Comparative Efficacy Of Prebiotic Mannan-Oligosaccharide And Diclazuril In Chickens Experimentally Infected With *Eimeria tenella*

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ABSTRACT

The aim of this study was to use the combination of diclazuril, in a therapeutic dose, plus prebiotic mannan-oligosaccharide (MOS) to control *Eimeria tenella* infection in Hubbard chickens. One hundred and fifty, one-day old apparently healthy Hubbard chicks were divided into 5 equal groups (30 each). The first group was served as a negative control. The other groups were infected with $1x10^5$ sporulated oocysts of *E. tenella* on the 14th day of age. The second group was considered a positive control (infected and non-treated). The third group was fed ration mixed with standard dose of diclazuril (1 ppm). The fourth group was fed ration mixed with prebiotic mannan-oligosaccharide (MOS) (1g / kg ration). The fifth group was fed ration mixed with diclazuril plus MOS in the same dose and duration. Our results revealed that the combinations of diclazuril plus MOS elicited better anticoccidial effect. These combinations showed a significant increase in body weight, body weight gain, R.B.Cs, W.B.Cs, serum total protein, albumin and globulin. These results were confirmed by the histopathological pictures. It could be inferred that, the anticoccidial effect of diclazuril was enhanced by MOS.

INTRODUCTION

Avian coccidiosis is a protozoan disease caused by apicomplexon intracellular protozoan of the genus *Eimeria*. Currently, the pathogenic species in chickens are *Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, E. mitis,* and *E. mivati.* Coccidiosis is the major parasitic disease of poultry.

Prebiotic compound was defined as non digestible feed ingredient that beneficially affects the host by stimulating the growth and / or activity of one or a limited number of colonic microflora and thus improves gut health. Prebiotics are hydrolyzed in the upper gastrointestinal tract and are able to favorably alter the colonic microflora *(1)*.

Mannan-oligosaccharide (MOS) is one of prebiotics that present in the cell wall of yeast (YCW) and has been shown to alter microbial population in livestock. Yeast cell wall contains a high affinity ligand for bacteria and offers a competitive binding for bacteria *(2)*. The dietary supplementation of MOS (0.05 % of YCW) increased local, cellular and humoral immune responses, and reduced oocyst output in the feces in chickens during natural exposure to coccidian parasite *(3)*. Supplemental MOS also has been shown to increase the production of immunoglobulin A in rats *(4)*, dogs *(5)*, and turkeys *(6)*. Yeast cell wall containing MOS reduces intestinal *Salmonella* concentrations by 26 % in broiler chicks *(7)* compared with that fed an unsupplemented diet. MOS also was used to improve growth performance of young chicks from 0 to 21 day of age *(8)*.

Prebiotic oligo-saccharide (MOS) at 1g / kg of diet for 18 weeks increased the concentration of bifidobacteria and decreased the concentration of *Clostridium perferingens* in turkeys *(9)*. It also improved live weight when compared with the controls and was similar to turkeys fed a diet containing bacitracin methylene disalicylate *(10)*. MOS improved the growth performance, metabolizable energy and amino acid digestibility and reduced the cecal population of *Clostridium perferingens* in chicks *(11)*.

MOS increased the production of IgA concentrations in the intestinal and tracheal mucosa. Moreover, MOS supplementation reduced the number of Eimeria spp. oocysts excreted in the faeces *(12)*. It is thought that

execratory IgA attached the parasite surface and prevented its binding to the intestinal epithelium and also reduced the development of sporozoites and merozoites and prevented invasion within host cell ***(13, and 14).*** In addition, MOS increased secretion of intestinal IgA which was responsible for Eimeria spp. infections and tracheal IgA which systemically responsible for respiratory infections, thus MOS has an immuno-regulatory effect at local and systemic level ***(15).***

The anticoccidial efficacy of diclazuril against *Eimeria spp.* in broiler chickens was firstly reported in 1989 ***(16).*** Diclazuril is highly and more efficacious anticoccidial drug than the other chemical and ionophorous anticoccidials in broiler chickens ***(16-20).*** Meanwhile, diclazuril did not interfere with development of immunity to *E. brunetti, E. maxima, E. necatrix* and *E. tenella* ***(21-23).***

Coccidial infections have been controlled, to a great extent, with the use of anticoccidial drugs; however, the increase in resistance to many of these products has raised concerns about the need for new anticoccidial alternatives ***(24).*** Coccidial and some bacterial infections were also controlled by the use of mannan-oligosaccharide (MOS) ***(3,8,11)***

Our study aims to use the prebiotic (dietary mannan-oligosaccharide (MOS) to control *Eimeria tenella* infection in Hubbard chickens. The effects of MOS on growth performance, lesion scoring, some haematological, biochemical parameters and histopathological changes were also determined.

