Effect Of *Lactobacillus*-Based Probiotic On Parasitological And Biochemical Parameters In Broiler Chickens Infected With *E.maxima*

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ABSTRACT

This work was carried out to study the effect of supplementation of *Lactobacillus*-based probiotic in diets of broiler chickens infected with *E.maxima* on growth performance, parasitological and biochemical parameters. One hundred and sixty, one-day old male broiler Hubbard chicks were used. They were divided into equal eight groups which were allotted in their sterile wire-floored cages (twenty chicks pear each). Chicks of 1st and 3rd groups were fed rations supplemented with growth promoter ;probiotic; (Avi-bac) containing *Lactobacillus spp.* in a rate of ½ kg/ton, and chicks of 2nd and 4th groups were fed rations supplemented with anticoccidial drug ;clopidol; (Avicoccin) in a rate of 125gm clopidol/ton. While chicks of 5th and 6th groups were fed rations supplemented with both probiotic and clopidol together with the same previously mentioned doses. However, chicks of 7th group (positive control) and 8th group (negative control) were fed standard rations (without any supplementation). Chicks of 3rd , 4th , 6th and 7th groups were infected intra crop (using stomach tube) with $1x10^3$ sporulated oocysts of *E.maxima* at 21 days of age. Parasitological parameters were recorded ; oocyst outputs per group were counted for 10 consecutive days starting from 6th till 15th day PI. Also economical parameters; body weights, gains and feed conversion ratio (FCR) were measured weekly, as well as lesion scoring of small intestine which described at 7th day PI. Blood and serum samples were taken for haematological and biochemical studies. The results showed that oocyst production in fecal matter of chickens fed rations supplemented with probiotic alone and then infected with *E.maxima* was markedly decreased than that shedded from positive control group, although it was nearly similar to that of chickens infected with *E.maxima* and supplemented with either clopidol alone or both of them (two drugs together). Body weights, gains and FCR of chickens infected with *E.maxima* and supplemented with probiotic alone were improved. As well as, lesion scoring of chickens fed probiotic infected with *E.maxima* was significantly decreased. Erythrocyte (RBCs), total leucocyte (WBCs) counts, haemoglobin (Hb%), total protein , albumin and globulin were significantly decreased. These results clearly indicate that the probiotic bacteria (*Lactobcilli*) impacted the protection against *E.maxima* infection which reflected by marked lowering of oocyst production, improving the growth performance of chickens, decreasing the intestinal lesion scores and increasing levels of serum globulin and total protein as well as total leucocytic count.

INTRODUCTION

Chicken coccidiosis is an intestinal infection caused by the intracellular protozoan parasite of the genus *Eimeria*. It is the major parasitic disease of poultry, with substantial economic burden estimated to cost the industry more than $ .800 million in annual losses world-wide ***(1)***. Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis ***(2,3)***, although there is a rising problem of drug resistant strain of *Eimeria*.

Gut mucosal surfaces play a key role in the exclusion and elimination of potentially harmful dietary antigens and enteric micro-organisms. The gut microflora are essential to maintaining healthy flocks and minimizing losses associated with various diseases and stressors. The use of probiotics for poultry is based on the knowledge that the gut flora is involved in resistance to enteric infections including *E.coli* ***(4)***,*Salmonella* ***(5,6)***, *Campylobacter* ***(6,7)***, *Clostridium* ***(8)***, and *Eimeria* ***(9)***.

Feeding probiotics maintain the beneficial intestinal microflora and modulate the mucosal immune system enhancing the host's resistance to pathogens. The probiotic bacteria impacted

the local immune response as characterized by altered Intestinal Intraepithelial Lymphocyte (IEL) subpopulations and increased the bird's resistance to *E.acervulina* as reflected by 75% reduction of oocyst shedding ***(9).*** The probiotic continued to afford some measure of protection through immune modulation despite a fairly overhelming dose of *E.acervulina* ***(10).***

Probiotic are traditionally defined as viable microbial dietary supplement that have a beneficial effect in the prevention and treatment of specific pathogenic conditions. They have been shown to increase the natural defense mechanism of chickens. Chickens fed Probiotics grew rapidly, consumed less feed, showed lower costs, lower mortality rate and harboured less pathogenic bacteria ***(11,12).*** There are many mechanisms by which probiotics enhance intestinal health including stimulation of immunity, competition for limited nutrients, inhibition of epithelial invasion and production of antimicrobial substance ***(12).***

The growth and metabolism of many individual bacterial species inhabiting the large intestine depend primarily on the substances available to them. This has led to attempt to modify the structure and metabolic activities of the community through diet using probiotics and prebiotics. The best known probiotic is the lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*). These organisms are non pathogenic and non toxigenic, retain viability during storage and survive passage through the stomach and intestine ***(13)***.

Intestinal sections from infected non-treated chickens displayed partial desquamation of the villous epithelium, congestion and infiltration of lymphocytes in the mucosa and sub mucosa. The intestine of chickens received prebiotic, probiotic and synbiotic showed healthy intact villi, such villi were greatly elongated, branched and re-branched. The intestinal glands were hyperplastic ***(14).***

The present work was conducted to characterize the underlying mechanisms involved in the probiotic-enhanced local immunity to coccidial infection.

MATERIAL AND METHODS

Birds

One hundred and sixty (160), day-old male broiler chickens (Hubbard, Cairo Poultry Comp.) were randomly divided into eight groups; twenty (20) chicks per each. Chicks were allotted in wire- floored battery cages. The chicks were individually numbered using wing bangle and weighed at day-old. Body weights, weight gains and feed conversion rate (FCR) were calculated and tabulated. Lesion scores were described at 7^{th}- day post infection as described previously ***(15).***

Rations and water

Commercial starter rations from Cairo Poultry Company assumed to be balanced and 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. Rations were used after sterilization in hot air oven at 65 C° for 18 hr. Water was sterilized by boiling then cooling before offering to birds. They were offered *add-libitum* to the chicks.

