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Anticoccidial effect of mananoligosaccharide against experimentally induced coccidiosis in broiler

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Abstract The aim of this study was to find the effect of mananoligosaccharide (MOS) in comparison with amprolium hydrochloride on performance and integrity of gut in experimentally induced coccidiosis in broiler. A total of 300, day-old male broiler chickens (Ross 308) was randomly allocated to four treatments. Each group was further divided into five replicates of 15 birds each. Group A was kept as control; group B was contaminated with *Eimeria tenella*, while groups C and D were infected with *E. tenella* and treated with MOS (0.8 g/kg feed) and anticoccidial drug, amprolium hydrochloride (12 g/100 l water), respectively. The results showed that weight gain, feed intake, and feed conversion ratio (FCR) were significantly higher ($P < 0.05$) in infected + MOS-treated group compared to the other groups. The result of oocyte per gram (OPG) was significantly higher ($P < 0.05$) in the group infected with coccidiosis during 5th, 7th, 10th, and 12th day post infection (dpi). Furthermore, the OPG was significantly lower ($P < 0.05$) in infected groups treated with MOS and amprolium at the studied periods (5, 7, and 10 dpi). At 12 dpi, the infected group treated with MOS showed significantly lower OPG compared to the other groups suggesting the effectiveness of MOS in comparison to amprolium. The result of pinpoint hemorrhages, thickness of cecal wall, bloody fecal contents, and mucoid contents in the cecum were significant highly ($P < 0.05$) in birds fed with

infected oocytes. It was also noted that the differences were not significant in these parameters between amprolium and MOS-treated birds showing the effectiveness of the prebiotic agent. It was concluded from the results of the present study that MOS improved growth performance and reversed the lesions of *E. tenella*.

Keywords Coccidiosis · Birds · Monoligosaccharide · Feed efficiency · Lesions

Introduction

In commercial poultry, coccidiosis is one of the most expensive parasitic diseases (Tanweer et al. 2014). This disease reduces the effectiveness of poultry production by increasing the mortality, worsening feed conversion ratio, and negatively affecting the growth rate of birds (Lillehoj et al. 2004). Coccidiosis causes losses of about US\$127 million annually in US poultry industry, and similar losses may have been experienced by the poultry producers in other divisions of the world (Chapman 1999). The protozoan of the genus *Eimeria*, which is responsible for coccidiosis in avian, creates primary economic losses via high mortality (McDougald 2003). The *Eimeria* parasite reproduces in the intestinal region, causing tissue injury and ensuing low ingestion of feed and nutrient absorption, diminishes body weight growth, reduces the moisture level in the body, and leads to blood loss and vulnerability to many additional diseases (McDougald 2003; Tanweer et al. 2014).

To manage *Eimeria* infection in avian species, anticoccidial immunization and anticoccidial products in poultry ration are used. It is predicted that 95 % of the meat-producing birds are treated with anticoccidial drugs (Chapman 2005). There are some chemical preparations that are available in the market such as amprolium, nicarbazin, robenidin, diclazuril, zoalene,

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decoquinatone, and halofuginone that are extensively used in broiler production. In majority cases, farmers mainly rely on the prophylactic and therapeutic use of chemicals for the control of avian coccidiosis. Anticoccidial preparations added into the poultry feed may lead to increased chances of resistance against *Eimeria* strains (Ruff and Danforth 1966; Chapman 1994, 1997, 1998; Allen and Fetterer 2002). So, alternative therapies are needed, which may potentially encourage cecal immune system, lessen harmful microbes, and substitute the anticoccidial drugs.

Non-digestible polysaccharides like oligofructose and oligomannose (prebiotics), commonly known as prebiotics, increase the expression of valuable bacteria (*Bifidobacteria* and *Lactobacillus*) and diminish detrimental microbes (*Escherichia coli* and *Salmonella*) and also arouse the immunity of the bird (Hidaka et al. 1986; Gibson and Roberfroid 1995; Gibson et al. 1995; Gibson 1999; Gibson and Fuller 2000; Cummings and Macfarlane 2002). Prebiotics enhance the function of endogenous supporting organisms in the gut of birds (Bezkorovainy 2001) and can be used as a substitute for growth-encouraging antibiotics (Hatemink 1995). Mannan-oligosaccharide (MOS) is a starch material extracted from the cell component of the yeast, *Saccharomyces cerevisiae*, which eliminates harmful bacteria in the intestinal region of avian species (Sonmez and Eren 1999; Spring et al. 2000; Iji et al. 2001). MOS may advance intestinal conditions by intensifying height of the villi and integrity of the avian intestine (Loddi et al. 2002) and regulate intestinal and systemic resistance (Ferket et al. 2002). Also, MOS supplementation in the diet increases the concentration of *Bifidobacterium* and *Lactobacillus* species in the gut and decreases the population of *Enterobacteriaceae* (Fernandez et al. 2002).

To the best of our knowledge, there is no information on the comparison of MOS and amprolium against *Eimeria tenella* infection in broiler chickens. Therefore, the present research work was planned to investigate the impact of MOS and amprolium in experimentally induced *E. tenella* infection in broilers on the growth performance and intestinal integrity.