MATERIAL AND MEHODS

1.Birds

One hundred and fifty, one-day old apparently healthy Hubbard chicks (male and female) were purchased from Dakahleia Poultry Company and numbered by leg bangles. The birds were allotted in separate units of metal wire-floored battery after arranging them using the ranking method ***(25).***

2.Drugs

Diclazuril (Clinacox®; Schering Plough Comp.) was added to the ration in a dose of 1 ppm ***(20).*** Prebiotic (Nutricell-MOS®; Industrial Comercio Exprtacaoe Importacao Ltda (ICC, Sao Paulo, Brazil) was added to the ration in a dose of 1g / kg of ration ***(11).***

3. Ration and Water

Chicks were fed on commercial standard balanced ration from Cairo Poultry Company without supplementation of anticoccidial drugs. It contained crude protein not less than 21%, crude fat not less than 2.7%, crude fibers not more than 2.7% and metabolizing energy not less than 2950 Kcal / kg ration. The ration and fresh water were offered to the chicks *ad libitum*. The ration was sterilized in the oven at 65 °C for 18 hr to destroy the probable accidental sporulated oocysts of *Eimeria* which may contaminate the rations. The water was boiled then cooled before offered to the chicks.

4. Experimental design

One hundred and fifty, apparently healthy, one-day old Hubbard chicks were used. They were divided into five equal groups of 30 chicks each. The first group was served as a negative control (non-infected and non-treated). The other groups were directly inoculated intra-crop, using stomach tube, with $1x10^5$ sporulated oocysts of *E. tenella* on the 14^{th} day of age ***(23).*** The second group was served as a positive control (infected and non-treated). Chicks in the three remaining groups were fed ration mixed with the tested drugs for six successive weeks. The third group was fed ration mixed with standard dose of diclazuril 1ppm (1 mg / kg ration) ***(20).*** The fourth group was fed ration mixed with Prebiotic (dietary mannan-oligosaccharide) at the dose of 1 g/kg ration ***(11).*** The fifth group was fed ration mixed with both of diclazuril (1 mg / kg ration) plus Prebiotic [dietary mannan-oligosaccharide (1g/kg ration)]. The design was shown in Table (1).

Table 1. Experimental design

Group No.	Number of chicks per group	Infected with $1x10^5$ sporulated oocysts of *E. tenella* /chick	Treatments		
			Diclazuril 1 ppm (1mg/kg ration)	Prebiotic (MOS) (1g/kg ration)	Diclazuril 1 ppm + Prebiotic (MOS) (1g/kg ration)
1	30	-	-	-	-
2	30	+	-	-	-
3	30	+	+	-	-
4	30	+	-	+	-
5	30	+	-	-	+
Experimental infection					
Time of infection		On the 14^{th} day of age			
Infective dose per chick		$1x\ 10^5$ sporulated oocysts of *Eimeria tenella*			
Route of infection		Directly inoculated intra-crop by the stomach tube			
Sampling					
Parameters	**No. of samples per group**	**Time of sampling**			
Oocysts output	All birds	Daily from the 5^{th} To the 14^{th} day PI			
Lesion scoring	5	On the 7^{th} day PI			
Growth performance	All birds	At one-day old and then weekly till 6 weeks			
Haematological	5	On the 14^{th} day PI			
Biochemical	5	On the 14^{th} day PI			
Histopathology	5	On the 8^{th} PI			

PI= Post infection

5. Oocyst inoculation

A field strain of *E. tenella* oocysts has been previously recognized and identified in the Poultry Diseases Dept., Faculty of Vet. Med., Moshtohor, Banha University, since 1999. The strain was isolated from the caeca of naturally infected chickens by the single oocyst isolation technique described by ***(26)***. The parasite was repeatedly passed in one-day old chicks every two months. The oocysts were preserved in 2.5% potassium dichromate solution. The chicks were inoculated intra-crop (using stomach tube) with ($1x10^5$) sporulated oocysts of *E. tenella* on the 14^{th} day of age. Ten grams of the faeces were collected daily for ten successive days post inoculation (PI), starting from the 5^{th} to the 14^{th} day PI. The collected droppings were preserved in pot. Dichromate (2.5%) till to be counted, using the McMaster technique ***(27)***.

6.Growth performance parameters

The chicks were individually marked and weighed at one-day old, then body weight was recorded weekly till the end of the experiment (the sixth week). Body weight gains and feed conversion ratio (FCR) were calculated weekly.

7.Haematological and biochemical examinations

Blood samples were collected from the jugular vein of five chicks of each group. The samples were collected on the 14^{th} day post infections. Each sample was divided into two parts: the first part was collected in tube containing EDTA (1mg / ml fresh blood) for haematological study which included erythrocytic (RBCs) and total leucocytic counts (WBCs) ***(28)***. The second part was allowed to separate the serum and kept at -20 °C till biochemical analysis to determine the serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) enzyme activity ***(29)***, total protein ***(30)***, albumin ***(31)***, globulins (difference between total protein and albumin), serum uric acid ***(32)*** and creatinine ***(33)***.

