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RESEARCH ARTICLE

Effects of Ochratoxin A Feeding in White Leghorn Cockerels on Hematological and Serum Biochemical Parameters and its Amelioration with Silymarin and Vitamin E

Muhammad Fakhar-ud-Din Ahmad, Muhammad Kashif Saleemi*, Muhammad Zargham Khan, Faqir Muhammad[1], Zahoor-ul-Hassan[2], Aisha Khatoon, Sheraz Ahmed Bhatti, Rao Zahid Abbas[3], Farzana Rizvi and Ishtiaq Ahmed[4]

Department of Pathology, [1]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad; [2]Department of Animal Sciences, KPK Agricultural University, Peshawar; [3]Department of Parasitology, University of Agriculture, Faisalabad; [4]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
*Corresponding Author: drkashif313@yahoo.com

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ABSTRACT

The objective of the present study was to evaluate the hematobiochemical effects tempted by ochratoxin A (OTA) in White Leghorn (WL) cockerels and to evaluate the effect of silymarin (SL), vitamin E (VE) and their combination against OTA persuaded pathological alterations in cockerels. A total of 240 day-old WL cockerels were divided into 12 groups A-L having 20 birds in each group and group A was control. Two doses of OTA 1000 and 2000 µg/kg of feed were given to cockerels up to 7 weeks. Silymarin was administered at a dose rate of 10000 mg/kg and Vitamin E at a dose rate of 200 mg/kg alone and in combinations with two doses of OTA. In OTA treated groups total erythrocytes counts, leukocytes count, PCV and Hb were decreased as compared to control, SL and VE groups. Albumen and serum total proteins in OTA treated groups were significantly lower as compared to control, SL and VE groups. Serum alanine transferase was significantly increased in OTA fed groups in comparison with control, SL and VE groups. Creatinine and urea were increased in OTA treated groups but were almost normal in SL and VE groups. Results showed that OTA had severe effect on liver and kidney but SL and VE treated groups had normal liver and kidneys showing its hepatoprotective effects. However, at higher dose of OTA this ameliorative effect was partially observed. These agents may be recommended as a remedy for ochratoxicosis.

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INTRODUCTION

Ochratoxin A is an important mycotoxin produced by different *Aspergillus* and *Penicillium* species. The presence of ochratoxin A (OTA) in animal feeds raises concerns in poultry and livestock industry due to subclinical intoxications and poor growth in animals (Gentles *et al*., 1999; Zia *et al*., 2010). OTA present in the poultry feeds in low to moderate levels induces immunosuppression, decreased bodyweight gains and increased susceptibility to infectious diseases (Saleemi *et al*., 2010; Mukhtar *et al*., 2010). OTA has gained considerable attention due to its intrinsic toxicity and its frequent occurrence in feed commodities used in livestock feeds. The main target organ of OTA in poultry, as in other species, appears to be the kidney, although liver, gastrointestinal tract, lymphoid organs, skeletal system, hematopoietic tissues and the reproductive organs can also be effected (Paterson and Lima, 2010). At higher toxic levels the birds show clinical signs of disease, mortality, nephritis (Elaroussi *et al*., 2006), hepatitis (Kumar *et al*., 2004). Ochratoxicosis developing from high degree of contamination of feeds with OTA is a common condition in different avian species. OTA induces degenerative changes and an increase in the weight of kidneys and liver (Stoev *et al*., 2011).

OTA is frequently present in poultry feeds and its ingredients (Saleemi *et al*., 2012). Its levels in the feeds are kept minimum by different methods including use of ingredients having low levels of OTA, proper storage and use of toxin binders to bind the preformed mycotoxins and rendering them unabsorbable from the gut. All these methods work with a variable degree of efficiency.

