MIN GmbH, Herzogenburg, Austria) on d 21 lesion scores of Cobb 500 male broiler challenged with *Eimeria* species on d 15. Data are presented as least squares means \pm SEM; bars lacking a common letter (a–d) differ significantly. There was a significant effect of treatment on lesion scores in the duodenum (A), the site of *Eimeria acervulina* infection ($P < 0.0001$), in the jejunum (B), the site of *Eimeria maxima* infection ($P < 0.0001$), but no significant lesions were observed in the ceca (C), the site of *Eimeria tenella* infection ($P = 0.0473$). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

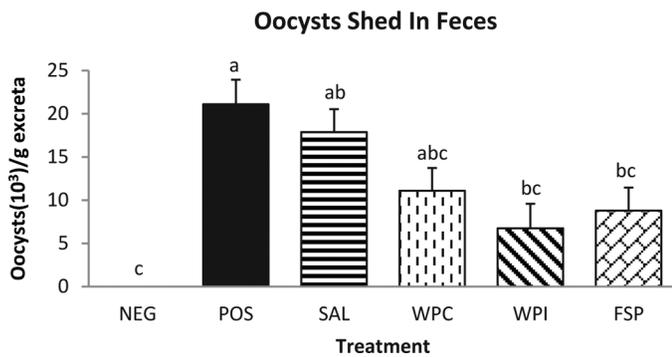


Figure 2. Effect of administration of probiotics (PoultryStar, BIO-MIN GmbH, Herzogenburg, Austria) on d 20 to 24 oocyst shedding in feces of Cobb 500 male broiler chicks challenged with *Eimeria* species on d 15. Data are presented as means \pm SEM; bars lacking a common letter (a-c) differ significantly. There was a significant effect of treatment ($P = 0.0014$). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

whereas the probiotic treatments gained a comparable weight as the SAL birds ($P < 0.0001$).

FI. As shown in Figure 5A, there was a significant treatment effect on FI from DOH to d 15 ($P = 0.0050$), where SAL birds consumed more feed per bird per day than POS, WPC, and WPI birds. From d 15 to 21, the NEG group consumed significantly more feed compared with all other treatments (Figure 5B). Also, the SAL birds consumed more feed than the WPC birds ($P < 0.0001$).

FCR. There was a significant effect of treatment on FCR during DOH to d 15 (Figure 6A). Treatment groups that received probiotics in drinking water had significantly lower FCR than the SAL group ($P = 0.0017$). Figure 6B presents the significant effect of treatment noted from d 15 to 21, with the NEG group exhibiting a significantly lower FCR compared with all other treatment groups ($P < 0.0001$). The POS group had a higher FCR compared with the SAL and NEG groups ($P < 0.0001$). The WPI treatment had a significantly higher FCR than the SAL group and the negative control ($P < 0.0001$), whereas treatment groups that received a low dose of probiotics continuously in drinking water and in feed did not differ significantly from SAL-treated group.

DISCUSSION

In this study, the effects of probiotic products in the feed and water of broilers and their resistance to an *Eimeria* infection were investigated. Birds in the WPC treatment group had less severe duodenal and jejunal lesion scores, indicating a healthier intestine. While present in the challenge inoculum, the dose of *E. tenella* may not have been sufficient to cause extensive damage to the site of infection, resulting in the absence of lesions in the ceca. Similar to present results, Lee et al. (2010) reported that birds given a strain of

