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RESEARCH ARTICLE

Ochratoxin Induced Pathological Alterations in Broiler Chicks: Effect of Dose and Duration

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ABSTRACT

The present study was designed to evaluate the toxicopathological effects of feeding of ochratoxin contaminated feeds to broiler chicks for 21 and 35 days. Two experiments were conducted simultaneously. In these experiment six groups each having 75 chicks were maintained and offered feeds containing 0, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg OTA. Half of the birds from each group of both experiments were killed on days 21 and 35, respectively. Remaining birds of all the groups were switched to basal feed and killed on day 42 of the experiment. Birds killed in both experiments showed a significant decrease in the feed intake and body weight in OTA fed groups. OTA associated clinical signs and behavioral alterations included diarrhea, depression, increased water intake and ruffled feathers. The highest mortality was 12 and 20 percent observed in birds fed 0.4 and 0.8 mg/kg OTA, respectively. OTA fed birds showed a significant increase in the relative weights of liver and kidneys while decrease in weight of bursa of Fabricius and thymus. Gross lesions in liver and kidneys included enlargement, paler discoloration, friable consistency and hemorrhages. Microscopic changes in the kidneys included congestion and tubular epithelial cell necrosis. Liver showed vacuolar degeneration along with individual cell necrosis in birds fed 0.2-0.8 mg/kg OTA. Birds killed on day 35 of the intoxication showed changes similar to those observed in 21 days old birds with the exception of increased severity of these alterations in 0.4 and 0.8 mg/kg OTA groups. In conclusion, present study suggested that OTA induced pathological alterations were dependent upon dose and duration of exposure.

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INTRODUCTION

Mycotoxins are the secondary fungal metabolites produced by toxigenic fungi (Ahmad *et al*., 2012; Saleem *et al*., 2012; Söyler *et al*., 2012; Sultana *et al*., 2013). The toxicity of mycotoxins is dependent on the nature, dose, duration and route of exposure (Fink-Gremmels, 1999; Jubeen *et al*., 2012). Ochratoxins mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* are divided into three distinct types namely ochratoxin A (OTA), ochratoxin B (OTB) and ochratoxin C (OTC). OTA is the most toxic and common contaminant of cereals, cereals based food and feeds (Xiao *et al*., 1996; Li *et al*., 1997). OTA is also characterized as class 2-B carcinogen (Anonymous, 1993). Ochratoxin A is known to have nephrotoxic, hepatotoxic and immunotoxic effects along with growth suppressive activities in poultry and animals (Kumar *et al.*, 2003; Hassan *et al.*, 2011; Hassan *et al.*, 2012b). Presence of OTA in the poultry feeds have been reported throughout the world (Dalcero *et al.*, 1998) and Pakistan (Hanif *et al.*, 2006). The data about the toxicological effects of OTA in broilers has been reported using 0.5-8.0 mg/kg OTA in feeds (Elaroussi *et al.*, 2006; Hanif *et al.*, 2008), while a meager information is available about ochratoxicosis using low levels. Keeping in view the persistent presence of OTA at low levels in poultry feeds, the present experimental study was designed to investigate the pathological effects of 0.05-0.8 mg/kg OTA fed for 21 and 35 days to broiler chicks.

MATERIALS AND METHODS

Preparation of OTA contaminated feed: Lyophilized spores of OTA producing fungus *Aspergillus ochraceus* (CECT 2948) were used for the production of OTA on broken wheat grains according to the modified method of Trenk *et al.* (1971) as described by Hassan *et al.* (2010; 2012a). OTA produced on fermented wheat grains was

extracted and quantified by HPLC using fluorescence detection method. Basal feed was a corn and soy meal based feed having 21% total proteins and 3000 Kcal/kg metabolizable energy. It was ensured that the levels of OTA and aflatoxin were less than 1.0 µg/kg. For preparation of experimental feeds, the basal feed was amended with fermented wheat to provide desired OTA concentrations in different experimental feeds. Prior to feeding, each experimental feed was subjected to HPLC analysis to ensure the OTA level.

Experimental induction of ochratoxicosis in broiler birds: A total of 900, one day old broiler chicks were randomly divided into 12 equal groups used for two experiments conducted simultaneously. In both experiments six groups were maintained and offered feed containing 0, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg OTA for 21 and 35 days, respectively from day one of age. In first experiment different groups were offered experimental feeds for up to 21 days and then switched to ochratoxin free basal feed for up to 42 days. Similarly in second experiment, chicks were offered experimental feeds for 35 days and then switched over to basal feed for up to 42 days of the experiment. All the birds of both experiments were killed on day 42 and samples were collected.

