

## Journal of the Hellenic Veterinary Medical Society

Vol 74, No 3 (2023)



**Effects of ochratoxin on the performance, haematobiochemical profile, macroscopic and histopathological lesions in quails (*Coturnix coturnix Japonica*)**

*F Ahmad, H Khan, K Khan, FA Khan, N Ahmad, M Saeed, T Ayasan*

doi: [10.12681/jhvms.30561](https://doi.org/10.12681/jhvms.30561)

Copyright © 2023, F Ahmad, H Khan, K Khan, FA Khan, N Ahmad, M Saeed, T Ayasan



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

### To cite this article:

Ahmad, F., Khan, H., Khan, K., Khan, F., Ahmad, N., Saeed, M., & Ayasan, T. (2023). Effects of ochratoxin on the performance, haematobiochemical profile, macroscopic and histopathological lesions in quails (*Coturnix coturnix Japonica*): Pathological effects of ochratoxin on the performance and haematobiochemical profile of quails (*Coturnix coturnix Japonica*). *Journal of the Hellenic Veterinary Medical Society*, 74(3), 5953–5960.  
<https://doi.org/10.12681/jhvms.30561>

## Effects of ochratoxin on the performance, haematobiochemical profile, macroscopic and histopathological lesions in quails (*Coturnix coturnix Japonica*)

F. Ahmad<sup>1</sup>, H. Khan<sup>2</sup>, K. Khan<sup>3,\*</sup>, F.A. Khan<sup>4</sup>, N. Ahmad<sup>5</sup>, M. Saeed<sup>6</sup>, T. Ayasan<sup>7</sup>

<sup>1,2,4,6</sup> College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

<sup>1</sup> Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, USA

<sup>3</sup> Department of Animal Sciences, Shaheed Benazir Bhutto University Sheringal, Pakistan

<sup>5</sup> Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

<sup>7</sup> Osmaniye Korkut Ata University, Kadirli Faculty of Applied Sciences, Osmaniye, Turkey

**ABSTRACT:** Mycotoxins are ubiquitous in the environment and occur naturally in human food and animal feed. Therefore, in this study the performance, haematobiochemical profiles, macroscopic and histopathological lesions in quails caused by ochratoxin (OTA) were examined. The OTA was obtained by culturing the spore of *Aspergillus ochraceus*. Sixty healthy male quails were selected and distributed into 12 replicates (5 quails per replicate) in a completely randomized block arrangement. Each experimental diet was assigned to 4 replicate groups. Quails in first group were fed on standard basal diet (CP 27%) without the addition of OTA (OTA-0; negative control), while in other groups OTA was incorporated in basal diet at 1 mg/kg (OTA-1) and 2 mg/kg (OTA-2) of basal diet, respectively. The feeding trial continued for 21 days experimental period. Clinical signs observed in OTA-fed birds were diarrhea, broken feathers, increased water intake, and depression. In addition, lower ( $p < 0.05$ ) feed intake was reported in OTA-fed quails. Likewise, weight gain was reduced ( $p < 0.05$ ) in OTA-fed groups. Gross lesions of hypertrophy, hemorrhages, paleness and friability were detected in the liver and kidney of OTA-treated birds. Microscopic examination of kidney and liver showed degeneration and sloughing of tubular epithelium in the kidney, narrowing of the lumen of kidney tubules, and hepatic fatty infiltration and necrosis of liver parenchyma. The serum Alanine transaminase, Aspartate transaminase, urea, and creatinine levels of OTA-fed birds were higher ( $p < 0.05$ ) than OTA-0. However, serum total protein and albumin were lower ( $p < 0.05$ ) in OTA-treated groups in a dose- dependent manner. Likely, red blood cell count, packed cell volume, and hemoglobin concentration were lower ( $p < 0.05$ ) in OTA-treated groups. The results of this study indicate that OTA at  $\geq 1$  mg/kg feed is nephrotoxic and hepatotoxic, and cause hematobiochemical disorder in quails, which adversely affect their growth performance and may eventually lead to economic losses.