Treatments

The drugs which supplemented to the rations were probiotic (growth promoter) or clopidol (anticoccidial drug). The probiotic was Avi-bac, USA origin, it is a concentrated source of naturally occurring micro-organisms containing a synergistic blend of lactic acid bacteria and enzymes for use in poultry feed or water. Avi-bac contains lactic acid bacteria ; *Lactobacillus acidophilus* , *L.planterum* and *L.bervis* {$1.6x10^9$ CFU/gm(1.6 billion)}, amylase (224 BAU/gm), beta-glucanase (144 BGU/gm) and hemicellulase(16 HCU/gm). Avi-bac was added to rations at a rate of ½ kg/ton in 1^{st}, 3^{rd}, 5^{th} and 6^{th} groups. Whereas clopidol (Avicoccin, Pharma Swede Company) was added to rations of 2^{nd}, 4^{th}, 5^{th} and 6^{th} groups at a rate of 125gm clopidol/ton. Chicks of 3^{rd}, 4^{th}, 6^{th} and 7^{th} groups were infected with $1x10^3$ sporulated oocysts of *E.maxima* at 21days of age. The 7^{th} group was regarded as a positive control (infected

with *E.maxima*, not supplemented). However, the 8th group was negative control (not infected, not supplemented). Rations were supplemented with probiotic and /or clopidol along the experimental period (35 days-old).

Eimeria oocysts

The sporulated oocysts of *E.maxima* (the largest oocysts) were submitted from Parasitology Department of AHRI (Animal Health Research Institute) which obtained from single oocyst isolation and preserved in 2.5% potassium dichromate solution ***(16)***. They were washed by distilled water 2-3 times through centrifugation at 1500 r.p.m. for 10 minutes. Birds of 3rd, 4th, 6th and 7th groups were inoculated intra crop (using stomach tube) with $1x10^3$ sporulated oocysts of *E.maxima* at 21days of age. Daily oocyst outputs were counted from 6-15 days post infection (PI) by examination of 10 gm of fecal matter per group by salt floatation technique after preservation in 2.5% pot. dichromate solution and then counted by Mc Master technique ***(17)***.

Haematological studies

Blood samples from slaughtered chickens at 28 days-old (1 week PI) were collected on sodium salt of EDETA. Erythrocytes (RBCs) and total leucocytes (WBCs) were counted ***(18)***. Haemoglobin (Hb%) was estimated ***(19)***.

Biochemical analysis

Serum was separated after clotting of blood samples and the clear supernatant serum was kept at -20C° for biochemical analysis. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) ***(20)***, serum urea ***(21)***, creatinine ***(22)***, total protein ***(23)*** and Albumin ***(24)*** were determined. Serum globulin was determined by subtraction of obtained serum albumin from total protein ***(25)***.

Statistical analysis

Differences among experimental treatments were tested by analysis of variance; ANOVA: ***(26, 27)*** using the computer software program called SPSS, Ver., 11, ***(28)***.

RESULTS

Parasitological parameter (oocyst production) and growth performance of chickens (body weights, body weight gains and feed conversion ratio; FCR) as well as lesion scoring were recorded and tabulated as shown in Tables 1-5 and haematological and serum biochemical studies were shown in Table 6.

Daily oocyst outputs per gram (OPG) of feces from chickens were counted for 10 days starting from 6th day to 15th day PI. The data were depicated in Table 1. There were no significant differences among values of means of chickens fed rations supplemented with either probiotic and/or clopidol, then infected with *E.maxima* at 21days-old. Means of daily oocyst output of infected with *E.maxima* and fed rations supplemented with either probiotic alone, clopidol alone or both of them together were significantly decreased in all times (6th to 15th day PI) when compared with that of those infected and not supplemented (positive control) at $P \leq 0.05$.

Peak of oocyst production of positive control (chickens fed standard rations without supplementation and infected with $1x10^3$ sporulated oocysts of *E.maxima*) was obvious at 9th day PI ($176.33 \times 10^3 \pm 14.82$), and another peak was noticed at 13rd day PI ($145.33 \times 10^3 \pm 5$ 62). While that peak of chickens infected and supplemented with either probiotic and/or clopidol was noticed at 8th day PI at $P \leq 0.05$ ($34.33 \times 10^3 \pm 2.62$, $23.50 \times 10^3 \pm 2.62$ or $27.67 \times 10^3 \pm 3.20$, respectively), but there was no another peak of oocyst shedding in these chickens. Overall mean of oocyst output of chickens infected with *E.maxima* and supplemented with either probiotic and/or clopidol was significantly decreased than that of those of positive control chickens (infected, not supplemented) at $P \leq 0.05$ ($13.267 \times 10^3 \pm 1.503$, $10.600 \times 10^3 \pm 1.218$ or $12.267 \times 10^3 \pm 1.503$ vs $98.967 \times 10^3 \pm 6.862$, respectively).

Economical parameters were estimated and represented as shown in Tables 2-4. Table [2] showed means of body weights of chickens and differences among them every week. At 28 days old (1week PI), the mean of body weight of chickens infected with *E.maxima* and not supplemented was significantly marked decreased than that of other groups. Mean of body weight of chickens fed rations supplemented with *Lactobacillus*-based probiotic and infected with $1x10^3$ sporulated oocysts of *E.maxima* at 21days- old was

significantly increased than that of positive control (chickens fed standard rations without supplementation and infected with $1x10^3$ sporulated oocysts of *E.maxima*) at P≤0.05 (1120.00gm ±3.89 vs 927.50gm ±3.56, respectively). While it was not significantly decreased than that of chickens which fed rations supplemented with either clopidol alone or probiotic plus clopidol together after infection with *E.maxima* (1120.00gm ±3.89 vs 1131. 00gm ±7.50 or 1126.50gm ±2.99, respectively). At 35 days of age (2weeks PI), the mean of body weight of chickens fed rations supplemented with *Lactobacillus*-based probiotic and infected with *E.maxima* was significantly increased than that of positive control chickens (1520.50gm ±9.73 vs 1164.75gm ±3.13, respectively). But there were no significant differences of values between it and that of chickens fed rations supplemented with clopidol alone or both of probiotic and clopidol together and then infected with *E.maxima* (1520.50gm ±9.73 vs 1528.50gm ±7.19 or 1536.25gm ±6.65, respectively). Means of body weight of chickens fed rations supplemented with *Lactobacillus*-based probiotic and not infected with *E.maxima* were significantly increased than that of those of other groups even negative control group at P≤0.05.

