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EFFICACY OF HUMIN FEED IN COUNTERACTING THE TOXIC EFFECT OF OCHRATOXIN FED TO QUAIL

(With 5 Tables and 18 Figures)

By

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(Received at 24/5/2007)

كفاءة الهيومين كمضاد للتأثير السام للأوكراتوكسين في السمان

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أجريت التجربة علي ٨٠ طائر من السمان عمر ٢١ يوم وقد قسمت الطيور عشوائيا إلي أربعة مجموعات كل منها عشرون طائر. وقد تم التغذية كالآتي: المجموعة الضابطة غذيت عليقه عادية دون إضافات والثانية غذيت عليقه عادية مضاف إليها ١٦ جزء في المليون من الأوكراتوكسين والثالثة عليقه عادية مضاف إليها ١٦ جزء في المليون أوكراتوكسين +٢جم/كيلو جرام من حمض الهيومك والمجموعة الرابعة عليقه عادية مضاف إليها ٢جم/كيلو جرام من حمض الهيومك واستمرت التجربة لمدة ٤ أسابيع وقد تبين الآتي: حدث هناك نقص في وزن الجسم نتيجة انخفاض معامل التحويل في المجموعة الثانية مقارنة بالمجموعة الضابطة والمجموعات الأخرى هذا بالإضافة إلي أنه حدث نقص في كرات الدم الحمراء والهيماتوكريت والهيموجلوبين بينما لم يحدث تغير في العد الكلي لكرات الدم البيضاء بين المجموعات المختلفة وقد حدث نقص بنسبة خلايا اللمفوسيت في طيور المجموعة الثانية هذا من ناحية وعلي الجانب الأخر حدثت زيادة في الآلينين أمينو ترانسفيريز والأسبرتيت أمينو ترانسفيريز وحمض البوليك والكرياتينين في نفس المجموعة الثانية المغذاة علي العلف المضاف إلية الاوكراتوكسين وذلك نتيجة التأثير السام علي الكبد والكلي وقد أكدت ذلك الدراسة الهستوباثولوجية للكبد والكلي. وقد حدث تحسن ملحوظ في معدل الأداء والأوزان وكذلك الفحوص البيوكيميائية والدموية والهستوباثولوجية نتيجة إضافة الهيومين للعليقة وذلك للحد من الآثار السلبية لسموم الأوكراتوكسين.

SUMMARY

Eighty 21-days-old quails were randomly distributed into four experimental groups each of 20 birds and fed ration containing 0 (control), 16 ppm ochratoxin, 16 ppm ochratoxin + 2gm/kg humin feed and 2gm/kg humin feed. Each treated group was replicated twice of 10 quails. Quails fed for a period of 4 weeks 16 ppm, ochratoxin showed

reduced body weight, poor feed conversion ratio compared with control and other groups as well as reduces total RBCs count, hematocrite value and hemoglobin content. No differences were found among leukocytes counts in all groups when compared to control, a lower percentage of lymphocytes was observed in birds fed 16 ppm ochratoxin. GOT, GPT, uric acid and creatinine were higher in birds fed 16 ppm ochratoxin indicated the hepatotoxic and nephrotoxic effect of ochratoxin in quails. Histopathological examination of kidneys, liver, spleen and bursa of birds fed 16 ppm ochratoxin confirm the toxic effects of ochratoxin in quail. The performance indices, hematological, biochemical and histopathological results indicated that humin feed is efficient in counteracting the toxic effects of ochratoxin fed to quail.

Key words: *Humin feed, ochratoxin, quail.*

INTRODUCTION

Humin substances are the most widespread and ubiquitous natural non living organic materials (Steffen *et al.*, 2002).

Now-a-days, quails industry has widely distributed among Egyptian people, it has mainly kept for meat production, since their meat is palatable, cheap, popular and considered as the smallest avian species size for meat production (Panda and Singm, 1990).

Kubena *et al.*, (1989) reported that feeding diet containing ochratoxin A (OTA) for broiler, depressed body weight as well as feed utilization was reduced.

Ochratoxin is immunosuppressive, gentotoxic, teratogenic, carcinogenic and nephrotoxic in all animal species (Baudrimont *et al.*, 2001).

Regarding this economic importance, continues search for new antimycotoxic drugs is a necessity.

Humic acid (HA); a class of compounds resulting from decomposition of organic matter, has an antibacterial effect and inhibit fungal growth, thus decrease levels of mycotoxin in feed as well as improve the health status of animal (Islam *et al.*, 2005).

The possibility of using humin feed to control ochratoxicosis as well as its effects on the performance of quail motivated for the present study.

Thus, the objective of this study was to investigate and describe the effects of ochratoxin on performance hematological, biochemical and histopathological parameters of young quail as well as use of humin feed for counteracting the toxic effects of ochratoxin in quails.

MATERIALS and METHODS

Birds and experimental design:

Eighty unsexed Japanese quails, 21-days-old, were randomly assigned to 4 groups. Birds were placed in one battery of eight wire cages (10 birds per cage), and allowed to feed and water ad-libitum and all birds were monitored daily any healthy problems were recorded.

Group I: Fed on basal diet free from ochratoxin contamination or/and humin medication.

Group II: Feed on basal diet contaminated with 16 ppm ochratoxin (OT) and not medicated, [Ochratoxin were obtained from Nuclear Research Center Atomic Energy Authority, Enshas, Egypt].