**Keywords:** Ochratoxin, Quails, Hematology, Serology, Histopathology

*Corresponding Author:*

Kamran Khan, Department of Animal Sciences, Shaheed Benazir Bhutto University Sheringal, Pakistan  
E-mail address: dr.kamran@sbbu.edu.pk

*Date of initial submission: 15-06-2022*  
*Date of acceptance: 21-12-2022*

## INTRODUCTION

Several species of fungi produce mycotoxins that can contaminate food and feed at considerable levels in different parts of the world (Eskola et al. 2019). It is known for a long time that mycotoxin in foodstuff has adverse effects on human and animal health leading to huge economic losses (Wu and Mitchell 2016; Pitt and Miller 2017). In addition, it also impedes international trading and diverts resources towards research to overcome the mycotoxin problems (Stoev, 2013). Currently, over 300 mycotoxins are recognized and reported, however, the most important, frequent and problematic are the *Aspergillus*, *Penicillium* and *Fusarium* (Marin et al. 2013). Globally, about 25% foods have been contaminated with mycotoxins annually leading to significant burden to agriculture (Mitchell et al. 2016; Ostry et al. 2017).

Continuous long-term exposure to mycotoxin-contaminated feed may cause serious endocrinological and developmental disorders, malignant tumors, and other diseases in animals ((Frazzoli et al., 2017; Peles et al. 2019). The most frequently occurring mycotoxin in nature that can contaminate food and feed are zearalenone, ochratoxin, T-2 toxin, aflatoxin, fumonisin, and deoxynivalenone (Eskola et al. 2019). Therefore, research is flourishing to develop fungal-resistant plant species to protect them from mycotoxin contamination.

Ochratoxin is particularly important because of carcinogenic, neurotoxic, hepatotoxic, immunosuppressive effect, genotoxic and hemotoxic biological disorders (Shipton, 2014; Cetin, 2020). The major obstacle to the growth of the poultry industry is the immune suppressive effects of mycotoxins (Zaghini et al. 2007). Notably, ochratoxin A (OTA) contamination of cereal grains, corn, and oilseeds affects animal performance through damage to kidneys (Richard et al. 2003). Humans are primarily exposed to OTA by ingesting of contaminated cereals and animal meat (Duarte et al., 2010). The maximum permitted level of OTA intake established by the European Union is 5 ng/g in raw cereal and 3 ng/g in processed cereals (Commission Regulation EC, 2005).

Importantly, OTA has primarily nephrotoxic effect due to its strong affinity towards ATP-dependent transporters being present in the luminal membrane of kidney (Schricks et al. 2006), and also its prolonged half-life (4.1 h) in poultry (Galtier et al. 1981). The OTA has a multiple range of toxico-pathological effect in different types of poultry birds. The detrimental

effect in term of LD50 are 0.5, 2.14, 3.4, 4.63, and 16.5 mg/kg body weight for duckling, broiler chicks, white leghorn chicks, Turkey chicks and Japanese quail chicks respectively (Patil et al. 2014). Kumar et al. (2004) reported that OTA has structure similarity with phenylalanine and has more nephrotoxic effect than hepatotoxic effects in poultry birds at same dose rate of OTA.

Keeping in view the impact of OTA on public health perspective it is imperative to develop control strategy of OTA contamination in the whole feed and food chain. Therefore, the present study is designed to evaluate the effect of different doses of OTA (1 and 2 mg/kg basal diet) on performance, haematobiochemical profile, and histopathological lesion of quails, in order to address the problem for the future control strategy of mycotoxicosis in poultry industry.

## MATERIAL AND METHOD

### *Ethical consideration*

This research study was approved in the Departmental Board of Studies meeting of The University of Agriculture, Peshawar on the recommendation of its ethical committee (January, 2020).

### *Ochratoxin description and quantification*

The spores of *Aspergillus ochraceus* (link: Fries. A (CECT 2948) used in this study were obtained from the University of Agriculture, Faisalabad (UAF). Spores were cultured on slants of Potato Dextrose Agar (PDA), and then incubated at 37 °C for two weeks. The inoculums were prepared by adding 3 ml of sterile distilled water containing 0.0001 ml of Triton X-100. Ochratoxin was produced according to the standard technique developed by Trenk et al. (1971). For the quantification of OTA a high performance liquid chromatographic (HPLC) technique was used as described by Bayman et al. (2002).