8. Histopathological studies

One centimeter of the caeci of the inoculated chicks with *E. tenella* was removed immediately after slaughtering on the 8th day PI, slit opened longitudinally and fixed in 10% neutral buffered formalin for 10 days. Five micron thick paraffin sections were stained with H&E *(34)* and examined microscopically. The developmental stages (schizonts, gametocytes and oocysts) were counted per microscopic field from stained sections.

9.Statistical analysis

The data were analyzed by F-test (one-way ANOVA) *(35)* using a computer program **(SPSS 11)** *(36)*.

RESULTS

The overall mean of oocysts output per gram faeces of the infected Hubbard chicks with *E. tenella* and treated with diclazuril and mannan-oligosaccharide (MOS) was significantly decreased when compared with the infected chicks and treated with diclazuril (1 ppm) or MOS (1 g /kg ration) as well as the positive control. The reduction rate of the infected chickens with *E. tenella* and treated with diclazuril and mannan-oligosaccharide (MOS) was excellent when compared with the positive control or infected chickens and treated with diclazuril (1 ppm) or MOS (1 g / kg ration) alone (Table, 2).

Table 3 showed the effect of diclazuril and / or mannan-oligosaccharide (MOS) on lesion scoring of the infected broilers with *E. tenella*. Lesion scores of the infected chickens with *E. tenella* and treated with either diclazuril (1 ppm) or mannan-oligosaccharide (MOS) (1 g / kg ration) were not significantly changed when compared with each other. Meanwhile, they were significantly decreased when compared with the positive control group.

The growth performance parameters were evaluated weekly and the data were showed in Table 4. The recorded results elicited a significant increase in body weight and body weight gain of the groups infected and treated with either diclazuril (1 ppm) or MOS (1 g / kg ration) when compared with the negative control group (non infected and non treated). The group infected and treated with diclazuril plus MOS elicited a significant increase in body weight and body weight gain when compared with the other groups.

The obtained results revealed that the group infected and treated with diclazuril plus MOS showed a significant decrease in feed conversion ration (FCR) when compared with the other groups.

The haematological and biochemical parameters in the chickens experimentally infected with ($1x10^5$) sporulated oocysts of *E. tenella* were evaluated on the 14th day PI and the data were represented in Table 5. The obtained haematological data revealed a significant increase in Red blood corpuscles (R.B.Cs) and White blood cells (W.B.Cs) of the groups infected and treated with MOS only or diclazuril plus MOS when compared with the other groups.

The obtained biochemical data elicited a significant increase in ALT and AST enzyme activities of the positive control group (infected non treated group) when compared with the other groups. The groups infected and treated with MOS only or diclazuril plus MOS showed a significant increase in serum total protein, albumin and globulin when compared with the other groups.

No significant changes in serum uric acid levels on the 14th day PI in all experimental groups (Table 5).

On the 8th day PI, microscopically, numerous second-generation schizonts, gametocytes and mature oocysts of *E. tenella* were seen in the epithelial lining of the caecal Lieberkohn glands of positive control (Fig.1). There were few number of the second-generation schizonts, gametocytes and mature oocysts in the epithelial lining of the Lieberkohn glands of the caeca, besides slight necrosis and degenerative changes in the epithelial cells in the presence of leukocytic infiltration in the lamina propria in the infected chickens with *E. tenella* and treated with diclazuril (1 ppm) (Fig.2), and MOS (1 g / kg ration) (Fig.3). The chickens infected with *E. tenella* and treated with diclazuril and mannan-oligosaccharide (MOS) showed few different developmental stages, slight necrosis and degenerative changes in the epithelial cells, besides leukocytic infiltration (Fig.4).

Table 2. Effect of MOS and /or Diclazuril on oocysts output of broiler chickens infected with *E. tenella* (n=12). (Mean ±SE)

