Effect of probiotic, prebiotic and symbiotic on toxicosis of ochratoxins in broilers

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تأثير المعزز، السابق والمؤازر الحيوي على الأثر السام لسموم الأوكرا في فروج اللحم

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جامعة واسط / كلية التربية الأساسية / قسم العلوم/ فرع علوم الحياة

المستخلص

هذه الدراسة أعدت لتقييم التأثير الوقائي للمعزز الحيوي متعدد العتر والسابق والمؤازر الحيوي ضد الأصابة المستحثة لسموم الاوكرا في عليقة فروج اللحم من عمر 1-35 يوم. خاصية التقييم شملت التحليلات البايوكيميائية والدموية بالاضافة لدراسة الافات المرضية المرافقة.

مائة وخمسون فروج لحم بعمر 1 يوم قسمت لخمسة مجاميع كل مجموعة عوملت بعليقة تختلف عن الاخرى. مجموعة الإصابة بسموم الاوكرا أظهرت انخفاض معنوي ($P < 0.05$) بنتائج تحليلات الدم مقارنة بالمجاميع المعاملة بالمعزز، السابق، المؤازر الحيوي ومجموعة السيطرة كذلك فأن نتائج التحليلات البايوكيميائية لمجموعة الإصابة بسموم الاوكرا أظهرت زيادة معنوية ($P< 0.05$) واضحة بمستوى حامض اليوريك والكرياتنين في مصل الدم مع قلة بالبروتين الكلي لمصل الدم، الالبومين والكلوبيولين.

التغيرات النسجية المرضية في الكلى للمجاميع 3، 4، 5 تشير لعدم وجود تغييرات مرضية عند مقارنتها بالمجموعة 2 التي تظهر تنخر النبيبات البولية العلوية ووجود احتقان شديد مع تخلل لمفاوي. الطحال في مجموعة الإصابة أظهر قلة بالنسيج اللمفاوي (قلة تنسج لمفاوي) مع تنخر الخلايا اللمفاوية وقلة عددها عند مقارنتها مع مجموعة السيطرة وباقي المجاميع المعالجة بكل من المعزز والسابق والمؤازر الحيوي والتي أظهرت قوام نسجي طبيعي للطحال متمثل بوضوح اللب الاحمر والابيض مع وجود الخلايا اللمفاوية الناضجة ،أيضاً توجد مراكز جرثومية في طحال المجموعة المعالجة بالمؤازر الحيوي. الغدة الزعترية في المجاميع 3 ، 4 و 5 كانت ذات قوام نسجي طبيعي عند مقارنتها مع مجموعة الاصابة بسموم الاوكرا والتي أظهرت تنخر في الجريبات اللمفاوية مع نزف. بالنهاية استنتجنا بأن كل من المعزز والسابق والمؤازر الحيوي كان فعالاً بأزالة وتخفيف التأثيرات السامة لسموم الاوكرا وكان المؤازر الحيوي أكثر فعالية من المعزز والسابق الحيوي.

Abstract

This study was conducted to evaluate the prophylactic effect of multistrain probiotic in drinking water, prebiotic in diet and both of them (symbiotic) when afford with a diet containing ochratoxins and fed to broiler chicks from 1 to 35 days old. The criteria of evaluation include hematological and biochemical analysis, in addition to associated lesions. A total 150 Rose day old broiler chicks were separated into five groups that each one received different diets. Ochratoxicosis group showed significant ($P<0.05$) reduction in hematological parameters in comparison with probiotic, prebiotic, symbiotic and control

groups. The biochemical analysis of ochratoxicosis group showed considerable ($P<0.05$) increase in the serum uric acid and creatinine levels with reduction in serum total protein, albumin and globulins. Pathological observations in kidney of broilers in groups 3, 4 and 5 revealed no pathological changes as compared with group 2 that showing necrosis of proximal renal tubules, severe congestion and lymphocytic proliferation. Spleen of ochratoxicosis group showing poor lymphoid tissue and depletion with necrosis of lymphocytes when compared with control and treated groups (probiotic, prebiotic and symbiotic) that appeared normal architecture represented by white and red pulp with mature lymphocytes, also there are germinal centers in symbiotic treated group. Normal thymus architecture in groups 3, 4 and 5 when compared with ochratoxicosis group that showed necrosis in the lymphoid follicles and hemorrhage. Finally, it was conducted that the probiotic, prebiotic and symbiotic are effective in the amelioration of toxic effects of ochratoxins. Symbiotic are more effect than probiotic or prebiotic alone.

Introduction

Mycotoxins are produced by many fungal species like the genus of *Aspergillus* (*A. flavus, A. ochraceous* and *A. parasticus*), *Fusarium*, *penicillium* and others are normal contaminants of poultry feed (1). The occurrence of mycotoxicosis in poultry is quite common in many countries like Iraq. Mycotoxicosis causes significant economic losses.