At 21-28 days of age (1week PI), the mean of body gain of chickens fed rations supplemented with probiotic and infected with *E.maxima* was significantly increased than that of chickens fed standard rations and infected with *E.maxima*; positive control; at P≤0.05 (439.80gm ±5.03 vs 302.20gm ±7.24, respectively). At the end of experiment (35 days-old), the mean of body gain of this group was also significantly increased than that of positive control group at P≤0.05 (400.50gm ±9.55 vs 237.25gm ±7.64 , respectively). Whereas, the mean of body gain of chickens fed rations supplemented with probiotic alone and not infected with *E.maxima* was significantly increased than that of other groups but not significantly decreased than that of those fed rations supplemented with both of probiotic and clopidol together and without infection with *E.maxima* (463.50gm ±4.96 vs 464.75gm ±12.73, respectively). The data were reported in Table 3.

Table 4 showed that at 28 days-old, mean of FCR of chickens fed rations supplemented with *Lactobacillus*-based probiotic and infected with *E.maxima* were significantly decreased than that of those fed standard rations without supplementation (positive control) at P≤0.05(1.850 ±0.021 vs 2.310 ±0.123, respectively). Also at the end of experiment (35 days-old), mean of FCR of chickens fed rations supplemented with probiotic and infected with *E.maxima* was significantly decreased than that of positive control chickens (2.225 ±0.037 vs 3.148 ±0.067, respectively). However, there were no significant differences among values of means of FCR of chickens fed rations supplemented with probiotic alone or probiotic and clopidol together and infected with *E.maxima* as well as those fed standard rations without supplement (negative control) at P≤0.05 (2.225 ±0.037 vs 2.220 ±0.039 or 2.218 ±0.068, respectively).

At 7th day PI, the mean of lesion scores in mid part of small intestine of chickens infected with *E.maxima* and fed rations supplemented with probiotic was not significantly increased than that of those infected and supplemented with either clopidol alone or both of them together at P≤0.05 (1.40 ±0.25 vs 1.20 ±0.20 or 1.00 ±0.32, respectively). Mean of lesion scoring of chickens of positive control group was significantly increased than that of other groups (3.80 ±0.20). The data were represented in Table 5.

Haematological and serum biochemical parameters in chickens were estimated and shown in Table 6. Erythrocytes (RBCs), leucocytes (WBCs) counts and haemoglobin (Hb%) in chickens fed rations supplemented with probiotic and infected with *E.maxima* were significantly increased compared to those in positive control, but they were not significantly changed compared to those in negative control group.

Serum urea and creatinine in chickens fed rations supplemented with probiotic and infected with *E.maxima* were not significantly

decreased compared to those in positive control. However, AST and ALT levels were significantly decreased than those in positive control and not significantly changed than that in negative control group. The levels of total protein, serum albumin and globulin in chickens fed rations supplemented with probiotic and infected with *E.maxima* were significantly increased compared to those of positive control but significantly decreased than those of negative control group.

Table 1. Effect of supplemented probiotic and/or clopidol on daily means (n=6) of oocyst output in fecal matter of chickens infected with 1x10^3 of *E.maxima* sporulated oocysts. (no.x10^3/gm fecal matter)

Group / Day PI	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P≤0.05
6th day	0.00^{c} ±0.00	0.00^{c} ±0.00	1.00^{b} ±0.37	0.83^{bc} ±0.31	0.00^{c} ±0.00	0.50^{bc} ±0.22	3.50^{a} ±0.57	0.00^{c} ±0.00	0.833*
7th day	0.00^{d} ±0.00	0.00^{d} ±0.00	17.83^{b} ±1.99	17.00^{b} ±1.48	0.00^{d} ±0.00	9.83^{c} ±1.58	51.50^{a} ±6.38	0.00^{d} ±0.00	7.187*
8th day	0.00^{c} ±0.00	0.00^{c} ±0.00	34.33^{b} ±2.62	27.33^{b} ±3.12	0.00^{c} ±0.00	23.50^{b} ±2.62	151.00^{a} ±10.25	0.00^{c} ±0.00	23.500*
9th day	0.00^{c} ±0.00	0.00^{c} ±0.00	26.67^{b} ±2.63	23.67^{b} ±3.20	0.00^{c} ±0.00	22.33^{b} ±1.89	176.33^{a} ±14.82	0.00^{c} ±0.00	22.333*
10th day	0.00^{c} ±0.00	0.00^{c} ±0.00	21.00^{b} ±3.27	18.67^{b} ±2.20	0.00^{c} ±0.00	18.50^{b} ±1.89	130.33^{a} ±6.10	0.00^{c} ±0.00	18.500*
11th day	0.00^{b} ±0.00	0.00^{b} ±0.00	10.17^{b} ±2.18	9.17^{b} ±0.87	0.00^{b} ±0.00	9.00^{b} ±0.58	84.67^{a} ±8.97	0.00^{b} ±0.00	10.167*
12th day	0.00^{c} ±0.00	0.00^{c} ±0.00	9.33^{b} ±1.26	9.50^{b} ±1.26	0.00^{c} ±0.00	7.67^{b} ±1.26	74.67^{a} ±6.11	0.00^{c} ±0.00	7.667*
13th day	0.00^{c} ±0.00	0.00^{c} ±0.00	6.00^{bc} ±1.32	7.83^{b} ±0.70	0.00^{c} ±0.00	6.50^{bc} ±1.34	145.33^{a} ±5.62	0.00^{c} ±0.00	6.500*
14th day	0.00^{b} ±0.00	0.00^{b} ±0.00	5.00^{b} ±1.07	5.83^{b} ±0.60	0.00^{b} ±0.00	3.50^{b} ±0.76	97.33^{a} ±7.47	0.00^{b} ±0.00	91.500*
15th day	0.00^{b} ±0.00	0.00^{b} ±0.00	1.33^{b} ±0.21	1.17^{b} ±0.40	0.00^{b} ±0.00	0.83^{b} ±0.17	75.00^{a} ±5.88	0.00^{b} ±0.00	73.667*
Overall means	0.00^{c} ±0.00	0.00^{c} ±0.00	13.267^{b} ±1.503	12.100^{b} ±1.238	0.00^{c} ±0.00	10.600^{b} ±1.218	98.967^{a} ±6.862	0.00^{c} ±0.00	10.600*