Ochratoxin A not only induces disease in chicken but also it may accumulate in the eggs and meat of the poultry and thus enters in human food chain. Therefore, all the efforts should be made to protect the commercial poultry birds from the toxic effects of ochratoxin. Another important strategy in case of clinical ochratoxicosis is to reduce the damage and losses by alleviating the deleterious effects of OTA by different hepato-protective and renal protective substances. At present different chemical substances and plant extracts are being used globally to treat the liver, kidney damage and immunosuppression caused by a variety of toxicants. Currently many hepatoprotective substances are being used to prevent liver damage due to these mycotoxins. Among these natural substances, silymarin is an important antitoxin compound. Silymarin, an extract from seeds and fruits of *Silybum marianum*, is one of the most important natural hepato-renal protective substances containing a mixture of flavonoids isomers such as silibinin, isosilibinin, silidianin and silichristin (He *et al*., 2004). This plant is naturally present in northern areas of Pakistan including Kashmir. It is locally used by people as hepatoprotective substance in its crude form. In present study, purified SL in powdered form obtained from China was used. There is scanty information available on use of SL in poultry and livestock as antagonistic substance of mycotoxins. Therefore current study was planned to explicate the effects of SL in the cockerels to improve their health status in the presence of OTA in feed. However, no report is available about health improving effects of SL in cockerels in terms of their increased vigor to counter the injurious effects of OTA.

Vitamin E is an essential vitamin possessing an antioxidant activity. Vitamin E given at higher than recommended doses increase the number of antibody producing cells and raises antibody titers in chickens and mice (Hossain *et al*., 1998). Both VE and SL have an antioxidant effects and when given together may enhance the immunoprotective and immunostimulatory properties of each other (Horvath *et al.*, 2001). Keeping in view the OTA induced pathological changes and hepatoprotective effects of SL and VE in different species, the present project was designed to study OTA induced hematobiochemical alterations in WL cockerels and protection provided by SL and VE.

MATERIALS AND METHODS

Birds feed and housing: A total of 240, day-old, WL cockerels free from any clinical ailment were procured from a local hatchery and kept on rice husk litter material under standard housing and management conditions. Birds were fed on corn soybean based feed having 22 % total protein and provided feed and water *ad libitum.*

Experimental design: After two days of acclimatization, these birds were divided into 12 groups from A to L having 20 birds in each group. The detailed layout of experiment is presented in Table 1. Duration of the experiment was 7 weeks. Ten birds from each group were sacrificed at 21 days of age and remaining birds at the end of experiment. Blood was collected with and without anticoagulant for hematological and serum biochemical studies.

Table 1: Layout of experiment

Groups	No. of birds	Treatment
A	20	Control
B	20	OTA I (1000 µg/kg feed)
C	20	OTA 2 (2000 µg/kg feed)
D	20	SL (10000 mg/kg feed)
E	20	VE (200 mg/kg feed)
F	20	VE+ SL
G	20	OTA 1+VE
H	20	OTA 2+VE
I	20	OTA 1+SL
J	20	OTA 2+SL
K	20	OTA +SL+VE
L	20	OTA 2+SL+VE

Parameters studied: Blood samples with EDTA were collected on the day of slaughtering and used for evaluation of following hematological studies. Erythrocyte counts were made with the help of hemocytometer using the technique described by Natt and Herrick (1952). Hemoglobin and PCV were determined according to method described by Sharaf *et al*. (2010). Serum total protein was determined by Biuret method (Oser, 1976). Serum albumin concentration was determined by bromocresol green dye binding method (Anonymous, 1984). Serum creatinine was determined by Jaffe Reaction (Bosnes and Taussky, 1945) using commercially available kit of Diasis Diagnostic System, Turkey (DDS Lot # 8116). Serum alanine transferase was determined colorimetric method of Reitman and Frankel (1957) by using the commercially available kit of Diasis Diagnostic System, Turkey (DDS Lot # 7275). Serum urea concentration was determined by using commercially available kit (DDS Lot # 7580).

Statistical analysis: Data thus obtained subjected to statistical analysis by using factorial test different means compared by using Duncan's multiple range test (DMR). The level of significance was $P \leq 0.05$.

RESULTS

Hematological parameters: At 3rd week of experiment, all the hematological findings were non-significant, therefore, not present in the table. At week 7 of experiment erythrocyte count of group B (OTA1) and group C (OTA2) was significantly lower than the control group while of group G (OTA1+VE), group H (OTA2+VE), group I (OTA1+SL), group J (OTA2+SL), group K (OTA1+ SL+VE) and group L (OTA2+SL+VE) was significantly different from control group. In groups D (SL), group E (VE) and group F (SL+VE), erythrocyte count was nonsignificantly different from the control group (Table 2).