Group III: Fed on basal diet contaminated with 16 ppm ochratoxin (OT) and medicated with humin feed®* (2 g/kg feed).

Group IV: Fed on basal diet free from ochratoxin but supplemented with humin feed (2 g/kg feed).

Chemical:

(1) Humin feed: Is water soluble sodium humate granules supplied from Humin Tech. Comp., Germany.

(2) Ration: Chemical composition of basal fed of quails:

Nutrient	*%*
Crude protein%	24.0
Metabolizable energy (Kcal/lb)	1320
Calcium %	1.0
Available phosphate%	0.50
Lysine %	1.4
Methionine + cystine %	0.90

*Source of additional information by *(McNaughton and Haymes, 1978)*

All birds in the 4 groups were weighed at 21-days-old, then weekly feed intake and body weight were recorded and feed conversion ratio also calculated as performance indices.

Hematological and biochemical analysis:

At the end of the experimental period (7 weeks age) blood samples were randomly collected from 3 quails of each replicate cage (total 6 quail per treatment). 2 ml of blood was collected without anticoagulant to obtain serum for analysis of hepatic and renal function, another 2 ml of blood was collected with EDTA for determination of

* Humin feed is water soluble sodium humate granules supplied from Humin.Tech.Com. Germany

hematological parameters. The total numbers of RBCs and leukocytes were determined using Natt-Herricks staining solution (Natt and Herricks, 1952). Differential leukocytic counts were made on slides film stained with Gemsa stain according to Dacie and Lewis, (1975).

The hemoglobin (Hb) were estimated according to Vankampen and Zylistra (1961) using commercial Randox Kit. The packed cell volume (PCV) was recorded by microhematocrit method (Swarup *et al.*, 1986). Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically according to the formula of Benjamin, (1970).

Sera were processed for determine GOT and GPT according to Reitman and Frankel, (1957). Total protein was estimated according to Peters, (1968). Albumin was determined according to Frank, (1950). Uric acid according to the method of James and White, (1971). While creatinine was determined according to Henry, (1974).

Histopathological examination:

At the end of the experimental period (7 weeks age) birds sacrificed, liver, kidney, heart, spleen and bursa Febrecus were taken, fixed in 10% neutral buffered formalin, sectioned and stained with H&E stain and examined microscopically for showed any histopathological changes (Woods and Ellis, 1994).

Statistical analysis:

The results obtained were analyzed using ANOVA test SAS Institute, (1992).

RESULTS

Table 1: The average body weights (g) of quails in the different experimental groups

Age/week	Control	Och. 16 ppm	Och. 16 ppm + Humin 2g/kg	Humin 2g/kg
3rd	55.5 ± 0.1	54.0 ± 0.14^{N}	55.5 ± 0.14^{N}	55.0 + 0.85^{N}
4th	78.5 ± 0.65	75.5 ± 1.20^{N}	78.5 ± 0.92^{N}	79.5 ± 1.01^{N}
5th	110.5 ± 1.14	104.5 ± 0.65**	112.5 ± 0.57^{N}	115.0 ± 1.06*
6th	135.0 ± 1.84	115.0 ± 1.49***	139.0 ± 1.27^{N}	142.0 ± 0.85**
7th	145.0 ±1.27	120.0 ±1.53***	149.0 ± 1.90^{N}	155.0 ± 0.85***
Dif. than .C. at the end	100%	89.7%	104.1%	106.9%

N: Non significant *: Significant **Highly significant ***Very highly significant

Table 2: The average cumulative feed consumption (g) of quails in the different experimental groups.

Age/ week	Control	Och. 16 ppm	Och. 16 ppm + Humin 2g/kg	Humin 2g/kg
3rd	115 ± 0.57	112 ± 0.35**	115.0 ± 1.06N	115 ± 1.27N
4th	157.0 ± 0.85	154.0 ± 1.27N	157.0 ± 1.14N	158 ± 0.85N
5th	225 ± 1.30	215.0 ± 1.23***	225 ± 1.84N	225 ± 1.14N
6th	270 ± 1.58	235.0 ± 1.58***	275 ± 1.58N	275 ± 1.14*
7th	290 ± 2.12	245 ± 1.7***	295 ± 1.46N	300 ± 1.27**
Total	1057	961	1067	1073

N: Non significant *: Significant **Highly significant ***Very highly significant

Table 3: The feed conversion ration in quail in the different experimental groups.

Age/week	Control	Och. 16 ppm	Och. 16 ppm + Humin 2g/kg	Humin 2g/kg
3rd	2.072 ± 0.01	2.07 ± 0.01N	2.07 ± 0.02N	2.09 ± 0.02N
4th	2.00 ± 0.01	2.04 ± 0.02N	2.00 ± 0.04N	1.98 ± 0.02N
5th	2.03 ± 0.02	2.06 ± 0.02N	2.00 ± 0.03N	1.95 ± 0.02*
6th	2.00 ± 0.02	2.04 ± 0.03N	1.97 ± 0.02N	1.93 ± 0.01*
7th	2.00 ± 0.03	2.04 ± 0.02N	1.97 ± 0.02N	1.93 ± 0.02*
Dif. Than C. at the end	-	+ 2 %	- 1.5 %	- 2.5 %

N: Non significant *: Significant

Table 4: Hemogram of quails in control and three treated groups.