Materials and methods

This study was approved by the Departmental Committee on Issues and Welfare of Animal, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan. A total of 300, day-old male broiler chickens (Ross 308) was purchased from the local market. On the day of arrival (day 1), the chicks were weighed and randomly allocated to four treatments. Each group was further divided into five replicates of 15 birds each. Group A was kept as control; group B was contaminated with *E. tenella*, while groups C and D were infected with *E. tenella* and treated with MOS (0.8 g/kg feed; Taha Biochemical Industry, Peshawar,

Pakistan) and anticoccidial drug amprolium hydrochloride (12 g/100 l water; Taha Biochemical Industry, Peshawar, Pakistan), respectively. The feed was formulated according to the recommendation of the NRC (1994) as shown in Table 1.

Induction of coccidial infection

The oocysts were isolated from the infected guts as described by Tanveer et al. (2014). Briefly, the ceca of the infected birds were collected from the poultry postmortem room of Veterinary Research Institute Peshawar, Pakistan. The cecal contents of the gut were collected and soaked overnight in 2.5 % potassium dichromate solution. The suspension was filtered and centrifuged at 1500 rpm for 3 min. The supernatant was discarded, and the sediment was resuspended in a saturated solution of sodium chloride and centrifuged at 1500 rpm for 3 min. The sediment containing oocysts was separated and kept in incubator at 30 °C for 24–72 h. The sporulated oocysts were stored at 4 °C in potassium dichromate solution, and their number was adjusted to 20,000–30,000 oocysts per 2 ml of inoculum (Hodgson 1970; Long et al. 1976). The chicks in all replicates except (uninfected, untreated) were treated orally with 20,000–30,000 oocysts per chick on day 14 of experimental trial.

Treatment of MOS and amprolium

MOS was acquired from the market and was given in the feed from first day to the last day. Amprolium hydrochloride was

Table 1 Composition of basal diets

| Ingredients | Percent |
|---------------------------------|---------|
| Maize | 60.7 |
| Soybean meal | 35.6 |
| Vegetable oil | 0.5 |
| Limestone | 1.4 |
| Dicalcium phosphate | 1.2 |
| DL methionine | 0.15 |
| Salt | 0.4 |
| Vitamin + trace mineral premix | 0.3 |
| Total | 100 |
| Calculated nutrient composition | |
| Protein (%) | 22 % |
| Metabolizable energy, kcal/kg | 2960 |

Each kilogram of premix consisted pyridoxine, 1 mg; folic acid, 0.4 mg; molybdenum, 0.32 mg; ethoxyquin, 25 mg; choline chloride, 60 mg; dl- α -tocopherol acetate, 4 mg; iodine, 0.2 mg; thiamine, 0.3 mg; Ca pantothenate, 3 mg; cyanocobalamin, 3 μ g; biotin, 0.02 mg; Mn, 15 mg; Zn, 10 mg; iron, 4 mg; Cu, 1 mg; Co, 0.06 mg; Se, 0.02 mg; cholecalciferol, 0.018 mg; *trans*-retinol, 0.66 mg; menadione, 0.4 mg; riboflavin, 1.6 mg; and niacin, 6 mg

administered 1 week after challenging the birds with *E. tenella*. The experiment lasted for 35 days. All the broiler chicks were kept in an open-sided house in cages. Sawdust was used as bedding substance. Similar feeders, drinkers, and other vital materials were provided in each pen to maintain identical environment.

Performance traits

Gain in body weight in individual bird was recorded on the termination of each week. Birds were weighed at the start of the study and then at the termination of each week. Initial weight was subtracted from final weight to calculate gain in body weight. Total body weight gain was computed at the termination of the trial. Starter ration was provided from day 1 to 21 days. While finisher ration was presented for the remaining period of the study. Feed intake on a daily basis was considered in preinfection and postinfection period by subtracting the amount of feed rejected from the feed offered. Total feed intake was computed for each week. Feed conversion ratio (FCR) was computed on a weekly basis for each group by using the following formula: $FCR = \text{feed intake} / \text{weight gain}$.

Number of oocysts/gram of feces

Five fecal samples per replicate were collected on days 5, 7, 10, and 12 of coccidial infection for oocyst counting. Fecal and litter samples were collected in the evening, and the samples were held for a night in a refrigerator. The oocysts in each sample were counted the next day, and the number was expressed per gram of feces. The counting of oocysts was made by modified McMaster technique as described by Hodgson (1970). Briefly, a 10 % (w/v) fecal sample was suspended in a salt solution (151 g NaCl blended into 1 l of water). After shaking carefully, 1 ml of the suspension was combined with 9 ml of a salt solution (311 g of NaCl mixed into 1 l of distal water). Then, the suspension was introduced into the McMaster chamber by means of a micropipette, and the number of oocysts was totaled.

Gross lesions

On days 5, 7, 10, and 12 of coccidial infection, two birds were randomly selected from each replicate for lesion scoring. A 0–4 lesion scoring classification of Tanweer et al. (2014) was used. The lesions were comprised of petechial hemorrhages, thickening of cecal wall, bloody fecal contents, and mucoid discharge. Based upon the severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions), or 4 (extremely severe lesions) was computed for each bird.

