

Evaluation Of Some Probiotic And Prebiotic In Prevention Of Paratyphoid Infection In Broiler Flocks

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ABSTRACT

Six salmonella (*Sal.*) isolates were recorded from 110 broiler chicks aged 1-30 days –old collected from different localities at Sharkia Governorate. These isolates were serotyped into two *Sal. Enteritidis* (*S.E.*), one *Sal. typhimurium* and three untyped. *In vivo* studies were carried out on 300 broiler chicks. They were divided into 6 equal groups. Group A served as control (non infected, non treated) the groups B and C were infected I/M with 0.25 ml broth containing 103.3 c.f.u *Sal. Enteritidis* at the age of one week .Group B (a positive control, non treated). Groups C and E medicated 48 hrs before infection with probiotic 1gm/kg of ration where groups D and F medicated 48 hrs before infection with prebiotic 1g/kg ration. At the age of 2 weeks, the obtained results concerning clinical signs, mortality rate, reisolation of *Sal.* and body gain showed that probiotic or prebiotic was highly effective agent in the control of the experimental infection of salmonellosis. Moreover, chicks infected with *Sal. Enteritidis* serum AST, ALT, creatinine, uric acid, globulin and heamogram were significantly increased but albumin level were decreased significantly. These parameters were improved towards the normal in chicks infected with *Sal. Enteritidis* and treated with probiotic or prebiotic 48 hrs before infection.

INTRODUCTION

Alternative to antibiotic growth promotants have become important in commercial chickens mainly because of apprehensions about the possible development of resistant bacteria.

The use of probiotics for growth promotion of poultry as substitute for antibiotics to avoid development of drug resistant microorganism and drug residues in poultry products has become a subject of interest. Probiotic, are live microbial feed supplement such as bacteria or yeast which has been shown to be responsible for improved growth rate, feed conversion, fertility and hatchability in poultry (1-3). Probiotic act by increasing normal gut flora on the expense of pathogenic organism (4). The mos is dived from the outer wall of yeast and its evaluation in diets for breeders is of particular interest because it not only shifts gastrointestinal microflora balance toward beneficial organisms (5, 6), but also has immuno-modulatory propertie (7, 8).

Supplementation of poultry diet with mannligosaccharide Mos results in improved production in terms of body weight gain and

feed conversion (9). In addition, the Mos supplementation resulted in a significant improvement in antibody response in broilers and layers (10).

This work was aimed to investigate effects of probiotics and prebiotic on the performance, biochemicals and haematological values of broilers.

MATERIAL AND METHODS

Probiotic

- (a) Probiotic (Bioplus 2B) highly concentrate probiotic (*Bacillus liecheniformis* DSM 57ug and *Bacillus subtilis* DSM57ug in ratio 1:1) produced by Biochem. Co.
- (b)Prebiotic (Bio- Mos) produced by Altech Co.

Samples and cultivation

One hundred and ten specimens from liver, small intestine and large intestine were collected from both diseased and freshly dead balady chicks with an average age 1-30 days old from private farms at different localities at the Sharkia Governorate. The specimens were cultured on peptone broth and Selenite F. both (Oxoid) for 24hr. at 37°C.

Bacterial colonies were selected for further morphological and biochemical identification (11).

Broiler chicks

A total of three hundred and five, one day broiler chicks were brooded on deep litter were feed on balanced ration (Table 1). Five chicks were randomly sacrificed and bacteriologically examined to confirm that free from Salmonella. The birds were divided randomly into six equal groups (A, B, C, D, E and F).

Group A was left as a control (its ration and drinking water was free from any growth promoter) where group B was infected with *Sal.* and remain non treated "positive control".

Groups C and D were infected with Salmonella and treated with prebiotic (Bio plus 2B) 19/kg ration and ration containing 19/kg prebiotic.

Group E was fed on ration containing 19/kg (probiotic) Bio plus 2B where group F was fed on ration containing 19/kg prebiotic (Bio Mos). The route of infection each chicks, infected group received *Sal. enteridis* by I/M infection of infective dose 0.25 ml of $10^{3.3}$ c.f.u (12).

Treatment started 48 hrs before infection and it feed continued up to the end of the experiment. Broiler chickens in all groups were weighed weekly and body gain for each group was calculated and feed consumption rate was estimated at the end of experiment (40 day old).