Mycotoxins, especially ochratoxins cause severe renal damage (2,3) and severe hepatic necrosis and fatty change (1), in addition to immunosuppersion (4,5).

Mycotoxicosis cases lead to an increase in serum enzymes activities of transferases, alkaline phosphatase, also in uric acid and creatinine levels with a reduction in total serum protein, albumin, globulins, glucose, calcium and phosphorus (6,7,8,9). Microcytic normochromic anemia associated with leukocytosis, hetrophilia, lymphopenia and monocytopenia have been observed in mycotoxicosis (10, 11, 12).

Measures used by livestock farmers to protect animals from the toxic effects of mycotoxins includes grain testing, using of mould inhibitors, fermentation, physical separation, thermal inactivation, irradiation, ammoniation (1) and ozone degradation (13) and most of these methods are impractical or potentially unsafe because of the formation of toxic residues or alteration of nutrient contents, flavor and odor of the product. Furthermore these methods are both time consuming and costly. Thus new practical and effective method to eliminate mycotoxicosis is the use of adsorbents in diet and water, which adsorb mycotoxins in the gastrointestinal tract of animals and reduce bioavailability and toxicity. At present time the use of probiotic is the method of choice. Probiotic is added to feed and water contaminated with mycotoxins, and to remove mycotoxins during the digestive process allowing the mycotoxins to pass harmlessly through animal (14, 15, 16, 17, 18). Multi strain probiotic that contain live microbes which establish, enhance or re-establish essential micro flora in the gut. Probiotic in drinking

water combined with mycotoxin in the gut, then prevent absorption and alters their structure (19). Mannanoligosaccharide is a prebiotic belongs to a class of non-digestible carbohydrates. Mannan oligosaccharide is also a highly effective prebiotic that stimulates the growth of beneficial micro biota in the gut. The mannanoligosaccharide is fermented by action of *Bifidobacteria, Lactobacilli, Pediococcus and Enterococcus* in colon to produce short chain fatty acids like acetic acid, propionic acid and butyric acid beside hydrogen, hydrogen sulphide, carbon dioxide and methan gases. Moreover, lactate, pyruvate, succinate and formate are formed (20,21, 22). The acetate, propionate and butyrate that are not metabolized in colonocytes are absorbed from the colon and transported via the portal circulation to the liver. These short chain fatty acids are transported by blood to various tissues, where they undergo further metabolism (17,23). Symbiotic refers to synergistic effect of both probiotic and prebiotic as additives in diet or drinking water.

Materials and methods

Animals and Experimental Design

A total of 150 Rose broiler chicks one day old were purchased from commercial hatchery and housed in well-isolated floor pens under continuous florescent lighting. They all received the ration have all requirements according to the National Research Council (24) table (1) but the ration of groups 2, 3, 4 and 5 were contaminated with ochratoxins by using contaminated yellow corn. They randomly assigned to 5 equal groups and treated from 1 to 35 days old as following:

Group 1: the control diet.

Group2: ochratoxins contaminated diet at level of 110 part per billion (ppb).

Group 3: multi strain probiotic supplemented in dose of 3g/150 bird/day in drinking water with ochratoxins contaminated diet at level of 110 ppb.

Group 4: prebiotic (mannanoligosaccharide) 0.2% in ochratoxins contaminated diet at level of 110 ppb.

Group 5: symbiotic (multi strain probiotic supplemented in dose of 3g/150 bird /day in drinking water and 0.2% mannanoligosaccharide in ochratoxins contaminated diet at level of 110 ppb. The experimental diet and water were available *ad libitum*. At the age of 42 days old the study was terminated to examine the blood and hematological profile and chemistry, and to perform the pathological examination to the kidney, spleen and thymus.

Ochratoxins contaminated diet was examined by Enzyme linked Immuno-sorbent Assay (ELISA) that detected the level of ochratoxins.

Multi strain probiotic **Poultry Star®** of Biomin® Company contains four genus of bacteria *Lactobacillus, Pediococcus, Enterococcus and Bifidobacterium*. This product contains a minimum of $5x10^{12}$ CFU/kg; it was applied in accordance to instructions of manufacturer. Mannanoligosaccharide was obtained from the marine sciences center in Basrah city.

Table (1): Ingredients and chemical composition of the experiment's diets.