Parameters	Control	Ochratoxin (16 ppm)	Ochratoxin (16 ppm) + Humin (2g/kg)	Humin (2g/kg)	L.S.D.
R.B.Cs (x 10^6/mm^3)	7.49 ± 0.51^A	5.09 ± 0.28^C	6.31 ± 0.21^B	7.43 ± 0.14^A	0.954
Hematocrite (%)	40.90 ± 0.64^A	35.19 ± 0.35^C	37.14 ± 0.71^B	41.64 ± 0.45^A	1.665
Hemoglobin (g/dl)	7.16 ± 0.32^A	6.13 ± 0.28^B	6.56 ± 0.39^A	7.38 ± 0.42^A	1.075
M.C.V. (um^3)	55.43 ± 3.13^B	69.85 ± 3.23^A	58.97 ± 0.87^B	56.09 ± 0.88^B	6.989
M.C.H. (pg)	9.64 ± 0.41^B	12.07 ± 0.13^A	10.36 ± 0.28^B	9.92 ± 0.48^B	1.053
M.C.H.C. (%)	17.48 ± 0.51^A	17.40 ± 0.61^A	17.61 ± 0.73^A	17.68 ± 0.83^A	2.037
Leucocytes (x 10^3 mm^3)	3.59 ± 0.42^A	3.28 ± 0.20^A	3.35 ± 0.25^A	4.04 ± 0.39^A	0.978
Lymphocytes (%)	60.80 ± 0.71^A	25.90 ± 0.64^C	44.60 ± 0.42^B	59.03 ± 0.63^A	1.824
Heterophilis (%)	35.90 ± 0.50^C	71.80 ± 1.77^A	53.80 ± 0.71^B	36.0 ± 0.85^C	3.211
Monocytes (%)	3.30 ± 0.23^B	2.30 ± 0.18^B	1.60 ± 0.17^C	4.97 ± 0.69^A	1.149

*Means with the same letter in each row are not significantly different at $p \leq 0.05$.
*L.S.D. → least significant difference.

Table 5: Liver and kidney functions of quails in control and different experimental groups.

Parameters	Control	Ochratoxin (16 ppm)	Ochratoxin (16 ppm) + Humin (2g/kg)	Humin (2g/kg)	L.S.D.
T. protein (g/dl)	4.26 ± 0.18^A	3.46 ± 0.14^B	4.01 ± 1.49^A	4.47 ± 0.33^A	0.958
Albumin (g/dl)	2.49 ± 0.29^A	2.20 ± 0.23^A	2.34 ± 0.28^A	2.53 ± 0.37^A	0.948
GOT (u/L)	70.40 ± 1.77^C	173.30 ± 2.33^A	116.80 ± 3.54^B	68.30 ± 0.92^C	7.017
GPT (u/L)	11.0 ± 0.25^C	20.90 ± 1.06^A	14.60 ± 0.92^B	8.40 ± 0.28^D	2.178
Uric acid (mg/dL)	3.70 ± 0.35^A	4.48 ± 0.35^A	4.06 ± 0.28^A	3.65 ± 0.42^A	1.066
Creatinine (mg/dL)	0.53 ± 0.18^A	0.99 ± 0.11^A	0.64 ± 0.13^A	0.52 ± 0.09^B	0.388

*Means with the same letter in each row are not significantly different at ≤ 0.05
*L.S.D. → least significant difference.

