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Original Research Article

Effect of Probiotics on Serum Biochemical and Blood Constituents in Chicken Challenged with *Salmonella enterica* Subsp Typhimurium

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Abstract

Purpose: *To examine the effects of two commercial probiotics (Toyocerin and CloSTAT) on serum enzyme activities, and hematological and biochemical indices of broiler chickens challenged with Salmonella enterica serovars Typhimurium (ST).*
Methods: *The chicks received one of the following treatments at 0 day of age: T1 = control group, unmedicated diet, unchallenged birds, (negative control); T2 = unmedicated diet + bacterial challenge (positive control); T3 = medicated diet with neoxyval (0.05 g/kg diet) + bacterial challenge (NEOX); T4 = toyocerin (1 g/kg diet) + bacterial challenge (Toyocerin); and T5 = CloSTAT (1 g/kg diet) + bacterial challenge (COLS). Blood samples were withdrawn from 7 selected chicks in each treatment at 7, 21, 28, 35 and 42 days of age, and analyzed for total protein, albumin and globulin concentration, and the albumin: globulin ratio computed. Glutamate-oxaloacetate transaminase (GOT) and glutatamate pyruvate transaminase (GPT) levels in serum were measured on days 7 and 42.*
Results: *The results revealed that albumin ($p < 0.001$), globulin ($p < 0.001$) and albumin: globulin ratio ($p < 0.001$) were influenced by the time of blood collection. Globulin increased significantly after ST challenge while albumin decreased significantly. Glutamate-oxaloacetate transaminase (GOT) after ST challenge was affected by treatment ($p < 0.05$). Higher levels were obtained from birds which had received NEOX or positive control, compared to negative control group.*
Conclusion: *Salmonella challenge affects serum albumin, globulin and GOT enzyme. The results obtained suggest that the probiotic, Toyocerin, mitigates the negative effects of Salmonella challenge.*

Keywords: *Bacillus subtilis PB6, Bacillus Cereus var. toyoi, Probiotics, Liver enzymes, Hematology, Salmonella enterica*

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INTRODUCTION

Salmonella enterica serovars typhimurium (ST) is an enteric bacterium that can colonize chickens; it is a common serovar causing salmonellosis in broiler chickens [1,2]. The extent of *Salmonella* infection after oral inoculation can be systemic and often comprises infection of the liver [3]. After oral inoculation of adult Leghorn hens with doses of 109 cells of *Salmonella enteritidis*, it was recovered from 53 % of liver samples after 5-weeks post-inoculation [4].

A significant decline in growth performance was reported in broilers as a result of *Salmonella* [5,6]. The reduction in performance was ascribed

to decrease in feed intake in broilers due to mucosal damage, diarrhea and systemic infection [7,8]. The liver is one of the main systemic organs affected by *Salmonella* and as a result of damaged liver cells, liver enzymes increase in the blood.

Currently, probiotics are used in the poultry industry as an alternative to antimicrobial growth promoters [9]. Probiotics have been reported to prevent the colonization of the gut by pathogenic bacteria like *Clostridium perfringens* and *Salmonella* through the mechanism of competitive exclusion [9,10]. *Bacillus subtilis* is capable of producing an antimicrobial factor against many bacteria [11]. Other reports showed that *Bacillus cereus* var. toyoi based probiotics reduced the prevalence of *Salmonella* in poultry [12]. There is scarcity of information on the effects of probiotics on serum hematological, biochemical indices and liver enzymes activity in broiler chickens challenged with oral salmonella. Therefore, in the present study, the objective was to evaluate the effect of two strains of probiotics (*Bacillus subtilis* PB6 and *Bacillus cereus* var. toyoi) on hematological and biochemical indices of ST challenged broiler chickens.

EXPERIMENTAL

Animals, husbandry and treatment

The study was conducted under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia. The experiment was carried out from 0 to 42 days of age by utilizing a total of two hundred, Cobb 500 broiler chicks which were allotted into 50 experimental cages with four chicks per cage. The chicks had been vaccinated for Marek's disease, Newcastle and infectious bronchitis. Feed and water were provided *ad libitum* and the birds were maintained at 24 h light schedule. A typical isocaloric and isonitrogenous starter (0 - 14 d) and finisher (15 - 42 d) diets based on corn-soybean meal diets were formulated in mashed form which met or exceed the recommendations in commercial practice (Tables 1, 2 and 3).