Calculated chemical analysis

Metabolizable Energy (kcl/kg)	3133
Crude protein %	20
Calcium %	1
Available Phosphorus %	0.60
Lysine %	1.17
Methionine %	0.61
Fibers %	3.1

Each kg of Premix* contains 8000000 IU vitamin A, 1500000 IU vitamin D_3, 1400 mg vitamin E, 2000 mg vitamin k_3, 1250 mg vitamin B_1, 2800 mg vitamin B_2, 8000 mg Niacin, 4000 mg Ca-D-pantothenate, 2000 mg B_6, 6 mg B_{12}, 400 mg folic acid, 18 mg Biotin, 20000 mg vitamin C, 50000 mg Choline chloride, 80000 mg Manganese,60000 mg Iron,60000mg Zinc,5000mg Copper, 200mg Cobalt, 1000mg Iodine,150mg Selenum.

Hematological and Biochemical Examinations

Two blood samples were collected from each bird in all groups via wing vein puncture at 35 days old of age. The first blood portion (2ml) was collected in heparinised and evacuated tubes for hematological study in accordance with standard technique of the red blood cells counting, hemoglobin concentration Hb, packed cell volume pcv, white blood cells count and differential leukocytes count (25). The second portion was collected in plain centrifuge tubes, and the serum was separated for measuring the quantities of the total serum protein and albumin (26). Serum globulins were calculated as the differences between total proteins and albumin. Creatinine (27) and uric acid was also measured (28).

Ingredients	Ingredients %
Animal protein (40 % protein)	8%
Soybean meal (45 % protein)	17%
Yellow corn	42%
Wheat	32.2%
Calcium ($CaCo_3$)	0.6%
Premix*	0.1%
Salt (Nacl)	0.1%
Total	100%

Pathological Examination

Specimens were collected from the kidney and lymphoid organs (thymus and spleen). They were fixed in 10% buffered neutral formalin. Paraffin sections (thickness: 5 microns) were prepared and were stained by Hematoxylin and Eosin stains (H&E) (29) and examined microscopically.

Statistical analysis

The data of the present investigation were statistically analyzed by using one way analysis of variance (ANOVA). The means showing significant differences in ANOVA were compared using the Duncan multiple range test at $P < 0.05$. The comparison between groups for major microscopic observations in the kidney was performed by using the chi-square (x^2) test.

Results:

Hematological and Biochemical Findings

The toxic effects of ochratoxins were apparently shown in significant alteration in the hematological values (table 2). The birds in group 2 showed a

significant reduction ($P < 0.05$) in the total erythrocytes count, hemoglobin concentration and packed cell volume, associated with cases of leukopaenia, hetropaenia, lymphopaenia and insignificant change in the eosinophils and monocytes count when compared to groups 3, 4, 5 and control. The biochemical analysis showed a significant elevation ($P < 0.05$) in the values of uric acid and creatinine in group 2 with reduction in the total serum protein, albumin and globulin when compared with others (table 3). On the other hand, the albumin /globulin **A/G** ratio showed significant changes except in group 2 birds which increased significantly ($P < 0.05$).

Table (2): Hematological parameters mean of the different broilers groups.

	Hematological profile					Differential leukocytes count ($x10^3/\mu l$)			
Groups	**RBCs** $x10^6/\mu l$	**Hb%**	**PCV**	**MCV**	**WBCs** $x10^3/\mu l$	**H**	**L**	**E**	**M**
1	3.11 [a]	10.66 [a]	31.75 [a]	102.9 [a]	23.15 [a]	6.90 [a]	15.51 [a]	0.52 [a]	0.22 [a]
	± 0.17	± 0.53	± 1.49	± 5.10	± 1.58	± 0.44	± 1.11	± 0.04	± 0.01
2	2.62 [b]	8.09 [b]	22.53 [b]	85.9 [b]	18.21 [c]	5.35 [b]	12.18 [c]	0.48 [a]	0.20 [a]
	± 0.13	± 0.44	± 1.37	± 5.16	± 1.10	± 0.32	± 0.74	± 0.03	± 0.01
3	3.13 [a]	10.41 [a]	29.08 [a]	92.9 [a]	22.96 [b]	6.95 [a]	14.34 [b]	0.51 [a]	0.21 [a]
	± 0.17	± 0.57	± 1.60	± 5.11	± 1.32	± 0.41	± 0.86	± 0.03	± 0.01
4	3.19 [a]	10.43 [a]	29.25 [a]	91.7 [a]	22.18 [b]	7.04 [a]	14.38 [b]	0.56 [a]	0.20 [a]
	± 0.19	± 0.62	± 1.56	± 5.9	± 1.34	± 0.44	± 0.97	± 0.04	± 0.01
5	3.18 [a]	10.52 [a]	29.00 [a]	91.2 [a]	23.79 [a]	7.09 [a]	15.94 [a]	0.54 [a]	0.20 [a]
	± 0.17	± 0.58	± 1.60	± 5.12	± 1.67	± 0.50	± 1.12	± 0.04	± 0.01

Means in the same column with different superscript letters (a, b and c) are significantly different ($P < 0.05$).

