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Clinicopathological and Immunological Effects of Multistrain Probiotic on Broiler Chicken Vaccinated Against Avian Influenza Virus

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Abstract: Avian influenza virus considered one of the most important avian viruses causing economic losses in poultry industry. In February 2006, highly pathogenic avian influenza (HPAI) virus was firstly recognized in Egypt and since this time the disease had become enzootic in poultry throughout much of the country. In the present work, a total of 160 one day old commercial (Cobb) broiler chicks were used for studying clinicopathological and immunological effects associated with the use of multistrain probiotic (Protexin®) on vaccinated chicks against avian influenza virus. To evaluate probiotic immunological effect, geometric mean haemagglutination inhibition antibody (GM-HI) titers of chicken against avian influenza virus (AIV) H5N2 was carried. Hematological and serum biochemical parameters were carried also, to evaluate its clinicopathological effects. Immunological and clinicopathological results revealed, the used multistrain probiotic (Protexin®) has immunostimulatory, hepatostimulatory and hepatoprotective effects on vaccinated chicks against avian influenza virus H5N2.

Key words: Clinical Pathology · Immunology · Avian Influenza Virus · Probiotic

INTRODUCTION

The term "influenza" originally referred to epidemics of acute, rapidly spreading catarrhal fevers of humans caused by viruses in the family *Orthomyxoviridae* [1]. Today, orthomyxo viruses are recognized as the cause of significant numbers of natural infections and diseases, usually of the upper respiratory tract in human, horses, domestic pigs and various bird species [2]. Infection of domestic poultry by avian influenza viruses (AIV) typically produces syndromes ranging from asymptomatic infection to respiratory disease and drops in egg production to severe systemic disease with nearly 100% mortality [3].The latter form of disease resulted from the infection by highly pathogenic avian influenza (HPAI) virus. In February 2006, HPAI virus was firstly recognized in Egypt and since this time the disease had become enzootic in poultry throughout much of the country. Birds are susceptible to infection with influenza viruses belonging to any of the 15 hemagglutinin (HA) subtypes and there is no way to predict their exposure to any particular one. It is not practical to use preventive vaccination against all possible subtypes [4]. In poultry industry, probiotics have been established in feeding practice in recent years to support the gut microflora and to maintain the health of animals [5&6]. Probiotics are single or mixed cultures of microbes have beneficial effects on host health [7]. These probiotics, when fed, improve properties of indigenous microflora [8] and feed conversion ratio [9] which reflected on the performance of poultry [10]. Probiotics after residing intestinal tract, their metabolites can act as immunomodulatory agent by activating specific and non-specific immune responses in chicks, which help in prevention and control of various infectious diseases [11]. Protexin® is a multistrain probiotics used in poultry feed [12]. It contains naturally occurring nine different species of beneficial microflora which are generally regarded as safe by the American food and drug administration [13]. Protexin® is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts (*Lactobacillus plantarum, Lactobacillus bulgaricu, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacteriumbifidum, Streptococcus thermophilus, Enterococcus faecium, Aspergillusoryza, Candida pintolopesii)* [12]. The present work aimed to study clinicopathological and immunological effects of Protexin® on broiler chicks vaccinated against avian influenza virus (H5N2).

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MATERIALS AND METHODS

Chicks and Experimental Design: A total of 160 one day old commercial (Cobb) broiler chicks were used in this study. All of them were reared on floor housed system and were fed *ad libitum* on a balanced commercial ration. The chicks were divided equally into 4 groups as follow; A, B, C and D each group contains 40 chicks. Group A, fed on a balanced ration and considered as a control group. Group B, fed on a balanced ration with probiotic supplementation (Protexin®) at a rate of 150 gm/ton ration. Group C, fed on a balanced ration and was vaccinated against AIV. Group D, fed on a balanced ration with probiotic supplementation at a rate of 150 gm/ton ration and was vaccinated against AIV. The experiment continued for 6 weeks through which collection of samples was performed weekly.

Vaccination: Chicks of groups C and D were vaccinated with inactivated H5N2 avian influenza vaccine (Intervet International BV Boxmeer-Holland) by subcutaneous route (S/C) at 7^{th} and 28^{th} day of age. Group A, acted as a control group neither vaccinated nor probiotic treated.