At week 7 of experiment total leukocyte count of groups B, C, G, H, I, J and K was significantly higher than the control group. In groups D, E, F and L leukocyte count was non-significantly higher from the control group.

At week 7 of experiment, PCV value of groups B, C, G, H, I, J, K and L was significantly lower than the control group while in groups D, E and F, PCV was non-significantly lower than the control group. At week 7 of

experiment Hb value of groups B, C, G, H, I, J, K and L was significantly lower than the control group while of groups D, E and F it differed non-significantly.

Table 2: Hematological parameters of cockerels administered various levels of ochratoxin A, Silymarin and Vitamin E at 49th day of experiment

Group	Hb (g/dl)	PCV (%)	RBC (10^6/µL)	WBC (10^3/µL)
A	11.67±0.47	28.00±0.60	2.73±0.08	24.53±0.92
B	9.11±0.32*	21.69±0.87*	2.18±0.15*	27.16±2.27*
C	8.50±0.37*	19.04±0.89*	1.91±0.17*	27.13±1.82*
D	10.91±0.76	26.29±1.25	2.61±0.12	25.43±1.27
E	11.66±0.79	26.81±1.76	2.71±0.21	24.71±1.70
F	12.00±1.08	27.76±2.29	2.85±0.28	24.86±0.90
G	10.39±0.48*	24.17±1.29*	2.39±0.18*	26.79±0.81*
H	10.11±0.56*	24.14±1.38*	2.35±0.14*	26.50±1.04*
I	9.93±0.62*	23.76±1.50*	2.30±0.22*	27.57±1.72*
J	9.20±1.00*	22.14±2.91*	2.24±0.33*	26.57±1.13*
K	9.51±0.79*	24.39±1.24*	2.38±0.19*	26.43±1.13*
L	8.91±0.82*	23.06±1.67*	2.26±0.23*	26.00±2.08

Values (Mean ± SD) in each column bearing asterisk differ statistically from control P≤0.05.

Serum biochemical parameters: At 21 days of experiment total protein value of groups B and C was significantly lower than the control group. While in other groups (D to L) total protein value was non-significantly different from the control group. At 7th week of experiment groups treated with OTA1 and OTA2 (B and C), the total protein value was non-significantly lower than the control group while of other groups (D to L), it was non significantly different from the control group (Table 3).

At 3rd week of experiment in groups B, C, F, H, I and L albumen concentration was significantly lower than the control group. In group D, K, E, G and J the albumin concentration was non-significantly different from control group.

At 7th week of experiment in groups B, C treated with OTA1 and OTA2 respectively, and in groups G, I and K albumen concentration was significantly lower than the control group. In group D, E, F, L, H and J albumin concentration was non-significantly lower from the control group.

At 21 days of experiment in groups B, C, G, H, J, I and K was significantly higher than the control group. In groups D, E, and F creatinine concentration was non-significantly higher from the control group. In group L, it was significantly lower than the control group (Table 3).

At week 7 of experiment in group B, C, G, H, J, and group I, the creatinine concentration was significantly higher than the control group while in group K and group L the creatinine concentration was significantly lower than control group. In group D, E and F the creatinine concentration was non-significantly higher from the control group.

At week 3 of experiment, the ALT concentration in group B, C and H treated with OTA1, OTA2 and OTA2 + VE respectively was significantly higher than the control group while in groups J, I, K, L, G, D, E and F its concentration was non-significantly different from control group. At week 7 of experiment, ALT concentration in groups B, C, G and group H was significantly higher than the control group while of all other groups J, D, L, F, K, I, E was non-significantly higher from the control group.

At week 3 of experiment, urea concentration in groups B, C, G, H, J, K and L was significantly higher than the control group while in groups D, E, F and group I it was non-significantly higher from control group. At week 7 of experiment urea concentration in groups B, C, G, H, I and L was significantly higher than the control group while in groups D, E, F, I and J it was non-significantly higher than the control group.