### *Source, husbandry, and management of quails*

A total of 150 quails (*Coturnix coturnix Japonica*), day-old were procured from the hatchery. After incubation, 60 healthy male quails were selected and were grouped into 12 replicates groups (5 birds/ replicate) and each experimental diet was assigned to 4 replicate groups. The experimental layout is presented in Table 1. Quails of each replicate group was placed in separate cage, which was kept in the same shed having 1 square feet space and wood shavings were used as litter. Quails in first group were fed on standard

**Table 1.** Experimental layout of the study

Total no. of quails	Groups*											
	OTA-0				OTA-1				OTA-2			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
60	5	5	5	5	5	5	5	5	5	5	5	5

\*All quails received basal diet

In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 groups basal diet was supplemented with ochratoxin at 1 mg and 2 mg per kg of basal diet

basal diet without the addition of OTA (OTA-0; negative control). In the testing groups, OTA was added to the basal diet at 1 mg/kg (OTA-1) and 2 mg/kg (OTA-2), respectively. The feeding trial continued for 21 days after one week of acclimatization period to the diets. The basal diet was formulated according to the recommended nutrient requirements (CP 27%) of birds according to NRC (1994). The ingredients composition of basal diet comprises of maize (57.7%), SBM (36.3%), Di calcium phosphate (1.65%), NaCl (0.57%), DL Methionine (0.16%), Anticoccidial (0.04%), mixture of vitamin (0.25%) and mineral (0.15%).

The experimental diet to each group was given *ad-libitum* and had free access to clean drinking water all the time. Quails were vaccinated against ND, IB and IBD per approved schedule.

### **Clinical signs and growth performance**

Clinical signs like thriftiness, anorexia, and changes in normal behavior were noted throughout the trial. On daily basis, the feed intake by the quails was recorded for each experimental group. The amount of feed intake (g) per bird per day was calculated by the total amount of feed intake (g) per group divided by the quantity of birds in a group. The body weight gain of quails per group was determined on weekly basis. Likewise, feed conversion ratio of the birds was calculated on weekly basis. The total amount of weekly feed intake (g) was divided by the total body weight gain (g) in a week.

### **Collection of blood**

Before euthanasia (after three weeks of feeding trial), 3 ml of blood was collected from all the experimental birds from the wing vein for hematological and serological profiles. Half of the blood was used for hematological study for which 1.5 ml was transferred under aseptic conditions to EDTA containing sterile tubes. The remaining blood was transferred to a gel containing sterile tubes for serological studies.

### **Hematobiochemical profile**

For hematological profile, the blood was collected in EDTA containing clean tubes. The complete blood counts include hemoglobin level (Hb), packed cell volume (PCV), total erythrocyte count (TEC). The erythrocytic indices include mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). The hematological analysis was carried out as described by Jalees et al., 2011. For the serological profile, the blood contained in the gel tube was centrifuged for 10 minutes at 3000 rpm. The separated serum was then placed in Eppendorf tubes and stored at -20 °C until further process. The obtained serum was subjected to different biochemical tests. Liver function test i.e., Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total serum protein test, serum albumin level and Kidney function tests i.e., blood urea nitrogen (BUN) and creatinine were performed by commercial kits.

### **Necropsy and gross lesions**

At the end of experimental feeding trials (3 weeks), all birds from all groups were euthanized and gross lesions in the kidney and liver were recorded. The lesions were ranked as 0 (absence), 1 (presence), 2 (mild) and 3 (severe) scales. The scores of specific lesions appearing in quails of the same group were added together to obtain the individual lesion score. Then these lesion scores of a particular organ were added to get individual lesion scores for a particular organ. By summation of all lesion scores of a particular organ in a group, a cumulative lesion score was obtained.

### **Histopathological lesions**

Pieces of kidney and liver were removed and placed in 10% buffered formalin for histopathological examination. Samples of the tissues were trimmed and then washed in running tap water overnight. Then the tissue was dehydrated in ascending grades of alcohol, cleared in xylene and embedded finally in

paraffin. Tissue sections of the kidney and liver were cut at 5µm using a rotary microtome and stained with hematoxylin and eosin for microscopic examination (Bancroft and Gamble, 2007).