• (*): Significance at P≤0.05. •**n**=60(**for Overall means**).

•**LSD:** Least significance difference among means at P≤0.05.

•Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOVA •Infection of birds at 21-day-old.

Table 2. Effect of supplemented probiotic and/or clopidol on means (n=20) of body weight (gm) per chicken infected with $1x10^3$ of *E.maxima* sporulated oocysts.

Group / Age (days)	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P ≤0.05
1 day old	41.25[a] ±0.40	41.05[a] ±0.44	41.35[a] ±0.46	41.55[a] ±0.56	41.00[a] ±0.42	41.45[a] ± 0.50	41.20[a] ±0.40	41.40[a] ±0.45	0.600 (NS)
7 days old	175.60[a] ±1.85	144.45[b] ±1.68	171.00[a] ±3.56	144.35[b] ±1.78	171.45[a] ±2.14	172.25[a] ±1.91	150.05[b] ±1.75	148.55[b] ±3.18	5.600*
14 days old	352.65[a] ±5.20	310.05[a] ±2.22	350.55[a] ±5.36	310.55[a] ±2.56	352.40[a] ±4.63	355.00[a] ±4.35	313.20[b] ±3.06	314.00[b] ±2.88	36.550*
21 days old	690.35[a] ±3.24	616.60[b] ±7.09	680.20[a] ±4.26	619.75[b] ±7.27	681.30[a] ±3.65	684.35[a] ±2.47	625.35[b] ±5.20	626.95[b] ±5.87	53.250*
28 days old	1121.00[a] ±6.40	1133.75[bc] ±5.71	1120.20[c] ±3.89	1131.00[bc] ±7.50	1215.00[a] ±9.61	1126.50[bc] ±2.99	927.50[d] ±8.52	1143.25[b] ±2.70	23.250*
35 days old	1688.00[a] ±3.88	1556.50[b] ±4.52	1520.50[c] ±9.73	1528.50[c] ±7.19	1679.50[a] ±5.80	1536.25[c] ±6.65	1164.75[d] ±3.13	1561.00[b] ±2.78	20.250*

- **NS:** Non significance at P ≤0.05. • (*):Significance at P ≤0.05. •**n**=20
- Means with different alphabetical superscripts in the same row are significantly different at P ≤0.05.
- **LSD:** Least significance difference among at P≤0.05(in the same row).
- Data were analysed by One Way ANOVA.
- Infection of birds at 21-day-old.

Table 3. Effect of supplemented probiotic and/or clopidol on means (n=20) of body weight gain (gm) per chicken infected with $1x10^3$ of *E.maxima* sporulated oocysts.

Group / Age (day)	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P≤0.05
0 day	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	0.000 (NS)
0-7 days	134.35^{a} ±1.94	103.40^{b} ±1.58	129.65^{a} ±2.46	102.80^{b} ±1.76	130.45^{a} ±2.36	130.80^{a} ±2.11	108.85^{b} ±1.81	107.15^{b} ±1.78	6.050*
7-14 days	177.05^{ab} ±5.71	165.60b^{c} ±4.38	179.55^{a} ±4.52	166.25^{bc} ±3.11	180.95^{a} ±4.19	181.75^{a} ±4.17	163.15^{c} ±3.63	165.45^{bc} ±2.86	11.450*
14-21days	337.70^{a} ±4.55	306.55^{c} ±7.91	329.65^{ab} ±4.51	309.15^{c} ±7.37	323.90^{ab} ±6.23	324.35^{ab} ±6.65	312.10^{bc} ±4.72	312.95^{bc} ±4.90	17.350*
21-28days	530.65^{ab} ±6.53	517.20^{ab} ±7.54	439.80^{c} ±5.03	511.25^{b} ±8.03	533.70^{a} ±8.23	442.15^{c} ±6.58	302.20^{d} ±7.24	316.30^{bc} ±7.14	19.400*
28-35days	463.50^{a} ±4.96	422.75^{b} ±6.96	400. 50^{bc} ±9.55	397.50^{c} ±7.28	464.75^{a} ±12.73	409.75^{bc} ±6.71	237.25^{d} ±7.64	417.75^{bc} ±3.33	22.250*

•**NS:** Non significance at P<0.05. •Sig.(*):Significance at P ≤0.05. •**n**=20

•Means with different alphabetical superscripts in the same row are significantly different at P ≤0.05.

•**LSD:** Least Significant difference among means at P ≤0.05.

•Data were analysed by One Way ANOVA. •Infection of birds at 21-day-old.

Table 4. Effect of supplemented probiotic and/or clopidol on means (n=5) of FCR per chicken infected with 1x10^3 of *E.maxima* sporulated oocysts.