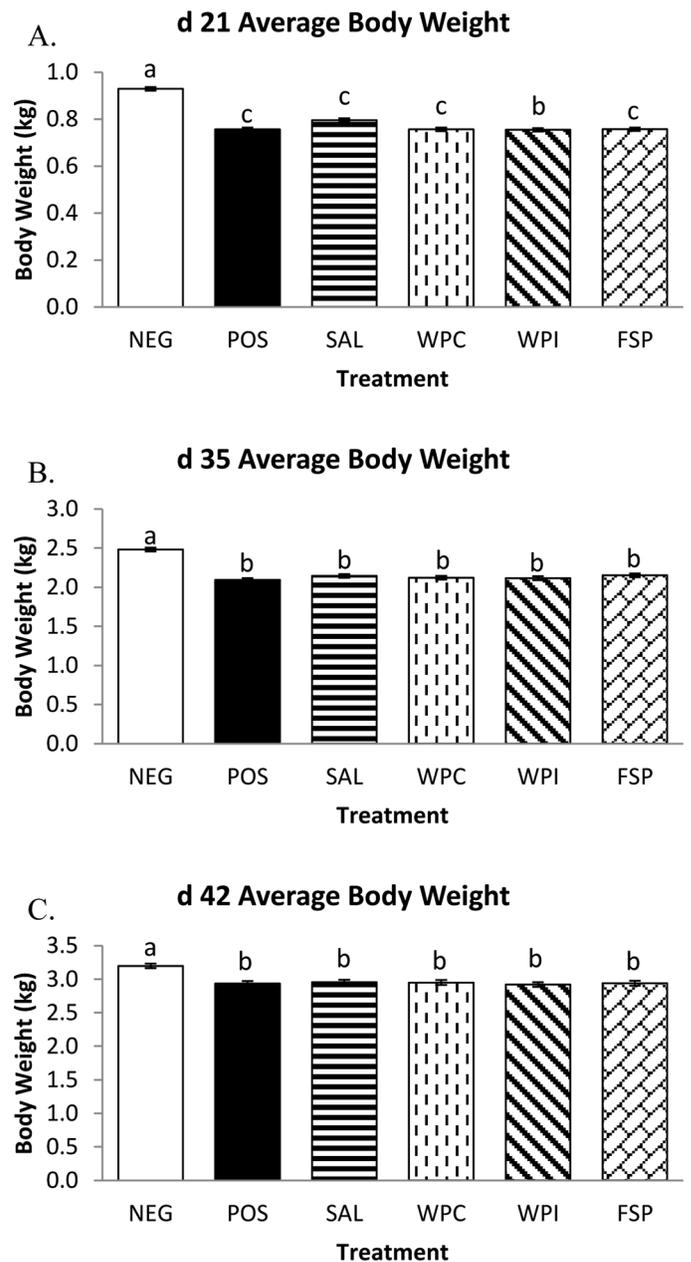


Figure 3. Effect of administration of probiotics (PoultryStar, BIO-MIN GmbH, Herzogenburg, Austria) on BW of Cobb 500 male broiler chicks. Data are presented as least squares means \pm SEM; bars lacking a common letter (a-c) differ significantly. There was a significant effect of treatment ($P < 0.0001$) on d 21 (A), on d 35 (B), and on d 42 (C). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

a *Bacillus*-based direct-fed microbial had significantly lower lesion scores in the gastrointestinal tract than birds given the nonsupplemented diet following an *E. maxima* challenge. Studies investigating necrotic enteritis in broilers found birds given 2 different blends of direct-fed microbials had significantly reduced intestinal lesions due to necrotic enteritis than birds in the positive control (McReynolds et al., 2009). Fewer and less severe lesion scores are indicative of less damage to the epithelium of the intestine, leading to infected

