Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

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

Somaia S Abu-Akkada[1]* and Ashraf M Awad[2]

[1]Department of Parasitology; [2]Department of Avian and Aquatic Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt
*Corresponding author: somaia_abuakkada@yahoo.com

ARTICLE HISTORY (15-095)

Received: February 22, 2015
Revised: April 15, 2015
Accepted: May 03, 2015

Key words:
Probiotic
Prebiotic
Coccidiosis
Eimeria tenella
Broilers

A B S T R A C T

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 ($4x10^4$) 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.

To Cite This Article: Abu-Akkada SS and AM Awad, 2015. Protective effects of probiotics and prebiotics on *Eimeria tenella*-infected broiler chickens. Pak Vet J, 35(4): 446-450.

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; Mahmood *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 third week till the completion 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 $4x10^4$ 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 PI. 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

Table 1: The composition of probiotic and prebiotic additives used in the experiment

Probiotic (Lacto G)® each 1 kg contains		Prebiotic (Immunolin)® each 1L contains		
Component	Quantity	Component		Quantity
Lactobacillus acidophilus	206 x10^8 cfu/kg	Mannanoligosaccharide		25000 mg
Lactobacillus plantarum	126 x10^8 cfu/kg	B- glucan		25000 mg
Lactobacillus casei	206 x10^8 cfu/kg	Sorbitol		50000 mg
Lactobacillus bulgaricus	206 x10^8 cfu/kg	Edible sugar		23500 mg
Bifidobacterium bifidum	100 x10^8 cfu/kg	Nigellone		100000 mg
Streptococcus thermophiles	410 x10^8 cfu/kg	Echincea extract		100000 mg
Streptococcus faecium	540 x10^8 cfu/kg	Vitamins	Vit E	5000 IU
Saccharomyces boutardii	100 x10^8 cfu/kg		Vit C	50000 IU
Aspergillus oryzae	532 x10^7 cfu/kg	Organic minerals	Zinc	4000 mg
Torulopsis spp.	532 x10^7 cfu/kg		Selenium	200 mg
Amylase Enzyme	100000 u/kg		KCl	25000 mg
Xylanase Enzyme	15750 u/kg		Na HCO_3	20500 mg
Lactose Up to	1 kg		Cobalt	100 mg
		Aminoacids		100000 mg
		Propyline glycal		50000 mg

Table 2: Effect of probiotic (Lacto G) ® and prebiotic (Immunolin) ® on weight gain and feed conversion ratio of broiler chickens challenged with *E. tenella* at different periods of the experiment

Group	Weight gain (g)			Feed conversion ratio (FCR)		
	BW gain-1 (21–28 days)	BW gain-2 (28–35 days)	Total BW gain (21-35 days)	FCR-1 (21–28 days)	FCR-2 (28–35 days)	Total FCR (21-35 days)
1	415± 59^a	443±59^a	859±111^a	1.77±0.23^b	1.79±0.24^c	1.78±0.22^b
2	414±124^a	447±122^a	861±144^a	1.92 ±0.63^c	1.92 ±0.63^c	1.63±0.28^b
3	422±72^a	375±81^b	797±131^b	2.13±0.32^b	2.13±0.32^b	2.09±0.24^a
4	340±74^b	373±55^b	714±78^b	2.43±0.60^b	2.43±0.60^b	1.98±0.35^a
5	357±58^b	367±147^b	724±78^b	3.25±3.89^a	3.25±3.89^a	1.93±0.34^a
6	445±65^a	440±61^a	886±144^a	1.79 ±0.29^c	1.79 ±0.29^c	1.65±0.24^b

Values (mean±SE) with different letters within the same column are significantly (P<0.05) different. Groups 1) probiotic non-challenged; 2) prebiotic non-challenged; 3) probiotic challenged; 4) prebiotic challenged; 5) non-treated challenged (positive control) and 6) non-treated non-challenged (negative control).

Table 3: Effect of probiotic (Lacto G)® and prebiotic (Immunolin)® on mortality %, lesion scores and dropping scores in broiler chickens challenged with *E. tenella*

Groups	Mortality %	Lesion score	Dropping score
1	0	0.00±0.00	0.00±0.00
2	0	0.00±0.00	0.00±0.00
3	0	1.33±0.58^b	1.00±0.00^c
4	20	3.00±1.00^a	3.00±0.00^a
5	20	3.00±0.00^a	3.00±0.00^b
6	0	0.00±0.00	0.00±0.00

Values (mean±SE) with different letters within the same column are significantly (P<0.05) different. Groups 1) probiotic non-challenged; 2) prebiotic non-challenged; 3) probiotic challenged; 4) prebiotic challenged; 5) non-treated challenged (positive control) and 6) non-treated non-challenged (negative control).

