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Pathological Responses of White Leghorn Breeder Hens Kept on Ochratoxin A Contaminated Feed

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ABSTRACT

Mycotoxins are among the most important environmental contaminants. In the present study, ochratoxin A (OTA) was produced by propagation of *Aspergillus ochraceus* and fed to breeder hens. For this purpose, 84 breeder hens were divided into seven groups (A-G). Group A served as control, while groups B, C, D, E, F and G were fed OTA at 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg/Kg feed, respectively for 3 weeks. Clinical signs, feed intake, feed conversion ratio and egg mass production were recorded on daily basis, while body weight was recorded on weekly basis. Lesions on visceral organs and serum biochemical parameters were determined. Significant decrease in feed intake, body weight and egg mass production was found in the OTA treated groups compared to control ($P<0.05$). Among different groups, diarrhea, unthriftiness, water intake and depression increased with increase in dietary OTA levels. Enlargement and hemorrhages on liver and kidney were more severe in birds fed higher dietary OTA levels. Serum ALT, urea, creatinine and total protein levels were significantly higher in OTA treated groups. It was concluded that production performance, pathological alterations and serum biochemical changes determined became more severe with increase in dietary levels of OTA.

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INTRODUCTION

Mycotoxins, the secondary metabolites of toxigenic fungi, are unavoidable contaminants in foods and feeds exerting injurious effects upon animal and human health. The most significant mycotoxins in naturally contaminated foods and feeds are aflatoxins, ochratoxins, zearalenone, T-2 toxin, deoxynivalenol and fumonisins (Devegowda *et al*., 1998).

Ochratoxin A (OTA) is produced by some species of *Aspergillus* and *Penicillium* including *Aspergillus ochraceus, A. carbonirus, A. niger* and *Penicillium verocosum*. The presence of OTA has been reported in a wide variety of poultry feeds (Dalcero *et al*., 1998; Rosa *et al*., 2006) and feed ingredients like corn, wheat and rice (Zinedine *et al*., 2006, Liu *et al*., 2007). In Pakistan, OTA has also been reported in poultry feed and ingredients (Zafar *et al*., 2001; Hanif *et al*., 2006; Sultana and Hanif, 2009). OTA exerts its toxicological effects in different species of birds and animals. In chicken, pathomorphological, immune and serum biochemical alterations produced by OTA have been reported (Stoev *et al*., 2002; Kumar *et al*., 2004; Koynarski *et al*., 2007). Residues of ochratoxin have been detected in organs and tissues of poultry (Niemiec *et al*., 1994), swine (Matrella *et al*., 2006) and rats (Rached *et al*., 2007). In Pakistan, OTA has been detected in edible chicken tissues like liver and muscles (unpublished data). However, little information is available about the clinical ochratoxicosis experimentally produced in chicken under local environmental conditions. Such information is necessary for the diagnosis and comparative studies of field cases of ochratoxicosis in the local environment. The present paper describes the toxico-pathological and serum biochemical effects of OTA in the White Leghorn breeder hens.

MATERIALS AND METHODS

Production of ochratoxin

Ochratoxin A was produced from *Aspergillus ochraceus* (CECT 2948, Culture Collection Center, University De Valencia, Spain) by culturing on wheat grain with a modified method of Trenk *et al*. (1971). Briefly, 80g wheat grains was soaked in 80 ml distilled water in a 1000 ml Erlenmeyer flask for 2 hours and autoclaved for 20 minutes at 121°C. An inoculum

prepared from 2 weeks old slant culture of *A. ochraceus* was mixed with the autoclaved wheat in the flask. The flask was incubated for 14 days at 28°C in dark and shaken once daily to break the mycelial growth. On day 15, the flask was autoclaved at 121°C for a while to destroy the mycelial growth. Ochratoxin A was extracted from the fermented wheat in acetonitrile-water and quantified by HPLC and fluorescent detection method (Bayman *et al*., 2002).