Histological examination

Cecal tonsils were collected from three birds per replicate on 12th day post infection and immediately fixed in 10 % buffered formalin. Samples were processed according to the standard protocol as adopted by Tanweer et al. (2014). After washing, tissues were placed in ascending grade of alcohol for dehydration with a decreasing time period in automatic tissue processor (Tissue-Tek® Sakura, Japan). Following dehydration, the tissue was placed in paraffin and tissue blocks (Tissue-Tek® TEC™ Sakura). Tissue blocks were sectioned by using microtome (Accu-Cut® SRM™ 200 Sakura) with thickness of about 4–5 μm . For staining of slide section, hematoxylin and eosin (H&E) staining was used. Randomly three views per slide per sample for a total 30 readings per treatment were recorded.

Statistical analysis

The data were statistically analyzed with the standard procedure of analysis of variance (ANOVA) using a completely randomized design. Means were compared for significance of difference by least significant difference (LSD) as described by Steel and Torrie (1981). Statistical package SAS (1998) was used to perform the above analysis on a personal computer.

Results

The effect of feeding MOS and amprolium on weight gain and feed intake is given in Tables 2 and 3, respectively. The results showed that weight gain and feed intake were significantly higher ($P < 0.05$) in infected + MOS-treated groups compared to the other groups. The result of FCR indicated that it was significantly improved in a group of birds treated with MOS and coccidial oocysts as shown in Table 4 during the whole experiment except week 2 where the treatment did not show a significant difference.

As shown in Table 5, the result of oocyte per gram (OPG) was significantly higher ($P < 0.05$) in the group infected with coccidiosis during 5th, 7th, 10th, and 12th day post infection. The result also indicated that the OPG was significantly lower in infected groups treated with MOS and amprolium at the studied periods (5, 7, and 10 day post infection (dpi)). At 12 dpi, the infected group treated with MOS showed significantly low OPG compared to other groups suggesting the effectiveness of MOS in comparison to amprolium.

The result of pinpoint hemorrhages, thickness of cecal wall, bloody fecal contents, and mucoid contents in the cecum are given in Table 6. As expected, these lesions were significant highly ($P < 0.05$) in birds fed with infected oocytes. It is also pertinent to note that the differences were not significant in

Table 2 Effect of MOS and amprolium hydrochloride on weight gain (g) of broiler birds challenged with *Eimeria tenella*

| Groups | Second week | Third week | Fourth week | Fifth week | Overall mean |
|------------------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|------------------------------|
| Control | 448.00 ^b ± 0.95 | 463.29 ^b ± 0.36 | 505.33 ^b ± 1.39 | 651.92 ^b ± 11.15 | 2069.50 ^b ± 8.83 |
| Infected | 447.88 ^b ± 1.12 | 455.73 ^c ± 0.34 | 496.58 ^c ± 0.46 | 594.37 ^d ± 8.49 | 1996.60 ^d ± 8.82 |
| Infected + MOS treated | 469.15 ^a ± 0.26 | 484.81 ^a ± 0.49 | 518.97 ^a ± 1.25 | 677.99 ^a ± 17.05 | 2153.90 ^a ± 16.01 |
| Infected + amprolium hydrochloride | 449.13 ^b ± 0.93 | 461.34 ^b ± 0.37 | 503.68 ^b ± 0.70 | 539.39 ^c ± 14.08 | 2057.50 ^c ± 14.31 |

Means in the same column with different superscripts are significantly different ($P < 0.05$)

these parameters between amprolium and MOS-treated birds showing the effectiveness of the prebiotic agent.

As shown in Fig. 1, the infected birds showed glandular structure infiltrated with coccidial oocyst and leukocytic infiltration. Birds treated with MOS showed caecal section of broiler showing intact and strong epithelial layer.

Discussion

Weight gain was significantly affected by MOS supplementation in broiler birds. Higher body weight gain of broiler birds may be due to the fact that prebiotics such as MOS increase the number of healthy bacteria such as *Bifidobacteria* and *Lactobacilli* in the intestinal tract of the chicks (Baurhoo et al. 2007). Prebiotics are passed undigested through the upper part of the intestines, while in the lower region of intestines, prebiotics act selectively as a substrate to stimulate the development of microbes that are beneficial to host health (Grizard and Barthomeuf 1999). Improved microfloral condition of the gut determines a better nutrient adjustment and lessens nutrient loss (Miles et al. 1991). Our results are similar to the previous findings which reported better weight gain in meat-type birds by adding MOS in the diet (Iji et al. 2001; Xu et al. 2003; Mohamed et al. 2008; Bozkurt et al. 2009; Koc et al. 2010). Feed intake in broilers was significantly influenced by MOS supplementation in the diet of broiler chicks. Improved feed intake in the MOS-supplemented group may be due to the healthy microbial habitation in the gut, leading to healthy intestine, and resulted in better feed absorption of the birds (Thong song et al. 2008). Our results are in agreement with the previous reports who found better feed intake in

broiler by adding MOS in the feed (Xu et al. 2003; Pelicano et al. 2004; Mohamed et al. 2008; Bozkurt et al. 2009; Koc et al. 2010).