Blood Samples for Clinicopathological and Serological Examinations: Blood samples from 10 chicks of each group were collected at weekly intervals. Two blood samples were taken from each bird (wing vein). The first blood sample was anticoagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and used for evaluating hemogram. The second blood sample was collected in a clean centrifuge tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non hemolysed supernatant serum was harvested for biochemical studies and haemagglutination inhibition (HI) test for determining serum antibody titers against AIV [14].

Hematological and Serum Biochemical Studies

Hematological Studies: Total erythrocyte and leukocyte counts were done using an improved Neubauer hemocytometer. Packed cell volume (PCV %) was estimated by microhematocrit technique. Hemoglobin concentration was colorimetrically determined using cyanmethemoglobin method. Differential leukocytic count was performed on Giemsa stained blood smears [15].

Serum Biochemical Studies: Serum samples were prepared to assay the following biochemical studies; serum total proteins was determined by the Biuret reaction according to Weichselbaun [16], serum albumin was determined according to Dumas and Biggs [17] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. A/G ratio was obtained by subdividing values of serum albumin by those of serum globulins. Colorimetric determination of aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities was performed according to Reitman and Frankel [18] and Tietz [19], respectively. Blood glucose level was determined as described by Trinder [20]. Serum uric acid was determined according to Fossati *et al.* [21]. Serum creatinine was assayed using the method described by Fabiny and Ertingshausen [22]. Serum total cholesterol was determined according to Allain *et al.* [23]. Serum high density lipoprotein cholesterol (HDL-c) was determined according to Warnick *et al.* [24]. Serum low density lipoprotein cholesterol (LDL-c) was calculated according to Friedewald *et al.* [25] with the following equation; LDL= Total cholesterol-HDL-Triglycerides/5. Serum very low density lipoprotein cholesterol (VLDL-c) was determined according to Friedewald *et al.* [25]. Serum totaltriglycerides were determined according to Wahlefeld [26]. Serum calcium and inorganic phosphorus concentrations were determined according to Biggs and Moorhead [27] and Goodwin [28], respectively. The above mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

Statistical Analysis: Values were expressed as mean ± SD. Statistical comparisons among the means of different experimental groups were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance.

RESULTS AND DISCUSSION

Immunological Results: Geometric mean haemagglutination inhibition antibody (GM-HI) titers against avian influenza virus (H5N2) of chicken in various experimental groups at weekly intervals are summarized in Table, 1.

The immune response after vaccination is an elegant tool for studying the effect of probiotic in both in human and animal species [29]. In our study, for evaluating the effect of probiotic on immune system, experimental chicks were vaccinated with inactivated H5N2 avian influenza vaccine by S/C route at 7^{th} and 28^{th}day of age.

Table 1: Avian influenza virus (H5N2) geometric mean haemagglutination inhibition antibody (GM-HI) titers of chicken in various experimental groups at weekly intervals.

	GM-HI			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)
0	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00
2	0.00	0.00	11.00	14.00
3	0.00	0.00	17.00	25.00
4	0.00	0.00	23.00	44.00
5	0.00	0.00	39.00	71.00
6	0.00	0.00	42.00	82.00

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

The results showed, GM-HI titer against avian influenza virus (H5N2) in chicks at 0 and 1st week were zero in all groups. These results may be due to absence of maternal antibodies which indicates the parent stock was not vaccinated with avian influenza vaccine (H5N2) and also, the first vaccination was done on the day 7. GM-HI titer of chicks in experimental groups A, B, C and D at 2nd week were 0, 0, 11 and 14, respectively indicating the development of titer post vaccination in sera of all treatment groups except in A and B groups (unvaccinated groups). At 3rd week, GM-HI titer of chicks in experimental groups A, B, C and D were 0, 0, 17 and 25, respectively. At 4th week, GM-HI titer of chicks in experimental groups A, B, C and D were 0, 0, 23 and 44, respectively. At 5th week, GM-HI titer of chicks in experimental groups A, B, C and D were 0, 0, 39 and 71, respectively. At 6th week, GM-HI titer of chicks in experimental groups A, B, C and D were 0, 0, 44 42 and 82, respectively. At 7th week, GM-HI titer of chicks in experimental groups A, B, C and D were 0, 0, 44 and 86, respectively.