Birds and experimentation

Eighty four White Leghorn layer breeder hens of 40 weeks of age, free from salmonella and mycoplasma infections, were procured from a breeder farm. Birds were acclimatized for one week on basal layer feed having 16% crude protein and 2900 Cal/Kg energy. The basal ration used was checked to not to contain >1 ng/g OTA and aflatoxin B1. Birds were divided into seven groups (A-G) having 12 birds in each and offered OTA contaminated feed @ 0, 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg/Kg, respectively for 21 days. Afterwards, the birds of all groups were switched over to basal feed and experiment was terminated at 28th day.

Parameters studied

Clinical parameters

Feed intake of each group was daily determined, while body weight of birds was determined at the end of each week. Clinical signs of toxicosis were recorded on daily basis. A subjective evaluation of the clinical signs was performed based upon the absence, presence, extent and severity and each sign was assigned a score from 0 to 3. The score of a particular sign in each group was summed up at the end of trial.

Necropsy of the birds

At the end of toxin feeding (21st day), six birds from each group were humanely killed and gross lesions on different organs were recorded. Different organs including liver, kidneys, spleen and gizzard were removed and weighed. Prior to killing, blood was collected from the wing vein of each bird and allowed to clot for serum separation. Serum collected from each bird was used for determination of biochemical parameters.

Serum biochemical parameters

Serum samples collected from birds of each group at the end of the experiment were used to determine ALT, creatinine, urea, total proteins and albumin concentrations using commercially available kits (Diasys Diagnostic system GmbH, Germany). Serum globulin concentration was determined by subtracting serum albumin concentration from that of serum total proteins.

Production performance parameters

Egg mass production was calculated by adding up the mass (g) of total eggs per group per day. Feed conversion ratio (FCR) per gram of egg for different groups was calculated by using the following formula:

$$FCR = \{(\text{Feed (g)/hen per day}) \div (\text{Egg (g)/hen per day})\}$$

Statistical analysis

The data were analyzed by analysis of variance and different group means were compared by Duncan's multiple range test. A statistical software package "MSTATC" was used for statistical analysis. Cumulative scores for clinical signs were compared with control group on arithmetical difference basis.

RESULTS

Body weights of hens fed different levels of OTA are shown in Table. 1. The body weights differed non significantly among the groups in week 1 and 2, whereas in week 3 of experiment the body weights of groups D, E, F and G were significantly lower than that of control ($P \leq 0.05$).

Table 2 shows feed intake of hens fed different levels of OTA. In week 1 of the experiment, groups E, F and G had significantly lower feed intake whereas other groups were non significantly different from control. In week 2, groups F and G showed significantly lower values than control group. In the week 3, only group G had significantly lower feed intake than control, while all other groups were non significantly different.

In week 1, egg mass production of all groups, except B, was significantly lower than group A (Table 3). In weeks 2 and 3, all the groups had significantly lower values than that of group A and the decrease was significant with increase in dietary OTA levels.

Feed conversion ratios of the hens fed OTA contaminated feed for 21 days have been shown in Table 4. In week one of the toxin feeding, group F had significantly higher value than control, while all other groups had non significant difference. In week 2, groups E, F and G had significantly higher FCR value than control, while differences in all other groups were non significant. In week 3, the values of FCR of all treatment groups were significantly higher than that of control.

Table 5 shows scores of clinical signs of breeder hens fed OTA for 21 days. Hens in group A remained alert throughout the length of the experiment and responded well upon tapping the cages. Feeding of OTA to hens resulted in development of depression which became more severe with increase in the dietary level of toxin. Feeding of OTA resulted in decreased attraction to feed and this sign became more severe with increased dietary OTA concentration. Birds in the group G showed maximum interest in water than all other groups. The consistency of fecal material varied from semisolid to watery in groups A-G in the dietary concentration related manner. Group G showed sever watery diarrhea throughout the length of experiment. Feathers of hens of group A were shiny and well formed throughout the length of experiment, while the hens fed OTA resulted in the ruffled and broken feather in dose related manner.

Livers of the hens of group A were normal in size, color and consistency, while the hens fed OTA contaminated feed showed enlargement, pale discoloration (Fig. 1), friable consistency of liver and presence of hemorrhages on the surface. These alterations became more severe with increase in dietary OTA levels.