Days PI	Oocysts output (---x10^5 oocysts/gram faeces)					
	Groups					LSD at P ≤ 0.05)
	-Ve control	+Ve control	Dicl	MOS	Dicl + MOS	
5	0.00 ^{d} ± 0.00	16.00 ^{a} ± 1.05	7.75 ^{b} ± 0.33	8.92 ^{b} ± 0.54	4.67 ^{c} ± 0.61	3.08*
6	0.00 ^{e} ± 0.00	36.92 ^{a} ± 1.79	17.08 ^{c} ± 0.65	20.33 ^{b} ± 0.77	12.50 ^{d} ± 0.57	3.25*
7	0.00 ^{e} ± 0.00	94.17 ^{a} ± 1.55	36.08 ^{c} ± 1.29	46.08 ^{b} ± 1.01	21.92 ^{d} ± 1.20	10.00*
8	0.00 ^{e} ± 0.00	169.00 ^{a} ± 3.27	69.67 ^{c} ± 2.34	78.80 ^{b} ± 1.14	42.17 ^{d} ± 0.88	8.33*
9	0.00 ^{e} ± 0.00	141.83 ^{a} ± 2.56	54.50 ^{c} ± 1.18	61.08 ^{b} ± 1.68	30.67 ^{d} ± 0.89	6.58*
10	0.00 ^{e} ± 0.00	121.08 ^{a} ± 1.37	30.50 ^{c} ± 0.42	37.50 ^{b} ± 1.03	19.67 ^{d} ± 0.77	7.00*
11	0.00 ^{d} ± 0.00	102.17 ^{a} ± 1.49	21.50 ^{b} ± 0.87	23.58 ^{b} ± 0.82	10.58 ^{c} ± 0.79	10.58*
12	0.00 ^{d} ± 0.00	86.17 ^{a} ± 2.40	7.25 ^{c} ± 0.45	13.08 ^{b} ± 0.58	1.67 ^{d} ± 0.56	5.58*
13	0.00 ^{c} ± 0.00	69.33 ^{a} ± 1.58	3.17 ^{b} ± 0.63	3.67 ^{b} ± 0.58	0.00 ^{c} ± 0.00	3.17*
14	0.00 ^{c} ± 0.00	58.38 ^{a} ± 1.63	1.33 ^{bc} ± 0.26	2.50 ^{b} ± 0.38	0.00 ^{c} ± 0.00	2.50*
Overall mean	0.00 ^{d} ± 0.00	88.81 ^{a} ± 4.20	25.47 ^{b} ± 2.01	30.19 ^{b} ± 2.23	14.35 ^{c} ± 1.25	11.12*
Reduction %	0.00	0.00	71.32	66.01	83.84	---

-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa. - Dicl = Diclazuril at dose of (1 mg / kg ration) -MOS = Mannan-oligosaccharide (prebiotic) at dose of (1 g / kg ration). - LSD = Least significance difference at P ≤ 0.05 (in the same row). - (*) = Significance at P ≤ 0.05.

Table 3. Effect of MOS and /or Diclazuril on lesion scoring of broiler chickens infected with *E. tenella* (n=5). (Mean ±SE)

Days PI	Lesion Scoring					
	Groups					LSD at P ≤ 0.05)
	-Ve control	+Ve control	Dicl	MOS	Dicl + MOS	
The 7th day PI	0.00 ^{d} ± 0.00	3.80 ^{a} ± 0.13	2.00 ^{b} ± 0.21	2.30 ^{b} ± 0.15	1.50 ^{c} ± 0.17	0.50*

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Table 4. Effect of MOS and/or Diclazuril on some growth performance parameters (body weight, body weight gains (gm) and FCR) of broiler chickens infected with *E. tenella* (n=5). (Mean ±SE)

Time	Growth performance parameters	Growth Performance Parameters					
		Groups					LSD At $P \leq 0.05$)
		-Ve control	+Ve control	Dicl	MOS	Dicl + MOS	
One-day	Body weight (gm)	$47.30^{a} \pm 0.57$	$47.41^{a} \pm 0.51$	$46.88^{a a} \pm 0.64$	$47.53^{a} \pm 0.57$	$46.97^{a} \pm 0.63$	NS
0-7	Body weight (gm)	$131.98^{b} \pm 2.63$	$133.44^{b} \pm 3.15$	$137.86^{b} \pm 4.51$	$142.40^{a} \pm 1.45$	$151.44^{a} \pm 2.01$	10.42*
	Body gain (gm)	$84.68^{b} \pm 2.15$	$86.03^{b} \pm 3.12$	$90.98^{b} \pm 2.26$	$94.87^{a} \pm 3.01$	$104.47^{a} \pm 4.22$	10.19*
	FCR	$1.60^{a} \pm 0.05$	$1.62^{a} \pm 0.03$	$1.34^{b} \pm 0.06$	$1.32^{bc} \pm 0.02$	$1.28^{c} \pm 0.03$	0.28*
8-14	Body weight (gm)	$355.74^{b} \pm 11.05$	$358.63^{b} \pm 12.11$	$361.42^{b} \pm 11.55$	$394.35^{a} \pm 9.66$	$422.11^{a} \pm 8.66$	38.61*
	Body gain (gm)	$223.76^{b} \pm 12.45$	$225.19^{b} \pm 10.21$	$223.56^{b} \pm 9.63$	$251.95^{a} \pm 11.54$	$270.67^{a} \pm 13.40$	28.39*
	FCR	$1.76^{a} \pm 0.03$	$1.84^{a} \pm 0.06$	$1.55^{b} \pm 0.05$	$1.51^{bc} \pm 0.03$	$1.431^{c} \pm 0.0.2$	0.25*
15-21	Body weight (gm)	$614.41^{c} \pm 14.62$	$577.57^{d} \pm 16.42$	$667.35^{b} \pm 20.45$	$655.74^{b} \pm 14.22$	$708.33^{a} \pm 16.30$	36.84*
	Body gain (gm)	$258.67^{c} \pm 9.81$	$218.94^{d} \pm 8.65$	$305.93^{b} \pm 7.84$	$261.39^{b} \pm 10.41$	$286.22^{a} \pm 9.78$	24.83*
	FCR	$1.90^{b} \pm 0.04$	2.11^{a} 0.07	$1.76^{c} \pm 0.03$	$1.73^{c} \pm 0.05$	$1.62^{d} \pm 0.06$	0.11*
22-28	Body weight (gm)	$925.87^{c} \pm 13.33$	$844.61^{d} \pm 12.85$	$974.86^{b} \pm 20.45$	$995.24^{ab} \pm 19.05$	$1127.46^{a} \pm 20.64$	81.26*
	Body gain (gm)	$311.46^{c} \pm 11.40$	$267.04^{d} \pm 7.36$	$307.51^{b} \pm 9.63$	$339.5^{ab} \pm 9.85$	$419.13^{a} \pm 8.45$	28.04*
	FCR	$2.23^{b} \pm 0.06$	$2.53^{a} \pm 0.08$	1.91^{c} 0.03	$1.89^{c} \pm 0.04$	$1.737^{d} \pm 0.07$	0.30*
29-35	Body weight (gm)	$1286.41^{c} \pm 20.11$	$1172.75^{d} \pm 19.55$	$1385.63^{b} \pm 26.35$	$1419.36^{b} \pm 24.61$	$1611.24^{a} \pm 25.67$	225.61*
	Body gain (gm)	$360.54^{c} \pm 8.77$	$328.14^{d} \pm 11.25$	$410.77^{b} \pm 12.45$	$424.12^{b} \pm 10.66$	$483.78^{a} \pm 14.50$	59.66*
	FCR	$2.39^{b} \pm 0.02$	$2.67^{a} \pm 0.04$	$1.98^{c} \pm 0.03$	$1.94^{c} \pm 0.02$	$1.81^{d} \pm 0.04$	0.28*
36-42	Body weight (gm)	$1658.62^{c} \pm 22.47$	$1512.47^{d} \pm 24.60$	$1887.68^{b} \pm 23.56$	$1911.48^{b} \pm 20,49$	$2103.45^{a} \pm 27.61$	215.77*
	Body gain (gm)	$372.21^{c} \pm 13.05$	$339.72^{d} \pm 14.25$	$502.05^{b} \pm 11.67$	$492.12^{a} \pm 12.47$	$492.21^{a} \pm 11.30$	9.93*
	FCR	$2.62^{b} \pm 0.03$	$2.80^{a} \pm 0.06$	$2.07^{c} \pm 0.07$	$2.04^{c} \pm 0.06$	$1.92^{d} \pm 0.07$	0.18*