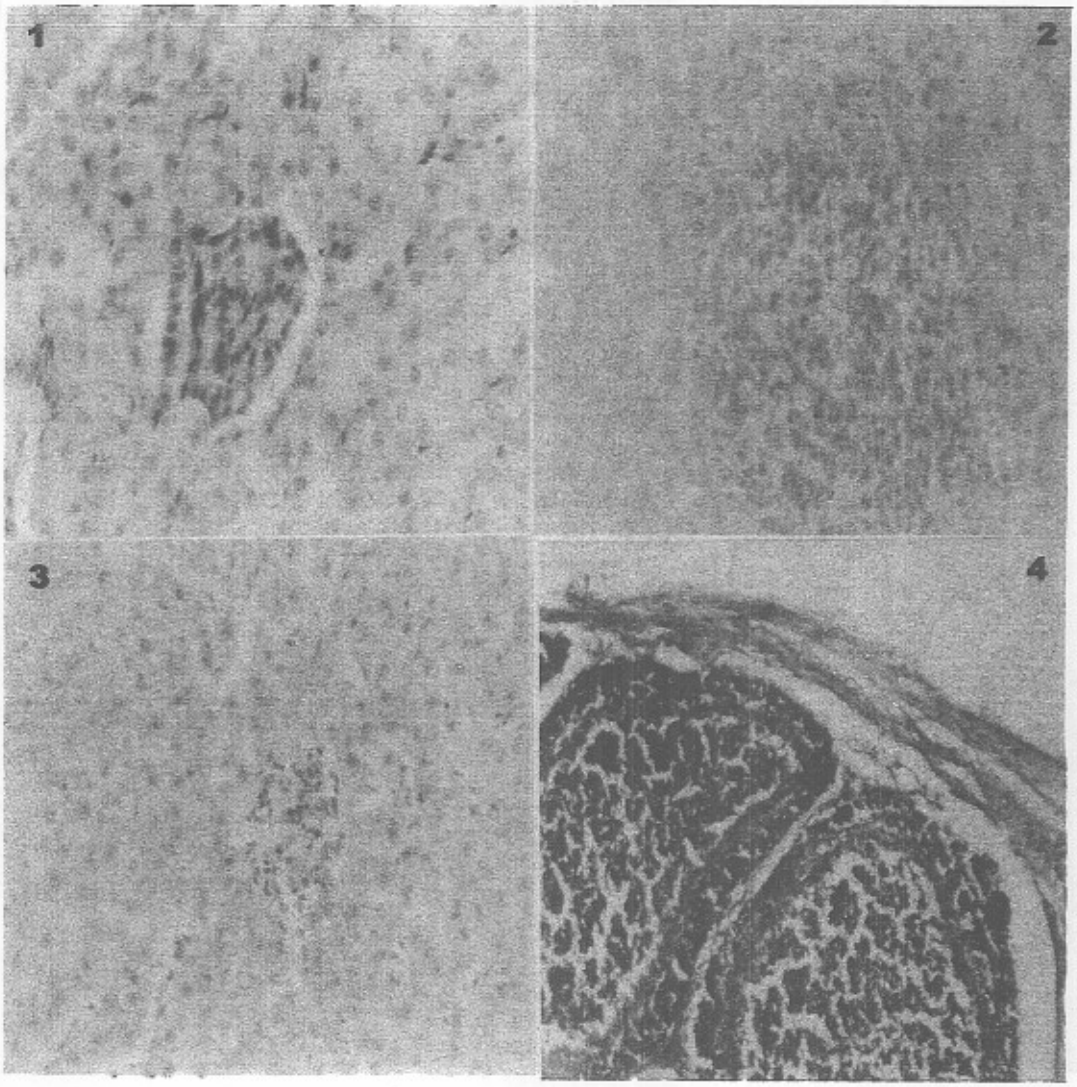

Fig. 1: Kidney of quail (Humin®) showing mild vacuolar degeneration in some tubular epithelium (H&E x 150)

Fig. 2: Liver of quail (Humin®) showing interstitial leukocytic aggregations and mild degenerative changes (H&E x 150)

Fig. 3: Liver of quail (Humin®) showing single or multiple vacuoles in he hepatic cells and congested blood vessels (H&E x 150)

Fig. 4: Bursa Fabricus of quail (Humin®) showing mild hyperplasic lymphoid follicles (H&E x 150)