From the above results we observed that, GM-HI titer in group D which received probiotic and vaccine was significantly higher than those of group C whose birds received vaccine only. These results indicate that, the used multistrain probiotic Protexin® has positive effect on immune response of birds against avian influenza virus (H5N2) vaccine. These findings are in agreement with those reported by Zulkifli *et al.* [30], Dalloul *et al.* [31] and Eman *et al.* [32]

Clinicopathological Findings

Erythrogram: Mean values of erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] of different experimental groups are illustrated in Tables 2, 3.

Erythrogram mean values of different experimental groups, in comparison to those of control group A showed, insignificant changes in group C and insignificant increases in groups B and D from the 1st week. This could be attributed to hepatostimulatory and hepatoprotective effects of probiotic leading to production of more RBCs by bone marrow under control of erythropoietic factors released by hepatic cells [33].

Leukogram: Mean values of leukogram [total leukocyte count (TLC), neutrophil, lymphocyte and monocyte counts] of different experimental groups are illustrated in Tables 4, 5.

Compared to control group, results showed significant leukocytosis due to significant lymphocytosis started from the 2nd week in all experimental groups. Lymphocytosis of group B was a result of immunostimulatory effects of probiotic [34], while in groups C and D was a result of vaccination. Lymphocytosis which observed in group D was higher than those in group C due to its treatment with probiotic.

Table 2: Erythrogram of different experimental groups (means ± SD).

	RBCs count (x10^6/µl)				PCV (%)				Hb concentration (g/dl)			
Weeks	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	1.92±0.61	1.97±0.68	1.82±0.52	1.87±0.58	25.17±2.71	26.03±2.37	23.91±2.48	24.73±2.94	10.84±2.60	11.32±2.76	10.62±1.52	11.38±2.31
1	2.00±0.54	2.14±0.71	1.90±0.46	2.21±0.61	25.87±2.14	28.12±1.52	24.58±2.98	26.71±2.02	11.34±1.10	12.45±1.43	11.12±1.69	11.91±2.25
2	2.12±0.50	2.25±0.38	2.01±0.43	2.33±0.61	26.6±1.13	28.69±1.97	25.27±1.12	27.26±1.92	11.36±2.11	12.49±2.27	11.13±1.96	11.93±1.33
3	2.20±0.57	2.37±0.34	2.09±0.67	2.42±0.57	27.13±2.28	29.83±2.59	25.78±1.13	28.34±2.8	11.96±2.40	13.20±1.02	11.72±1.92	12.56±1.41
4	2.40±0.58	2.61±0.68	2.28±0.31	2.64±0.70	28.74±1.95	31.11±1.42	27.30±2.54	29.55±2.03	12.76±2.62	14.46±1.83	12.50±1.36	13.39±1.22
5	2.54±0.65	2.64±0.62	2.42±0.84	2.70±0.64	30.07±1.87	31.73±1.08	28.56±2.95	30.14±1.60	13.44±1.13	14.45±2.35	13.17±1.36	14.11±2.87
6	2.66±0.66	2.82±0.61	2.53±0.61	2.93±0.57	29.84±2.02	32.59±2.53	28.35±2.070	30.96±2.73	13.59±1.82	15.21±2.63	13.32±1.63	15.74±2.25
LSD	0.30				3.65					1.84		

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Table 3: Values of MCV and MCHC of different experimental groups (means ± SD).

	MCV (fl)				MCHC (%)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	124.72±8.04	130.95±9.47	126.03±12.14	133.45±12.35	38.86±5.16	42.96±3.44	39.27±4.29	43.77±4.41
1	122.57±14.24	128.70±13.46	123.86±9.83	131.15±10.64	39.58±2.25	43.74±5.58	39.99±3.72	44.58±4.03
2	119.34±14.53	125.31±15.05	120.60±13.18	127.69±13.53	38.54±3.43	42.60±3.49	38.95±2.13	43.41±4.82
3	117.40±8.91	123.27±8.75	118.64±9.31	125.62±11.72	39.78±5.72	43.96±2.01	40.20±5.21	44.80±5.37
4	113.82±11.94	119.51±9.73	115.02±12.13	121.79±12.82	40.06±3.57	44.28±4.92	40.48±3.60	45.12±4.13
5	112.31±9.66	117.92±14.08	113.49±11.22	120.17±14.21	40.33±3.28	44.57±5.13	40.75±4.13	45.42±5.48
6	106.41±11.44	111.73±14.47	107.53±12.30	113.86±13.95	41.11±2.61	45.43±5.14	41.54±2.64	46.30±4.32
LSD	13.20				1.60			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Table 4: Total leukocyte count (TLC) and heterophil count of different experimental groups (means ± SD).