Clinical signs and gross lesions: Birds treated with OTA1 (group B) and OTA2 (group C) were dull and depressed throughout the length of experiment. Birds died during the experiment and slaughtered at the day 21 and

Table 3: Serum biochemical parameters of cockerels administered various levels of ochratoxin A, Silymarin and Vitamin E

Group	Albumen (g/100ml)	Total protein (g/100ml)	ALT (IU/µL)	Creatinine (mg/100ml)	Urea (mg/100ml)
1st Killing (Day 21)					
A	1.62±0.13	4.06±0.13	11.26±3.13	0.83±0.04	11.67±1.65
B	0.88±0.24*	3.75±0.11*	32.66±9.18*	1.23±0.06*	25.90±2.74*
C	0.88±0.10*	3.75±0.12*	41.37±8.67*	1.32±0.05*	35.77±3.20*
D	1.53±0.37	3.99±0.13	10.56±5.03	0.87±0.04	11.90±2.01
E	1.43±0.26	4.10±0.19	8.83±4.26	0.86±0.04	12.32±1.89
F	1.15±0.14*	4.11±0.12	8.48±3.43	0.87±0.04	13.42±2.41
G	1.33±0.24	3.97±0.16	18.96±7.99	1.08±0.08*	23.49±3.76*
H	1.11±0.39*	4.11±0.16	28.17±4.33*	1.05±0.08*	24.49±5.15*
I	1.16±0.09*	4.04±0.15	9.34±4.38	1.03±0.03*	16.20±3.59
J	1.27±0.26	3.86±0.06	12.71±3.58	1.04±0.05*	22.10±1.49*
K	1.49±0.13	4.08±0.12	10.33±0.99	1.01±0.11*	16.36±2.54*
L	1.12±0.10*	4.01±0.12	9.18±1.99	0.94±0.12*	19.39±1.98*
2nd Killing (Day 49)					
A	1.88±0.09	4.11±0.08	8.93±1.40	0.82±0.03	11.96±1.37f
B	1.42±0.2*	3.81±0.21	29.64±7.37*	1.31±0.04*	28.13±1.54*
C	1.43±0.18*	3.92±0.17	38.72±10.50*	1.35±0.05*	37.06±6.45*
D	1.72±0.14	4.23±0.07	11.26±2.50	0.84±0.05	13.38±1.26
E	1.73±0.14	4.14±0.33	7.65±2.96	0.80±0.03	13.17±1.05
F	1.68±0.14	3.92±0.09	9.29±1.35	0.80±0.06	12.58±2.15
G	1.18±0.69*	3.78±0.29	17.26±4.71*	1.03±0.05*	17.63±0.93*
H	1.66±0.14	3.99±0.12	22.35±5.97*	1.11±0.04b	21.17±3.43*
I	1.39±0.14*	4.05±0.30	8.03±2.54	1.04±0.06*	14.17±1.63
J	1.62±0.16	4.10±0.26	13.00±3.87	1.10±0.02*	23.54±4.85*
K	1.16±0.17*	3.98±0.39	8.35±3.50	0.95±0.03*	15.63±1.98
L	1.68±0.12	4.25±0.27	9.86±1.52	0.97±0.06*	17.79±2.53*

Values (Mean ± SD) in each column at a specific day bearing asterisk differ statistically from control P≤0.05.

49 showed swollen liver and kidneys in OTA contaminated groups. Intestinal mucosa was congested in these OTA fed groups. It indicated pathological changes induced by OTA. In control, SL and VE groups birds were active and no such changes were observed on necropsy examination.

DISCUSSION

Keeping in mind this important issue the present study was designed to investigate toxico-pathological effects of OTA in WL cockerels and their amelioration with silymarin and Vitamin E (α tocopherol). In present study, hematological parameters the RBCs count of groups B, C, G, H, I, J, K and L were significantly lower from control group while of groups D, E and F were non-significantly different from control group A. Similar results of decreased RBCs count in response to OTA feeding has been reported by different researchers (Mohiuddin *et al*., 1993; Stoev *et al*., 2011). Packed cell volume and hemoglobin values of all OTA treated groups was significantly lower from control group, while D, E and F groups were non-significant different from control group. Similar to our findings, anemia (decrease RBCs, PCV and Hb) have been reported in OTA intoxicated broiler birds by Elaroussi *et al*. (2006). This is related to OTA intoxications through feed.