Sampling

Blood samples were collected from five birds in each group on EDTA for hematological study. Serum samples were collected for determination of ALT, AST (13), total protein (14), albumin by colorimetric method (15) and uric acid (16).

At the end of experiment 10 birds of each group were slaughtered for

bacteriological isolation from internal organs. Analysis of data was carried out statistically (17).

RESULTS

Bacteriological examinations

Bacteriological examinations resulted of 110 diseased and dead chicks strains presence of 6 stains of Salmonella spp. with ratio of 5.5% which identified as three untyped Salmonella.

Experimental infected chicks with *Sal. Enteritidis* showed clinical signs manifested by depression, loss of appetite, pasty vents and lose of body weight. Post-mortem examination of both freshly dead and sacrificed experimentally infected chicks revealed that congested liver and caseous plagues in ceca with server enteritis.

The clinical symptoms and P/M lesion were milder with both probiotic and prebiotic and completely disappeared after treatment for 7 successive days.

Infected and non treated chicks (positive control) showed 40% mortalities.

Probiotic and prebiotic reduced mortality from 40% to 6% and 8% respectively in comparison to infected not treated chicks.

The obtained data revealed a significant ($P \leq 0.05$) improvement in terms of life, body weight gain and feed conversion of broiler chicken fed on ration containing probiotic and prebiotic when compared with untreated groups (Table 4).

Probiotic and prebiotic had significant effect on serum ALT, AST and uric acid of treated broiler (Table 5). On the other hand, a significant ($P \leq 0.05$) increase in serum protein level in groups treated with probiotic and prebiotic was recorded. A significant increase of erythrocyte and lymphocytes counts were noticed in all treated groups (Table 6).

Table 1. Composition of experimental ration

<i>Items</i>	<i>Starter ration (ton)</i>	<i>Grower finisher (ton)</i>
Yellow corn	600	663
Soya bean meal 44%	300	250
Corn gluten 6%	60	60
Dica. ph	15	15
Diomethonin	1	1
Lysine	1	1
Fish meal 68%	28	15
NaCl	3	3
Primex	3	3
Total	1000 kg	1000 kg
Calculated C.P	23%	21%
ME/kg(kcal)	3100	3200

Crude CP: Protein

ME: Metabolism energy

Table 2. Mortality rate and frequency of reisolation of *Sal. enteritidis* from infected broiler chicks and probiotic and prebiotic treated chicks

<i>Group</i>	<i>Parameter</i>	<i>Mortality</i>		<i>Frequency of reisolation</i>
		<i>NO</i>	<i>%</i>	
(A) Control		0	0	0
(B) Positive control		20	40	100%
(C) Infected and treated with probiotic		3	6	10%
(D) Infected and treated with prebiotic		4	8	10%
(E) Treated with probiotic		0	0	0
(F) Treated with prebiotic		0	0	0

Table 3. Effect of oral administration of probiotic (1g/kg ration) and prebiotic (1g/kg ration) on mean body gain, feed conversion ratio (FCR) of *Sal. enteritidis* infected broilers (Mean \pm S.E.) (n = 5)

Group	Parameter	Week after infection			6 weeks old		FCR
		Initial body weight (gm)	Body weight (gm)	Body weight gain (gm)	Body weight (gm)	Body weight gain (gm)	
(A) Control		51 \pm 5.0	703 \pm 20 ^c	561 \pm 15	1887 \pm 111	1845 \pm 71 ^c	1.96
(B) Positive control		52 \pm 1.4	360 \pm 12 ^d	501 \pm 12	1355 \pm 216	1350 \pm 120 ^d	2.3
(C) Infected and treated with probiotic		51 \pm 1.3	709 \pm 12 ^b	658 \pm 25	2000 \pm 185	195 \pm 12 ^b	1.8
(D) Infected and treated with rebiotic		53 \pm 1.2	712 \pm 15 ^b	659 \pm 22	1989 \pm 120	1915 \pm ?? ^b	1.87
(E) Treated with probiotic		50 \pm 1.3	728 \pm 22 ^a	678 \pm 28	2122 \pm 70	2172 \pm 63 ^a	1.76
(F) Treated with prebiotic		52 \pm 1.3	725 \pm 30 ^a	673 \pm 33	2196 \pm 80	2144 \pm 70 ^a	1.78

Different letters in the same row indicated significant changes (P < 0.05)

Table 4. Effect of probiotic (Bioplus 2B) and prebiotic (Bio-Mos) on some biochemical parameters of Salmonella infected 4 weeks broiler chicks (Mean \pm S.E.) (n = 5)