### Statistical analysis

Data obtained were subjected to statistical analysis using ANOVA with statistical software Statistix (Version 8.1).

## RESULTS

Quails in the negative control group (OTA-0) were alert, active, showed normal feed and water intake and normal consistency of feces. Unlike the negative control group, quails fed OTA-treated feed were depressed, showed inappetence and increased water intake with watery feces. These signs were more prominent in OTA-2 as compared to OTA-1.

The feed intake of quails treated with different levels OTA is shown in Table 2. There was a decrease in feed consumption with the increase in OTA concentration in feed throughout the experimental period and a significant difference ( $p < 0.05$ ) was recorded among the treatment groups.

Weight gain and OTA concentration in feed had a negative correlation; body weight gain of quails was lower with increasing concentration of OTA, which was statistically significant ( $P < 0.05$ ) among the treatment groups (Table 3).

The feed conversion ratios of quails fed with graded levels of OTA for 3 weeks are presented in Table 4. During the first week of the experiment, there was no significant difference among the groups, while in second and third week of experiment increase in FCR was recorded and the statistical difference was

**Table 2.** Ingredient composition (%) of the basal diet

Ingredient	Composition
Maize	57.7
SBM	36.3
Di calcium phosphate	1.65
NaCl	0.57
DL Methionine	0.16
Anticoccidial	0.04
Mixture of vitamin	0.25
Mineral	0.15

**Table 3.** Feed intake (mean  $\pm$  SE) of quails fed diets with different concentration of OTA

Groups*	week 1	week 2	week 3	Total
OTA-0	89.9 <sup>a</sup> $\pm$ 1.57	128.5 <sup>a</sup> $\pm$ 1.79	140 <sup>a</sup> $\pm$ 1.15	391.6 <sup>a</sup> $\pm$ 2.61
OTA-1	82.4 <sup>b</sup> $\pm$ 1.53	121 <sup>b</sup> $\pm$ 2.00	129.7 <sup>ab</sup> $\pm$ 0.88	368.3 <sup>b</sup> $\pm$ 3.64
OTA-2	76.6 <sup>c</sup> $\pm$ 0.70	116 <sup>b</sup> $\pm$ 2.08	120.3 <sup>b</sup> $\pm$ 5.48	345.2 <sup>c</sup> $\pm$ 4.94
Significance	***	*	**	***

In the same column, value carrying different superscript letter (a, b, c) differ significantly ( $P < 0.05$ ); \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

\*All quails received basal diet; In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 groups basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet

**Table 4.** Body weight gain (mean  $\pm$  SE) of quails fed diets with different levels of OTA

Groups*	week 1	week 2	week 3	Total
OTA-0	24.3 <sup>a</sup> $\pm$ 0.38	26.5 <sup>a</sup> $\pm$ 0.61	35.7 <sup>a</sup> $\pm$ 0.97	99.6 <sup>a</sup> $\pm$ 1.61
OTA-1	21.2 <sup>b</sup> $\pm$ 0.33	23.1 <sup>b</sup> $\pm$ 0.19	26.2 <sup>b</sup> $\pm$ 1.27	85.2 <sup>b</sup> $\pm$ 1.92
OTA-2	19.1 <sup>c</sup> $\pm$ 0.67	20.6 <sup>c</sup> $\pm$ 0.62	21.5 <sup>c</sup> $\pm$ 0.68	75.3 <sup>c</sup> $\pm$ 1.67
Significance	***	***	***	***

In the same column, value carrying different superscript letter (a, b, c) differ significantly ( $p < 0.05$ ); \*\*\*,  $p < 0.001$

\*All quails received basal diet

In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 groups basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet.



observed between OTA-treated groups and control.