Group / Age (day)	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P≤0.05
7 days	1.256^{abc} ±0.016	1.284^{ab} ±0.013	1.252^{abc} ±0.014	1.240^{bc} ±0.017	1.242^{bc} ±0.018	1.288^{a} ±0.011	1.254^{abc} ±0.012	1.230^{c} ±0.010	0.042*
14 days	1.576^{b} ±0.009	1.598^{ab} ±0.010	1.590^{ab} ±0.010	1.650^{a} ±0.035	1.606^{ab} ±0.015	1.602^{ab} ±0.021	1.630^{ab} ±0.028	1.612^{ab} ±0.017	0.060*
21 days	1.918^{a} ±0.016	1.940^{a} ±0.009	1.962^{a} ±0.012	1.944^{a} ±0.015	1.960^{a} ±0.012	1.950^{a} ±0.017	1.928^{a} ±0.016	1.940^{a} ±0.018	0.042 (NS)
28 days	1.776^{d} ±0.015	1.992^{b} ±0.021	1.850^{c} ±0.021	1.998^{b} ±0.014	1.760^{d} ±0.030	1.835^{c} ±0.026	2.310^{a} ±0.123	1.820^{c} ±0.050	0.045*
35 days	2.186^{d} ±0.053	2.344^{b} ±0.031	2.225^{c} ±0.037	2.360^{b} ±0.024	2.192^{d} ±0.056	2.220^{c} ±0.039	3.148^{a} ±0.067	2.218^{c} ±0.068	0.021*

•NS: Non significance at P<0.05. • (*): Significance at P≤0.05. •**n**=5

•Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •**LSD:** Least significant difference among means at P≤0.05(in the same row).

•Data were analysed by One Way ANOVA. •Infection of birds at 21-day-old.

Table 5. Effect of supplemented probiotic and/or clopidol on means (n=5) of Lesion scoring at (7th day PI) of birds infected with 1x10^3 of *E.maxima* sporulated oocysts.

Group / Day PI	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P≤0.05
At 7th day PI	0.00^{c} ±0.00	0.00^{c} ±0.00	1.40^{b} ±0.25	1.20^{b} ±0.20	0.00^{c} ±0.00	1.00^{b} ±0.32	3.80^{a} ±0.20	0.00^{c} ±0.00	1.000*

• (*):Significance at P<0.05. •**n**=5 •Infection of birds at 21-day-old.

•Means with different alphabetical superscripts in the same row are significantly different at P≤0.05. •Data were analysed by One Way ANOV.

•**LSD:** Least significant difference among means at P≤0.05(in the same row).

Table 6. Effect of supplemented probiotic and/or clopidol on some haematological and serum biochemical parameters in chickens infected with *E.maxima* sporulated oocysts (n=5).

Group / Parameter	Probiotic (1)	Clopidol (2)	Probiotic + *Eimeria* (3)	Clopidol + *Eimeria* (4)	Probiotic + Clopidol (5)	Probiotic + Clopidol + *Eimeria* (6)	+Ve Control (7)	-Ve Control (8)	LSD at P≤0.05
Erythrocytes (RBCs) (cell $\times 10^6$/ml)	3.32^{a} ±0.086	2.88^{b} ±0.080	2.82^{b} ±0.058	2.28^{c} ±0.051	3.30^{a} ±0.071	2.65^{b} ±0.063	2.15^{c} ±0.071	2.90^{b} ±0.055	0.200*
Total Leucocytes (WBCs) (cell$\times 10^3$/ml)	19.72^{c} ±0.242	19.28^{cd} ±0.307	22.42^{a} ±0.570	20.86^{b} ±0.330	20.68^{b} ±0.285	21.30^{b} ±0.230	21.18^{b} ±0.376	18.25^{d} ±0.341	1.040*
Haemoglobin (Hb) (gm%)	10.58^{a} ±0.218	9.52^{b} ±0.291	9.50^{b} ±0.188	8.26^{c} ±0.191	10.26^{a} ±0.181	8.90^{b} ±0.212	8.28^{c} ±0.222	9.46^{b} ±0.280	0.740*
Serum Urea (mg/dl)	8.78^{b} ±0.242	9.80^{ab} ±0.267	9.92^{ab} ±0.516	9.20^{b} ±0.460	8.80^{b} ±0.215	9.84^{ab} ±0.611	11.04^{a} ±0.681	10.16^{ab} ±0.458	1.360*
Serum Creatinine (mg/dl)	1.32^{a} ±0.080	1.34^{a} ±0.081	1.36^{a} ±0.051	1.42^{a} ±0.049	1.36^{a} ±0.075	1.40^{a} ±0.089	1.38^{a} ±0.107	1.28^{a} ±0.128	0.020 **(NS)**
AST (µ/L)	48.68^{d} ±0.917	51.12^{d} ±1.329	66.62^{c} ±1.552	68.98^{bc} ±1.688	48.58^{d} ±0.376	72.62^{b} ±1.303	77.14^{a} ±2.052	51.72^{d} ±0.344	4.520*
ALT (µ/L)	39.54^{e} ±0.411	43.40^{d} ±0.640	51.86^{bc} ±1.082	54.48^{b} ±1.383	39.66^{e} ±0.530	50.88^{c} ±0.940	58.20^{a} ±1.842	41.36^{de} ±0.604	3.600*
Total Protein (gm/dl)	5.60^{a} ±0.055	5.15^{a} ±0.068	4.40^{b} ±0.217	4.04^{bc} ±0.179	5.20^{a} ±0.110	4.60^{b} ±0.230	3.58^{c} ±0.240	5.43^{a} ±0.103	0.520*
Serum Albumin (gm/dl)	3.12^{a} ±0.139	3.01^{a} ±0.130	2.54^{b} ±0.163	2.58^{b} ±0.116	3.18^{a} ±0.086	2.63^{b} ±0.130	2.18^{c} ±0.139	3.18^{a} ±0.062	0.360*
Serum Globulin (gm/dl)	2.48^{a} ±0.159	2.14^{ab} ±0.144	1.86^{c} ±0.108	1.46^{d} ±0.075	2.02^{bc} ±0.213	1.97^{bc} ±0.114	1.40^{d} ±0.105	2.16^{ab} ±0.075	0.380*

- **NS:** Non significance at P ≤0.05. • (*):Significance at P ≤0.05. •**n**=5
- Means with different alphabetical superscripts in the same row are significantly different at P≤0.05.
- **LSD:** Least significance difference among at P≤0.05(in the same row).
- Data were analysed by One Way ANOVA.
- Infection of birds at 21-day-old.