	TLC (x103/µl)				Heterophil count (x103/µl)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	18.03±2.40	19.92±3.15	18.22±3.46	20.30±3.64	4.18±1.52	4.22±1.11	4.26±1.38	5.20±1.17
1	18.96±2.91	20.96±2.67	19.16±4.80	21.36±4.72	4.75±1.86	4.80±1.33	4.85±1.83	4.86±1.11
2	17.80±4.16	19.67±3.49	17.98±4.55	20.05±3.26	4.36±1.32	4.40±1.42	4.45±1.14	4.71±1.17
3	17.43±2.84	19.27±4.70	17.61±4.44	19.63±4.73	3.98±1.28	4.02±1.96	4.06±1.03	4.21±1.82
4	17.82±2.43	19.69±4.41	18.00±4.93	20.07±2.53	4.44±1.53	4.48±1.88	4.53±1.88	4.75±1.67
5	18.56±3.91	20.52±3.66	18.76±2.42	20.91±4.64	4.60±1.52	4.64±1.65	4.69±1.52	4.57±1.25
6	18.68±2.86	20.64±2.63	18.87±2.65	21.03±4.97	4.95±1.16	5.00±1.55	5.05±1.78	4.74±1.93
LSD	1.81				0.94			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Table 5: Lymphocyte and monocyte counts of different experimental groups (means ± SD).

	Lymphocyte count (x103/µl)				Monocyte count (x103/µl)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	12.78±2.66	12.89±1.26	12.92±1.62	12.95±1.29	1.26±0.13	1.19±0.18	1.27±0.14	1.26±0.14
1	13.18±2.73	13.86±1.67	13.91±1.08	13.94±2.54	1.24±0.14	1.12±0.14	1.24±0.11	1.18±0.13
2	12.38±2.24	15.23±2.78	16.75±2.42	18.12±2.64	1.25±0.17	1.19±0.17	1.25±0.12	1.22±0.16
3	12.40±1.72	15.84±1.64	17.43±2.52	18.85±2.95	1.24±0.16	1.12±0.18	1.23±0.14	1.21±0.08
4	12.33±1.73	16.07±2.43	17.68±1.77	19.12±1.97	1.25±0.22	1.29±0.20	1.25±0.17	1.28±0.06
5	12.99±1.91	16.34±1.42	17.97±1.06	19.44±1.81	1.19±0.13	1.25±0.16	1.18±0.16	1.23±0.21
6	12.70±1.63	16.74±1.61	18.42±2.23	19.92±2.17	1.23±0.17	1.19±0.14	1.24±0.08	1.25±0.16
LSD	2.06				0.21			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Serum Biochemical Evaluation: Statistical analysis of different serum biochemical parameters of different experimental groups is illustrated in Tables 6 -12.

Compared to control group, protein profile results showed, no significant changes were observed in albumin concentration while, significant hyperproteinemia due to hyperglobulinemia with significant decrease in A/G ratio started from 2nd week in all experimental groups was recorded. Hyperglobulinemia of group B resulted from immunopotentiating effect of probiotic [35], while in groups C and D was a result of vaccination. Hyperglobulinemia which observed in group D was higher than those in group C due to its treatment with probiotic.

Table 7: Levels of serum globulins and A/G ratio of different experimental groups (means ± SD).

	Globulins (g/dl)				A/G ratio			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	1.07±0.06	1.19±0.03	1.22±0.06	1.35±0.04	1.32±0.12	1.28±0.07	1.25±0.06	1.17±0.09
1	1.17±0.05	1.29±0.04	1.29±0.04	1.46±0.04	1.31±0.07	1.20±0.08	1.19±0.05	1.05±0.08
2	1.23±0.07	1.35±0.06	1.39±0.07	1.54±0.06	1.15±0.11	1.09±0.12	1.06±0.08	0.95±0.10
3	1.12±0.04	1.24±0.10	1.27±0.04	1.40±0.04	1.25±0.08	1.25±0.07	1.21±0.07	1.10±0.09
4	1.12±0.08	1.24±0.11	1.28±0.90	1.41±0.05	1.39±0.07	1.22±0.09	1.18±0.11	1.07±0.07
5	1.20±0.06	1.33±0.12	1.36±0.11	1.50±0.05	1.29±0.12	1.22±0.05	1.18±0.12	1.07±0.08
6	1.22±0.04	1.35±0.13	1.39±0.08	1.53±0.04	1.27±0.07	1.14±0.06	1.10±0.10	0.99±0.11
LSD	0.13				0.14			

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents probiotic treated group.