Serum ALT of OTA-2 group was significantly higher ( $p < 0.05$ ) than those of OTA-0 and OTA-1 groups. However, no difference ( $p > 0.05$ ) was recorded between OTA-0 and OTA-1 group. Quails fed on OTA-0 diet had lowered ( $p < 0.05$ ) AST value than OTA-1 and OTA-2. The urea value of OTA-2 group was higher ( $p < 0.05$ ) as compared to OTA-1 and OTA-0. Creatinine values did not vary ( $p > 0.05$ ) between the tested groups, whereas it differ ( $p < 0.05$ ) in OTA-0. Nonetheless, the total protein and albumin concentration were significantly ( $p < 0.05$ ) reduced with increased in OTA concentration in feed (Table 5).

The values of hematological parameters are shown in Table 6. The number of TEC decreased ( $p < 0.05$ ) in OTA-2 group as compared to OTA-0 and OTA-1. With increase in concentration of OTA

in quails diet the PCV value was decreased ( $p < 0.05$ ). Quails fed on OTA-2 diet had lower ( $p < 0.05$ ) concentration of hemoglobin. On the other hand, MCV value was higher ( $p < 0.05$ ) in OTA-2 group. While the concentration of MCH and MCHC did not vary ( $p > 0.05$ ) among the dietary groups.

On postmortem examination of the OTA-0 group, no gross changes were detected in the liver and kidney, which were of normal size and shape. The liver in OTA-1 group was slightly enlarged and pale while that of group OTA-2 was greatly enlarged and hemorrhagic. The kidneys of OTA-treated groups were slightly enlarged and pale in color. Some areas of hemorrhages were also found on the kidneys of OTA-2 group (Table 7).

Liver and kidney of negative control (OTA-0) birds showed normal and healthy histoarchitecture,

**Table 5.** Feed conversion ratio (mean  $\pm$  SE) of quails fed diets with different levels of OTA

Groups*	Week 1	Week 2	Week 3	Total
OTA-0	3.71 $\pm$ 0.11	4.82 <sup>b</sup> $\pm$ 0.05	3.92 <sup>b</sup> $\pm$ 0.08	3.93 <sup>c</sup> $\pm$ 0.06
OTA-1	3.91 $\pm$ 0.13	5.26 <sup>a</sup> $\pm$ 0.05	5.01 <sup>a</sup> $\pm$ 0.27	4.33 <sup>b</sup> $\pm$ 0.07
OTA-2	4.02 $\pm$ 0.10	5.66 <sup>a</sup> $\pm$ 0.19	5.62 <sup>a</sup> $\pm$ 0.21	4.59 <sup>a</sup> $\pm$ 0.05
Significance	Ns	**	**	***

In the same column, value carrying different superscript letter (a, b, c) differ significantly ( $p < 0.05$ ); ns = non-significant, \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$

\*All quails received basal diet; In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 group's basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet

**Table 6.** Serological profile (mean  $\pm$  SE) of quails fed diets with different level of OTA

Groups*	ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine ( $\mu$ mol/l)	Total protein (g/dl)	Albumin(g/dl)
OTA-0	31.3 <sup>b</sup> $\pm$ 5.49	104.1 <sup>b</sup> $\pm$ 3.59	33 <sup>b</sup> $\pm$ 1.96	27.1 <sup>b</sup> $\pm$ 0.78	6.10 <sup>a</sup> $\pm$ 0.15	3.34 <sup>a</sup> $\pm$ 0.19
OTA-1	41.8 <sup>b</sup> $\pm$ 3.54	130.2 <sup>a</sup> $\pm$ 1.70	36.9 <sup>b</sup> $\pm$ 0.61	36.8 <sup>a</sup> $\pm$ 1.83	4.93 <sup>b</sup> $\pm$ 0.11	2.58 <sup>b</sup> $\pm$ 0.26
OTA-2	59.1 <sup>a</sup> $\pm$ 3.65	136.6 <sup>a</sup> $\pm$ 6.36	43.8 <sup>a</sup> $\pm$ 1.25	41.1 <sup>a</sup> $\pm$ 3.73	4.20 <sup>c</sup> $\pm$ 0.18	2.07 <sup>b</sup> $\pm$ 0.06
Sig.	*	**	**	*	***	**

In the same column, value carrying different superscript letter (a, b, c) differ significantly ( $p < 0.05$ ); \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase