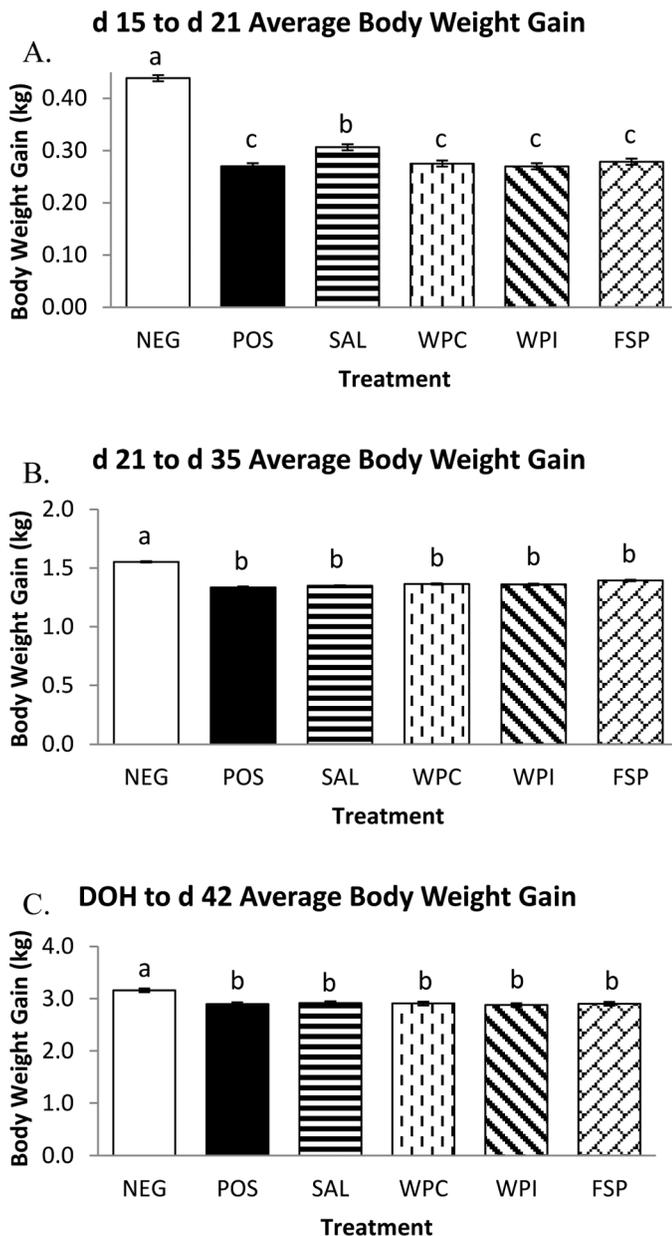


Figure 4. Effect of administration of probiotics (PoultryStar, BIO-MIN GmbH, Herzogenburg, Austria) on BW gain of Cobb 500 male broiler chicks. Data are presented as least squares means \pm SEM, bars lacking a common letter (a–c) differ significantly. There was a significant effect of treatment ($P < 0.0001$) on BW gain from d 15 to 21, the 6 d immediately following challenge (A), from d 21 to 35 (B), and through the course of the trial from day of hatch (DOH) to d 42 (C). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

birds having a greater chance of recovery from disease. Numerous studies have found probiotic supplementation leads to significant reductions in numbers of other intracellular pathogens present in the intestine, such as *Salmonella* Enteritidis and *Campylobacter jejuni* (Higgins et al., 2007; Ghareeb et al., 2012). The pathogen load reductions could be due to multiple mechanisms of action employed by direct-fed microbials, depending on strains present in various products employed in

