Protective Effects of Probiotics and Prebiotics on *Eimeria tenella*-Infected Broiler Chickens

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ABSTRACT

The current work was carried out to explore protective properties of commercially available probiotic (Lacto G®) and prebiotic (Immunolin®) preparations on performance traits, lesion scores, shedding of oocysts and some immunological parameters in *Eimeria tenella* infected broiler chickens. A total of 108 one-day-old broiler chicks were randomly divided into six groups: 1) probiotic non-challenged; 2) prebiotic non-challenged; 3) probiotic challenged; 4) prebiotic challenged; 5) non-treated challenged (positive control); 6) non-treated non-challenged (negative control). Chicks were challenged with *E. tenella* oocysts (4x10⁴) at 21 days of age. Supplementation with probiotics or prebiotics did not significantly improve the body weight or feed conversion ratio of birds compared to control. No mortality was recorded in the 3rd group while a high mortality rate (20%) was detected in the 4th and 5th groups. Lesion scores were significantly reduced in the 3rd group, while the 4th did not show any improvement in comparison with the infected group. The total number of oocysts starting from 7th up to 13th day post-challenge (PC) was significantly reduced in the 3rd and 4th groups in comparison with the control infected group. It could be concluded that there was a protecting effect of the probiotic and prebiotic preparations used, that helped to reduce the negative effects of coccidiosis but was not associated with an improved growth performance.

INTRODUCTION

Coccidiosis is one of the most important parasitic diseases of poultry causing death and impaired development rate in poultry industry (Lee et al., 2007). Conventional control of this problem depends mainly on anticoccidial drugs that are a significant cost to the industry. Moreover, development of resistance in most of the poultry parasites has endangered the economics of the poultry industry (Abbas et al., 2014). This requires new measures and unconventional control approaches (Zaman et al., 2012; Bachaya et al., 2015). Therefore, recent studies have given main consideration to feed additives of natural origins (i.e., herbal remedies, probiotic microorganisms, and prebiotic preparations) as alternative means for the control of poultry diseases (Abbas et al., 2012; Giannenas et al., 2012; Taherpour et al., 2012; Mahmoud et al., 2014a, 2014b). These supplements may be used to optimize the health of animals by positive manipulation of the gastrointestinal tract i.e., balancing the intestinal microbial community, improving intestinal histomorphology, and stimulating specific and nonspecific immunity (Mountzouris et al., 2011).

Probiotics are live micro-organisms which, when administered, confer a health benefit to the digestive tract. The adding of probiotics to the food improves growth rate and feed utilization in broilers (Mahmood et al., 2014c). Many bacterial species have been acted as probiotics, as species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Lactococcus*, *Streptococcus* and different yeast species (Mountzouris et al., 2007).

A prebiotic could be known as a non-digestible food component which benefits the host by motivating the growing of and/or stimulating the metabolism of a restricted number of bacteria in the digestive tract, therefore help the host's microbial equilibrium (Gibson and Roberfroid, 1995). Prebiotics are better than probiotics in an advantage that stimulated bacteria are normally existed in the gastrointestinal tract of the host and thus already adjusted to that environment (Snel et al., 2002). Mannanoligosaccharide (MOS) preparations, which are originated from the cell wall of the yeast...
Saccharomyces cerevisiae overwhelm pathogens in the intestinal tract of chickens and turkeys (Spring et al., 2000). Concerning coccidiosis, MOS supply enhances the growth of Bifidobacterium spp. and Lactobacillus spp in the intestinal tract and decrease the level of Enterobacteriaceae (Fernandez et al., 2002). An increase in Lactobacillus spp. and a reduction in Clostridium spp may decrease cecal coccidiosis in broilers (Elmusharaf et al., 2006).

The purpose of the present work was to explore the protecting effect of probiotic and prebiotic preparations in broiler chickens challenged with E. tenella, a highly pathogenic Eimeria species that causes cecal coccidiosis.

MATERIALS AND METHODS

Animals: A total of 108 one-day-old commercial broiler chicks were raised for 5 weeks in floor pens where feed and water were supplied without restriction. The chicks were fed a ration without anticoccidial drugs. All birds were nourished starter ration (23% protein) through the first 2 weeks of life and commercial grower ration (21% protein) from the 3rd week till the end of the trial.

Experimental design: All chicks were randomly allocated into six groups, 1st group: probiotic non-challenged; 2nd group: prebiotic non-challenged; 3rd group: probiotic challenged; 4th group: prebiotic challenged. The 5th and 6th groups represent the positive (non-treated challenged) and negative (non-treated non-challenged) control groups, respectively. Each group was classified into 3 replicates, 6 chicks each.