Group (C) represents untreated vaccinated group.

Group (D) represents treated vaccinated group.

Table 8: Values of serum total cholesterol and triglycerides of different experimental groups (means ± SD).

	T. Cholesterol (mg/dl)				Triglycerides (mg/dl)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	168.25±14.51	164.51±15.46	167.25±14.90	164.01±7.96	165.39±13.18	168.32±14.42	164.39±7.09	167.82±9.25
1	172.38±11.94	160.14±9.35	171.38±16.01	159.64±4.59	173.42±8.81	171.39±8.10	172.42±12.09	170.89±14.46
2	175.84±15.08	149.73±12.77	174.84±6.71	149.23±5.15	169.04±10.57	128.23±3.48	168.04±12.27	127.73±8.31
3	161.55±11.63	139.46±8.17	160.55±9.38	138.96±4.07	176.95±14.98	126.69±4.13	175.95±9.02	126.19±12.96
4	173.30±13.14	134.91±14.32	172.30±12.12	134.41±5.21	172.56±15.47	108.77±7.40	171.56±8.78	108.27±15.10
5	175.45±13.90	132.30±9.14	174.45±8.50	131.80±5.27	170.57±13.87	112.02±6.93	169.57±11.28	111.52±6.12
6	170.30±11.46	129.16±9.84	169.30±6.07	128.66±14.04	164.00±9.64	108.44±7.61	163.00±7.65	107.94±14.79
LSD	11.97				14.42			

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents probiotic treated group.

Group (C) represents untreated vaccinated group.

Group (D) represents treated vaccinated group.

Table 9: Levels of serum high density lipoprotein (HDL-c) and low density lipoprotein (LDL-c) cholesterol of different experimental groups (means ± SD).

	HDL cholesterol (mg/dl)				LDL cholesterol (mg/dl)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	74.12±1.17	73.58±1.31	73.62±1.80	73.08±1.05	62.45±2.33	58.07±3.19	61.45±2.67	57.57±3.17
1	72.79±0.94	73.57±1.42	72.29±3.35	73.07±2.60	66.31±3.40	53.09±4.01	65.31±8.55	52.59±5.34
2	72.78±1.85	73.27±1.80	72.28±3.11	72.77±1.83	70.65±2.66	51.62±2.23	69.65±6.56	51.12±7.26
3	73.65±0.83	72.59±1.43	73.15±3.32	72.09±2.24	53.91±1.63	42.33±1.67	52.91±9.52	41.83±8.37
4	73.12±1.14	73.99±1.03	72.62±1.63	73.49±2.63	67.06±3.03	39.96±3.62	66.06±8.65	39.46±6.36
5	72.83±1.61	73.54±1.41	72.33±3.46	73.04±2.34	69.91±2.45	37.15±2.45	68.91±5.60	36.65±7.65
6	73.40±1.65	74.02±1.60	72.90±1.76	73.52±1.02	65.50±3.27	34.25±4.30	64.50±4.54	33.75±5.60
LSD	4.68				5.24			

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents probiotic treated group.

Group (C) represents untreated vaccinated group.

Group (D) represents treated vaccinated group.

Table 10: Activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of different experimental groups (means ± SD).