DISCUSSION

The poultry industry is increasingly using probiotic as a natural method of preventing infections in chickens, rather than using antibiotics which leave residues in meat and build up resistance in the bacteria. Some investigations were designed to examine the effects of feeding a *Lactobacillus*-based probiotic on the intestinal immune responses of broiler chickens over the course of an *E.acervulina* infection and to determine the potential protection, it might provide to the birds against the pathogens. The susceptibility to Eimeria was assessed on the bases of the number of oocysts obtained from droppings collected for four days PI ***(10)***.

Our study revealed marked lowering of numbers of oocyst production in chickens fed rations supplemented with *Lactobacillus*-based probiotic and infected with $1x10^3$ sporulated oocysts of *E.maxima* in comparison of that of positive control. Fecal oocyst output of these birds was reduced by 86.59%. This may be due to the effects of *Lactobacillus spp.* on lining epithelial cells of small intestine through many mechanisms by which the probiotics enhance intestinal health, including stimulation of immunity, competition for limited nutrients, inhibition of epithelial and mucosal adherence, inhibition of epithelial invasion and production of antimicrobial substances. These findings are similar to that previously cited ***(12)***. However, *Eimeria* being an intracellular parasite, must invade the host cell in order to replicate. Firstly, it must adhere to epithelial cell surface. Gut-adapted probiotic bacteria may complete for adhesion sites and occupy common receptors on the epithelial cells, this would retard penetration and infiltration by *Eimeria* oocysts and consequently, their replication and shedding. Several investigators recorded nearly the same findings.

These results demonstrate an immunoregulatry effect of dietary probiotic on the local immune system in broiler chickens (e.g., stimulating and increasing of Intestinal Epithelial Lymphocytes "TEL" in lamina propria of small intestine), this improved resistance of oocyst shedding. This findings are consistent with that previously reported ***(9)***. In this study probiotic significantly reduced the fecal oocyst load by 86.59%, the reduction in shedding was as great as recently described ***(9)***, which obtained a 75% reduction. This finding may be due to small dose of infection, where the dose in our study was $1x10^3$ sporulated oocysts of *E.maxima* per bird, and the dose which was used was $1x10^4$ sporulated oocysts of *E.acervulina* per bird ***(9)***. While the double dose of *E.acervulina* ($2x10^4$ sporulated oocysts per bird) was used ***(10)***, the reduction of shedding of fecal oocysts was 14%. So the challenge dose must be regarded in our mind to be studied in the future investigations.

Economical parameters as body weight, body gains and feed conversion ratio of chickens fed rations supplemented with *Lactobacillus*-based probiotic either infected or non infected with *E.maxima* were improved when compared to that of positive control. This may be due to beneficial effect of *Lactobacillus acidophilus* colonies in the intestine of chickens which improves the bioviability of essential nutrients ***(29)***, as well as due to improvement of the state of intestinal villi responsible for absorption of nutrients ***(30)***. Also dietary probiotic decreased urease activity in the small intestinal contents of young chickens and thus may be beneficial for improving animal health and growth especially during early life ***(31)***. FCR was improved in chickens fed rations supplemented with probiotic and similar to that of negative control when compared to other groups. Our findings are nearly agreed with those recorded by several cited work ***(32,33)*** who found that *L.acidophillus* inoculation to one day-old broiler chickens improved weight gains and FCR.

Probiotic treatment lowered lesion scoring as nearly as similar to clopidol. This may be due to its preventing effect of invasion of infective stages (sporozoites and merozoites) of *E.maxima* through occupying the receptors of epithelial cells in small intestine, that

leading to prevention of its multiplication, consequently decreasing lesion scoring ***(9,10)***.

Our findings revealed a significant increase in RBCs count and Hb% in chickens received probiotic compared with control group. Probiotic increased absorption in small intestine and that compensate the losses of essential nutrients caused by *E.maxima* infection. Improvement of hematological parameters resulted from improvement of absorption of essential nutrients, and enhancement of vitamin B synthesis and/or its absorption. The probiotic increased the bioviability of essential nutrients in small intestine of chickens through increasing bacterial population which enhancing vitamin B synthesis and/or absorption ***(29,30)***.

The results of our work, showed an increase in serum total protein, albumin and globulin values, as well as, total leucocytic count in chickens received *Lactobacillus*-based probiotic. The increase in serum total protein in these results mainly due to the increase of globulin values which may indicate the improvement of humeral immunity ***(34-36)***. The improvement of serum total protein, globulin and WBCs count in our study indicates the immunostimmulatory effect of probiotic on chickens.

In conclusion, *Lactobacillus*-based probiotic is a cheap product, can be used as immunostimulator before and after *Eimeria* infection to enhance the immune response of chickens against coccidiosis to reduce its harmful effect, as well as, its growth promotor effect. The protection level against *E.maxima* infection may depend upon the challenge dose, the probiotic application still produced an immune response that had a discernible and describable effect on coccidial proliferation. In further investigation more than one challenge dose will be designed and administered to define the conditions and limits under which it might serve as a sole substitute for traditional approaches to coccidiosis control.

REFERENCES

1-Williams,R.B. (1998): Epidemiology aspects of the use of live anticoccidial vaccines for chickens. International Journal of Parasitology, 28: 1089-1098.

2-Chapman,H.D. (1999): Anticoccidial drug and their effects upon the development of immunity to *Eimeria* infection in poultry. Avian Pathology,28: 521-535.