Feed conversion ratio in broilers was significantly affected by MOS supplementation. Better feed conversion ratio in MOS-fed group may be due to the healthy intestinal tract of the birds and better nutrient utilization (Loddi et al. 2002). Decline in pathogenic microbial stock that struggle for nutrients in the gut of the bird may be helpful in enhancing FCR (Oyofe et al. 1989; Springs et al. 2000). In this study, birds which receive prebiotics have enhanced feed intake and body weight gain which ultimately gave a better FCR.

Oocyst count per gram of feces was significantly affected during 5th, 7th, 10th, and 12th dpi. The highest oocyst count was recorded in the infected untreated group which was decreased with supplementation of MOS and amprolium hydrochloride in *E. tenella*-infected birds. The decreased shedding of oocyst in the droppings of infected birds might be due to the fact that MOS depressed the growth of harmful bacteria (Fernandez et al. 2002). MOS has the capability to attach its mannose receptors to the intestine rather than metabolized to short chain fatty acids, which prevents the colonization of harmful pathogens in the gut (Crittenden and Playne 1996). MOS is fermented to lactic acid which reduces the pH of intestine, making it unfavorable for certain pathogenic bacteria (Chio et al. 1994; Gibson and Wang 1994). Williams (1995) found that there is an inverse relationship between the immune condition of chickens and the expelling of parasitic oocysts. Like our result, Elmusharaf et al. (2006, 2007) reported a reduced number of parasitic eggs in birds which were given MOS. Kamran et al. (2012) supplemented broiler diets with probiotic, prebiotics, butyric acids, and their

Table 3 Effect of MOS and amprolium hydrochloride on feed intake (g) in broiler birds challenged with *Eimeria tenella*

| Groups | Second week | Third week | Fourth week | Fifth week | Overall mean |
|------------------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|
| Control | 462.20 ^b ± 0.16 | 752.61 ^c ± 0.17 | 907.35 ^b ± 0.64 | 1053.5 ^b ± 1.45 | 3176.7 ^b ± 1.80 |
| Infected | 463.03 ^b ± 1.53 | 751.75 ^c ± 0.33 | 902.88 ^c ± 4.56 | 1001.30 ^d ± 1.09 | 3118.96 ^c ± 3.54 |
| Infected + MOS treated | 473.69 ^a ± 1.88 | 767.27 ^a ± 0.90 | 924.88 ^a ± 9.49 | 1070.6 ^a ± 0.41 | 3239.4 ^a ± 10.93 |
| Infected + amprolium hydrochloride | 461.68 ^b ± 0.88 | 757.69 ^b ± 0.87 | 907.75 ^b ± 0.31 | 1040.3 ^c ± 0.81 | 3171.4 ^b ± 0.93 |

Means in the same column with different superscripts are significantly different ($P < 0.05$)

Table 4 Effect of MOS and amprolium hydrochloride on feed conversion ratio of broiler birds challenged with *Emeria tenella*

| Groups | Second week | Third week | Fourth week | Fifth week | Overall mean |
|------------------------------------|-------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Control | 1.03 ± 0.00 | 1.62 ^b ± 0.00 | 1.80 ^b ± 0.01 | 1.60 ^c ± 0.03 | 1.52 ^c ± 0.01 |
| Infected | 1.03 ± 0.00 | 1.65 ^a ± 0.00 | 1.82 ^a ± 0.01 | 1.68 ^a ± 0.03 | 1.56 ^a ± 0.01 |
| Infected + MOS treated | 1.01 ± 0.00 | 1.58 ^c ± 0.00 | 1.78 ^c ± 0.02 | 1.58 ^c ± 0.04 | 1.50 ^d ± 0.01 |
| Infected + amprolium hydrochloride | 1.03 ± 0.01 | 1.64 ^a ± 0.00 | 1.80 ^b ± 0.00 | 1.65 ^b ± 0.01 | 1.54 ^b ± 0.00 |

Means in the same column with different superscripts are significantly different ($P < 0.05$)

combinations and reported lower oocyst shedding in the feces as compared with control group which supports our findings.

Lesion scores were significantly affected by MOS in the diet of broiler birds at all recorded stages in the present study. All the lesion scores were reported in the highest magnitude in infected untreated birds which were reduced by supplementing MOS and anticoccidial drug (amprolium hydrochloride) in the infected birds. The reduced lesions in the MOS-supplemented group may be due the fact that it is obtained from the cell wall of yeast *Sacchromyces cerevisiae* which is helpful in reducing harmful microbes and inducing the production of healthy microbes like *Lactobacillus* and *Bifidobacterium* variety (Fernandez et al. 2002). *Lactobacillus* bacteria produce compounds like bacteriocin and reuterin which have an inhibitory effect on certain pathogens (Lee et al. 2000). The reduction in pathogens reduces the clinical infection and macroscopic lesion in the gut of the chickens. MOS supplementation in the diet of chicken enhances the level of IgA immunoglobins in the intestines, which can reduce the number of bacteria by binding it to the specified antigens, thus prevents its entry to the mucosal membrane of the intestine and also reduces the severity of lesions (Kulkarni et al. 2010). Yeast cell wall (MOS) in the diet of birds boosts cell-mediated immune responses, which is a vital feature for the security against coccidial infections (Dzierszynski et al. 2008) and ultimately diminished lesions in the intestinal tract. Our result are supported by Moemen et al. (2011) who reported reduced lesion score in necrotic enteritis-infected chickens after feeding MOS. Kamran et al. (2012) reported lower cecal lesion with probiotics, prebiotics, and their combination as compared

to infected untreated group. Our results are in accordance with Sun et al. (2005) who reported lower lesion in mixed *Emeria* infection when fed with MOS.