RBC: red blood cells, **Hb:** hemoglobin, **PCV:** packed cell volume, **MCV:** mean corpuscular volume, **WBC:** white blood cells, **H:** heterophils, **L:** lymphocyte, **E:** eosinophil. **M:** monocytes.

Table (3): Biochemical parameters mean of the different broilers groups.

Groups	total protein (g/dl)	albumin (g/dl)	globulins (g/dl)	A/G (ratio)	uric acid (mg/dl)	creatinine (mg/dl)
1	3.76 [a]	1.99 [a]	1.77 [a]	1.12 [a]	3.91 [a]	1.48 [a]
	± 0.21	± 0.13	± 0.13	± 0.08	± 0.29	± 0.13
2	2.84 [c]	1.56 [b]	1.28 [c]	1.22 [b]	4.88 [b]	1.92 [b]
	± 0.15	± 0.09	± 0.07	± 0.0 4	± 0.23	± 0.10
3	2.91 [b]	1.55 [b]	1.36 [b]	1.14 [a]	4.18 [a]	1.53 [a]
	± 0.18	± 0.1	± 0.09	± 0.07	± 0.28	± 0.14
4	3.05 [a]	1.59 [b]	1.46 [b]	1.08 [a]	3.98 [a]	1.52 [a]
	± 0.23	± 0.08	± 0.10	± 0.06	± 0.27	± 0.15
5	3.49 [a]	1.73 [a]	1.76 [a]	1.14 [a]	4.16 [a]	1.51 [a]
	± 0.22	± 0.12	± 0.14	± 0.08	± 0. 30	± 0.13

Means in the same column with different superscript letters (a, b and c) are significantly different ($P< 0.05$).

A/G (ratio): albumin/globulin ratio.

Pathological findings

Birds in groups 3, 4, 5 and control showed no lesions (tables 2, 3 and 4).

Group 2

Macroscopically, the kidneys were swollen and pale. The thymus was small in size, while the spleen appeared normal.

Microscopically (table 4), in kidneys cortex there were coagulative necrosis of glomerular and renal tubules (75%) varied from scattered foci in the mild cases to large area in the severe cases with prominence leukocytic infiltration and aggregation interior Bowman's capsules, in addition to severe congestion of interlobular blood vessels in cortex and capillaries in medulla (100%) with interstitial hyperplasia (90%) (figure1).

The lymphoid organs (spleen and thymus) showed moderate depletion and necrosis of lymphocytes (figure 2 and 5), while in birds of groups 3, 4, 5 and control showed normal architecture of both spleen and thymus (figure 3 and 6), also there is formation of germinal centers in spleen of group 5 (figure 4).

Table (4): Frequency of major microscopic observations in the kidneys.

G	No. of Chicks	Inflammation & congestion +	-	lesions (%)	Hyperplasia +	-	lesions (%)	Necrosis +	-	lesions (%)	Fatty change +	-	lesions (%)
1	20	0	20	0.0 ^{e}	0	20	0.0 ^{e}	0	20	0.0 ^{e}	0	20	0.0 ^{d}
2	20	20	0	100.0 ^{a}	18	2	90.0 ^{a}	15	5	75.0 ^{a}	10	10	50.0 ^{a}
3	20	6	14	30.0 ^{b}	4	16	20.0 ^{b}	5	15	25.0 ^{c}	2	18	10.0 ^{c}
4	20	4	16	20.0 ^{c}	3	17	15.0 ^{c}	6	14	30.0 ^{b}	3	17	15.0 ^{b}
5	20	2	18	10.0 ^{d}	1	19	5.0 ^{d}	1	19	5.0 ^{d}	0	20	0.0 ^{d}
Chi-square(x^2)		102.50*			97.543*			69.819*			49.949*		
Probability		0.00001			0.00001			0.00001			0.00001		

Means in the same column with different superscript letters (a, b, c, d and e) are significantly different ($P< 0.05$).

* high significant differences.