Group	Parameter	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT (u/L)	AST (u/L)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group A		3.34 \pm 0.15 ^b	1.75 \pm 1.14 ^b	2.2 \pm 0.11 ^b	12 \pm 1.2 ^b	93 \pm 5.12 ^a	3.4 \pm 0.7 ^d	0.64 \pm 0.09
Group B		3.96 \pm 0.06 ^d	0.8 \pm 0.03 ^d	3.16 \pm 0.1 ^d	31 \pm 2.5 ^d	146 \pm 8.3 ^c	7.49 \pm 0.2 ^a	0.81 \pm 0.01
Group C		3.55 \pm 0.09 ^c	1.18 \pm 0.22 ^c	2.37 \pm 0.2 ^c	14 \pm 2.5 ^c	112 \pm 2.1 ^b	4.32 \pm 0.3 ^b	0.71 \pm 0.08
Group D		3.58 \pm 1.1 ^c	1.29 \pm 0.1 ^c	2.28 \pm 0.17 ^c	14 \pm 2.1 ^c	114 \pm 2.1 ^b	4.38 \pm 0.20 ^b	0.66 \pm 0.07
Group E		3.22 \pm 0.2 ^a	1.9 \pm 0.1 ^a	1.32 \pm 0.16 ^a	10 \pm 1.6 ^a	92 \pm 2.8 ^a	3.8 \pm 0.12 ^c	0.58 \pm 0.08
Group F		3.18 \pm 0.16 ^a	1.92 \pm 0.16 ^a	1.26 \pm 0.12 ^a	11 \pm 1.1 ^a	93 \pm 1.9 ^a	3.77 \pm 0.12 ^c	0.60 \pm 0.1

Different letters in the same row indicated significant changes (P < 0.05)

Table 5. Effect of probiotic and prebiotic on haemogram of Sal. infected broiler chicks (Mean \pm S.E.) (n = 5)

Group	Parameter	RBCs ($10^6/\text{mm}^3$)	PCV (%)	Total leucocytic count ($10^3/\text{mm}^3$)	Deferential leucocytic count				
					L%	H%	B%	E%	M%
Group A		3.22 ± 0.08^b	44 ± 1.4^b	15 ± 600	50 ^a	42	1	2	5
Group B		2.87 ± 0.5^d	35 ± 1.2^d	15.5 ± 700	38 ^c	54	2	1	5
Group C		3.12 ± 0.4^c	38 ± 2.1^c	16 ± 800	45 ^b	47	1	1	6
Group D		3.04 ± 0.5^c	37 ± 3.2^c	16.5 ± 500	46 ^b	48	1	2	3
Group E		3.34 ± 0.4^a	47 ± 0.8^a	16.5 ± 500	54 ^a	42	1	2	2
Group F		3.36 ± 0.3^a	48 ± 0.2^a	16 ± 800	53 ^a	41	0	1	5

Different letters in same row indicated significant changes ($p \leq 0.05$)

DISCUSSION

Avian salmonellosis is a problem of economic concern to all phases of the poultry industry from production to marketing (18).

Bacteriological examination of 110 diseased and dead broiler chicks aged 1-30 days old collected from Sharkia province revealed that 6 salmonella isolates with isolation rate 5.5%. These isolates were identified into two *Sal. enteritidis*, one *Sal. Typhimurium* and three untyped isolates. These findings are similar to that previously cited (19).

The clinical signs observed on infected and non treated chicks were depression, pasty vents and loss of body weight. Similar symptoms were previously recorded (20).

The infected group and treated chicks with probiotic (Bio plus 2B) and prebiotic (Bio-Mos) at dose 1g/kg ration 48 hrs before infection and after infection reduced clinical signs and decreased mortality rate from 40% to 6%.

These findings indicating the effectiveness of probiotic and prebiotic in both treatment and prevention of Salmonella infection.

Numerous reports have indicated that effectiveness of probiotic and prebiotic in treatment of Salmonella infection (21).

The obtained result revealed that probiotic and prebiotic improved the body weight gain and feed conversion of broiler. Supplementation of broiler chickens with probiotic for 6 weeks, resulted in high body weight and feed conversion efficiency (4, 22). *Hopper and Mawar*, (21) noticed that probiotic improved the feed conversion rate in growing broilers. The growth promoting effect of the present probiotic product may be due to microbial constituent (*Bacillus subtilis*) which produce natural lactic acid that helps in maintaining an optimum low pH to inhibit growth of undesirable bacteria leading to optimal engine activity (23).