Histopathological examination of the cecal tonsil revealed that MOS supplementation greatly enhanced the intestinal integrity. The enhancement in the intestinal structure may be due to the reason that MOS increases the humoral immunity by presenting the pathogenic organism or their cysts to immune cells as antigen which clears them from the intestines. Also, the MOS fermentation produces short chain fatty acids like butyrate, propionate, and acetate, which make the pH of intestine acidic, making the environment unfavorable for certain pathogenic bacteria (Brouns et al. 2002). Butyrate is necessary for the maintenance of intestinal epithelium and increases the immunity in the gut of chickens (Peuranen et al. 2006). In the infected untreated group, ruptured epithelium and glandular structures of the intestines having parasitic eggs may be due to the lower butyric acid concentration (Yang et al. 2007). Brouns et al. (2002) reported that a shortage of butyrate is associated with poor intestinal integrity and functions leading to lower immune response. Yang et al. (2009) reported the highest number of macrophages in prebiotic-supplemented animals which engulf the harmful pathogen thus enhance immunity and maintain intestinal integrity. Our results are supported by Zikic et al. (2008) who reported improved villus height in the gut of broilers when prebiotics were supplemented in their feed. Yang et al. (2007) also reported an increased villus height and improvement in the intestinal mucosa by prebiotic supplementation which is in line with our findings. Our results are in agreement with Baurhoo et al. (2007) who reported higher villus height as compared

Table 5 Effect of mannanoligosaccharide and amprolium hydrochloride on oocyst per gram of feces (OPG) expressed as $\log_{10}(X+1)$ in *Emeria tenella*-challenged birds

| Groups | OPG count | | | |
|------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | 5 dpi | 7 dpi | 10 dpi | 12 dpi |
| Control | 0.00 ^c ± 0.00 | 0.00 ^c ± 0.00 | 0.00 ^c ± 0.00 | 0.00 ^d ± 0.00 |
| Infected | 5.28 ^a ± 0.24 | 6.29 ^a ± 0.05 | 6.10 ^a ± 0.32 | 5.86 ^a ± 0.37 |
| Infected + MOS treated | 3.37 ^b ± 0.26 | 3.15 ^b ± 0.09 | 2.17 ^b ± 0.61 | 1.67 ^c ± 0.61 |
| Infected + amprolium hydrochloride | 4.25 ^b ± 0.52 | 3.46 ^b ± 0.18 | 3.09 ^b ± 0.06 | 2.99 ^b ± 0.34 |

Means in the same column with different superscripts are significantly different ($P < 0.05$)

Table 6 Effect of mannanoligosaccharide and amprolium hydrochloride on cecal lesion scoring in *Eimeria tenella*-challenged broiler birds

| Days (post infection) | Control | Infected | Infected + MOS treated | Infected + amprolium hydrochloride |
|---------------------------------|--------------------------|--------------------------|---------------------------|------------------------------------|
| Pinpoint hemorrhages | | | | |
| 5 | 0.00 ^c ± 0.00 | 1.67 ^a ± 0.08 | 0.49 ^{bc} ± 0.26 | 0.64 ^b ± 0.28 |
| 7 | 0.00 ^c ± 0.00 | 2.69 ^a ± 0.18 | 0.07 ^c ± 0.03 | 0.62 ^b ± 0.27 |
| 10 | 0.00 ^c ± 0.00 | 3.55 ^a ± 0.06 | 0.09 ^{bc} ± 0.02 | 0.15 ^b ± 0.04 |
| 12 | 0.00 ^b ± 0.00 | 2.93 ^a ± 0.26 | 0.29 ^b ± 0.18 | 0.04 ^b ± 0.03 |
| Thickness of cecal wall | | | | |
| 5 | 0.00 ^c ± 0.00 | 1.29 ^a ± 0.19 | 0.28 ^{bc} ± 0.25 | 0.68 ^{ab} ± 0.24 |
| 7 | 0.00 ^b ± 0.00 | 2.29 ^a ± 0.37 | 0.34 ^b ± 0.17 | 0.37 ^b ± 0.32 |
| 10 | 0.00 ^b ± 0.00 | 2.69 ^a ± 0.31 | 0.45 ^b ± 0.24 | 0.12 ^b ± 0.05 |
| 12 | 0.00 ^b ± 0.00 | 1.96 ^a ± 0.36 | 0.26 ^b ± 0.15 | 0.31 ^b ± 0.05 |
| Bloody fecal contents | | | | |
| 5 | 0.00 ^b ± 0.00 | 2.33 ^a ± 0.23 | 0.34 ^b ± 0.22 | 0.56 ^b ± 0.18 |
| 7 | 0.00 ^c ± 0.00 | 2.98 ^a ± 0.01 | 0.35 ^b ± 0.03 | 0.68 ^b ± 0.02 |
| 10 | 0.00 ^c ± 0.00 | 2.87 ^a ± 0.10 | 0.15 ^{bc} ± 0.09 | 0.24 ^b ± 0.01 |
| 12 | 0.00 ^c ± 0.00 | 2.84 ^a ± 0.10 | 0.17 ^{bc} ± 0.07 | 0.30 ^b ± 0.07 |
| Mucoid contents in cecum | | | | |
| 5 | 0.00 ^c ± 0.00 | 1.66 ^a ± 0.20 | 0.61 ^b ± 0.14 | 0.61 ^b ± 0.19 |
| 7 | 0.00 ^c ± 0.00 | 2.78 ^a ± 0.12 | 0.46 ^b ± 0.13 | 0.72 ^b ± 0.13 |
| 10 | 0.00 ^b ± 0.00 | 3.31 ^a ± 0.20 | 0.25 ^b ± 0.09 | 0.39 ^b ± 0.20 |
| 12 | 0.00 ^c ± 0.00 | 2.72 ^a ± 0.21 | 0.32 ^{bc} ± 0.12 | 0.63 ^b ± 0.17 |