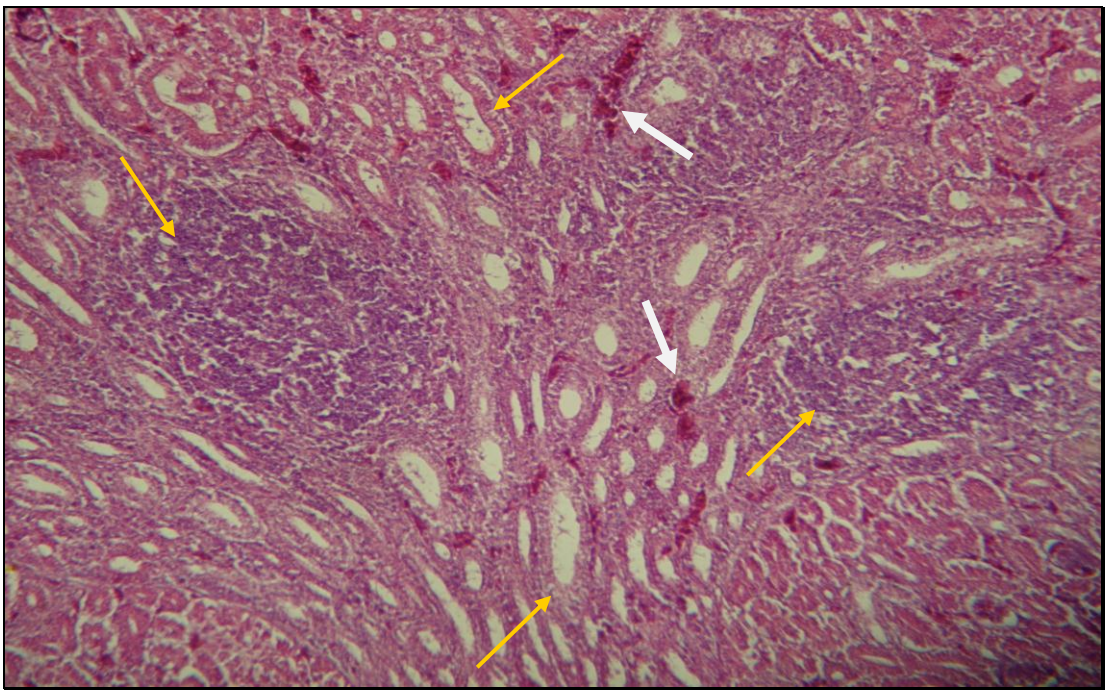

Figure (1): Prominence leukocytes' aggregation with glomerular coagulative necrosis and thickened epithelium of renal tubules with necrosis and sloughing (yellow arrows), also severe congestion in cortical blood vessels and medullar capillaries in a bird from group 2 (white arrows) (H&E 600 X).

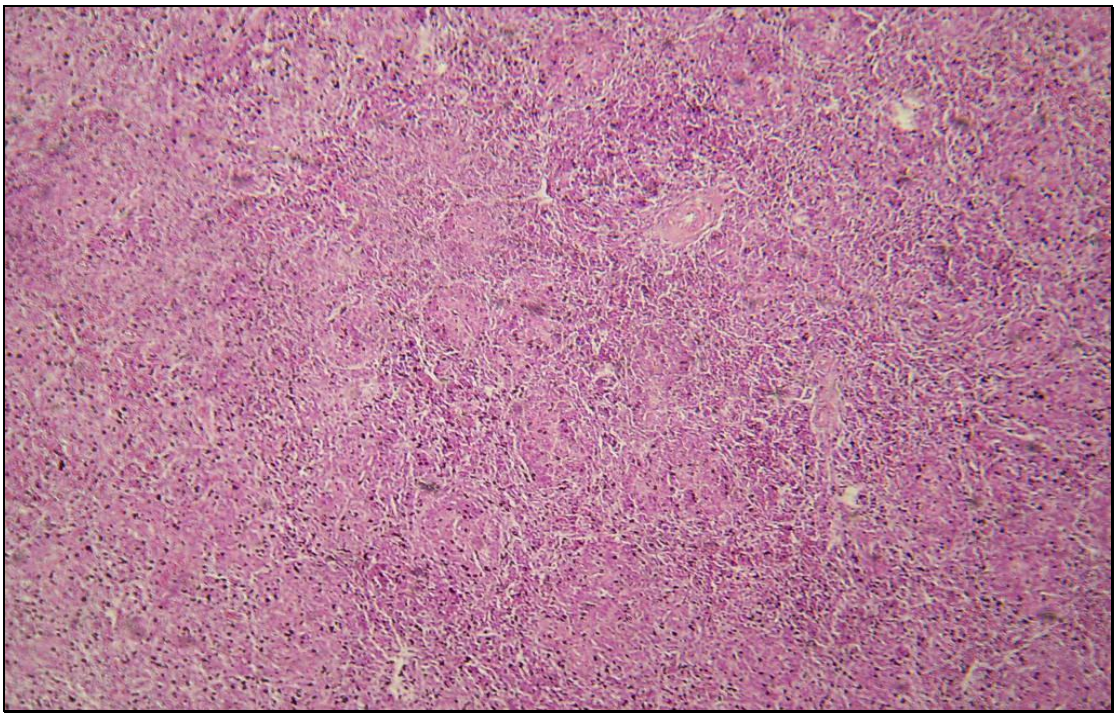

Figure (2): Spleen of a bird from group 2 showing poor lymphoid tissue and depletion and necrosis of the lymphocytes (H&E stain; X 125).