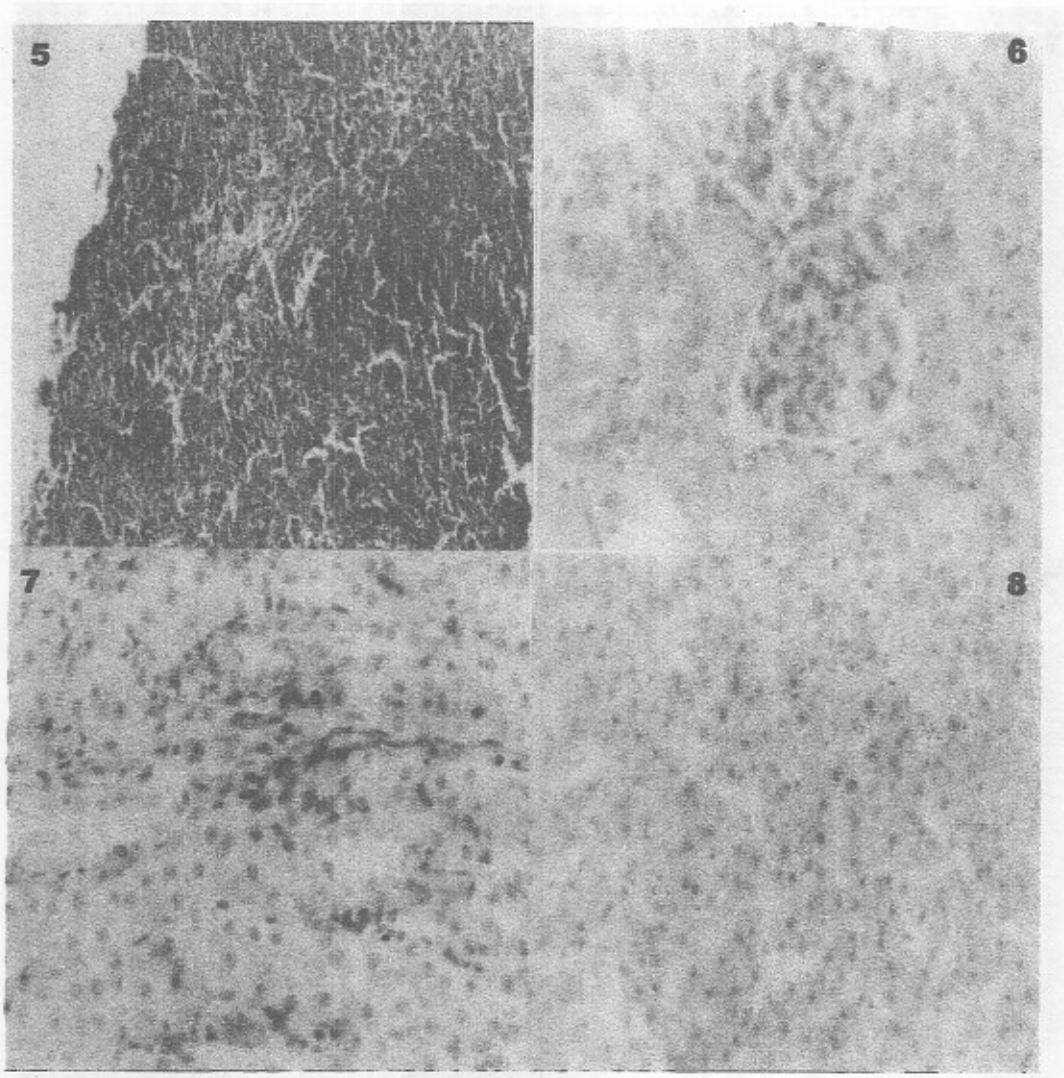

Fig. 5: Spleen of quail (Humin®) showing some activated splenic white pulps (H&E x 150)

Fig. 6: Kidney of quail (Humin® with ochratoxin) showing hypercellularity of some glemeruli (H&E x 150)

Fig. 7: Kidney of quail (Humin® with ochratoxin) showing degenerative tubular epithelium and mild interstitial fibroblastic proliferative (H&E x 150)

Fig. 8: Liver of quail (Humin® with ochratoxin) showing degenerative hepatic cells (H&E x 150)

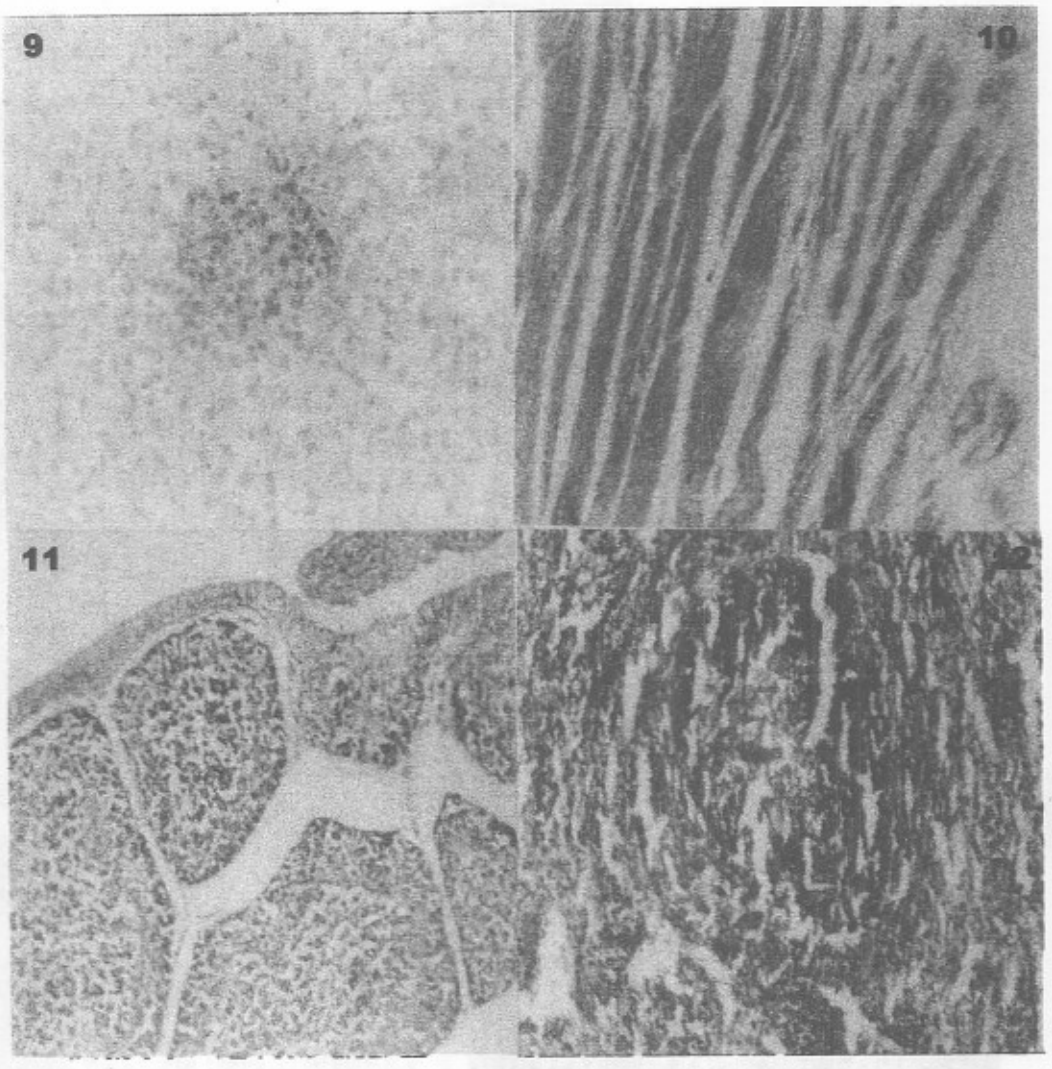

Fig. 9: Liver of quail (Humin® with ochratoxin) showing focal interstitial round cell aggregation (H&E x 150)