Similarly, kidneys of the hens of experimental groups were bulging out of sockets and hemorrhagic (Fig. 2). Severity of kidneys lesions increased in the dose related manner.

Table 6 shows relative organs weights of hens fed different levels of OTA. Values of relative weight of liver of groups E, F and G were significantly higher than groups A and B. Relative weight of kidneys of all treatment groups was non-significantly different from group A (control). Spleen relative weight differed non significantly among all groups. Gizzard relative weight increased significantly in all groups, except B, compared with the control group.

Serum biochemical parameters of hens fed different levels of OTA are shown in Table 7. Values of ALT in all groups were significantly higher than that of group A. Serum urea levels of groups E, F and G were significantly higher than those of groups A, B, C and D which in turn were non significantly different from each other. Serum creatinine concentrations of groups E, F and G were significantly higher than those of groups A, B and C, while group D was non significantly different from group A, B and C. Serum total proteins decreased significantly in groups F and G compared with control. Serum albumin levels of all the groups differed non significantly from each other but the values of globulin of groups D, F and G were significantly lower than control group.

Fig. 1: Photograph of liver of a hen fed OTA contaminated feed at 3 mg/Kg. Liver is large and light in color.

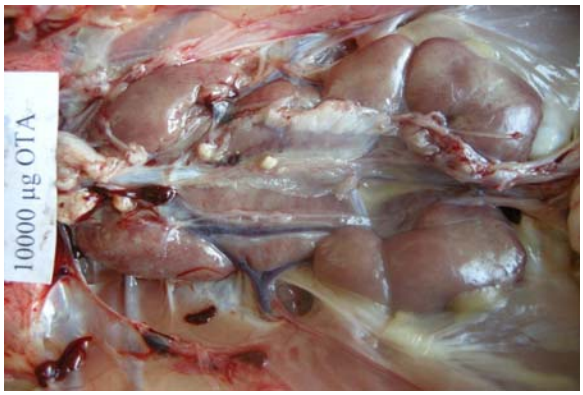

Fig. 2: Photograph of kidneys of a hen fed OTA @ 10 mg/Kg. Kidneys are swollen and bulging out of sockets.

DISCUSSION

Clinical signs of OTA toxicity observed in the present study comprising of dullness, diarrhea, increased water intake and ruffled feathers have also been reported by Hofacre *et al*. (1985). Increase in water intake and loose droppings as observed in the present study have also been reported by Chang *et al*. (1981) after feeding 4 and 8 mg/Kg OTA to broiler chicks. Paleness of comb and wattles were observed in the laying hens fed OTA contaminated feed (Sawale *et al*., 2009), however, no such signs were observed in the present study. Scoring of the clinical signs of different groups suggested an increase in the severity of clinical ochratoxicosis with increase in dietary OTA level. Decrease in the egg mass was recorded in OTA fed breeder hens. Similar findings have also been reported earlier (Niemec *et al*., 1994; Verma *et al.,* 2003).

In the present study, the weights of kidneys and liver of hens fed OTA were significantly increased. Denli *et al.* (2008) also recorded increase in liver weights of laying hens due to ochratoxicosis. Relative weight of spleen of hens differed non significantly among the groups in the present study, whereas a decrease in relative weight of spleen was reported in broiler chicks (Stoev *et al*., 2002). An increase in the weights of gizzards of hens fed different levels of OTA to layer hens in the present study has also been reported in broiler chicks (Elaroussi *et al*., 2006).

Liver of hens killed at the end of the experiment was enlarged, pale, friable and in some cases had pinpoint hemorrhages on the surface. Kidneys were swollen, bulging out of bony sockets and had hemorrhages on the surface. Similar lesions have been reported in laying hens by Sakhare *et al.* (2007) and Sawale *et al*. (2009) and also in broiler chicks by Kumar *et al*. (2004). Results of the present study were suggestive of an increase in severity of the lesions with increase in dietary concentration of OTA.