	AST (U/L)				ALT (U/L)				ALP (U/L)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	152.58±11.13	156.64±13.61	152.08±9.77	156.14±14.05	31.23±1.32	29.54±1.62	30.73±1.82	29.04±2.32	133.94±10.87	130.63±11.67	133.44±9.98	130.13±9.24
1	153.97±14.15	157.41±11.11	153.47±13.78	156.91±11.67	29.45±1.19	28.11±2.50	28.95±3.63	27.61±2.07	137.04±8.95	130.36±7.07	136.54±6.24	129.86±8.72
2	156.66±13.73	154.47±12.86	156.16±13.12	153.97±11.35	31.15±1.76	28.95±2.03	30.65±4.04	28.45±1.59	139.63±11.33	133.22±9.34	139.13±8.23	132.72±9.04
3	155.08±9.75	154.17±13.67	154.58±12.73	153.67±10.38	29.69±1.97	29.61±2.16	29.19±5.77	29.11±4.54	138.91±8.75	135.92±9.22	138.41±9.56	135.42±8.66
4	156.72±14.13	149.23±12.41	156.22±11.42	148.73±10.05	31.41±1.77	30.05±2.15	30.91±3.25	29.55±3.95	137.72±9.83	133.86±7.65	137.22±9.06	133.36±8.74
5	155.38±13.65	157.27±12.54	154.88±14.08	156.77±13.84	30.91±1.36	27.89±1.63	30.41±3.27	27.39±4.33	139.34±10.47	136.97±9.81	138.84±6.92	136.47±10.96
6	154.50±13.23	156.84±10.76	154.00±11.47	156.34±12.86	30.64±2.76	28.63±1.52	30.14±5.54	28.13±1.06	135.47±8.55	132.22±8.64	134.97±4.83	131.72±7.27
LSD	16.13				2.98				15.55			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Table 11: Values of serum glucose, creatinine and uric acid of different experimental groups (means ± SD).

	Glucose (mg/dl)				Creatinine (mg/dl)							
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	244.82±7.73	253.14±12.63	244.32±9.69	252.64±10.34	0.32±0.06	0.29±0.04	0.27±0.04	0.24±0.04	5.81±0.50	5.78±0.66	5.83±0.33	5.76±0.54
1	245.05±10.71	254.08±9.60	244.55±9.72	253.58±6.62	0.34±0.03	0.27±0.02	0.29±0.06	0.22±0.05	6.23±0.58	6.04±0.63	6.25±0.82	6.02±0.72
2	254.87±12.81	252.91±15.69	254.37±7.92	252.41±6.32	0.31±0.04	0.29±0.02	0.26±0.06	0.24±0.07	5.87±0.34	5.75±0.75	5.89±0.83	5.73±0.34
3	247.16±9.51	247.70±13.54	246.66±8.66	247.20±5.83	0.31±0.05	0.28±0.03	0.26±0.04	0.23±0.06	5.91±0.56	5.92±0.63	5.93±0.42	5.90±0.53
4	247.42±9.91	250.91±10.19	246.92±8.67	250.41±7.55	0.32±0.03	0.29±0.04	0.27±0.05	0.24±0.06	6.03±0.44	5.97±0.60	6.05±1.13	5.95±0.33
5	254.96±10.22	246.59±12.79	254.46±8.72	246.09±5.92	0.33±0.05	0.29±0.02	0.28±0.06	0.24±0.03	6.55±0.46	6.02±0.53	6.57±1.04	6.00±0.60
6	248.71±15.57	247.92±14.43	248.21±3.66	247.42±11.55	0.33±0.03	0.29±0.03	0.28±0.07	0.24±0.04	5.91±0.54	5.87±0.64	5.93±0.34	5.85±0.46
LSD	15.54				0.13				1.30			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Table 12: Levels of serum calcium and phosphorus of different experimental groups (means ± SD).

	Calcium (mg/dl)				Phosphorus (mg/dl)			
Weeks (p.i)	Group (A)	Group (B)	Group (C)	Group (D)	Group (A)	Group (B)	Group (C)	Group (D)
0	7.59±0.62	7.37±0.67	7.60±0.52	7.38±0.57	5.85±0.73	5.77±0.76	5.86±0.64	5.87±0.60
1	7.85±0.57	7.27±0.71	7.86±0.44	7.28±0.62	6.06±0.55	5.75±0.44	6.07±0.43	6.08±0.29
2	7.79±0.51	8.99±0.37	7.80±0.45	9.00±0.60	6.23±0.73	5.94±0.63	6.24±0.35	6.25±0.27
3	7.62±0.58	10.15±0.33	7.63±0.65	10.16±0.55	6.26±0.57	6.12±0.65	6.27±0.45	6.28±0.26
4	7.48±0.59	10.56±0.70	7.49±0.34	10.57±0.73	6.10±0.63	5.98±0.57	6.11±0.41	6.12±0.25
5	7.47±0.67	10.40±0.62	7.48±0.84	10.41±0.63	6.21±0.66	6.19±0.61	6.22±0.43	6.23±0.33
6	7.61±0.66	10.92±0.62	7.62±0.63	10.93±0.55	5.95±0.56	5.87±0.52	5.96±0.35	5.97±0.43
LSD	0.55				0.40			

Group (A) represents control group (untreated, unvaccinated).
Group (B) represents probiotic treated group.
Group (C) represents untreated vaccinated group.
Group (D) represents treated vaccinated group.