Parameters studied

Clinical parameters: Clinical signs and behavioral alterations including, alertness, attraction to feed and water, consistency of feces and roughness/dullness of feathers. Each of the parameter was subjectively assigned the score from 0-5 according to its absence (0), presence and severity (1-5). All parameters of clinical signs were observed twice a daily and means of these two observations for each parameter were computed on daily basis representing the daily score of each parameter. These daily scores for each group were summed up at the end of experiment to obtain the cumulative score of each group. Feed intake of the birds and mortality of each group was recorded on daily basis and body weight on weekly basis.

Necropsy examination of the birds: In experiment 1, six birds randomly selected from each group were weighed and necropsied on days 5, 14, 21, 28, 31 and 42. In experiment 2, six birds were necropsied on days 5, 14, 21, 28, 31, 37 and 39. Each necropsied bird was examined for presence of gross lesions on different visceral organs. Absolute weights of different organs were recorded and relative organ weights (percent of the body weight) were calculated. Tissues of different organs were fixed in 10% neutral buffered formalin for histopathological examination.

Statistical analysis: The data obtained from the experiment were subjected to the analysis of variance test and the means of different treatment groups were compared by Duncan's Multiple Range test using MSTATC statistical software package. Cumulative score of clinical signs and gross lesions were compared by percent difference and confidence interval. The level of significance was $P \leq 0.05$.

RESULTS

Clinical parameters: Feed consumption (g/bird/day) of broiler chicks fed different dietary levels of OTA (Fig. 1) showed a significant decrease from week 3 in 0.2 mg/kg OTA and higher dose groups as compared with control group. In birds fed OTA for 21 days, the decrease in the feed consumption became non significant from control at day 42 of the experiment while it remained significantly lower in the birds fed OTA for 35 days. Cumulative feed intake (Table 1) after 21 and 35 days feeding of OTA decreased significantly with increased dietary OTA levels and duration of OTA feeding.

No clinical signs were observed in birds fed 0.05 mg/kg OTA. While mild changes were observed in 0.1 and 2.0 mg/kg OTA groups throughout the duration of experiment. Birds of 0.4 and 0.8 mg/kg OTA groups showed mild degree of depression, decreased interest in feed intake, soft watery feces and dull feathers (loss of shining) from day 16 of the experiment. These signs became more apparent on day 21 of the experiment and then decreased gradually after withdrawal of OTA from feed. In birds receiving OTA for 35 days (Fig. 2), severity of signs increased gradually and was more pronounced on day 35 and then subsided gradually. A mild decrease in the attraction to wards feed and more attraction to water was present in the birds fed 0.05 mg/kg OTA for 35 days while no such changes were observed in those fed for 21 days. The percent comparison of cumulative scores of clinical sign after 21 and 35 days of OTA feeding suggested an increase in score with increase in the duration of OTA feeding in all groups. Progression in clinical signs was observed in a dose related manners and its severity also increased with increase in duration of OTA dose and exposure.

The mortality of chicks of different groups kept on OTA contaminated feeds was 12 and 20% in groups given 0.8 mg/kg OTA for 21 and 35 days, respectively. Body weights of the broiler chicks decreased in dose related manner after 21 days of OTA feeding. A more severe decrease was observed after 35 days of feeding (Fig. 3) during week 4 and 5 in 0.8 mg/kg OTA followed by 0.4 mg/kg OTA group compared with control. The decrease in the body weights occurred in a dose related manner and was significantly lower in all groups. The comparison of percent decrease in body weight gain showed that decrease was more severe in birds kept on OTA contaminated feed for longer duration (Table 1).

Relative organ weights: The relative weight of liver and kidneys significantly increased in a dose related manner in groups receiving 0.2 mg/kg OTA or higher levels in feed. The relative weight of bursa of Fabricius and thymus in 0.2-0.8 mg/kg OTA groups was significantly lower than that of control. This decrease occurred in a dose and duration related manner. On day 42, the relative organ weights exhibited non significant differences from control group in experiment where birds received OTA for 21 days while the differences were significant in those kept on OTA contaminated diets for 35 days.