Chicks received the dietary treatments at 0 day of age as follows: T1 = control group, unmedicated diet, unchallenged birds, (negative control); T2 = unmedicated diet + ST (positive control); T3 = medicated diet with neoxyval (0.05 g/kg diet) + ST (NEOX);

Table 1: Dietary ingredients and chemical composition of starter diets (0 to 14 days)

Ingredient (%)	T1, T2¥	T3¥	T4¥	T5¥
Corn	63.01	63.01	62.91	62.91
Soybean meal	31.15	31.15	31.15	31.15
Palm oil	1.72	1.72	1.72	1.72
Dicalcium phosphate	1.96	1.96	1.96	1.96
Limestone	0.73	0.73	0.73	0.73
Salt	0.25	0.25	0.25	0.25
VM Mix[1]	0.5	0.5	0.5	0.5
DL-Methionine	0.25	0.25	0.25	0.25
Lysine-HCL	0.18	0.18	0.18	0.18
Threonine	0.07	0.07	0.07	0.07
Sodium bicarbonate	0.12	0.12	0.12	0.12
Choline chloride	0.05	0.05	0.05	0.05
Neoxyval	0	0.005	0	0
Toyocerin	0	0	0.1	0
Clostat	0	0	0	0.1
Total	***100***	***100***	***100***	***100***

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg; ¥ T1=control group, unmedicated diet, unchallenged birds, (negative control); T2=unmedicated diet + bacterial challenge (positive control); T3=medicated diet with neoxyval (0.05 g/kg diet) + bacterial challenge (NEOX); T4= toyocerin (1 g/kg diet) + bacterial challenge (TOYO); and T5=CloSTAT (1 g/kg diet) + bacterial challenge (COLS)

Table 2: Dietary ingredients and chemical composition of finisher diets (15 to 35 days)

Ingredient (%)	T1, T2	T3	T4	T5
Corn	70.09	70.09	69.99	69.99
Soybean meal	23.08	23.08	23.08	23.08
Palm oil	2.98	2.98	2.98	2.98
Dicalcium phosphate	1.87	1.87	1.87	1.87
Limestone	0.59	0.59	0.59	0.59
Salt	0.25	0.25	0.25	0.25
VM Mix[1]	0.5	0.5	0.5	0.50
DL-Methionine	0.25	0.25	0.25	0.25
Lysine-HCL	0.17	0.17	0.17	0.17
Threonine	0.07	0.07	0.07	0.07
Sodium bicarbonate	0.11	0.11	0.11	0.11
Choline chloride	0.04	0.04	0.04	0.04
Neoxyval	0	0.005	0.005	0
Toyocerin	0	0	0.1	0
Clostat	0	0	0	0.1
Total	100	100	100	100

[1]Same as in the starter diet (Table 1)

Table 3: Chemical composition of the diets

Composition	0 to 14 days	15 to 35 days
ME, kcal/kg	3000	3150
Crude protein, %	20.5	17.28
Methionine, %	0.55	0.51
Lysine, %	1.20	0.98
Sulfur amino acids, %	0.89	0.80
Threonine, %	0.85	0.73
Calcium, %	0.95	0.85
Phosphorus, %	0.41	0.38

T4 = toyocerin (1 g/kg diet) + ST (TOYO); and T5 = CloSTAT (1 g/kg diet) + ST (COLS). Neoxyval® (Sogeval Laboratory, France) was used as the reference antibiotic; each 1 g of the antibiotic contains 200 mg oxytetracycline® and 200 mg neomycin®. Toyocerin® is a product with an active ingredient consists of viable spores of a microorganism: *Bacillus cereus* var. toyoi (Rubinum, Spain). CloSTATTM is a probiotics which contains a unique strain of *Bacillus subtilis* PB6 (CloSTAT, Kemin Industries Inc., Des Moines, IA).

Challenge inoculum

At day 16 of age, chicks in treatments 2 to 5 were challenged with ST (3 x 10^9 CFU/ml). The strain used in this experiment was *Salmonella typhimurium* ATCC13311. Chicks were gavaged with 1 ml of cocktail containing 3 x 10^9 CFU/mL ST which was obtained commercially (MicroBiologics, Cloud, MN-U.S.A) according to the procedure described by [6].

Hematological and biochemical measurements

The following analyses were conducted by using enzymatic colorimetric kits: total protein (Biuret method), albumin (Bromoreesol green method), globulin concentration was calculated, thereafter, as the difference between total protein and albumin concentrations. Albumin: globulin ratio was calculated. Seven chicks per group were selected for blood collection via brachial venipuncture into plain tubes for serological analysis starting at days 7 (before bacterial challenge), 21, 28, 35 and 42 (after the challenge). Samples were centrifuged by using plain tubes at 5 ºC and 3,000 rpm for 10 min. Serum samples were stored in eppendorf tubes and stored at -80 ºC until further analysis, unless fresh sample is required for the analysis.