Values of serum lipogram of experimental groups in comparison to those of control group showed, insignificant changes in HDL-cholesterol concentration throughout the experiment in all groups. Significant decreases in values of serum total cholesterol, total triglycerides and LDL-c concentrations in groups B and D from the 2nd week till the end of experiment was observed. These decreases may be attributed to their treatment with probiotic which has the ability to assimilate cholesterol present in GIT for its own cellular metabolism thus reducing the amount absorbed [36].

Compared to control group, the activities of serum liver enzymes (AST, ALT and ALP) and the concentrations of blood glucose, serum creatinine and uric acid showed, insignificant changes throughout the experiment in all groups. Insignificant changes in serum phosphorus concentration throughout the experiment and significant hypercalcaemia in groups B and D from the 2nd week till the end of experiment were noticed. These changes in groups B and D may result from using probiotic w hich increase serum calcium concentration [37]. Also, this hypercalcaemia may be attributed to

hyperproteinemia as there is a linear relationship between total proteins and calcium concentration [38]. Hypercalcaemia present may also due to the probiotic used resulting in an enlargement of the absorption surface of mineral including calcium by promoting proliferation of enterocytes mediated by bacterial fermentation products predominantly lactate also, it increased expression of calcium-binding proteins [39].

CONCLUSION

From the present study, it is concluded that, the used multistrain probiotic (Protexin®) has immunostimulatory effect reflected on increasing the immune response of probiotic treated chicken against avian influenza vaccine. Protexin® has also, hepatostimulatory and hepatoprotective effects reflected on some changes on hematological and serum biochemical parameters of probiotic treated chicken.

REFERENCES

1. Kilbourne, E.D., 1987. Influenza. Plenum: NY, pp: 1-359.
2. Webster, R.G., W.J. Bean, O.T. Gorman, T.M. Chambers and Y. Kawaoka, 1992. Evolution and ecology of influenza A viruses. Microbiol. Rev., 56: 152-179.
3. Easterday, B.C., V.S. Hinshaw and D.A. Halvorson, 1997. Influenza. In B. W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, (eds.). Diseases of Poultry, 10th ed. Iowa State University Press: Ames, IA, pp: 583-605.
4. Swayne, D.E., 2008, Avian Influenza, John Wiley & Sons.
5. Original (in German) published in: DGS-Magazin (Magazinfür Geflügelwirtschaft und Schweine produktion), 2012, 64(14): 25-28.
6. Martins, F.S., R.M. Nardi, R.M. Arantes, C.A. Rosa, M.J. Neves and J.R. Nicoli, 2005. Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. J. Gen. Appl. Microbiol., 51: 83-92.
7. Soomro, A.H., T. Masud and H.A. Rathore, 2002.Application of probiotics culture. J. Am. Vet. Adv., 1: 40-42.
8. Havenaar, R. and J.H.J. Huisin't Veld, 1992. Probiotics; a general view in the Lactic Acid Bacteria, Vo1.1. The Lactic Acid Bacteria in Health and Disease; ed. B.J.B. Wood, Elsevier App. Sc. Barking, pp: 151- 170.
9. Rajmane, B.V., 2000. Efficacy of protexin on performance of broilers Parel Mumbai, Bombay Vet.College, Born. Vet., 14: 542.
10. Stavric, S. and E.T. Kornegay, 1995. Microbial probiotics for pigs and poultry biotechnology in animal feeds and animal feeding. R.J. Wallace and A. Chesson, eds. V.C.H. Weinheim, Germany, pp: 205-231.
11. Koenen, M.E., J. Kramer, R. van der Hulst, L. Heres, S.H. Jeurissen and W.J. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. Br. Poult. Sci., 45: 355-66.
12. Ayasan, T., B.D. Ozcan, M. Baylan and S. Canogullari, 2006. Inter. J. Poul. Sci., 5: 776-779.
13. Fuller, R., 1989. A review: Probiotics in man and animals. J. Appl. Bacter., 66: 365-378.
14. Lexander, D.A. and N.J. Chettle, 1977. Procedures for the haemagglutination and the haemagglutination inhibition test for Avian Infectious Bronchitis Virus. Avian. Pathol., 6: 9-17.
15. Feldman, B.F., J.G. Zinkl and N.C. Jain, 2000. “Schalm's Veterinary Hematology” 5 ed. Lea and Febiger, Philadelphia, U.S.A.
16. Weichselbaun, T.E., 1946. An accurate rapid method for determination of protein in small amounts of blood, serum and plasma. Am. J. Clin. Pathol., 7: 40.
17. Dumas, B.T. and H.G. Biggs, 1972. Standard Methods of Clinical Chemistry. Vol 7. Academic Press, New York, pp: 175.
18. Reitman, S. and S. Frankel, 1957. A colorimeteric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.
19. Tietz, N.W., 1986. Text Book of Clinical Chemistry. Philadelphia: WB Saunders.
20. Trinder, P., 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 22(2): 246.
21. Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3, 5-dichloro-2 hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