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Table 5. Effect of MOS and/or Diclazuril on haematological and biochemical parameters of broiler chickens infected with *E. tenella* (n=5). (Mean ±SE)

Parameters	Some haematological and biochemical parameters					
	Groups					LSD at P ≤ 0.05)
	-Ve control	+Ve control	Dicl	MOS	Dicl + MOS	
RBCs (cell x 10^6/ml)	2.91^{b} ± 0.02	2.32^{c} ± 0.04	2.95^{b} ± 0.06	3.38^{a} ± 0.02	3.35^{a} ± 0.03	0.44*
WBCs (cell x 10^3/ml)	19.31^{b} ± 0.21	21.45^{a} ± 0.45	19.04^{b} ± 0.72	20.94^{a} ± 0.36	21.09^{a} ± 0.31	1.90*
ALT (U/L)	26.56^{b} ± 0.74	36.56^{a} ± 0.62	25.71^{b} ± 0.11	26.20^{b} ± 0.67	26.40^{b} ± 0.46	10.85*
AST (U/L)	52.72^{b} ± 1.02	74.13^{a} ± 1.31	51.92^{b} ± 1.22	54.05^{b} ± 0.86	54.32^{b} ± 1.62	22.21*
Total Protein (gm/dl)	3.72^{b} ± 0.06	2.89^{c} ± 0.02	3.81^{b} ± 0.04	4.18^{a} ± 0.03	4.12^{a} ± 0.07	0.40*
Serum albumin (gm/dl)	2.12^{b} ± 0.03	1.76^{c} ± 0.04	2.10^{b} ± 0.05	1.49^{a} ± 0.04	1.53^{a} ± 0.02	0.61*
Serum globulin (gm/dl)	1.60^{b} ± 0.01	1.13^{c} ± 0.02	1.71^{b} ± 0.05	2.69^{a} ± 0.01	2.60^{a} ± 0.03	1.00*
Uric acid (mg/dl)	9.74^{a} ± 0.06	9.96^{a} ± 0.04	9.91^{a} ± 0.03	9.83^{a} ± 0.05	9.88^{a} ± 0.07	NS

-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa. - Dicl = Diclazuril at dose of (1 mg / kg ration) -MOS = Mannan-oligosaccharide (prebiotic) at dose of (1 g / kg ration). - LSD = Least significance difference at P ≤ 0.05 (in the same row). - (*) = Significance at P ≤ 0.05. - NS = Non significance at P ≤ 0.05.