The activities of glutamate-oxaloacetate transaminase (GOT) and glutatamate pyruvate transaminase (GPT) in the serum were measured according to the colorimetric method by using GOT-GPT assay kits, Asan Pharmaceutical). For this purpose blood samples were withdrawn from 7 selected chicks of each treatment at days 7 (before bacterial challenge) and 42 (after the challenge).

At the end of the trial, blood samples were withdrawn from 5 selected chicks of each treatment via brachial venipuncture into EDTA tubes for hematological analysis and were placed inside an ice box and transferred to the laboratory. Within 1 h after collection, the hematological parameters were determined (Maxcom Auto Hematology Analyzer (MC-6200, China.

Statistical analysis

Data were analyzed by using the general linear model procedure of SAS [13]. Five treatments were arranged in 10 replications in a randomized complete block design. Means of measurements

showing significant differences in analysis of variance (ANOVA) were tested using the difftype statement (PDIFF). Overall level for statistical significance was set at $p < 0.05$. All values are expressed as statistical means ± standard error of the mean (SEM).

RESULTS

The data related to serum biochemical indices are shown in Table 4. Total protein, albumin, globulin, and albumin : globulin ratio (A/G ratio) were similar among all groups ($p > 0.05$). The interaction term (treatment*week) was not significant for any parameters measured for serum biochemistry ($p > 0.05$).

On the other hand, albumin, globulin and their ratio (A/G ratio) were influenced by time of collection (week) ($p < 0.001$). Albumin concentration was the lowest when measured after the challenge (1.13 g/dl for week 3), then albumin concentration the uttermost at weeks five and six (1.94, 194 g/dl, respectively). In the contrary, globulin concentration increased right after the challenge (0.89 vs. 1.58 g/dl, for weeks one and three, respectively) and then started to decline after week four. On weeks five and six, the values were similar to the value before the challenge in week one (0.72, 0.75 and 0.89 g/dl, respectively). The A/G ratio dropped significantly after the challenge from 3.02 in week one to 0.87 in week three. In weeks five and six, A/G ratio was not different from week one.

The activities of glutamate-oxaloacetate transaminase (GOT) and glutatamate pyruvate transaminase in the serum are presented in Table 5. Treatment showed no effect on GOT or GPT in serum before the challenge (week one) ($p > 0.05$). However, after the challenge (week 6), serum from birds which had received NEOX or positive control had significantly higher GOT value as compared to negative control (274.4, 244.6 and 195.6 IU/L, for NEOX, positive and negative control, respectively) ($p < 0.05$). GOT for birds which had received CLOS was 230.6 IU/L. The lowest GOT value was obtained from birds which had received the negative control or TOYO (195.6 and 200.4 IU/L, respectively). On the other hand, week of collection showed a significant effect on GOT ($p < 0.01$), the mean for GOT in week six was 229 IU/L as compared to 199 IU/L in week one. In this study, the GPT values after the challenge were not affected by any treatments ($p > 0.05$).

Table 6 shows the effect of treatment on hematological parameters of broiler blood profile. The values of the blood parameters measured in this trial were comparable and treatment had no effect on blood hematological values ($p > 0.05$).

Table 4: Serum biochemical profile of broilers

Treatment	***Challenge***	**Total protein** (g/dl)	**Albumin** (g/dl)	**Globulin** (g/dl)	**A/G ratio**
Negative control	No	2.73	1.62	1.11	2.43
Positive control	Yes	2.86	1.72	1.13	1.93
Neoxyval®	Yes	2.79	1.70	1.09	2.29
Toyocerine®	Yes	2.57	1.63	0.94	2.4
Clostat®	Yes	2.76	1.58	1.19	2.02
SEM±		0.13	0.05	0.12	0.24
P value		ns	ns	ns	ns
Age (weeks)	*Challenge*				
1	No	2.68	1.78^{b}	0.89^{b}	3.02^{a}
3	Yes	2.71	1.13^{d}	1.59^{a}	0.87^{b}
4	Yes	2.96	1.46^{c}	1.52^{a}	1.21^{b}
5	Yes	2.77	1.94^{a}	0.72^{b}	3.35^{a}
6	Yes	2.69	1.94^{a}	0.75^{b}	3.20^{a}
SEM		0.11	0.04	0.11	0.19
p value		ns	***	***	***
*Treatment*Week*		ns	ns	ns	ns