22. Fabiny, D.L. and G. Ertingshausen, 1971. Automated reaction-rate method for determination of serum creatinine. Clin. Chem., 17: 696-700.
23. Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol.Clin. Chem., 20: 470-475.
24. Warnick, G.R., V. Benderson and N. Albers, 1983. Selected methods.Clin. Chem., 10: 91-99.
25. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of LDL cholesterol in plasma, without use of the preparative ultracentrifuge.Clin. Chem., 18: 499-504.
26. Wahlefeld, 1974. In: Methods of Enzymatic Analysis, Vol.5, Bergmeyer, H.U. Academic Press, New York, pp: 1831-35.
27. Biggs, H.G. and W.R. Moorhead, 1974. Clin. Chem., 20: 1458-60.
28. Goodwin, J.F., 1970. Clin. Chem., 16(9): 776-780.
29. Abdolahfam, M. and H. Ghahri, 2012. Immune response of broiler chicks fed yeast derived mannan oligosaccharides and humate against Newcastle disease. World Appl. Sci. J., 18(6): 779-785.
30. Zulkifli, I.N., N.M. Abdullah, Azrin and Y.W. Ho, 2000. Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci., 41: 593-597.
31. Dalloul, R.A., H.S. Lillehoj, T.A. Shellem and J.A. Doerr, 2003. Intestinal immunomodulation by vitamin A deficiency and lactobacillus-based probiotic in Eimeriaacervulina-infected broiler chickens. Avian Dis., 47: 1313-20.
32. Eman, R.H., K.M. Mahgoob, K.H.M.E. Lbayoumi, M.S. Zeinab, G. Amin and M.M. Hoda, 2012. Comparative studies between the effects of antibiotic (oxytetracycline), probiotic and acidifier on E. coli infection and immune response in broiler chickens. J. Am. Sci., 8(4): 795-801.
33. Sarma, M., Sapcota, S. Sarma and A.K. Gohain, A.K. Gohain, 2003. Herbal growth promoters on hematobiochemical constituents in broilers. Indian Vet. J., 80: 946-948.
34. Silva, E.N., 2000. "Probióticos e Prebióti cosnaalimentação de aves". In: ConferênciaApinco de Ciência e TecnologiaAvícolas; Campinas, São Paulo. Brasil. Campinas, FACTA, pp: 241-251.
35. Leedle, J., 2000. "Probiotica and DFMs - mode of action in the gastrointestinal tract". In: Simpósiosobreaditivosalternativosnaprodução animal; Campinas, São Paulo. Brasil.Campinas, CBNA, pp: 25-40.
36. Liong, M.T. and N.P. Shah, 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci., 88: 55-66.
37. Panda, A., M. Reddy, R.S. Rama and N. Praharaj, 2003. Production performance, serum /yolk cholesterol and immuno-competence of White Leghorn Layers as influenced by dietary supplementation with probiotic. Trop. Anim. Health Prod., 35: 85-94.
38. Lumeij, J.T., J.D. Remple and K.E. Riddle, 1993. Relationship of plasma total proteins and albumin to total calcium in peregrine falcons (Falco peregrinus). Avian Pathol., 22: 183-188.
39. Scholz-Ahrens, K.E., P. Ade, B. Marten, P. Weber, W. Timm, Y. Acil, C.C. Gluer and J. Schrezenmeir, 2007. Prebiotics, probiotics and synbiotics affect mineral absorption, bone mineral content and bone structure. J. Nutr., 137(3 suppl 2): 838S- 46S.