Probiotic and prebiotic supplementary treatments: Multispecies probiotic mix Lacto-G® (Dynovet, Egypt) and the prebiotic preparation Immunolin® (Dynovet, Egypt) were used at the commercial recommended rates. Lacto-G® was administered through drinking water at a concentration of 0.5g/L. water. Immunolin® was also administered through water at a concentration of 0.5 ml/L water. The ingredients of probiotic and prebiotic additives are summarized in Table 1. Each investigational group was administered the equivalent treatment from the 1st to the 35th days of age.

Challenge: Challenge of chickens was performed at 21 days of age by direct inoculation of 4x10⁷ sporulated oocysts of E. tenella into the crop via insulin syringe. The used E. tenella oocysts represented Behera isolate previously isolated from a single oocyst and described by Abu-Akkada and Awad (2012). Prior infection, feces were examined from all groups to confirm absence of coccidial infection. No oocysts were detected in all groups. The experiment was terminated after 2 weeks PC (35 days of age).

Mortality and performance parameters: Mortality due to coccidiosis was recorded daily in each replicate. All of the chicks were independently weighed every week starting from the 21st day of age till the end of the experiment (2 weeks PC) to assess weight gain during the infection period. Feed consumption and feed conversion ratio (FCR) were calculated on the day of challenge and weekly for 2 weeks after the challenge.

Lesion scores and dropping scores: From each group, three chicks were slaughtered 7 days PC. Lesions produced by E. tenella were scored according to Johnson and Reid (1970). Dropping scores were graded from 0–4, according to the consistency of the feces and the existence of mucus and/or blood (Morehouse and Barron, 1970).

Oocyst count: The number of oocysts per gram of feces (OPG) was assessed from 7th to 13th day PC. The OPG was counted by the McMaster counting technique as described by Long and Joyner (1976).

Weight of lymphoid organs: Lymphoid organs including bursa of Fabricius, thymus and spleen were removed surgically and weighed 7 days PC.

Phagocytic activity and phagocytic index: On the 7th day PC, blood samples were collected into heparinized tubes. Phagocytic activity (PA) and phagocytic index (PI) were evaluated as described by Kawahara et al. (1991). The number of phagocytized organisms was counted in the phagocytic cells and referred to as PA. PA = Percentage of phagocytic cells containing yeast.

Statistical analysis: The data were compared between groups using the Statistical Analysis System software (SAS, 2002). All of the data are offered as the mean ±SE. The data were statistically analyzed using analysis of variance. The data were considered significant at P<0.05.

RESULTS

The body weight gain from day 21 to 35 of age is shown in Table 2. E. tenella infection significantly decreased the body weight gain of all infected groups in comparison with the negative group of chicks. The decrease in growth was more noticeable in the control positive group that had the worst final BW gain. Unexpectedly, body weight gains in birds supplemented with the probiotic and prebiotic preparations and non-infected with E. tenella were not significantly increased compared to the negative control group. At the same time, no significant improvement was observed in BW gain in groups 3 and 4 compared to the infected controls.

There are significant differences in the total FCR among different groups (Table 2). Group 1, 2 and 6 had the least total FCR compared to other groups. While, groups 3 and 4 did not show any improvement in total FCR compared to the infected control group.

No mortalities were recorded in the control negative, 1st, 2nd and 3rd groups whilst a high mortality rate was detected in the infected group and group 4 (20%) (Table 3). The values of cecal lesion scores of the control infected group were the worst (Table 3). Challenged birds supplemented with probiotic (3rd group) exhibited significantly better lesion score (1.33) compared to those observed in the infected control group. The score of the control negative and treated non-challenged groups (1st and 2nd) was nil score. Concerning cecal mucosal scrapings, the 3rd group exhibited significantly better
starts to increase from the 7th day and continued till the 10th day PC. Starting from the 10th day PC, the count started to decrease until the 13th day PC. The total OPG was significantly the lowest in the 3rd group followed by the 4th compared to the control infected group.

Concerning weights of lymphoid organs (spleen, bursa and thymus), outcomes are shown in Table 5. No noteworthy variations on the weight of spleen in all experimental groups except for the 5th group that was significantly lower than other groups. There is a significant rise in the weight of bursa of Fabricius in probiotic treated groups compared to the control group. Probiotic administration significantly increased the weight of thymus compared to other groups. Results showed that all the lymphoid organs had higher values in chickens given probiotics as compared to those administered prebiotics however the variation was not always statistically significant. On the other hand, PI was significantly greater in the third group than in other groups while PA was significantly higher in the second group compared to other groups.