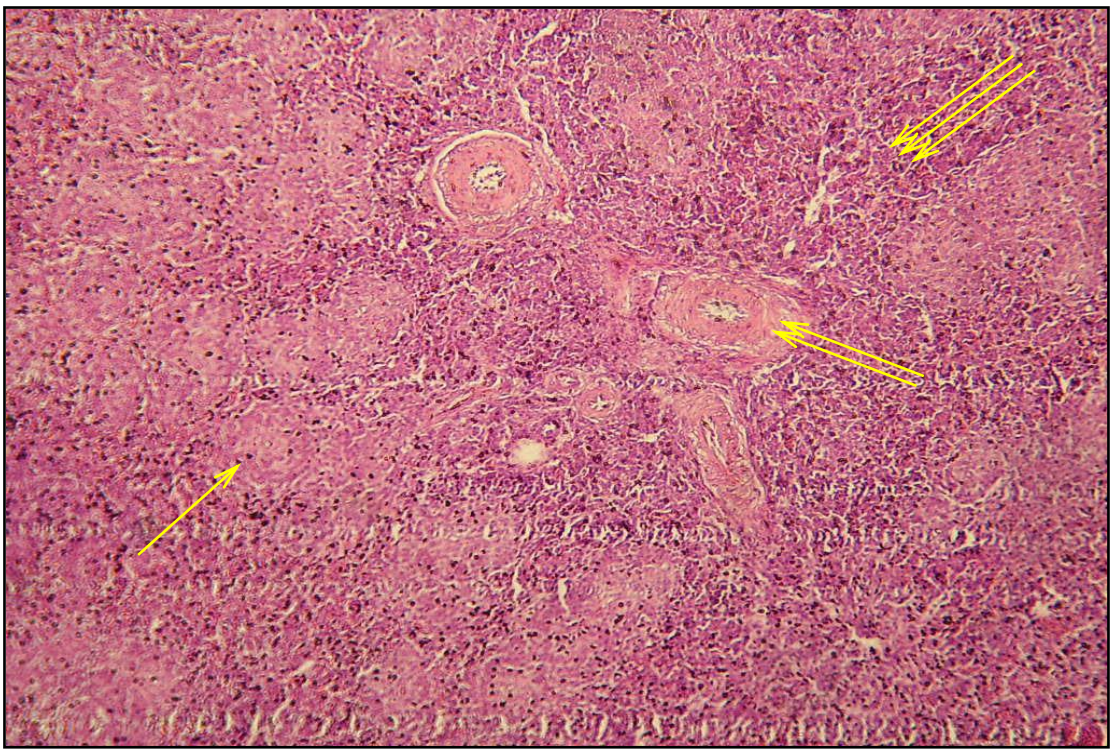

Figure (3): Spleen of groups 3, 4, 5 and control showing mature lymphocytes and white pulp (single arrow), trabecular artery (double arrow), and red pulp (triple arrow) (H&E stain X125).