In serum biochemical parameters total protein and albumen in group B (OTA1) and C (OTA2) were significantly lower from control group at day 21 and 49 of experiment. While in all the remaining groups total protein at day 21 and 49 was non-significantly different from control group. It is an important biochemical change induced by OTA in birds. But albumen of groups F, H, I and L were significantly lower from control group. Similar results have been reported by different workers (Hassan *et al*., 2010), where total protein and albumen decreased in a dose related manner and was minimum in higher OTA group (1.5 ppm) and maximum in control group. In present studies alanine amino-transferase (ALT) values of group B and C were significantly higher from control group, while all the remaining groups were non-significantly different from control group at day 21 of experiment. Sawale *et al*. (2009) and Hassan *et al*. (2010) have also been reported an increase in ALT concentration in dose related manner. Similarly at 49 day of experiment B, C, G and H were significantly higher from control group, while all the remaining groups were non-significantly different from control group at day 49 of experiment. Similar increasing trend in ALT values in OTA contaminated groups have also been reported by Wang *et al.* (2009) and Hassan *et al.* (2011). This increase in ALT level indicates acute hepatic necrosis. It is an important hepatotoxic effect of OTA.

In present study, creatinine values of groups B, C, G, H, J, I and K were significantly higher from control group at 21 and 49 days of experiment. Our findings of increased creatinine values coincide with the results previously reported in WL chicks and broilers (Kalorey *et al*., 2005; Sawale *et al*., 2009). In groups D, E, and F (SL, VE and combination of both) these values were non-significantly different from control group. It indicates amelioration of adverse effects of OTA with these agents like SL and VE. Group L was non-significantly different from control group. Urea concentrations in present study in groups B, C, G, H, J, K and L significantly higher from control group, while of D, E, F (Silymarin, Vit. E and both in combination) and I were non-significantly different from control group at 21 days of experiment. At 49 days groups B, C, G and H were significantly higher from control group, while D, E, F, I and J non-significantly different from control group. Similar to our findings (Stoev *et al*., 2002; Koynarski *et al*., 2007; Denli *et al*., 2008) reported significant higher serum uric acid concentration in OTA fed chickens.

The findings of present study may be concluded that birds kept on silymarin, VE and their combinations showed hemato-biochemical responses similar to those of control group. Birds fed SL, VE or their combination along with 1000 µg/OTA showed improvement in hemato-biochemical responses, almost similar to those of control birds suggesting a protective effect of SL against OTA. This protective effect, however, in birds fed SL, VE or their combination in birds fed 2000 µg/Kg OTA was partial. It indicates partial amelioration with these protective agents. These agents/substances may be used as protective agents in poultry to ameliorate the adverse effects of ochratoxin A.

REFERENCES

Anonymous, 1984. Manual of veterinary investigation laboratory techniques Volume 2, Reference Book 390. Ministry of Agriculture, Fisheries and Food, HMSO London.

Bosnes RW and HH Taussky, 1945. On the colorimetric determination of creatinine by Jaffe reaction. J Biochem, 158: 581-559.

Denli M, JC Blandon, ME Guynot, S Salado and JF Perez, 2008. Efficacy of a new ochratoxin- binding agent (Ocra Tox) to counteract the deleterious effects of ochratoxin A in laying hens. Poult Sci, 87: 2266-2272.

Elaroussi MA, FR Mohamed, EM El-Barkouky, AM Atta, AM Abdou and MH Hatab, 2006. Experimental ochratoxicosis in broiler chickens. Avian Pathol, 35: 263-269.

Gentles A, EE Smith, LF Kubena, E Duffus, P Johnson, J Thompson, RB Harvey, TS Edrington. 1999. Toxicological evaluation of cyclopiazonic acid and ochratoxin A in broilers. Poult Sci, 78: 1380-1384.

Hassan ZU, MZ Khan, A Khan and I Javed, 2010. Pathological responses of white leghorn breeder hens kept on ochratoxin A contaminated feed. Pak Vet J, 30: 118-123.

Hassan ZU, MZ Khan, A Khan, I Javed and MK Saleemi, 2011. Immunological responses of progeny of White leghorn layer breeder kept on ochratoxin A contaminated feed. J Immunotoxicol, 8: 122-130.

He Q, J Kim and RP Sharma, 2004. Silymarin protects against liver damage in BALB/c mice exposed to Fumonisins B1 despite increasing accumulation of free sphingoid bases. Toxicol Sci, 80: 335-342.