\*All quails received basal diet; In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 groups basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet

**Table 7.** Hematological profile (mean  $\pm$  SE) of quails fed diets with different level of OTA

Groups*	TEC ( $\times 10^6$ )	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
OTA-0	3.56 <sup>a</sup> $\pm$ 0.08	31.33 <sup>a</sup> $\pm$ 0.66	12.7 <sup>a</sup> $\pm$ 0.43	87.9 <sup>b</sup> $\pm$ 0.71	35.7 $\pm$ 1.23	40.6 $\pm$ 1.08
OTA-1	3.36 <sup>a</sup> $\pm$ 0.08	28.66 <sup>b</sup> $\pm$ 0.66	12 <sup>a</sup> $\pm$ 0.30	85.2 <sup>b</sup> $\pm$ 1.50	35.6 $\pm$ 0.47	41.8 $\pm$ 0.21
OTA-2	2.70 <sup>b</sup> $\pm$ 0.05	25 <sup>c</sup> $\pm$ 0.57	9.86 <sup>b</sup> $\pm$ 0.17	92.6 <sup>a</sup> $\pm$ 0.15	36.5 $\pm$ 0.19	39.5 $\pm$ 0.26
Significance	**	***	**	**	ns	ns

In the same column, value carrying different superscript letter (a, b, c) differ significantly ( $p < 0.05$ ); ns = non-significant, \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$

TEC, total erythrocyte count; PCV, packed cell volume; Hb, Hemoglobin level; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration

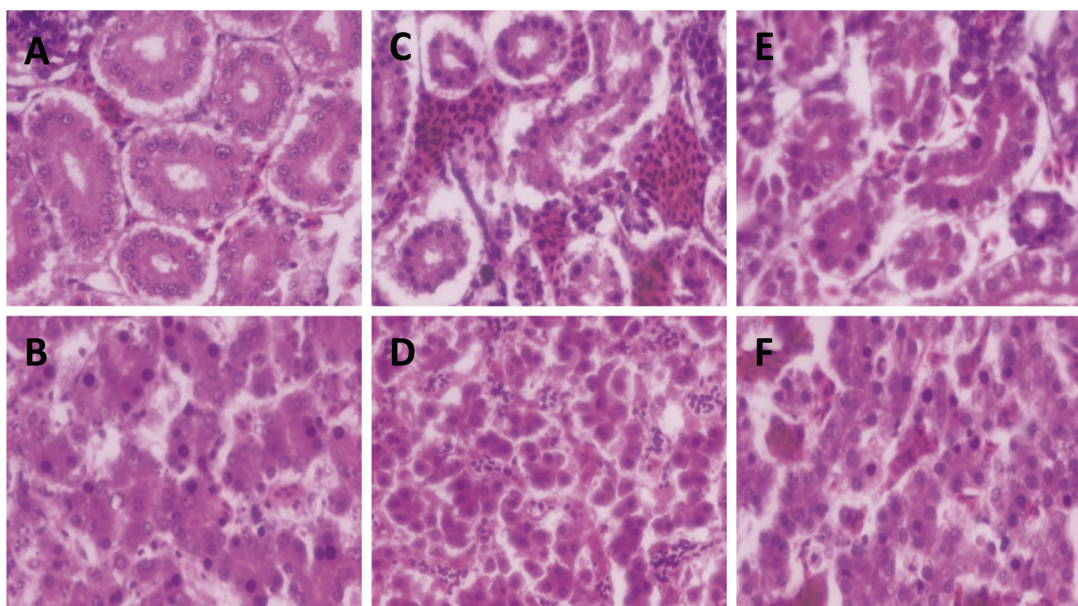
\*All quails received basal diet; In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 group's basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet

**Table 8.** Gross lesions score of different organs of quails fed diets with different levels of OTA

Organ	Lesion	Groups*			
		Max. score	OTA-0	OTA-1	OTA-2
Liver	Enlargement	0-3	0	10	14
	Pale discoloration	0-3	0	8	12
	Friable	0-3	0	9	12
	Hemorrhage	0-3	0	10	14
<b>Total liver score</b>		72	0	37	52
Kidneys	Enlargement	0-3	0	10	13
	Hemorrhage	0-3	0	9	12
<b>Total kidney score</b>		36	0	19	25
<b>Cumulative score (liver + kidney)</b>		108	0	56	77

0=absence, 1= present, 2= mild, 3= severe; Lesion scores of a particular organ were added to get individual lesion scores for a particular organ (liver and kidney). Moreover, a cumulative lesion score was obtained for each group by summation of all lesion scores of a particular organ in that group.