Means in the same row with different superscripts are significantly different ($P < 0.05$). Negative control (Group A), positive control (Group B), infected treated with MOS (Group C), and infected treated with amprolium hydrochloride (Group D)

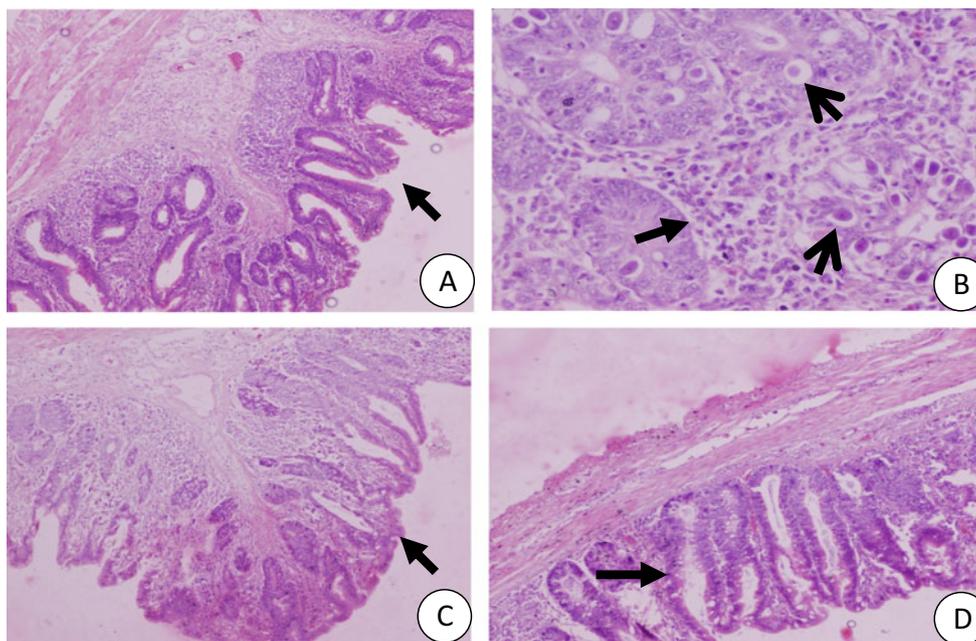


Fig. 1 **a** Cecal section of broiler showing normal epithelial layer (arrows) in birds in control group (H&E, 40×). **b** Section of broiler showing glandular structure infiltrated with coccidial oocyst (open arrows) and leukocytic infiltration (closed arrow) of a positive control group receiving infection but no MOS or treatment (H&E, 40×). **c** Cecal section of broiler showing intact and strong epithelial layer (arrow) of the

group receiving infection and given MOS treatment (H&E, 40×). **d** Cecal section of broiler showing repairing of epithelial layer (long arrows) and empty spaces in glandular region after oocyst elimination or control of oocysts receiving infection and treated with amprolium (H&E, 40×). Bar 50 μm for each

to control group when prebiotic like MOS was added into the birds' diet. Similarly, Xu et al. (2003) reported improved villus height by feeding fructooligosaccharides as prebiotics to broiler birds. Like our results, Houshmand et al. (2012) also found better intestinal structures with supplemental prebiotics in the diet of meat-type chickens.

Conclusion

From the results of the present study, it can be concluded that addition of mannanoligosaccharide can be used successfully in *Eimeria*-infected birds in comparison to amprolium hydrochloride to improve the growth performance and ameliorate the coccidial lesions.