**DISCUSSION**

The current study evaluated the protective effects of commercial probiotic and prebiotic preparations on performance traits, lesion scores, shedding of oocysts and some immunological parameters following an experimental infection of broiler chickens with *E. tenella*. Supplementation with probiotics or prebiotics did not significantly improve the performance of birds compared with those of the negative control. This is contrary to results stated by Yang et al. (2012) who indicated a significant increase in productivity with the use of these additives under unchallenged conditions. Otherwise, Lee
et al. (2007) reported that MitoGrow (as a probiotic) improved resistance to experimental *E. acervulina* infection by improved BW gain and decreased oocyst shedding in comparison with infected controls, but in *E. tenella*-infected birds, the oocyst output and weight gains of the MitoGrow-administered groups were not noticeably improved. They added that the difference between the two *Eimeria* species may be related to the infection sites specific for each species, where probiotic organisms may prefer settling site above the other. Cecal lesion scores on day 7 PC revealed that lesions of *E. tenella* in the infected birds administered probiotics were significantly decreased while lesions in those infected birds fed prebiotics did not have any improvement compared to the positive control group. Williams and Andrews (2001) found that lesions of *E. acervulina* lesions in the infected birds fed MOS were significantly decreased thus they reported that MOS preparation had anticoccidial activity against *E. acervulina* when included in the food.

Infected birds fed probiotics and prebiotics (groups 3 and 4) showed significant lesser total number of oocysts compared to the infected control. Thus, it can be suggested that these preparations have the advantage to decrease the severity of the infection where they reduced the oocyst output that is critical for the re-infection and the conservation of the immunity stimulated by the initial infection. It has been revealed before that MOS preparation decreased the schizonts in the lamina propria of the cecum of broilers infected with *E. tenella* (Elmusharaf et al., 2006). In another study with a *Pediococcus*- and *Saccharomyces*-based probiotic (MitoMax®) given to birds challenged with *E. acervulina* or *E. tenella*, less oocyst excretion and a better antibody response was detected compared to non-probiotic controls (Lee et al., 2007). It seems that reducing lesion scores and oocyst output by feed additives may be attributed to lower intestinal pH and conditions appropriate to increase the useful microflora (Taherpour et al., 2012).

The weight of spleen did not differ between chickens fed probiotic and prebiotic preparations and those of the control birds. This may point to the late response of spleen to the effect of probiotic or prebiotics as it is a secondary lymphoid organ which progresses its appropriate functions with age (Alkhalf et al., 2010). This is in agreement with the findings of Teo and Tan (2007) who reported no significant variances in the weights of the spleen in broilers fed probiotic in comparison with control groups. In contrast, Willis et al. (2007) stated that nourishing broilers on probiotic lead to rising in the relative weights of spleen of the treated group. Increase in the weight of bursa and thymus in the 1st group may be caused by increasing number of immune cells. These results are in agreement with that of Teo and Tan (2007) who stated that chickens given feed with *Bacillus subtilis* PB6 had a significantly heavier bursa weight compared with control groups.

Administration probiotics or prebiotics did not significantly rise the phagocytic activity or index measured in treated broilers although Dalloul et al. (2003) confirmed an increase in innate and acquired immunity against broilers infected with *Eimeria* and given a Lactobacillus-based probiotic.

The results of the present experiment showed that supplementing probiotic preparation (Lacto G®) to broilers prevented mortality, reduced oocyst output and reducing the severity of *E. tenella* lesions. While supplementation with prebiotic preparation (Immunolin®) had the effect of decreasing number of oocysts only. Although Bozkurt et al. (2014) recently stated that supplemental intake of probiotics and prebiotics by chickens on exposure to experimental coccidiosis alleviated the influence of disease and positively influenced growth and feed conversion efficiency. It could be concluded that the probiotic and prebiotic preparations used, have protective effects that was not associated with an improved growth performance. These results may possibly be changed by changing probiotic and prebiotic concentrations in water and the extent of *E. tenella* inoculation doses. Further research is necessary to confirm whether these products have anticoccidial activity when used at higher concentrations in combination with higher challenge doses.

**Conclusion:** It could be concluded that probiotics, and prebiotics improve the resistance of birds and to some extent protect against coccidiosis.

**Author’s contribution:** SSAA designed and executed the experiment and wrote the manuscript. AMA followed up the experiment and shared in analyzing the data. All authors interpreted the data, revised the manuscript and approved the final version.

**REFERENCES**