Table 4: Effect of probiotic (Lacto G)® and prebiotic (Immunolin)® on weight of lymphoid organs(spleen, bursa and thymus) of broiler chickens challenged with *E. tenella*

Groups	Weight (g) 7 days PC			PI	PA
	Spleen	Bursa	Thymus		
1	1.26±0.30^a	2.08±0.05^a	6.77±1.39^a	1.40±0.05^b	17.00±0.50^b
2	1.02±0.08^a	1.86±0.10^b	3.66±0.01^b	1.42±0.14^b	21.00±0.50^a
3	1.19±0.16^a	1.65±0.39^b	4.09±0.90^b	1.68±0.08^a	17.83±0.76^b
4	0.96±0.22^{ab}	1.07±0.04^c	3.48±0.01^b	1.28±0.03^b	16.00±0.00^b
5	0.75±0.24^b	0.82±0.15^c	4.83±1.00^b	1.40±0.05^b	16.00±1.50^b
6	1.31±0.02^a	2.69±0.32^a	6.40±0.40^a	1.33±0.06^b	17.00±2.00^b

PC=Post-challenge; PI=Phagocytic Index; PA=Phagocytic activity. Values (mean±SE) with different letters within the same column are significantly (P<0.05) different. Groups 1) probiotic non-challenged; 2) prebiotic non-challenged; 3) probiotic challenged; 4) prebiotic challenged; 5) non-treated challenged (positive control) and 6) non-treated non-challenged (negative control).

score (1) compared to those observed in infected control chickens (3). While the group of birds supplemented with prebiotics (4th group) did not show any improvement in cecal lesion or dropping scores compared with the control infected birds.

The oocyst count from 7th to 13th day PC is shown in Table 4. Generally, it could be noticed that oocyst count 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 3th 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

Table 5: Effect of probiotic (Lacto G) ® and prebiotic (Immunolin) ® on oocyst counts (per gram of feces) 7th to 13th post challenge in broiler chickens challenged with *E. tenella*

Groups	Experimental Days							Total
	7	8	9	10	11	12	13	
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	133±58^{c}	133±58^{c}	0.00±0.00	0.00±0.00	0.00±0.00	267±58^{c}
4	3600±100^{b}	3850±10^{b}	21033±58^{b}	132000±1000^{b}	7200±100^{b}	1500±50^{b}	583±29^{b}	169767±1220^{b}
5	21033±58^{a}	87900±100^{a}	276000±1000^{a}	335666±577^{a}	36100±100^{a}	10033±58^{a}	4000±100^{a}	770733±1656^{a}
6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values (mean±SE) with different letters within the same column are significantly (P<0.05) different. Groups 1) probiotic non-challenged; 2) prebiotic non-challenged; 3) probiotic challenged; 4) prebiotic challenged; 5) non-treated challenged (positive control) and 6) non-treated non-challenged (negative control).

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 Probiotic or prebiotic 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

Abbas RZ, DD Colwell, Z Iqbal and A Khan, 2014. Acaricidal drug resistance in poultry red mite (Dermanyssus gallinae) and approaches to its management. Worlds Poult Sci J, 70: 113-124.

Abbas RZ, DD Colwell, and J Gilleard, 2012. Botanicals: An alternative approach for the control of avian coccidiosis. Worlds Poult Sci J, 68: 203-215.

Abu-Akkada SS and AM Awad, 2012. Isolation, propagation, identification and comparative pathogenicity of five Egyptian field strains of *Eimeria tenella* from broiler chickens in five different provinces in Egypt. Res Vet Sci, 92: 92-95.

Alkhalf A, M Alhaj and I Al-Homidan, 2010. Influence of probiotic supplementation on immune response of broiler chicks. Poult Sci, 30: 271-280.

Bachaya HA, RZ Abbas, MA Raza, Z Iqbal, TU Rehman *et al*., 2015. Existence of coccidiosis and associated risk factors in broiler chickens in Southern Punjab, Pakistan. Pak Vet J, 35: 81-84.

Bozkurt M, N Aysul, K Kucukyilmaz, S Aypak, G Ege *et al*., 2014. Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. Poult Sci, 93: 389-399.