Serum enzymes concentration index depicts the function of the vital organs. OTA administrations to layer breeder hens also affected the concentration of different serum biochemical parameters. Concentrations of ALT, creatinine and urea were significantly higher in the groups fed OTA. Feeding of OTA increased the serum levels of ALT (Sawale *et al*., 2009), urea (Huff *et al*., 1988; Stoev *et al*., 2002) and creatinine (Huff *et al*., 1988; Bailey *et al*., 1989) in the White Leghorn and broiler chicks. Serum levels of total proteins and albumin were significantly lower in the groups fed OTA. Similar findings were also reported in feeding experiments of OTA (Huff *et al*., 1988; Singh *et al*., 1990).

In conclusion, the present study described clinical picture and pathological alterations in OTA fed White Leghorn breeder hens. Severity of clinical signs, gross pathological alterations and serum biochemical changes suggested an increase in severity of the ochratoxicosis with increase in dietary OTA levels.

Table 1: Body weights (g) of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

Group (OTA mg/Kg)	Weeks		
	1	2	3
A (0)	1623 ± 138.39	1656 ± 83.00	1680 ± 94.24a
B (0.1)	1607 ± 66.96	1664 ± 135.78	1649 ± 135.55ab
C (0.5)	1624 ± 49.66	1620 ± 93.98	1588 ± 74.93abc
D (1.0)	1621 ± 76.25	1601 ± 99.82	1542 ± 118.47bcd
E (3.0)	1613 ± 89.27	1579 ± 122.53	1554 ± 130.14bcd
F (5.0)	1563 ± 110.80	1548 ± 158.08	1508 ± 163.48cd
G (10.0)	1532 ± 189.32	1520 ± 215.19	1437 ± 163.15d

Values in each column followed by different letters are significantly different (P≤0.05).

Table 2: Feed intake (g) of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

Group (OTA mg/Kg)	Weeks		
	1	2	3
A (0)	115.57 ± 10.05a	112.66 ± 4.41a	110.00 ± 0.00a
B (0.1)	120.12 ± 20.33a	113.31 ± 11.19a	109.11 ± 2.34a
C (0.5)	106.76 ± 13.59ab	110.65 ± 24.10ab	110.00 ± 0.00a
D (1.0)	104.63 ± 11.33ab	103.92 ± 6.49ab	107.65 ± 1.52a
E (3.0)	89.89 ± 6.00bc	101.27 ± 8.49abc	106.09 ± 4.64a
F (5.0)	79.28 ± 7.99c	98.60 ± 3.79bc	104.88 ± 4.81a
G (10.0)	82.96 ± 28.46c	90.55 ± 6.11c	94.16 ± 19.69b

Values in each column followed by different letters are significantly different (P≤0.05).

Table 3: Egg mass production (g) of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

Group (OTA mg/Kg)	Weeks		
	1	2	3
A (0)	581.96 ± 42.63a	681.14 ± 74.12a	714.86 ± 164.11a
B (0.1)	521.69 ± 70.06ab	574.71 ± 41.40b	583.29 ± 42.50b
C (0.5)	488.00 ± 88.95b	574.54 ± 67.00b	528.00 ± 148.06bc
D (1.0)	511.28 ± 32.22b	535.57 ± 126.86b	437.86 ± 32.56cd
E (3.0)	381.38 ± 75.10c	386.57 ± 67.60c	348.57 ± 64.68de
F (5.0)	288.08 ± 50.56d	312.57 ± 71.01c	343.00 ± 87.84de
G (10.0)	309.02 ± 53.14d	327.35 ± 97.97c	230.77 ± 124.28e

Values in each column followed by different letters are significantly different (P≤0.05).