Fig. 10: Heart of quail (Humin® with ochratoxin) showing mild intermuscular edema (H&E x 80)

Fig. 11: Bursa of Fabricius of quail (Humin® with ochratoxin) showing slight lymphoid depletion (H&E x 150)

Fig. 12: Spleen of quail (Humin® with ochratoxin) showing mild atrophoid and depleced Malpighian body (H&E×150)

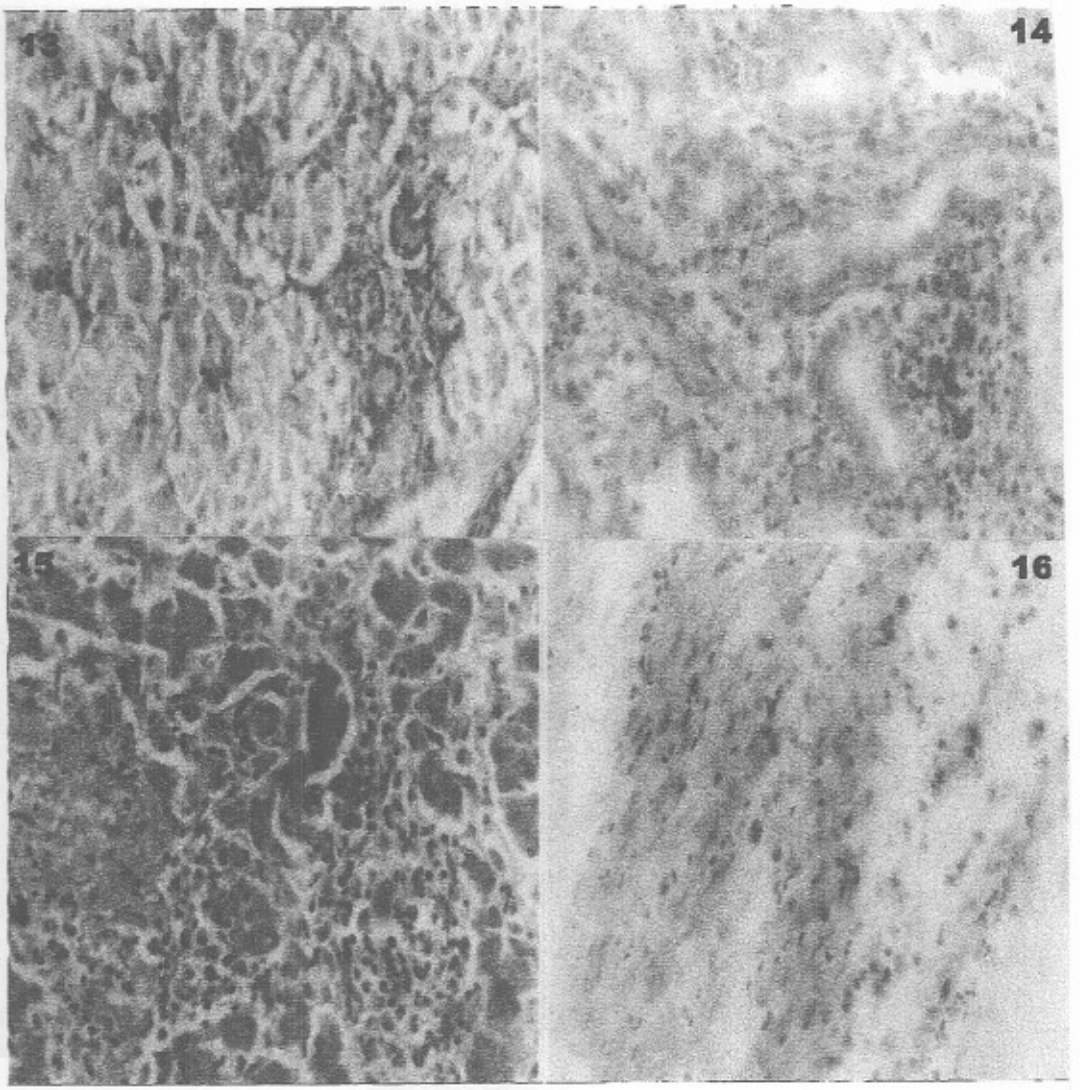

Fig. 13: Kidney of quail (ochratoxin) showing acute tubular necrosis and regenerative attempts (H&E x 80)

Fig. 14: Kidney of quail (ochratoxin) showing interstitial leukocytic aggregations and proliferations (H&E x 150)

Fig. 15: Liver of quail (ochratoxin) showing focal coagulative necrosis and interstitial round cell aggregaions (H&E x 150)

Fig. 16: Heart of quail (ochratoxin) showing partial hyalinization and intermuscular edema (H&E×150)