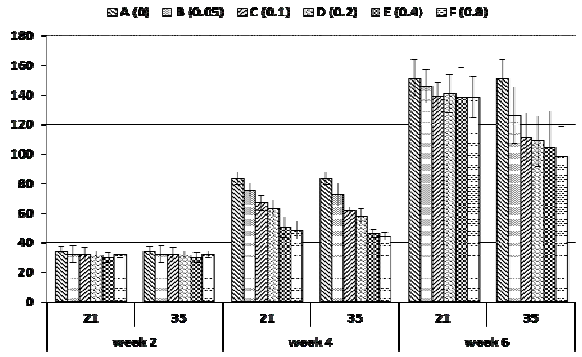

Fig. 1: Feed consumption (g/bird/day) of broilers fed OTA for duration of 21 and 35 days from day old birds (Mean±SD).

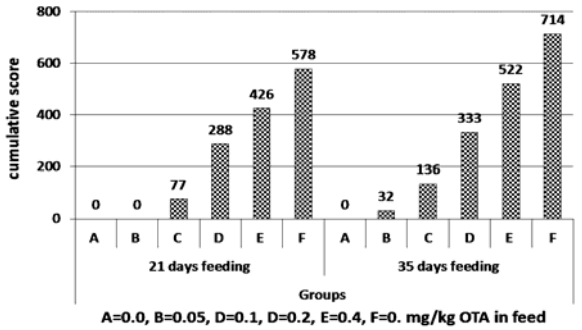

Fig. 2: Cumulative score of behavioral changes and clinical signs in broilers fed different levels of dietary OTA for 21 and 35 days from day 1 of age.

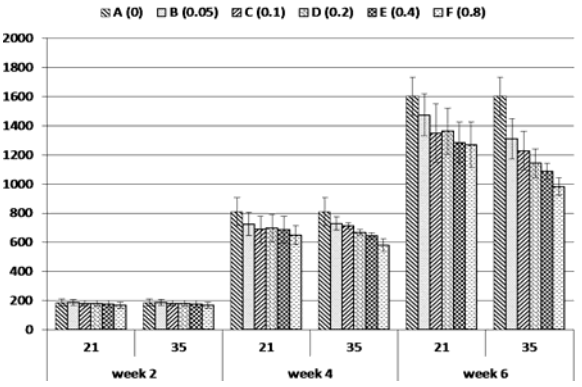

Fig. 3: Body weight (g) of broiler chicks given different levels of dietary OTA for 21 and 35 days from one day of age (Mean±SD).

Gross lesions: Gross lesions in visceral organs of OTA fed birds from different groups killed on day 21 and 35 days included hemorrhages on the subcutaneous tissue and muscles. Liver showed enlargement, pale discoloration, hemorrhages on the surface and friable consistency (Fig. 4). Kidneys of OTA fed groups were hemorrhagic and bulged out of sockets. Lesions were more intense in chicks fed 0.8 mg/kg OTA than other groups (Fig. 5). No significant changes could be observed in gross morphology of spleen, bursa of Fabricius and thymus of broiler chicks from different groups. All the changes increased in severity with increase in OTA dose related manner. Gross lesions observed in birds fed OTA contaminated feeds for 35 days were same as observed in those fed OTA for 21 days, however, the intensity and severity of the lesions was increased (Table 2).

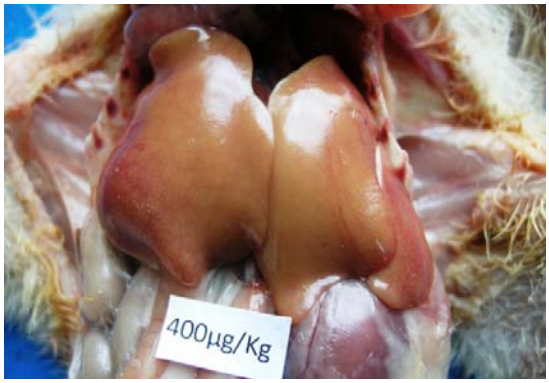

Fig. 4: A photograph of liver from broilers fed 0.4 mg/kg OTA for 21 days from one day of age. Liver is pale, enlarged and hemorrhagic.

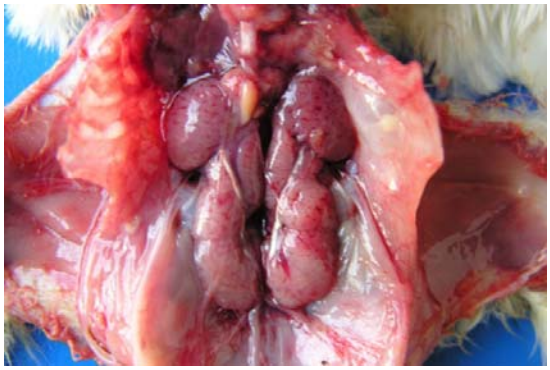

Fig. 5: A photograph of kidneys from broilers fed 0.8 mg/kg OTA for 21 days from one day of age. Kidneys are swollen, enlarged and bulging out from sockets.