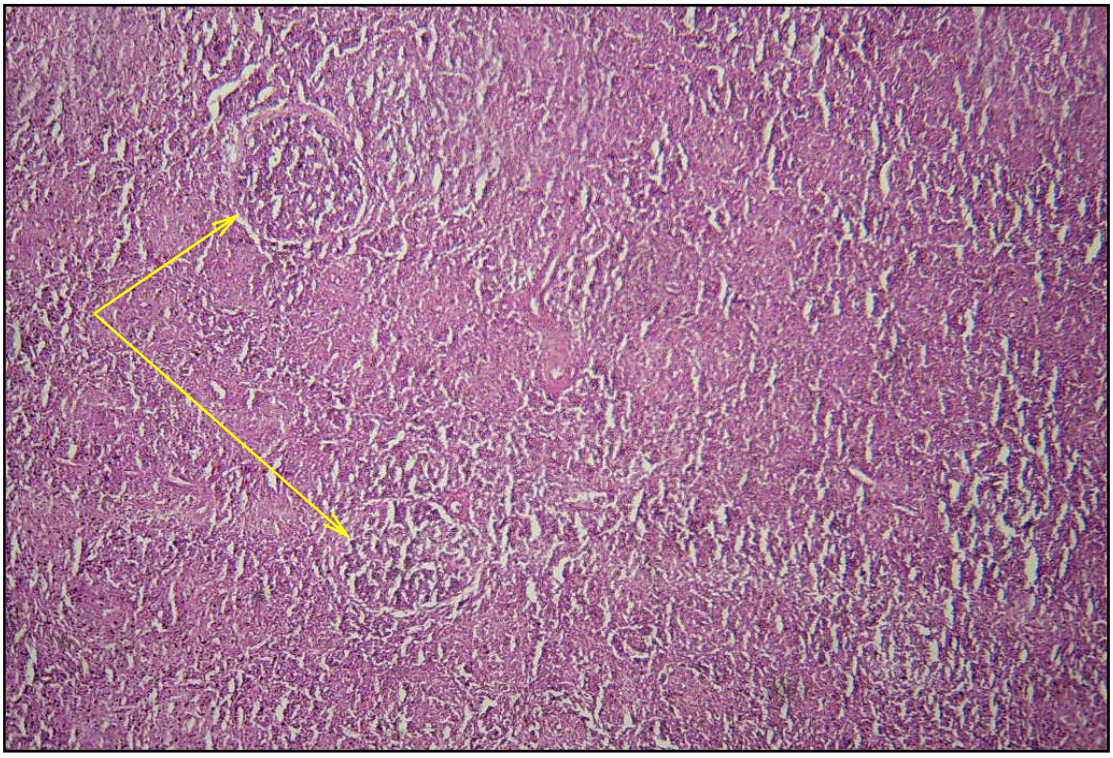

Figure (4): spleen of a bird from group 5 showing active white pulp and formation of germinal centers (arrows) (H&E stain X 125).

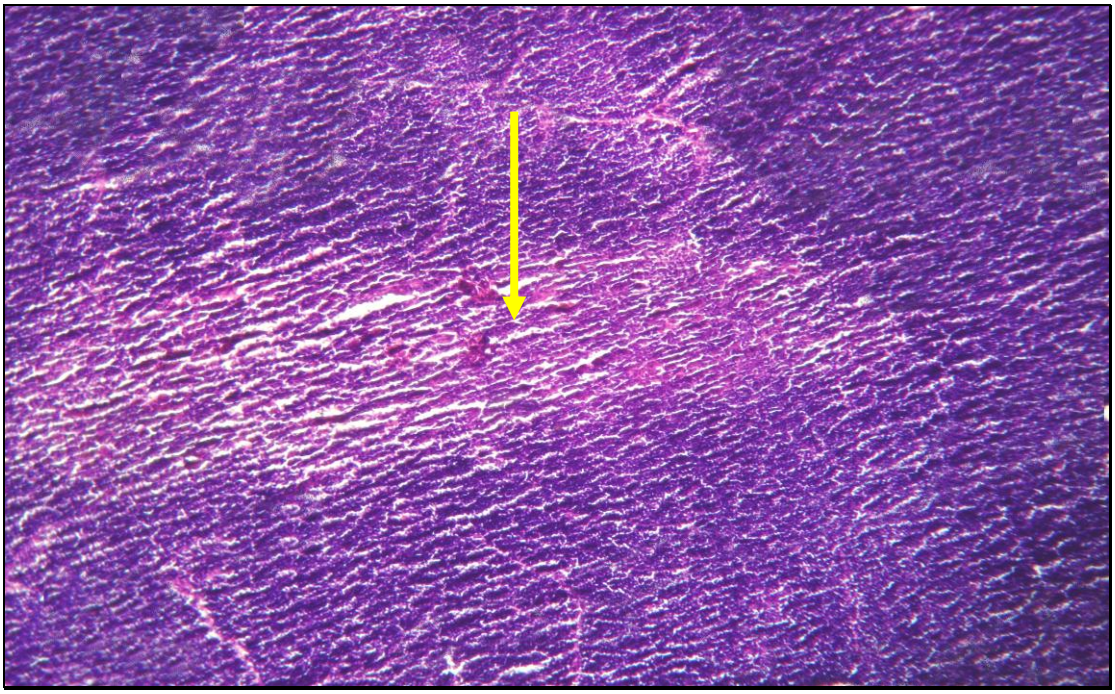

Figure (5): Thymus of a bird from group 2 showing necrosis in the lymphoid follicle with depletion of lymphocytes and hemorrhage (H&E 300X).