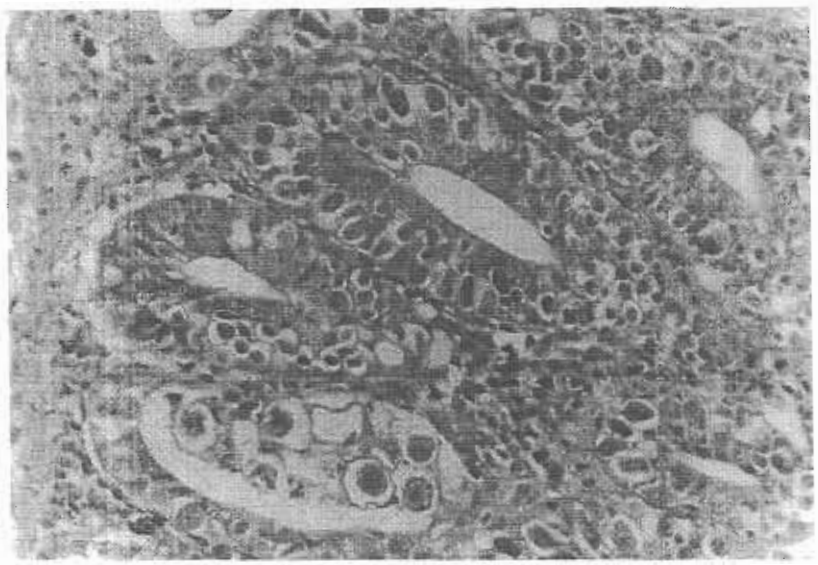

Fig. 1. On the 8th day PI, Caecum of chicks infected with *E. tenella* (positive control) showing numerous second-generation schizonts, gametocytes and mature oocysts in the epithelial lining of the Lieberkohn cells. H&E x 400.

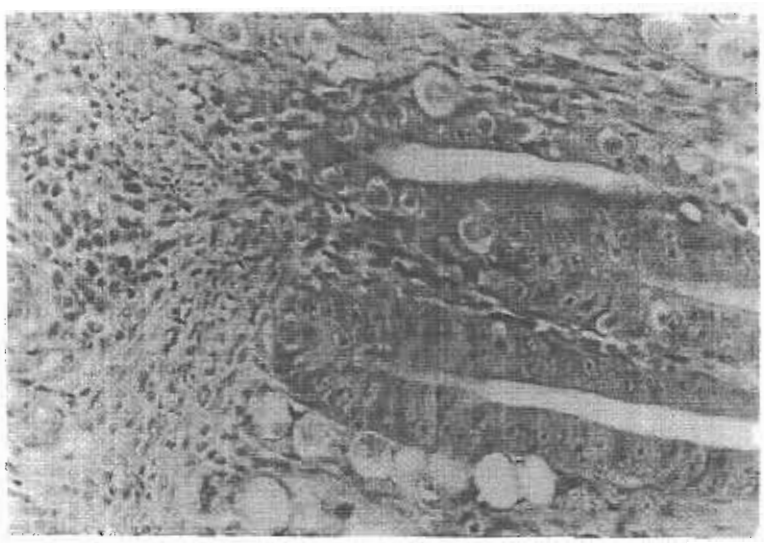

Fig. 2. On the 8th day PI, Caecum of chicks infected with *E. tenella* and treated with Diclazuril (1 ppm) showing few second-generation schizonts and gametocytes in the epithelial lining of the Lieberkohn cells. H&E x 400.

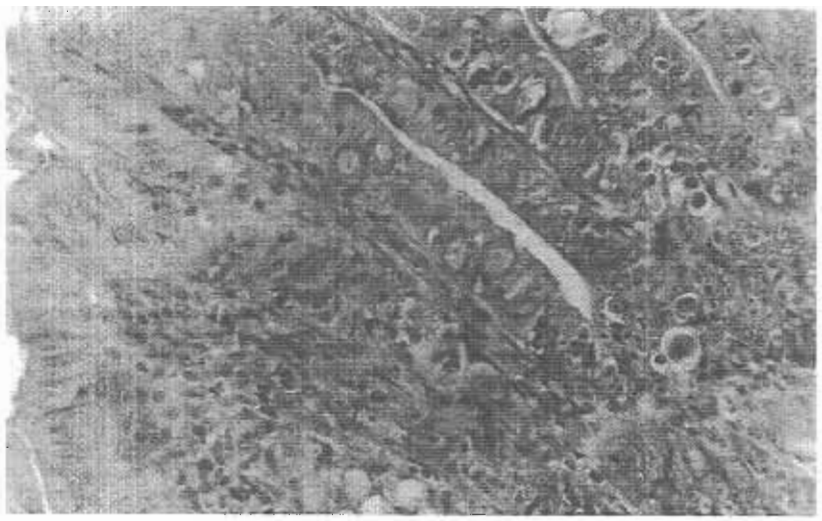

Fig. 3. On the 8th day PI, Caecum of chicks infected with *E. tenella* and treated with MOS (1 g / kg ration) showing few second-generation schizonts, gametocytes and mature oocysts in the epithelial lining of the Lieberkohn cells. H&E x 400.