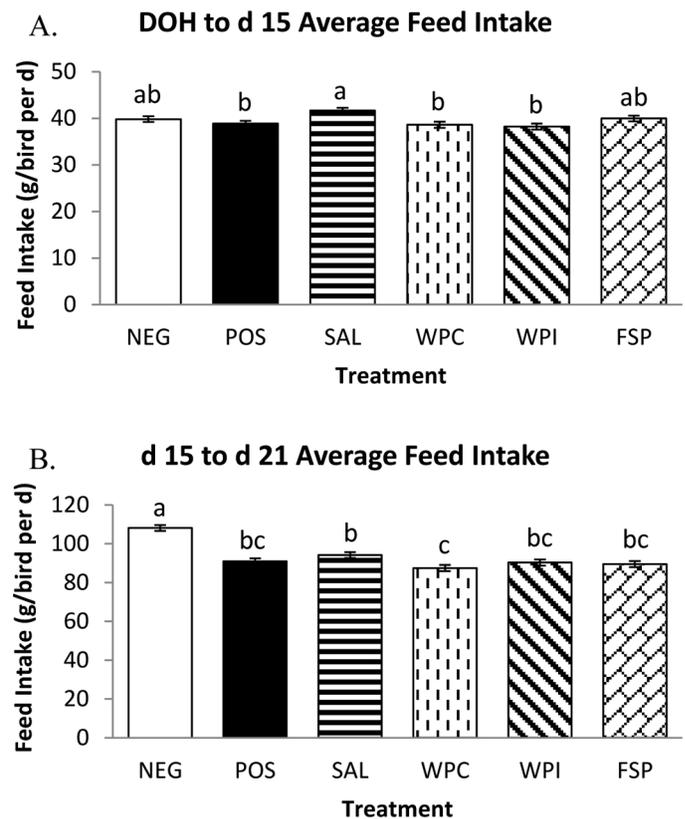


Figure 5. Effect of administration of probiotics (PoultryStar, BIO-MIN GmbH, Herzogenburg, Austria) on feed intake per bird per day of Cobb 500 male broiler chicks. Data are presented as least squares means \pm SEM. Bars lacking a common letter (a–c) differ significantly. A: There was a significant effect of treatment on feed intake from day of hatch (DOH) to d 15 (A, $P = 0.0050$) and from d 15 to 21 (B, $P < 0.0001$). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

those studies. Ultimately, the reduction in the presence of intracellular pathogens is indicative of a healthier intestine, with minimal damage done to the epithelium. An intact intestinal epithelium serves as the vital barrier preventing entry of potential pathogens and results in proper nutrient absorption and utilization, leading to optimal health and performance of the bird.

Birds receiving a high probiotic dose in the water supply on intermittent days (WPI) and in the feed (FSP) shed fewer oocysts in the feces than the positive control. Corroborating our findings, Dalloul et al. (2003, 2005) found that broilers provided a *Lactobacillus*-based probiotic in the feed shed significantly fewer *E. acervulina* oocysts compared with the challenged control. A reduction in oocysts shed in the feces indicates improved resistance of the bird to *Eimeria* species infection. Not surprisingly, the birds receiving the anticoccidial in the feed handled the challenge during the peak infection period better than probiotic-treated birds with regard to performance. However, as the study progressed, the probiotics helped the birds to overall perform as well as the birds receiving anticoccidial, and better than birds in the positive control.