Table 4: FCR of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

Group (OTA mg/Kg)	Weeks		
	1	2	3
A (0)	2.398 ± 0.32b	2.008 ± 0.25b	1.949 ± 0.52d
B (0.1)	2.842 ± 0.79ab	2.379 ± 0.32b	2.257 ± 0.19c
C (0.5)	2.703 ± 0.58ab	2.310 ± 0.44b	2.681 ± 0.79c
D (1.0)	2.470 ± 0.35b	2.426 ± 0.49b	2.966 ± 0.25bc
E (3.0)	2.917 ± 0.54ab	3.208 ± 0.49a	3.757 ± 0.68b
F (5.0)	3.414 ± 0.79a	3.946 ± 0.84a	3.894 ± 1.07b
G (10.0)	3.250 ± 1.18ab	3.605 ± 1.23a	5.993 ± 2.69a

Values in each column followed by different letters are significantly different (P≤0.05).

Table 5: Score of clinical signs of breeder hens fed different levels of OTA

Clinical sign and behavior	Score range	Groups (OTA mg/Kg feed)						
		A(0)	B(0.1)	C(0.5)	D(1.0)	E(3.0)	F(5.0)	G(10.0)
Alertness Normal – depressed	0-3	0	7	10	14	27	36	47
Attraction to feed Normal – less interest	0-3	0	9	13	13	21	32	43
Attraction to water Normal – more interest	0-3	0	4	12	15	26	46	52
Feces consistency Normal Formed – watery	0-3	0	0	5	35	29	45	56
Feather Normal Shiny – ruffled & Broken	0-3	0	0	0	7	13	21	33
Cumulative score		**0**	**20**	**40**	**84**	**116**	**180**	**231**

Table 6: Relative organ weights (% of the body weights) of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

OTA mg/Kg	A(0)	B(0.1)	C(0.5)	D(1.0)	E(3.0)	F(5.0)	G(10.0)
Liver	2.77 ± 0.51b	2.74 ± 0.23b	3.14 ± 0.20ab	3.28 ± 0.30ab	3.42 ± 0.45a	3.74 ± 0.63a	3.72 ± 0.29a
Kidneys	0.91± 0.08ab	0.85 ± 0.08b	0.94 ± 0.06ab	1.02 ± 0.08ab	1.05 ± 0.18ab	1.05 ± 0.16ab	1.11 ± 0.21a
Spleen	0.08 ± 0.03	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.10 ± 0.02	0.10 ± 0.01	0.08 ± 0.02
Gizzard	2.33 ± 0.31d	2.65 ± 0.26cd	2.82 ± 0.07bc	3.13 ± 0.18ab	3.09 ± 0.33ab	3.00 ± 0.31abc	3.35 ± 0.30a

Values in each row followed by different letters are significantly different (P≤0.05).

Table 7: Serum biochemical parameters of White Leghorn breeder hens fed different levels of OTA for 3 weeks (mean ± SD)

Group (mg/Kg)	ALT (U/l)	Urea (mg/dl)	Creatinine (µmol/l)	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)
A (0)	25.0 ± 5.00d	13.67 ± 1.53b	26.92 ± 3.57c	4.30 ± 0.30a	3.12 ± 0.13	1.18 ± 0.18a
B (0.1)	33.6 ± 2.08c	15.67 ± 1.53b	30.33 ± 5.51c	4.17 ± 0.07a	3.25 ± 0.25	0.91 ± 0.19ab
C (0.5)	37.6 ± 1.53bc	18.67 ± 3.06b	34.00 ± 5.29c	4.11 ± 0.14a	3.14 ± 0.12	0.97 ± 0.14ab
D (1.0)	37.6 ± 1.53bc	17.67 ± 5.51b	40.23 ± 4.69bc	3.85 ± 0.36ab	3.11 ± 0.10	0.74 ± 0.30bc
E (3.0)	39.6 ± 2.08abc	27.33 ± 3.06a	53.64 ± 5.47b	4.11 ± 0.10a	3.17 ± 0.08	0.94 ± 0.10ab
F (5.0)	46.6 ± 7.64a	30.33 ± 5.51a	77.32 ± 19.0a	3.42 ± 0.61b	2.94 ± 0.48	0.48 ± 0.19c
G (10.0)	43.3 ± 2.08ab	34.67 ± 5.13a	91.27 ± 9.24a	3.26 ± 0.02b	2.86 ± 0.15	0.40 ± 0.14c

Values in each column followed by different letters are significantly different (P≤0.05).

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