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References

- Allen PC, Fetterer RH (2002) Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin Micro Rev* 15:58–65
- Baurhoo B, Phillip L, Ruiz-Feria CA (2007) Effects of purified lignin and mannanoligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poult Sci* 86:1070–1078
- Bezkorovainy A (2001) Probiotics: determinants of survival and growth in the gut. *Am J Clin Nutr* 73(suppl):399–405
- Bozkurt M, Kucukyilmaz K, Catli AU, Cinar M (2009) The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. *South Afr J Anim Sci* 39(3):197–205
- Brouns F, Kettlitz B, Arrigoni E (2002) Resistant starch and “the butyrate revolution”. *Trends Food Sci Technol* 3:251–261
- Chapman HD (1994) Sensitivity of field isolates of *Eimeria* to monensin following the use of coccidiosis vaccine in broiler chickens. *Poult Sci* 73:476–478
- Chapman HD (1997) Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasite of the fowl. *Avian Pathol* 26:221–244
- Chapman HD (1998) Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. *Int J Parasitol* 28:1141–1144
- Chapman HD (1999) The development of immunity to *Eimeria* species in broilers given anti coccidial drugs. *Avian Pathol* 28(2):155–162
- Chapman HD (2005) Perspectives for the control of coccidiosis in poultry by chemotherapy and vaccination. Proceedings of the IXth International Coccidiosis Conference, Foz de Iguassu, Parana, Brazil. Apinco Foundation of Poultry Science and Technology, Campinas, São Paulo, Brazil, pp 99–103
- Chio KH, Namkung H, Paik IK (1994) Effects of dietary fructooligosaccharides on the suppression of intestinal colonization of *Salmonella typhimurium* in broiler chickens. *Korean J Anim Sci* 36:271–284
- Crittenden RG, Playne MJ (1996) Production, properties and applications of food-grade oligosaccharides. *Trends Food Sci Technol* 7:353–360
- Cummings JH, Macfarlane GT (2002) Gastrointestinal effects of prebiotics. *Br J Nutr* 87:145–151
- Dzierszinski FS, Hunter CA (2008) Advances in the use of genetically engineered parasites to study immunity to *Toxoplasma gondii*. *Parasite Immunol* 30:235–244
- Elmusharaf MA, Beynen AC (2007) Coccidiosis in poultry with emphasis on alternative anticoccidial treatments. *Annals of the world association on animal pathology. Ann World Assoc Anim Pathol (AWAAP)* 5:13–32
- Elmusharaf MA, Bautista V, Nollet L, Beynen AC (2006) Effect of a mannanoligosaccharide preparation on *Eimeria tenella* infection in broiler chickens. *Int J Poult Sci* 5:583–588
- Ferret PR, Parks CW, Grims JL (2002) Benefits of dietary antibiotic and mannanoligosaccharide supplementation for poultry. In: *Proc. Multi-State Poult. Feeding and Nutr. Conf., Indianapolis, IN. May 14–16.*
- Fernandez F, Hinton M, Van Gils B (2002) Dietary mannanoligosaccharide and their effect on chicken caecal microflora in relation to *Salmonella* Enteritidis colonization. *Avian Pathol* 31:49–58
- Gibson GR (1999) Dietary modulation of the human microflora using the prebiotics oligofructose and inulin. *J Nutr* 129:1438–1441
- Gibson GR, Fuller R (2000) Aspects of *in vitro* and *vivo* research approaches directed towards identifying probiotics and prebiotics for human use. *J Nutr* 130:391–395
- Gibson GR, Roberfroid MB (1995) Dietary manipulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 125:1401–1412
- Gibson GR, Wang X (1994) Bifidogenic properties of different types of fructooligosaccharides. *Food Microbiol* 11:491–498
- Gibson GR, Beatty ER, Wang X, Cummings JH (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterol* 108:975–982
- Grizard D, Barthomeuf C (1999) Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. *Reprod Nutr Dev* 39:563–588
- Hatemink R (1995) Non digestible oligosaccharides: healthy food for the colon. Proceedings Symposium Wageningen, 4–5 December, 1–177.
- Hidaka H, Eida T, Takiwaza T, Tokungo T, Tashiro Y (1986) Effects of fructooligosaccharide on intestinal flora and human health. *Bifid Microflora* 5:37–50
- Hodgson JN (1970) Coccidiosis oocysts counting technique for coccidiostat evaluation. *Expt Parasitol* 28:99–102
- Houshmand M, Azhar K, Zulkifli I, Bejo MH, Kamyab A (2012) Effects of non-antibiotic feed additives on performance, immunity and intestinal morphology of broilers fed different levels of protein. *S Afr J Anim Sci* 42:22–32
- Iji PA, Saki AA, Tivey DR (2001) Intestinal structure and function of broiler chickens on diet supplemented with a mannanoligosaccharide. *J Anim Sci* 81:1186–1192
- Koc F, Samli H, Okur A, Ozduven M, Akyurek H, Senkoylu N (2010) Effects of *Saccharomyces cerevisiae* and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulg J Agri Sci* 16(5):643–650
- Kulkarni RR, Parreira VR, Jiang YF, Prescott JF (2010) A live oral recombinant *Salmonella* enteric serovar Typhimurium vaccine expressing *Clostridium Perfringens* antigens confers protection against necrotic enteritis in broiler chickens. *Clin Vaccine Immunol* 17: 205–214
- Lee DJ, Drongowski RA, Coran AG (2000) Evaluation of probiotic treatment in a neonatal animal model. *Pediatr Surg Int* 16:237–242
- Lillehoj HS, Min W, Dalloul RA (2004) Recent progress on the cytokine regulation of intestinal immune responses to *Eimeria*. *Poult Sci* 83: 611–623