3-Allen,P.C. and Fetter,R.H. (2002): Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical Microbiolgy Review, 15: 58-65.

4-Jin,L.Z.; Ho,Y.W.; Abdullah,N. and Jalaludin,S. (1996): Influence of dried *Bacillus subtilis* and lactobacilli cultures on intestinal microflora and performance in broilers. Asian-Astralia Journal of Animal Sciences,9: 99-107.

5-Qin,Z.R.; Fukata,T.; Baba,E. and Arakawa,A. (1995): Effect of lactose and *Lactobacillus acidophilus* on the colonization of *Salmonella enteritidis* in chicks concurrently infected with *Eimeria tenella*. Avian Diseases,39: 548-553.

6-Stern,N.J.; Cox,N.A.; Bailery,M.E. and Musgrove,M.T. (2001): Comparison of mucosal comparative exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter spp.* Colonization in broiler chickens. Poultry Science,80: 156-160.

7-Morishita,T.Y.; Aye,P.P.; Harr,B.S.; Cobb,C.W. and Clifford,J.R. (1997): Evaluation of an avian-specific probiotic to reduce the colonization and shedding of *Campylobacter jejuni* in broilers. Avian Disease,41: 850-855.

8-LaRagione,R.M.; Narbad,A.; Gasson,M.J. and Woodward,W.J. (2004): In vivo characterization of *Lactobacillus johnsonii* FI_{9785} for use as a defined competitive exclusion agent against bacterial pathogens in poultry. Journal Applied Bacteriology,38: 197-205.

9-Dalloul,R.A.; Lillehoj,H.S.; Shellem,T.A. and Doerr,J.A. (2003): Enhanced Mucosal Immunity Against *E.acervulina* in Broilers Fed a *Lactobacillus*-Based Probiotic. Poultry Sciences,82: 62-66.

10-Dalloul, R.A.; Lillehoj, H.S.; Tamim, N.M.; Shellem, T.A. and Doerr,J.A.

(2005): Induction of local protective immunity to *E.acervulina* by a *Lactobacillus*-based probiotic. Comp. Imm. Microbiol. Infect. Dis.,28: 351-361.

11-Roberfroid, M.B. (2000): Prebiotics and probiotics: are they functional foods?. Am.J.Clin.Nutr.Jun.;71(6 suppl.): 16825-16875.

12-Rolfe,R.D. (2000): The role of probiotic cultures in the control of gastrointestinal health . Journal Nutrition Feb.;130(25 suppl.): 3965-4025.

13-Macfarlane,G.T. and Cummings,J.H. (1999): Probiotics and prebiotics: Can we benefit health through regulation of the activities of intestinal bacteria? Brit.Med.J.,318: 999-1003.

14-El-Nabtity,S.M.; Nasser,A.A.E. and Gehan Gad,N.A. (2003): Efficacy of probiotic and prebiotic in vaccinated chickens. Egypt.J.Agri.Res.,81: (2),753-769.

15-Jonhson,J. and Reid,W.M. (1970): Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology,28: 30-36.

16-Karim,M.J. and Tress,A.J. (1990): Isolation of five species of *Eimeria* in Bengladish. Trop. Ani. H. Prod.,22:153-159.

17-Long,P.L.; Joyner, L.P.; Millard,B.J. andNorton,C.C. (1976): A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Veterinaria Latina, 6: 201-217.

18-Wintrobe,M.M. (1965): Clinical and Haematology, 4th Ed., Lea and Febiger, Philadelphia, USA.

19-Schalm, O.W. (1986): Veterinary Haematology. 4th Ed., Lea and Febiger, Philadelphia, USA.

20-Reitman,S. and Franckle,S. (1957): A colorimetric determination of serum AST and ALT enzymes. Amer. J. Clin. Path., 28: 56-58.

21-Patton,G.J. and Crouch,S.R. (1977): Determination of urea (urease modified Berthelot reaction). Anal. Chem., 49: 464-469.

22-Husdan,H. and Rapaport,A. (1968): Chemical determination of creatinine with depolarization. Clin. Chem. ,14: 222-238.

23-Sonnenwirth,A. and Jarett,L. (1980): Gardwhols Clinical Laboratory Methods and Diagnosis. Vol.(1), 8th Ed., Mosby.

24-Webster,D. (1974): Colorimetric determination of serum albumin. Clin. Chem. Acta,53: 109.

25-Doumas,B.T. and Biggs,H.G. (1972): Determination of serum globulin. In: Standard Methods of Clinical Chemistry. 7th Ed., New York, Academic Press.

26-Duncan,D.R. (1955): Multiple range and multiple F tests. Biometrics,11:31-42.

27-Snedecor,G.W. and Cochran,W.G. (1969): Statistical methods ,sixth Ed., Iowa State University Press, Asmes, USA.

28-SPSS for Windows, Version:11 (19 September, 2001). Copyright © SPSS Inc. 1989-2001. All rights reserved.

29-Haddadin,M.S.Y.; Abdulrahim,S.M.; Odetallh,N.H. and Robiso,R.K. (1997): A proposed protocol for checking the suitability of Lactobacillus acidophilus cultures for use during feeding trials with chickens. Tropical Sci.,37: 16-20.

30-Jenkins,D.J.; Kendall,C.W. and Vuksan,V. (1999): Inulin,oligofructose and intestinal function. J.Nutr.,129: 1431-1433.

31-Yeo,J. and Kim,K.I. (1997): Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci. Feb.,76: 381-385.

32-Tortero,F. (1975): Influence of the implantation of L.acidophilus in chicks on the growth, feed conversion, malabsorption of fats syndrome and intestinal flora. Poult. Sci.,52: 197-203.

33-Aziz, M.A.; Zahra,A.A. and El-Dakroury, M.F. (2003): Comparative study on the antibiotics (Flavomycin & Enramycin) and the probiotics (Bio nutra & Dinaferm) as growth promotors in broilers. The 46th Annual conference of the Egyptian Society of Pharmacology & Experimental Therapeutics, Zagazig, Egypt, p.6.