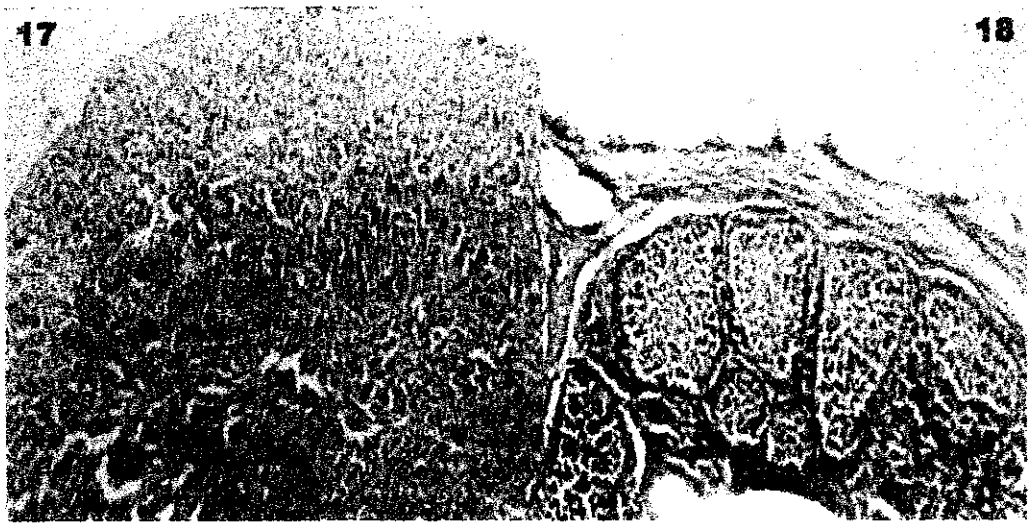

Fig. 17: Spleen of quail (ochratoxin) showing necrosis and depletions in white pulps (H&E x 80)

Fig. 18: Bursa of Fabricius of quail (ochratoxin) showing depletions of lymphoid follicles (H&E×80)

DISCUSSION

Growth performance and feed efficiency:

Mortality was only one bird in group fed 16 ppm ochratoxin after 3 weeks from start of treatment and this bird was necropsied and no note worthy macroscopic alterations were observed and the remaining birds in all groups did not show any signs of morbidity.

Stoltz *et al.,* (1988) reported that quail exhibited opisthatonus and leg, stretched fully back-wards is mycotoxicosis, the difference of our result than the results of Stoltz *et al.,* (1988) might be attributed to the different doses of ochratoxin.

The results in Tables 1, 2, 3 showed that body weight, feed consumption and consequently feed conversion rate were depressed in quails fed ochratoxin 16 ppm, mean-while birds fed ochratoxin 16 ppm and supplemented with humin feed 2 gm/kg and birds fed ration supplemented with humin feed only showed increase in body weight. Also feed consumption and feed conversion rate were improved, the improvement in body weight, and feed conversion were the highest in quails fed ration supplemented with humin feed 2 gm/kg feed only.

Farshid and Rajan, (1993) demonstrated significant reduction in body weight, bursa of Fabricius, spleen and thymus weights in the

ochratoxin treated Japanese quails. The efficiency of feed utilization was reduced in the OA and OA/t_2 combination in broiler (Kubena *et al.*, 1989) and our results agree with previous study.

The improvement in the performance in quails fed ochratoxin 16 ppm and supplemented with humin feed 2gm/kg than the quails feed ochratoxin 16ppm without humin feed may be attributed to that humin prevent the absorption of ochratoxin in the intestine of the birds or may be due to that humin neutralize the ochratoxin in the feed and prevent the ochratoxin adverse effects on the performance of the birds. These explination was supported by Kuhnert *et al.*, (1991) who reported that humic acid are able to form a protective film on the mucous epithelia of the gastrointestinal tract against infections and toxins.

Islam *et al.*, (2005) stated that, the macro-colloidal structure of humic acid ensures a good shielding on the mucous membrane of the stomach and gut, the peripheral capillaries and damaged mucous cells. As a result of this process, the resorption of toxic metabolites is reduced or fully prevented.

Bailey *et al.*, (1998) and Kocabagli *et al.*, (2002), studied the use 2.5g/kg feed of Farmagulator® Dry Humate on live performance, carcass weight and abdominal fat pad of broilers and indicated that feeding of humate during the growing period had the most beneficial effects in terms of growth and feed conversion. Islam *et al.*, (2005) stated that humic acid stabilize the intestinal flora and thus improved utilization of nutrients and this increase in live weight.

Hematological and biochemical analysis:

The effects of 16 ppm ochratoxine fed to quails on its hematology and biochemistry assays are presented in Tables (4 and 5). Quails fed 16 ppm ochratoxin for 4 weeks had reduced ($P < 0.05$). RBCs, Hb and hematocrit values. Ochratoxine had not adverse effect in leukocytic count while there was a significant decrease in the lymphocyte and heterophylis % when compared with the control birds. Also mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were significantly affected indicated microcytic hypochromic anemia. The results agree with Kubena *et al.*, (1989) who indicated that feeding ochratoxin (OA) to broiler chickens resulted in increase in relative liver, kidney, gizzard and pancreas weights, a microcytic hypochromic anemia and changed serum chemistry.

Butkeraitis *et al.* (2006) reported that reduction in quail RBCs and hematocrite which observed when fumonisin B_1, (FB_1) (mycotoxin)

fed to quails may have occurred as a result of FB_1 induced folate deficiency.

The decrease number in lymphocyte percentage indicated that ochratoxin had adverse effects on cellular immunity in quail.

In the other hand birds fed ochratoxin 16 ppm and supplemented with humin® feed 2gm/kg as well as birds supplemented with humin® feed alone showed no changes in hematological parameters than control birds and indicated that humin® feed counteracting with ochratoxin and that resulted normal hematological parameters in this group.