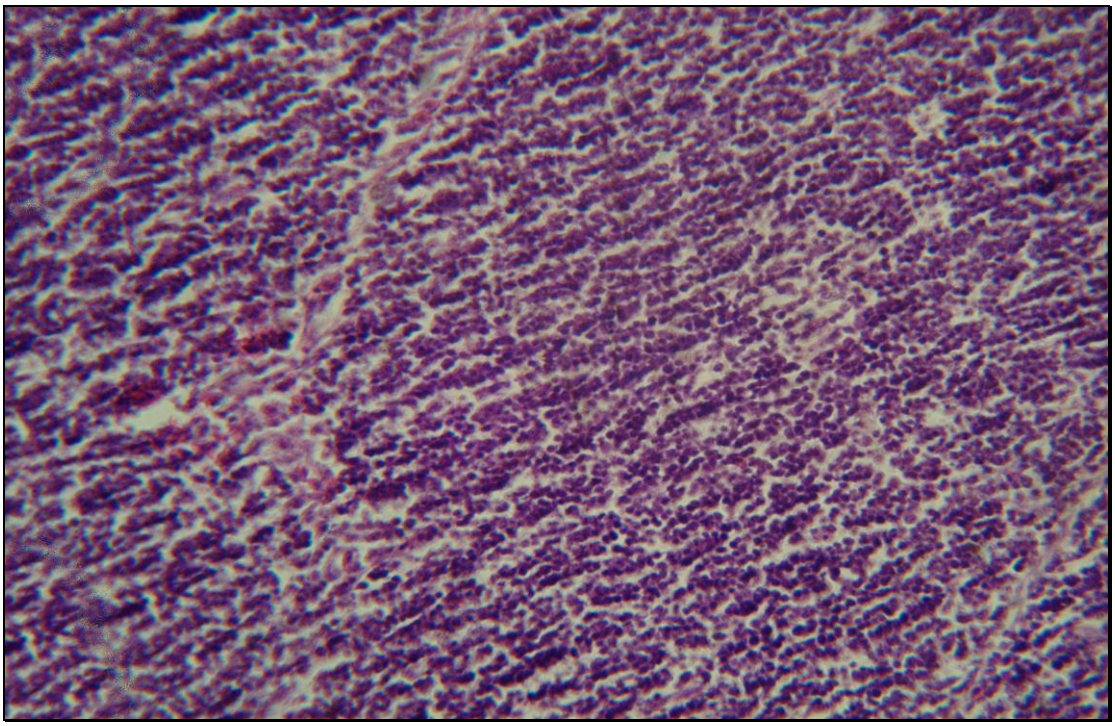

Figure (6): Normal thymus architecture and prominence active lymphoid follicle with proliferation of mature lymphocytes in groups 3, 4, 5 and control (H&E 300X).

Discussion

The toxicity of ochratoxins was expressed as significant alteration in the hematological and biochemical tests of broiler chickens. Microcytic normochromic anemia with a drastic decrease in the total leukocytes count, heterophils and lymphocytes were observed during ochratoxicosis cases. Previous findings according with the results obtained by other authors (10,12). The decrease in the total leukocytes count, heterophils and lymphocytes may be due to the toxic effect of ochratoxins on the circulating cells, sequestration of cells in tissues and/or effect of ochratoxins on the bone marrow and lymphoid tissues. The insignificant changes in the eosinophils and monocytes counts were similar to other findings (30).

Results of proteinogram showed a significant decline represented by the reduction of total serum protein associated with hypo-albuminaemia, and hypo-globulinaemia may be due to the interaction of ochratoxins with DNA, RNA and intracellular protein of chicken hepatocytes leading to impaired protein synthesis and leakage of albumin due to nephrotoxicity induced by ochratoxins (6, 8, 5). In regard to the renal function tests a significant increase in the serum levels of uric acid and creatinine was observed in the chicks that had been fed a diet containing mycotoxins. This revealed the toxic effect of ochratoxins on the renal tissues. However, findings of this study agree with other authors (10,11,12), who record similar increase in the creatinine and uric acid. Microscopically, the kidneys showed coagulative necrosis (75%) in the cortex especially in glomeruli and epithelium of renal tubules, also leukocytic proliferation, aggregation, severe congestion of cortical and medullar interlobular blood vessels. Similar lesions have been described by other workers (31, 14). They reported multi membranous glomerulonephritis during mycotoxicosis in broilers. The lymphoid tissues (spleen and thymus) revealed moderate depletion and necrosis of lymphocytes. Such findings confirm the immunosuppressive effect of mycotoxins (4). Individual cell necrosis and mild degenerative changes (25%, 30% and 5%) were observed in the kidneys of groups 3, 4 and 5 respectively in renal tubules and slight congestion of renal blood vessels (30%, 20% and 10%) in groups 3, 4 and 5 respectively. similar findings were reported for the combination of probiotic and prebiotic (symbiotic) proving their protection against intestinal pathogens, and their amelioration of severity of some inflammatory bowel diseases, and several kinds of toxicosis including mycotoxicosis (32,33,3,34,35,23).

The hematological and biochemical tests were not affected by the addition of multi strain probiotic to the drinking water, prebiotic to the ochratoxins contaminated diet but both of them that assisted in achieving the hematological and biochemical values close to the control levels, and alleviated the lesions associated with ochratoxins, especially the simultaneous addition of symbiotic. Similar findings have been reported by Agawane and Lonkar (2004) (36).

Conclusion

Finally, it can be concluded that the probiotic, prebiotic and combination of them are effective in alleviating the toxic effect of ochratoxins that may be present in poultry rations at levels up to 110 ppb. Symbiotic is highly effective compared to either probiotic or prebiotic alone.

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