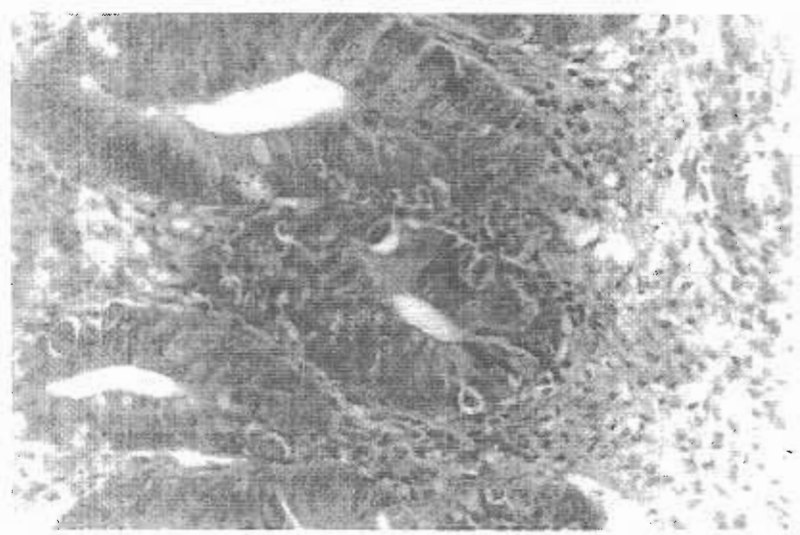

Fig. 4. On the 8th day PI, Caecum of chicks infected with *E. tenella* and treated with Diclazuril (1 ppm) plus MOS (1 g / kg ration) showing few second-generation schizonts and gametocytes in the epithelial lining of the Lieberkohn cells. H&E x 400.

DISCUSSION

The development of new strategies for control of coccidiosis is essential for the poultry industry. Chickens are highly at risk for coccidial infections due to environmental conditions during production. High poultry densities on floor pens and warm surroundings are favorable for a high transmission, replication and accumulation of *Eimeria spp.* ***(37)***. Moreover, the current practices for animal population create a strong selective pressure on coccidian parasites to develop anticoccidial drug resistance ***(38)***. Together, these factors make neonatal chickens an excellent animal model for the study and design of new and effective strategies for the control of coccidiosis.

In our study, the chickens fed ration supplemented with manna-oligosaccharide (MOS) and diclazuril and the infected with *E. tenella* showed low overall mean of oocyst output compared with those infected and treated with either diclazuril or MOS alone. The marked reduction of oocyst output may be due to the direct effect of diclazuril on the endogenous stages of *E. tenella* (sporozoites and merozoites), besides the indirect effect of MOS through increasing the intestinal microflora that occupying the specific receptors responsible for adherence and attachment of the invasive parasites (sporozoites and merozoites). These actions resulted in marked reduction of shedding oocysts of *E. tenella*. Our results are similar to the results recorded by several authors ***(3, 39, 40)***.

In the present study, dietary supplementation of MOS and diclazuril decreased the faecal oocyst output of *E. tenella* and cacal lesion scoring. Reduction percentage of the shedded oocysts in the faeces in chickens fed rations supplemented with manna-oligosaccharide (MOS) and diclazuril together and infected with *E. tenella* was 83.84 %, it was markedly decreased when compared with the other infected groups. These results may be due to the direct anticoccidial effect of diclazuril on the developmental stages of *E. tenella* (sporozoites and merozoites), besides the indirect of effect of dietary MOS on the parasite. Similar findings were previously recorded ***(3)***.

In our study, the marked reduction of oocyst output and consequently lesion scoring in the chickens infected with *E. tenella* and fed ration supplemented with manna-oligosaccharide (MOS) and diclazuril may be due to the prebiotic (MOS) which increased the production of IgA concentrations in the intestinal and tracheal mucosa. Moreover, MOS supplementation reduced the number of *Eimeria spp.* oocysts excreted in the faeces ***(12)***. Although the specific mechanisms of action by intestinal IgA on coccidial infection are still unknown, it is thought that execratory IgA attached the parasite surface and prevented its binding to the intestinal epithelium. Also IgA reduced the development of sporozoites and merozoites and prevented invasion within host cell ***(13,14)***. In addition, MOS increased secretion of intestinal and tracheal IgA. Intestinal IgA was stimulated by exposure to the parasite, while the tracheal IgA was systemically stimulated by MOS treatment, thus MOS has an immuno-regulatory effect at local and systemic level ***(15)***.

Chickens experimentally infected with *E. tenella* and fed ration supplemented with MOS (1 gm / kg ration) only or MOS plus diclazuril showed a significant increase in body weight and body weight gain. They also showed a significant decrease in FCR. These results could be attributed to the growth promoting effect of MOS, which attributed to the ability of MOS to enhance resistance to invading pathogens, improve bowel function and improve calcium bioavailability ***(41)***.