Lotosh, (1991) reported that RBCs and hemoglobin level remained on normal levels under the influence of humate® in comparison with control.

The date obtained from present study showed that birds fed 16 ppm ochratoxin without humin® feed had reduction in plasma protein and albumin. Serum GOT, serum GPT levels, uric acids and creatinine were increased. This indicated that ochratoxin has hepatotoxic and nephrotoxic effects on quail.

El-Yamany and Osfor, (2004) reported that there were highly significant changes in liver enzymes (alkaline phosphatase, GOT, GPT and bilirubin) also there were highly significant increases in serum level of urea and creatinine of birds fed with diets containing ochratoxins when compared with the negative control group.

The quail in this study which fed ochratoxin 16 ppm with humin feed 2 gm/kg and that supplemented with humin feed alone did not have any significant affects on liver and kidney functions.

Yalicin *et al.*, (2005) indicated that blood serum parameters were not affected by the supplementation of L. carnitine with or without humate.

Histopathological studies:

The results of the histopathological studies of the effect of humin in quails showed:

Kidneys:

The tubular epithelium were swollen and sometimes contain vacuoles (Fig. 1). Mild hydropic degeneration could be seen in the epithelium of some collecting tubules. The glomeruli were normal. Mild dilatation of inter-tubular capillaries and blood vessels could be seen.

Liver:

Interstitial leukocytic aggregations mainly lymphocytes with mild degenerative changes in the surrounding hepatic cells could be seen (Fig. 2). Other hepatic cells contain single or multiple clear vacuoles

beside congestion of hepatic vasculatur (Fig. 3). Some portal areas had leukocytic infiltration mainly round cells.

Heart:

It was apparently normal.

Lymphoid organs (spleen and bursa of Fabricius):

Bursal lymphoid follicles and splenic white pulps showed mild hyperplasia beside reduction in subcapsular splenic sinuses by lymphoid cells infiltration and presence of numerous lymphoid follicles in the interfollicular and subepithelial tissues of bursa (Figs. 4 & 5).

While in the group given humin + ochratoxin the histopathological studies showed:

Kidneys:

Hyper cellularity of some glomeruli with partial obliteration of some Bowman's spaces could be seen (Fig. 6). Some renal tubules had various degenerative changes varied from cloudy swelling to hydropic degeneration and mild interstium fibroblastic proliferation (Fig. 7).

Liver:

The majority of hepatic cells showed vacular degeneration with swelling of the hepatic cells (Fig. 8). Hyperplastic kuffer's cells in addition to interstitial and portal round cells aggregation were common (Fig. 9). The hepatic blood vessels and sinusoids were congested.

Heart:

Mild inter-muscular edema and congested capillaries were noticed (Fig. 10).

Lymphoid organs (spleen and bursa of Fabricius):

Both cortical and medullary zones of bursal lymphoid follicles revealed slight depletion beside inerfollicular edema and degenerative changes in covering epithelium (Fig. 11). The splenic Malpighian bodies were depleced and mild atrophied (Fig. 12).

The histopathological studies of quails given ochratoxin 16ppm showed:

Kidneys:

The kidneys revealed acute tubular necrosis manifested by pyknosis and karyorrhexis of their nuclei accompanied with dilated and engorged renal capillaries and regenerative attempts in some adjacent renal parenchyma (Fig. 13). Some tubules were dilated and had granular casts. Intersitial lymphocytic aggregations and fibroblastic proliferations in addition hemorrhages were encountered (Fig. 14). A few glomeruli were dilated and had thickened basement membrane.

Liver:

Focal coagulative necrosis or disassociated and shrunken hepatic cells beside interstitial round cell infelteration could be observed (Fig. 15). Some portal areas had proliferative bile ductules, fibroblastic proliferations and round cell infelteration.

Heart:

Some myocardial muscle fibers showed partial hyaline degeneration, inter and intramuscular edema and a few extravasated erythrocytes among degenerated muscle fibers (Fig. 16).

Lymphoid organs (spleen and bursa of Fabricius):

All bursal and splenic lymphoid elements revealed lymphoid depletion and necrosis. Atrophoid white pulps and depleted subcapsular sinuses were seen in spleen (Fig. 17). Edematous smooth muscles with reduction of lymphoid populations and both cortical and medullary zones of bursal lymphoid follicles were common (Fig. 18).

Maxwell *et al.*, (1987) reported that, ultrathin sections of liver and kidneys from 11 weeks old quail fed from one-day-old with diets containing ochratoxin A (4 and 8 mg/kg) were showed abnormal mitochondria and excessive numbers of lipids droplets were the main findings in the proximal convoluted tubules and glomeruli showing thickened basement membranes. Swollen mitochondria and variable glycogen deposit were the chief features present in livers.

Stoev, (1998) stated that ochratoxin A feeding for broiler caused clearly swilling and pronounced granular degeneration in the epithelium, proliferation of mononuclear cells and activation of capillary epithelium of lymphoid organs, degenerative changes and depletion of lymphoid cells in lymphoid organs (bursa of Fabricius, thymus and spleen) were found.

ACKNOWLEDGMENT

For histopathological investigation, the authors thanks Prof. Dr. Abd El-Meneem Aly, Professor of Pathology, Faculty of Veterinary Medicine, Zagazig University

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