For treatment, each mean is based on 35 birds. For week, each treatment is based on 7 birds. ^{ab}Means in the column with different superscripts differ significantly, ***$p < 0.001$

Table 5: The concentration of hepatic enzymes in serum before and after the bacterial challenge

	Before challenge		After challenge	
	GOT	GPT	GOT	GPT
Treatment	(IU/L)	(IU/L)	(IU/L)	(IU/L)
Negative control	201.6	4.33	195.6^{c}	3.6
Positive control	214.8	3.87	244.6^{ab}	4.93
Neoxyval	189.0	4.08	274.4^{a}	4.52
Toyocerine	199.0	4.25	200.4^{bc}	4.62
Clostat	190.8	4.72	230.6^{abc}	3.41
SEM ±	11.5	1.27	16.46	0.61
p value	ns	ns	*	ns

Age (weeks)	*Challenge*		
1	No	199^{b}	4.25
6	Yes	229^{a}	4.22
SEM		7.23	0.04
P-value		**	ns
*Treatment*Week*		ns	ns

Each mean is based on 7 replicates of birds: ^{abc}Means in the column with different superscripts differ significantly (*p < 0.05)

Table 6: Effect of different treatments on blood hematology

Parameter	Control	Negative	Neoxyval	Toyocerine	Clostat	SEM	*P-value*
WBC (x 10^{9}/l)	111.8	110.9	114.1	113.7	109.1	2.99	ns
RBC (x 10^{12}/l)	4.39	4.61	4.4	4.6	4.9	0.18	ns
HGB (g/dl)	40.8	40.9	39.3	40.8	41.7	1.09	ns
MCV (fl)	113.7	114	111.6	111.5	114.1	1.59	ns
MCH (pg)	92.9	88.9	89.7	89.7	84.7	2.23	ns
MCHC (g/dl)	81.7	78	80.4	80.4	74.2	2.17	ns
RDW-CV (%)	9.7	10.1	9.7	10.4	9.9	0.35	ns
RDW-CD (fl)	44.6	46.2	43.6	46.6	45.6	1.96	ns
HCT (%)	49.9	52.5	49.1	50.9	56.6	2.13	ns
PLT (x 10^{9}/l)	31.2	33.2	36.8	32.8	44.6	5.44	ns
MPV (fl)	11.6	11.5	11.4	11.6	11.6	0.24	ns
PDW	12.0	12.01	11.8	13.7	16.3	1.71	ns
PCT (%)	0.036	0.038	0.041	0.038	0.051	0.006	ns

WBC: white blood cell counts, RBC: total red blood cell counts, HGB: hemoglobin content, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: standard deviation in red cell distribution width, RDW-CV: coefficient variation of red cell distribution width, HCT: hematocrit, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit

DISCUSSION

The data related to serum biochemical indices indicated no differences between treatments in total protein, albumin, globulin, and A/G ratio among all groups. The findings of this trial are in agreement to the findings of [14] who reported no significant differences in serum biochemical indices examined as a result of probiotic supplementation.

The activities of glutamate-oxaloacetate transaminase (GOT) and glutatamate pyruvate transaminase in the serum were not affected by treatment before the bacterial challenge. However, after the challenge higher GOT values were found in birds which had received NEOX or positive control while probiotics lowered the GOT value especially for TOYO group. This finding agrees with [15] who reported that colonization of *Salmonella* in organs increases the liver enzyme activities in blood. Several studies have assessed the effects of *Salmonella* on the gene expression of chicken immune tissues or cells [16,17]. It was reported that the effects of *Salmonella* infection on the liver transcriptome profiles of broilers reflect a predominance of down regulation of genes with cell cycle and metabolic functions [18]. A clear sign of hepatic damage is the leaking of cellular enzymes into the plasma [19].

The values of the hematological parameters of broiler blood profile were comparable and treatment had no effect on blood hematological

values. Similar to our findings, it was reported that probiotics did not show any harmful changes on blood hematological parameters and it could improve health condition by enhancing concentration of hemoglobin, hematocrit and red blood cell count in broilers [20]. Conversely, probiotics lowered lymphocytes and neutrophil values when compared to the control [21].

CONCLUSION

Neither challenge nor treatment has a significant impact on blood hematological values. Albumin, globulin and their ratio (A/G ratio) were influenced by time of collection. Albumin concentration was lowest when measured after the challenge. On the contrary, globulin concentration increased right after the challenge. The results from this study suggest that the probiotic, TOYO, alleviates the negative effects of *Salmonella* challenge.

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