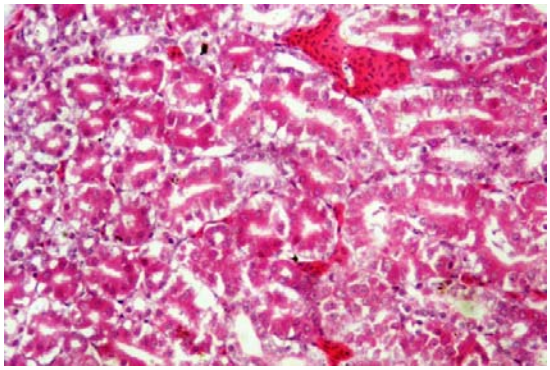

Fig. 6: A Microphotomicrograph of kidney from broilers fed OTA 0.4 mg/kg OTA contaminated for 21 days from one day of age. Degenerative changes along with mild congestion in the tubular epithelial cells (H and E Satin, 200X).

Histopathological findings: Histologically hepatocytes from birds given 0.4 and 0.8 mg/kg OTA showed moderate degree of vacuolar degeneration and congestion of the hepatic parenchyma. Sinusoidal spaces were not obliterated and appeared normal. Changes of less severe degree were observed in 0.2 mg/kg OTA group but no discernible in 0.1 and 0.05 mg/kg OTA groups. Microscopic changes in kidneys of 0.4 and 0.8 mg/kg OTA groups included moderate degree of congestion in the renal parenchyma and necrotic areas of tubular epithelial cells indicated by pyknotic and karyorrhectic nuclei at some places (Fig. 6). Mild degree of changes was observed in 0.2 mg/kg OTA group. A moderate to severe degree of necrotic changes were

Table 1: A comparative summary of the results indicating percent (%) variation of cumulative clinical parameters and relative organ weights compared with control after 21 and 35 days feeding of OTA

Parameters	Duration of OTA feeding (Days)	Groups (OTA mg/kg feed)					
		Group A (0)	Group B (0.05)	Group C (0.1)	Group D (0.2)	Group E (0.4)	Group F (0.8)
Feed intake	21	0	-5.54	-11.15	-13.79	-21.27	-23.27
	35	0	-13.51	-23.12	-27.39	-35.15	-36.62
Mortality	21	0	1.33	4.00	4.00	6.67	12.00
	35	0	2.67	4.00	6.67	10.67	20.00
Average body weight	21	0	-6.02	-11.45	-11.90	-14.70	-18.44
	35	0	-10.1	-14.56	-18.67	-22.40	-29.95
Clinical signs	21	0	0	7.33	27.43	40.57	55.04
	35	0	3.05	12.95	31.71	49.71	68.00
Gross lesions	21	0	14.12	22.69	13.66	17.28	31.17
	35	0	15.51	18.67	21.68	28.55	39.97
RW of liver	21	0	6.16	4.54	20.62	25.84	28
	35	0	4.02	10.04	22.09	34.14	35.74
RW of kidney	21	0	3.57	17.86	30.95	21.31	94.05
	35	0	31.67	70	73.33	66.67	108.33
RW of bursa of Fabricius	21	0	-4	-16	-48	-40	-36
	35	0	3.85	-38.46	-42.31	-46.15	-26.92
RW of thymus	21	0	-23.91	-15.22	-28.26	-56.52	-50
	35	0	-20.93	-6.98	-9.30	-44.19	-51.16

RW=Relative weight; (-) values indicates percent decrease from control while (+) indicates increase.

Table 2: Cumulative scores of gross lesions in different organs of broilers fed different levels of dietary OTA for 21 and 35 days from day one of age.