Chickens experimentally infected with *E. tenella* and fed ration supplemented with MOS (1 gm / kg ration) only or MOS plus diclazuril showed a significant increase in serum total protein, albumin and globulin. These results may be attributed to the addition of MOS caused improvement in the intestinal

environment leading to improvement of the digestion and absorption of the nutrients. The prebiotic MOS also limited the damage caused by the pathogenic parasites (*Eimeria spp.*) so, it may be increased the bioavailability of essential nutrients. A significant increase in serum total protein and globulins was recorded in the enramycin, probiotic and prebiotic treated groups ***(42,43)***.

All experimental groups were not showed any significant changes in serum uric acid level on the 14th day PI. These results indicated that, all experimental groups have no harmful effect on kidney functions in the chickens. Enramycin (2 g mg/kg feed) , probiotic (1 gm/ kg feed) and prebiotic (0.5%) had no significant effects on serum uric acid and creatinine in quails when given for seven successive weeks ***(43)***.

It could be concluded that, the anticoccidial effect of diclazuril was enhanced by the prebiotic MOS. Chickens given diet containing MOS and the therapeutic dose of diclazuril showed better anticoccidial efficacy when compared with diclazuril or MOS alone. We advice use the combination of diclazuril (1 ppm) plus MOS (1 g / kg ration) to control the avian coccidiosis and improve the growth performance. Further studies should be tried to investigate the combination of MOS and other anticoccidial drugs.

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الملخص العربى

دراسة مقارنة لكفاءة البريبيوتيك مانان-أوليجوسكارايد والديكلازوريل فى الدجاج المعدى معملياً بالأيميريا تينيللا

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معهد بحوث صحة الحيوان - فرع بنها
(قسم الكيمياء الحيوية والسموم وأمراض النقص الغذائى[1], قسم أمراض الدواجن[2])

تهدف هذه الدارسة لمعرفة تأثير خلط مضاد الكوكسيديا الديكلازوريل (بجرعته العلاجية) مع البريبيوتيك (مانان-أوليجوسكارايد) في العلف وذلك لعلاج كتاكيت الدجاج المعدية بطفيل الأيميريا تينيللا . اجريت هذه الدارسة علي عدد ١٥٠ كتكوت من نوع هبرد عمر يوم، واستمرت هذه التجربة لمدة ستة أسابيع متتالية. قسمت الكتاكيت إلى خمسة مجموعات متساوية يحتوي كل منها على ٣٠ كتكوت. استخدمت المجموعة الأولي كضابطة سالبة للتجربة (غير معدية وغير معالجة). تم تجريع باقي المجموعات بجرعة (١×١٠^5) حويصلة متجرثمة من الأيميريا تينيللا عند عمر ١٤ يوم. المجموعة الثانية استخدمت كضابطة موجبة (معدية وغير معالجة). المجموعة الثالثة عولجت بإضافة الديكلازوريل إلى العلف بالجرعة العلاجية الكاملة (١ ملليجرام / كيلوجرام علف). المجموعة الرابعة عولجت بإضافة البريبيوتيك (مانان- أوليجوسكارايد) إلى العلف بجرعة (١ جرام / كيلوجرام علف). المجموعة الخامسة عولجت بإضافة خليط من الديكلازوريل بجرعة (١ملليجرام / كيلوجرام علف) و البريبيوتيك (مانان- أوليجوسكارايد) بجرعة (١ جرام / كيلوجرام علف).

قد أظهرت النتائج أن خليط الديكلازوريل (بجرعته العلاجية) مع البريبيوتيك (مانان-أوليجوسكارايد) له تأثير جيد جدا كمضاد للكوكسيديا (الأيميريا تينيللا) أفضل من تأثير الديكلازوريل بمفرده وبجرعته العلاجية الكاملة (١ملليجرام / كيلوجرام علف). علاوة على ذلك، أظهر هذا الخليط زيادة معنوية في كل من وزن الجسم، ووزن الجسم المكتسب، وعدد كرات الدم الحمراء، والبروتين الكلي، والألبيومين والجلوبيولين في مصل الدم. ولقد تأكدت هذه النتائج بالدراسة الهستوباثولوجية لأنسجة الأعورين.

من هذه النتائج نستخلص الأتي:
استخدام خليط الديكلازوريل (بجرعته العلاجية) مع البريبيوتيك (مانان-أوليجوسكارايد) في علف دجاج اللحم له كفاءة عالية فى علاج عدوى الأيميريا تينيللا , وكان أفضل من استخدام مضاد الكوكسيديا الديكلازوريل بمفرده أو البريبيوتيك (مانان-أوليجوسكارايد) بمفرده. لذا نوصي باستخدام خليط الديكلازوريل (بجرعته العلاجية) مع البريبيوتيك (مانان-أوليجوسكارايد) لعلاج مرض الكوكسيديا فى الدجاج.