Horvath ME, RG Cabello, A Blazovics, MVD Looij, I Barta, G Muzes, P Gergely and J Feher, 2001. Effect of silibinin and vitamin E on restoration of cellular immune response after partial hepatectomy. J Ethnopharmacol, 77: 227–232.

Hossain SM, SL Barreto, AG Bertechini, AM Rios and CG Silva, 1998. Influence of dietary Vitamin E level on egg production of broiler breeders, and on the growth and immune response of progeny in comparison with the progeny from eggs injected with Vitamin E. Anim Feed Sci Tech, 73: 307-317.

Kalorey DR, NVS Kurkure, JS Ramgaonkar, PS Sakhare, S Warke and NK Nigot, 2005. Effect of polyherbal feed supplement "Growell" during induced aflatoxicosis, ochratoxicosis and combined mycotoxicosis in broilers. Asian-Aust J Anim Sci, 18: 375-383.

Koynarski V, S Stoev, N Grozeva, T Mirtcheva, H Daskalov, J Mitev and P Mantle, 2007. Experimental coccidiosis provoked by *Eimeria acervulina* in chicks simultaneously fed on ochratoxin A contaminated diet. Res Vet Sci, 82: 225-231.

Kumar A, N Jindal, CL Shukla, RK Asrani, DR Ledoux and GE Rottinghaus, 2004. Pathological changes in broiler chickens fed ochratoxin A and inoculated with *Escherichia coli*. Avian Pathol, 33: 413-417.

Mohiuddin SM, SMA Warasi, MV Reddey, 1993. Hematological and biochemical changes in experimental ochratoxicosis in broiler chickens. Indian Vet J, 70: 613-617.

Mukhtar A, AH Gilani and N Bhatty, 2010. Some nutritional and microbiological aspects of apples of common varieties available for household consumption. J Anim Plant Sci, 20: 253-257.

Natt MP and CA Herrick, 1952. A new diluent for counting the erythrocytes and leukocytes of chicken. Poult Sci, 31:735-737.

Oser BL, 1976. Hawks Physiological Chemistry. Tata Macgraw Hill Publications Co, New Dehli, India.

Paterson RRM and N. Lima, 2010. Toxicology of mycotoxins. Mol, Clin Environ Toxicol, 100: 31-63.

Reitman S and S Frankel, 1957. A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 28: 56-63.

Saleemi MK, MZ Khan, A Khan and I Javed, 2010. Mycoflora of poultry feeds and mycotoxins producing potential of *Asspergillus* species. Pak J Bot, 42: 427-434.

Saleemi MK, MZ Khan, A Khan, I Javed, ZU Hassan, MR Hameed, S Hameed and MA Mehmood, 2012. Occurrence of toxigenic fungi in maize and maize-gluten meal from Pakistan. Phytopathol Mediterranea, 51: 219-224.

Sawale GK, RC Gosh, K Ravikanth, S Maini and DS Rekhe, 2009. Experimental mycotoxicosis in layer induced by ochratoxin A and its amelioration with herbomineral toxin binder 'Toxiroak'. Int J Poult Sci, 8: 798-803.

Sharaf S, A Khan, MZ Khan, F Aslam, MK Saleemi and F Mahmood, 2010. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. Exp Toxicol Pathol, 62: 333-341.

Stoev SD, D Gundasheva, I Zarkov, T Mircheva, D Zapryanova, S Denev, Y Mitev, H Daskalov, M Dutton, M Mwanza and YJ Schneider, 2011. Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and fumonisin B1. Exp Toxicol Pathol, doi:10.1016/j.etp.2011.01.008

Stoev SD, H Daskolov, B Radic, A Domijan and M Peraica, 2002. Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology. Vet Res, 33: 83-93.

Wang GH, CY Xue, F Chen, YL Ma, XB Zhang, YZ Bi and YC Cao. 2009. Effects of combinations of ochratoxin A and T-2 toxin on immune function of yellow-feathered broiler chickens. Poult Sci, 88: 504-510.

Zia MA, KU Rahman, MA Sheikh and IA Khan, 2010. Chemically treated strain improvement of *Aspergillus niger* for enhanced production of glucose oxidase. Int J Agric Biol, 12: 964–966.