- Loddi MM, Nakaghi LSO, Edens F, Tucci FM, Hannas MI, Moraes VMB, Arika J (2002) Mannan oligosaccharide and organic acids on intestinal morphology integrity of broilers evaluated by scanning electron microscopy. In: Proceedings 11th European Poult. Con. Bremen Germany, pp. 121-126.
- Long PL, Joyner LP, Millard BJ, Norton CC (1976) A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. *Folia Vet Latina* 6:201-217
- McDougald LR (2003) Coccidiosis in diseases of poultry. 11th Ed. Iowa State Press, Blackwell Publishing Company, USA. 974-991
- Miles RD, Boot Walla SM (1991) Direct-fed microbial in animal production—a review. *J Vet Med Anim Hlth* 1994 5:308-316
- Moemen AM, Abdel-Aziz MS (2011) Effect of mannanoligosaccharides (Biomass) on necrotic enteritis infection in broiler chickens. *Int J Poultry Sci* 9(10):685-690
- Mohamed MA, Hassan HMA, El-Barkouky EMA (2008) Effect of mannan oligosaccharide on performance and carcass characteristics of broiler chicks. *J Agric Soc Sci* 4:13-17
- NRC (1994) Nutrient requirements of poultry, 9th edn. Natl. Acad. Press, Washington, DC
- Oyofe BA, Deloach JR, Corrier DE, Norman JO, Ziprin RL, Mollenhauser HH (1989) Effects of carbohydrates on *Salmonella typhimurium* colonisation in broiler chickens. *Avian Dis* 33:531-534
- Pelicano ERL, de Souza PA, de Souza HBA, Leonel FR, Zeola NMBL, Boiago MM (2004) Productive traits of broiler chickens fed diets containing different growth promoters. *Rev Bras Cienc Avicola* 6: 177-182
- Peuranen S, Kocher A, Dawson KA (2006) Yeast cell preparations prevent the attachment of enteropathogenic *Escherichia coli* on broiler gut mucus. *Reprod Nutr Dev* 46:S111
- Ruff MD, Danforth HD (1966) Resistance of coccidia to medications. Pages 427-430 in Proc. XX World's Poult. Congr. vol. 11.
- SAS (1998) User's Guide, Statistics. SAS Institute, Cary, NC.
- Sonmez C, Eren M (1999) Effect of supplementation of zinc bacitracin, mannanoligosaccharide, and probiotics into the broiler feed on morphology of the small intestine. *Vet Fac Dergist Uludag Univ* 18:125-138
- Spring P, Wenk C, Dawson KA, Newman KE (2000) The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. *Poult Sci* 79:205-211
- Steel RGD, Torrie JH (1981) Principles and procedures of statistics. McGraw Hill Book.Co.Inc. New York.
- Sun X, McElroy A, Webb KE, Sefton AE, Novak C (2005) Broiler performance and intestinal alterations when fed drug-free diets. *Poult Sci* 84:1294-1302
- Taherpour K, Moravej H, Taheri HR, Shivazad M (2012) Effect of dietary inclusion of probiotic, prebiotic and butyric acid glycerides on resistance against coccidiosis in broiler chickens. *J Poultry Sci* 49(1):57-61
- Tanweer AJ, Chand N, Saddique U, Bailey CA, Khan RU (2014) Antiparasitic effect of wild rue (*Peganum harmala L.*) against experimentally induced coccidiosis in broiler chicks. *Parasitol Res* 113: 2951-2960
- Thong Song B, Kalandakonond-Thongsong S, Chavanaikul V (2008) Effects of the addition of probiotics containing both bacteria and yeast or an antibiotics on performance parameters, mortality rate and antibiotics residue in broilers. *Thai J Vet Med* 38(1):17-26
- Williams RB (1995) Epidemiological studies of coccidiosis in the domestic fowl (*Gallus gallus*): IV. Reciprocity between the immune status of floor-reared chickens and their excretion of oocysts. *Appl Parasitol* 36:90-298
- Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ (2003) Effects of dietary fructo oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult Sci* 82:1030-1036
- Yang Y, Iji PA, Kocher A, Mikkelsen LL, Choct M (2007) Effects of mannanoligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. *J Appl Poult Res* 16(2):280-288
- Yang Y, Iji PA, Choct M (2009) Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poult Sci J* 65:97-114
- Zikic D, Peric L, Uscebrka G, Stojanovic S, Milic D, Nollet L (2008) Effect of prebiotics in broiler breeder and broiler diets on performance and jejunum morphology of broiler chickens. 1st Mediterranean Summit of WPSA, Book of Proceedings, Porto Carras, Greece, 879-882.