Dalloul RA, HS Lillehoj, TA Shellem and JA Doerr, 2003. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. Poult Sci, 82: 62-66.

Elmusharaf MA, V Bautista and AC Beynen, 2006. Effect of a mannanoligosaccharide preparation on *Eimeria tenella* infection in broiler chickens. Int J Poult Sci, 5: 583-588.

Fernandez F, M Hinton and B Van Gils, 2002. Dietary mannan-oligosaccharide and their effect on chicken caecal microflora in relation to *Salmonella Enteritidis* colonization. Avian Pathol, 31: 49-58.

Giannenas I, E Papadopoulos, E Tsalie, E Triantafillou, S Henikl *et al*., 2012. Assessment of dietary supplementation with probiotics, on performance, and intestinal morphology and microflora of chickens infected with *Eimeria tenella*. Vet Parasitol, 188: 31-40.

Gibson GR and MB Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr, 125: 1401-1412.

Johnson JK and WM Reid, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp Parasitol, 28: 30-36.

Kawahara E, T Ueda and S Nomura, 1991. In vitro phagocytic activity of white-spotted shark cells after injection with *Aermonas salmonicida* extracellular products. Gyobyo Kenkyu, Japan, 26: 213-214.

Lee SH, HS Lillehoj, RA Dalloul, DW Park, YH Hong *et al*., 2007. Influence of *Pediococcus* based probiotic on coccidiosis in broiler chickens. Poult Sci, 86: 63-66.

Long PL and LP Joyner, 1976. A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Vet Lat, 6: 201-217.

Mahmood MS, I Hussain, MF Ahmad, A Khan, RZ Abbas *et al*., 2014a. Immunomodulatory effects of *Pimpinella anisum* L. (Aniseed) in broiler chicks against Newcastle disease and infectious bursal disease viruses. Bol Latinoam Caribe Plant Med Aromat, 13: 458-465.

Mahmood MS, MF Ahmad, I Hussain, RZ Abbas, A Khan *et al*., 2014b. Growth promoting effect of *Pimpinella anisum* (Aniseed) in broiler chickens. Bol Latinoam Caribe Plant Med Aromat, 13: 278-284.

Mahmood K, SU Rahman, I Hussain, RZ Abbas, T Khaliq *et al*., 2014c. Non antibiotic strategies for the control of necrotic enteritis in poultry. Worlds Poult Sci J, 70: 865-879.

Morehouse NF and RR Barron, 1970. Coccidiosis: evaluation of coccidiostats by mortality, weight gains and fecal score. Exp Parasitol, 28: 25-29.

Mountzouris KC, P Tsirtsikos, E Kalamara, S Nitsch, G Schatzmayr *et al*., 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating caecal microflora composition and metabolic activities. Poult Sci, 86: 309-317.

Mountzouris KC, V Paraskevas, P Tsirtsikos, I Palamidi and T Steiner, 2011. Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim Feed Sci Technol, 168: 223-231.

SAS, 2002. Statistical Analysis System. Version 9, SAS Institute Inc., Cary NC, USA

Snel J, HJM Harmsen, PWJJ Van De Wielen and BA Williams, 2002. Dietary supplementedwith *Bacillus coagulans* as probiotic. Br Poult Sci, 39: 526-529.

Spring P, C Wenk, KA Dawson and KE Newman, 2000. The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella challenged broiler chicks. Poult Sci, 79: 205-211.

Taherpour K, H Moravej, HR Taheri and M Shivazad, 2012. Effect of dietary inclusion of probiotic, prebiotic and butyric acid glycerides on resistance against coccidiosis in broiler chickens. J Poult Sci, 49: 57-61.

Teo AY and HM Tan, 2007. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). J Appl Poult Res, 16: 296-303.

Williams RB and SJ Andrews, 2001. The origin and biological significance of thecoccidial lesions that occurs in chickens vaccinated with live attenuated anticoccidial vaccine. Avian Pathol, 30: 215-220.

Willis WL, OS Isikhuemhen and SA Ibrahim, 2007. Performance assessment of broilerchickens given mushroom extract alone or in combination with probiotics. Poult Sci, 86: 1856-1860.

Yang CM, GT Cao, PR Ferket, TT Liu, L Zhou *et al*., 2012. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function cecal microflora in broiler chickens. Poult Sci, 91: 2121-2129.

Zaman MA, Z Iqbal, RZ Abbas and MN Khan, 2012. Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. Parasitology, 139: 237-243.