34-El-Banna,R.; Azza,M.Kamel and Kamla,M.El-Saied (2001): Effect of Biogen and dry yeast on performance of broiler chickens. J.Egypt.Vet.Med.Associ.,61(6): 123-136.

35-Shoeib,H.K. and Madian,A.H. (2002): A study on the effect of feeding diets containing probiotics (Pronifer & Biogens) on growth performance, intestinal flora and haematological picture of broiler chicks. Assiut Vet. Med. J., 47(94): 110-128.

36-Khodary,R.M.; Rezk,H.I. and Assaf,I.M.M. (2004): Comparative study on Enramycin, Probiotic and Prebiotic as growth promoters in Quails. 7th Vet. Med. Zag. Conference (21-23 july, 2004), P: 699-711.

الملخص العربى

تأثير منشط النمو المعتمد على لاكتوباسيلس على المعايير الطفيلية و البيوكيميائية فى بدارى دجاج التسمين المصابة بالأيميريا ماكسيما

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إستخدمت فى هذه الدراسة مائة وستون كتكوتا من ذكور كتاكيت سلالة هابرد لبدارى دجاج التسمين عمر يوم، وقسمت هذه الكتاكيت إلى ثمانية مجموعات متساوية، ثم وضعت كل مجموعة فى أقفاص ذات أرضية سلك معقمة. وكانت معاملات هذه الكتاكيت كالتالى: كتاكيت المجموعتين الأولى والثالثة تغذت على علف مدعم بمنشط النمو (المحتوى على ميكروب اللاكتوباسيلس) بمعدل ½ كجم/طن، وكتاكيت المجموعتين الثانية والرابعة تغذت على علف مدعم بمضاد للكوكسيديا (كلوبيدول) بمعدل ١٢٥ جم/طن. بينما تغذت كتاكيت المجموعتين الخامسة والسادسة على علف مدعم بكل من منشط النمو (لاكتوباسيلس) ومضاد الكوكسيديا(كلوبيدول) بنفس الجرعات السابقة. أما كتاكيت المجموعتين السابعة (مجموعة ضابطة إيجابية) والثامنة (مجموعة ضابطة سلبية) تغذت على علف قياسى (بدون أية إضافات). وقد تم تجريع المجموعات الثالثة ،الرابعة، السادسة والسابعة عن طريق الفم باستخدام أنبوب اللى المعدى بجرعة (١× ١٠^{٣}) حويصلة متجرثمة من الأيميريا ماكسيما عند عمر ٢١ يوم . وقد تم قياس المعايير الطفيلية وهى عبارة عن عد حويصلات الأيميريا النازلة فى زرق الدجاج لكل مجموعة يوميا ولمدة عشرة أيام متتالية وذلك إبتداء من اليوم السادس وحتى اليوم الخامس عشر بعد العدوى. كذلك قيست المعايير الاقتصادية (أداء النمو) إسبوعيا متمثلة فى أوزان الطيور، الأوزان المكتسبة ومعامل التحويل الغذائى. هذا بالاضافة إلى وصف درجة الاصابة فى الأمعاء عند اليوم السابع من بداية العدوى.وقد أخذت عينتين دم من كل مجموعة، العينة الأولى على مانع للتجلط وذلك لعد كرات الدم الحمراء والبيضاء وكذلك قياس نسبة الهيموجلوبين.أما العينة الثانية لعمل السيرم (المصل) اللازم لقياس البروتين الكلى، والألبيومين والجلوبيولين ، ووظائف الكبد والكلى. وقد أوضحت النتائج أن إنتاجية حويصلات الأيميريا فى زرق الدجاج المغذى على علف مدعم بمنشط النمو وحده ومعدى بحويصلات الأيميريا المتجرثمة منخفضة بوضوح وذلك بمقارنتها بنظيرتها فى المجموعة الضابطة الايجابية، ولكنها تقريبا كانت مشابهة لتلك التى نتجت من الطيور المعدية والمعالجة بالكلوبيدول وحده أوبالكلوبيدول ومنشط النمو معا. أما أوزان الطيور، والأوزان المكتسبة ومعامل التحويل الغذائى قد لوحظ تحسنهم باستخدام منشط النمو مع العدوى ولكنها أيضا كانت مشابهة لتلك التى قيست فى المجموعات المغذاه على أعلاف بها كلوبيدول وحده (بدون عدوى)، كلوبيدول وحده (مع العدوى)،أوكلوبيدول ومنشط النمو معا (مع العدوى).كما أوضحت الدراسة أن هناك زيادة معنوية فى عدد كرات الدم الحمراء والبيضاء و نسبة الهيموجلوبين،وكذلك إرتفاع مستوى البروتين الكلى، والألبيومين والجلوبيولين فى الطيور التى تغذت على علف به منشط النمو مقارنة مع المجموعة الضابطة الإيجابية،بينما انخفض مستوى كل من (ALT & AST) معنويا. أما مستوى اليوريا والاكرياتينين لم يتغير معنويا. كما أوضحت النتائج أن بكتيريا النمو (لاكتوباسيلس) قد أدت الى زيادة المناعة ضد عدوى الأيميريا ماكسيما فى الدجاج وذلك بعد تسببها فى الانخفاض الملحوظ فى إنتاجية حويصلات الأيميريا فى زرق الدجاج وكذلك تحسن الأوزان ومعامل التحويل الغذائى ودرجة الاصابة فى الأمعاء وزيادة عدد كرات الدم البيضاء وكذلك ارتفاع مستوى الجلوبيولين والبروتين الكلى. وخلاصة هذه الدراسة أن منشط النمو المعتمد على ميكروب اللاكتوباسيلس له تأثير كمضاد للكوكسيديا ومحفز مناعى بالإضافة إلى كونه منشطا للنمو.