The obtained findings in this work showed a significant ($p \leq 0.05$) increase of body weight gain and feed conversion of broiler treated with a probiotic and prebiotic.

The positive effect of probiotic on growth might be attributed to induction of changes in the population and metabolic characteristic of gastrointestinal bacteria (24). The same conclusion was suggested *Hooge* (25) who mentioned that prebiotic in feed has significant effect on broiler performance trails.

In the current work, body gain and feed conversion were improved in probiotic and prebiotic used as growth promoter (26).

Results of sera analysis revealed that a significant ($p \leq 0.05$) increase of albumin level in broilers treated with probiotic and prebiotic.

Biochemical analysis of serum chicks infected with *Sal. enteritidis* showed that the serum AST, ALT and globulin were impressed towards normal levels as a result of treatment of infected chicks with probiotic and prebiotic.

The increase in serum AST and ALT activity after infection suggest a hepatocellular damage (27).

Treatment with probiotic displayed non significant changes in serum AST, ALT as well as total protein and albumin. Recent investigation recorded similar results (5,21). The authors mentioned that probiotic had adverse effects on liver enzymes where there was a rise with probiotic and prebiotic treatment.

Creatinine and uric acid levels were significantly increased in infected non treated chicks with Salmonella. Infected chicks with *Sal.* and treated with probiotic or prebiotic showed decrease in creatinine and uric acid level which returned to normal levels. These results coincided with study reported that the probiotic had no significant nephrotoxicity in rates (28).

A significant increase in total erythrocytic count and hematological picture lymphocytes of broiler received probiotic or prebiotic were recorded. This improvement

could be explained on the base of improved bio-availability of essential nutrient (29) and increase of bacterial population enhancing Vit. B synthesis and/or absorption (30).

The obtained result was reinforced with the study which detected improved erythrogram in chickens received pro / or prebiotic (31).

Finally it could be concluded that medication of Sal. infected broiler chicks at dose 1g/kg ration of probiotic and prebiotic improved body weight gain and feed conversion. Probiotic and prebiotic are considerable value in treatment and prevention of Salmonellosis in broilers.

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الملخص العربي

تقييم بعض البروبيوتك والبريبوتك فى الوقاية من عدوى الباراتفويد

فى قطعان التسمين

أشرف عبد الرحمن محمد الشافعى ، حلمى إبراهيم رجب البنا ، صفوت إبراهيم شلبى

معهد بحوث صحة الحيوان - فرع الزقازيق

تم عزل ٦ عترات من السالمونيلا من ١١٠ عينة من بدارى التسمين من عمر ١ إلى ٣٠ يوم من مراكز مختلفة بمحافظة الشرقية وتم تصنيفهم الى عدد أثنين معزولة سلمونيلا انترتيدس وواحد معزولة تيفي ميوريم وثلاثة معزولات لم يتم التعرف عليهم وكانت التجربة على ٣٠٠ كتكوت تسمين وقسمت بالتساوى إلى ٦ مجاميع وكانت المجموعة الأولى مجموعة ضابطة والمجموعة الثانية معدية بالسالمونيلا انترتيدس بالحقن العضلى ٠,٢٥ سم C.f.u. ١٠٣٠٣. وتم حقن المجموعتين الثالثة والرابعة بنفس الطريقة. وتم إضافة ١ جم/كجم عليقة من البروبيوتيك للمجموعة الثالثة والخامسة وكذلك تم إضافة ١ جم / كجم عليقة من البريبوتيك. وكان العلاج قبل العدوى بالسالمونيلا لمدة ٤٨ ساعة ومستمر حتى نهاية التجربة.

وعند مرور أسبوعين بعد الحقن كانت الأعراض واضحة على الكتاكيت فى المجموعة الثانية التى أخذت العدوى فقط بدون علاج. وتلاحظ فاعلية الوقاية بالبروبيوتيك والبريبوتيك فى الوقاية من العدوى.

بالإضافة إلى هذا كانت العدوى بالسالمونيلا تسبب زيادة معنوية فى قيم ALT, AST حمض البوليك والجلوبيولين وهناك نقص معنوى فى نسبة الألبومين بدأت هذه القيم بالتحسن فى المجاميع التى تم علاجها بالبروبيوتيك والبريبوتيك.