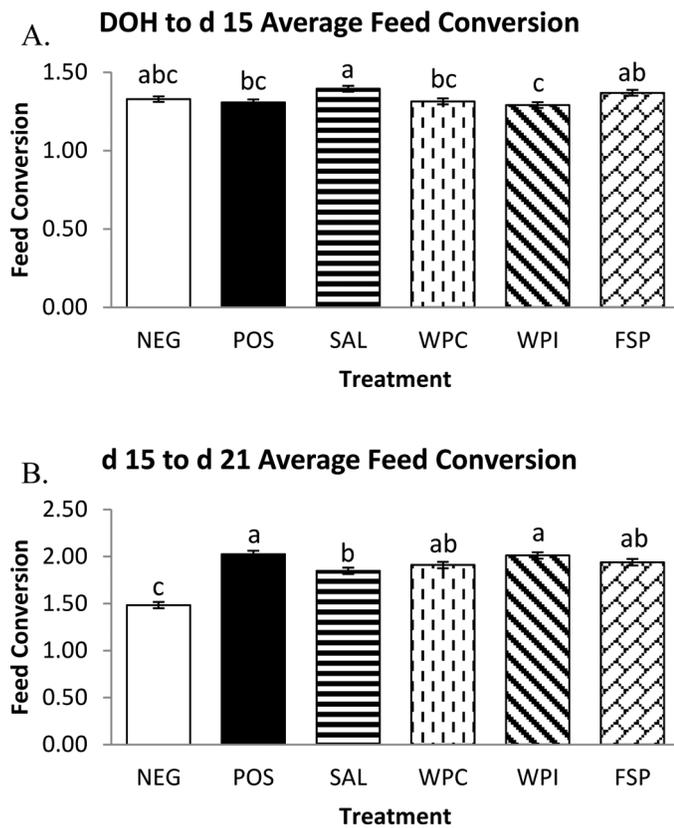


Figure 6. Effect of administration of probiotics (PoultryStar, BIOMIN GmbH, Herzogenburg, Austria) on feed conversion ratio of Cobb 500 male broiler chicks. Data are presented as least squares means \pm SEM; bars lacking a common letter (a–c) differ significantly. There was a significant effect of treatment on feed conversion ratio from day of hatch (DOH) to d 15 (A, $P = 0.0017$) and from d 15 to 21 (B, $P < 0.0001$). NEG = negative control; POS = positive control; SAL = salinomycin continuous in feed; WPC = probiotic at low-dose continuous water administration; WPI = probiotic at high-dose intermittent water administration; FSP = probiotic continuous in feed.

The reductions in BW and BWG due to the *Eimeria* challenge were not surprising because coccidial infections are known to cause significant damage to the intestinal mucosa and enterocytes during the progression of their lifecycle. This extensive damage causes nutrient malabsorption and subsequent reduced performance. Furthermore, parasitic infections result in nutrient resource allocation shifting from growth to immune response, which can also lead to noticeable differences in growth (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005). Supporting the current findings, Mountzouris et al. (2007) found that broilers receiving probiotic in the feed performed as well as broilers receiving the coccidiostat in terms of BWG and FCR over the duration of the trial. Numerous studies investigating probiotics as dietary additives in poultry have resulted in varying effects of probiotics on performance. Some studies reported that probiotic supplementation in the diet can improve BWG and FCR in chickens (Kabir et al., 2004; Nayebpor et al., 2007; Apata, 2008; Talebi et al., 2008; Ignatova et al., 2009; Sen et al., 2012), whereas others found no significant benefit to probiotic addition (Rahimi et al., 2011; Wolfenden et al., 2011). These

differences could be due to a variety of factors that can alter the efficacy of a probiotic such as strain(s) of bacteria used, composition and viability of the probiotic bacteria, and the preparation methods. Further, other factors may include probiotic dosage, method or frequency of application (or both), overall diet, condition and age of the birds, potential drug interactions, as well as environmental stress factors such as temperature and stocking density (Patterson and Burkholder, 2003; Mountzouris et al., 2007).

Concurrent with previous studies (Mountzouris et al., 2007; Karimi Torshizi et al., 2010), our data suggest that water administration of the probiotic product would be the method of choice, especially in a coccidiosis challenge situation. Although not examined in the current study, Karimi Torshizi et al. (2010) speculated that probiotics in water survive the demanding conditions in the upper gastrointestinal tract for a few reasons. The first possibility is a shorter transit time compared with solid feed. The second potential explanation is the water may limit the negative effects of gastric acid and digestive secretions on the microorganisms.

In conclusion, our data suggest that probiotic treatment (PoultryStar) helped alleviate the negative effects of the *Eimeria* species infection and may be used as a promising and beneficial anticoccidial. Poultry researchers are investigating the latest alternatives that will protect flocks from disease while not hindering performance or negatively affecting profit margins. Early establishment of beneficial microbiota by probiotics in poultry may lead to increased overall health and well-being while decreasing the need for prophylactic antibiotic use. Numerous studies have demonstrated that commensal intestinal microbiota inhibit pathogens and that probiotics can increase resistance to infection (Rolfe, 2000; Patterson and Burkeholder, 2003). Because PoultryStar is a product that contains multiple probiotic species of bacteria, there is a greater promise that such probiotics will be active in a wider range of conditions, similar to other multistrain probiotics, resulting in greater efficacy (Fuller, 1989; Dalloul et al., 2003, 2005; Timmerman et al., 2006). Future research evaluating pertinent gene expression within the intestinal and immune tissues, microbial profiles, histological changes, and other measurable parameters will provide further understanding of the probiotic effects and their mechanisms of action.

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