Organ/ Tissue	Groups										
	21 days feeding						35 days feeding				
	A	B	C	D	E	F	B	C	D	E	F
Subcutis Fat	0	8	8	13	19	30	10	14	21	39	39
Muscle	0	5	5	7	16	29	8	10	12	26	45
Liver	0	82	94	117	144	195	108	123	145	178	243
Kidneys	0	66	66	87	104	142	74	88	96	123	170
Bursa	0	3	0	0	8	9	0	3	5	8	11
Spleen	0	1	1	0	0	0	1	1	0	2	3
Thymus	0	0	3	0	3	0	0	3	3	4	7
Cumulative	0	165	177	224	294	404	201	242	281	370	518

observed in the bursa of Fabricius and thymus in birds fed 0.2 and 0.8 mg/kg OTA. Decreased thickness of cortex was observed in bursal follicles. The thickness of interfollicular connective tissue was increased as compared to control. No prominent alterations in the hepatic and renal parenchyma were observed in broiler chicks fed 0.05 and 0.1 mg/kg OTA for 21 days. Microscopic alterations in organs of different groups of broiler chicks fed 0.05-0.8 mg/kg OTA for 35 days were similar to those described for the chicks fed similar OTA levels for 21 days with exception of more severe degree of alterations in 0.4 and 0.8 mg/kg OTA groups.

DISCUSSION

Ochratoxin A and OTA producing fungi are known contaminants of food and feeds persistently reported in the poultry feed and feed ingredients throughout the world (Saleemi *et al.,* 2010; 2012). Many scientists described the potential toxico-pathological effects in poultry using 0.5mg/kg or higher levels of OTA. But present study focused on pathological effects of OTA in broiler chicken after feeding 0.05-0.8 mg/kg OTA. A significant reduction in the feed intake was observed in broiler chicks after 21 and 35 days feeding of OTA in all groups. Similar dose related decrease in feed consumption has been reported (Kumar *et al.,* 2003) but no information is available in the literature describing the decreased feed consumption associated with low OTA levels (0.05 and 0.10 mg/kg). This decreased feed intake also contributed in the decreased body weight along with other toxicological interventions in all OTA groups. Ochratoxin associated decrease in the body weight was reported by different workers (Elaroussi *et al.*, 2006; Hanif *et al.*, 2008) but present study showed a more severe decrease in body weight indicating that OTA induced decrease in body weight was not only related to the dose but also depends upon the duration of OTA feeding.

OTA associated clinical signs were in accordance with those described in layer chicken (Hassan *et al.,* 2012a). A subjective comparison of cumulative score of different groups suggested that clinical signs were directly related with dietary OTA levels and duration of exposure. No report by other authors in the accessible literature described the comparison of clinical signs of ochratoxicosis in broilers. Mortality pattern of OTA fed birds was similar to that reported by Kumar *et al.* (2003) and Elaroussi *et al.* (2006).

Increased relative weights of liver and kidneys were observed at lower dietary OTA levels compared with those reported earlier (Elaroussi *et al.,* 2008) by using high dietary OTA levels suggesting that these changes might be directly associated with route of elimination of OTA resulting in the more toxic effects and accumulation of OTA in these organs. While decrease in the relative weight of thymus and bursa could be because of the necrotic and degenerative changes in these organs ultimately resulting in the lower immune responses as described earlier (Stoev *et al.*, 2000). Gross enlargement of liver and kidney were in accordance with previous reports (Kumar *et al.*, 2004; Elaroussi *et al.*, 2008). Similar findings have been reported in layer chicks hatched from OTA inoculated eggs (Hassan *et al.,* 2012b).

No report about hemorrhages on breast and subcutaneous tissues is available in the literature. However, Ayed *et al.* (1991) reported hemorrhages on thigh muscles after feeding of 0.5 mg/kg OTA for 4 weeks. A subjective comparison of gross lesions suggested an increase in the severity with increase in the duration of exposure.

Microscopic alterations observed in liver and kidneys has been similar to those reported earlier (Koynarski *et al.*, 2007; Hanif *et al.*, 2008; Milićević *et al.*, 2011). However, Elaroussi *et al.* (2006) reported no vacuolar degeneration of hepatocytes following feeding of 0.4 and 0.8 mg/kg OTA to broiler birds. A comparison of histological lesion in birds fed OTA for 21 and 35 day suggested more severe changes associated with increase in dietary levels and duration of exposure. The necrotic and degenerative changes in lymphoid organs (Bursa of Fabricius and Thymus) were similar as described earlier (Stoev *et al.*, 2002; Elaroussi *et al.*, 2006; Hanif *et al.*, 2008). OTA induced damage to liver and kidneys might be attributed towards decreased feed consumption and body weight gain resulting in poor performance of broiler chicks contributing towards economic loss (Hanif *et al.*, 2008).

Conclusion: Present study demonstrated significant clinical and pathological changes in broiler chicks fed 0.05-0.8 mg/kg OTA for 21 and 35 days. The severity of the clinical and pathological alterations was related with dietary OTA levels and duration of exposure.

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