\*All quails received basal diet; In OTA-0 group no addition of ochratoxin to the basal diet; whereas, in OTA-1 and OTA-2 group's basal diet was supplemented with ochratoxin @ 1 mg and 2 mg per kg of basal diet

**Figure 1.** Photomicrograph of kidney and liver of quails fed with graded level of OTA.

H & E stain 400X. (A=kidney, B=liver): fed with 0 mg/kg OTA in feed; (C=kidney, D=liver): fed with 1 mg/kg OTA in feed; (E=kidney, F=liver): fed with 2 mg/kg OTA in feed.

histologically. In group OTA-1, we observed proliferation of mesenchymal cells in some glomeruli due to which urinary spaces were reduced. Tubular epithelial cells showed cloudy swelling and were detached from the basement membrane with pycnotic changes in the nuclei. In liver, hepatic sinusoids were dilated with congestion and cellular infiltrates around the blood vessels. Fatty changes along with pyknotic nuclei were also found in some high power fields. In group OTA-2, the histopathological changes were more pronounced than OTA-1 group with degeneration and denudation of tubular epithelial cells

in kidneys. The liver showed widespread areas of congestion and fatty change, which were severe in intensity. Hepatic sinusoidal spaces were increased and infiltration of leucocytes around the blood vessels was observed. Fatty changes in hepatic parenchyma and pyknotic nuclei in degenerating hepatocytes are more severe than OTA-1 group.

## DISCUSSION

Mycotoxins produced in food stuffs have adverse effects on human and animal since long, but the relationship between food, fungi and disease is recently

developed. Most frequently occurring mycotoxin in the environment are zearalenone, ochratoxin, T-2 toxin, aflatoxin, and fumonisin. In general, these mycotoxins are inevitable contaminants of food and feed (Alshannaq and Yu, 2017). Noteworthy, the ochratoxin, is important because of carcinogenic, neurotoxic, hepatotoxic, nephrotoxic, immunosuppressive effect, genotoxic and hemotoxic biological disorders (Eskola et al. 2019). Therefore, this study was conducted to evaluate the effects of different levels of ochratoxin on quails performance, haematobiochemical profile, and macroscopic and histological lesions of kidney and liver.

In this trial, the clinical signs observed in quails fed with OTA-contaminated diet included depression, decrease feed intake, increase water intake and watery feces. Similar results were reported in the literature in poultry feeding different concentrations of OTA (Elaroussi et al. 2006; Sawale et al. 2009; Hassan et al. 2012), whereas, Patial et al. (2015) reported same findings in quails. The feed conversion ratio of quails was negatively affected with increasing dose rate of OTA in feed. These findings are in line with the reported values of (Verma et al. 2003; Hassan et al. (2010). A subjective score of clinical signs increased with an increase in OTA concentration. Similar results were also reported by Hassan et al. (2010) in poultry. Macroscopically, the livers of OTA treated groups were pale in color, friable in consistency, enlarged and hemorrhagic as compare to the control group. Patial et al. (2015) also reported similar changes in the liver of OTA-fed quails. The kidneys were slightly bulging out from the socket in OTA treated group and showed enlargement. Some areas of necrosis were also present on surface of kidneys. Similar results have been reported in poultry (Sawale et al. 2009; Hassan et al., 2010). Gross lesion scoring increased with the increase in OTA concentration as has been reported in poultry by Hassan et al. (2010).

The serum biochemical profile of quails fed OTA was disturbed. There was a significant increase in serum ALT and AST as has been stated by Hassan et al. (2012) in a dose-dependent manner in birds. Sawale et al. (2009) also reported an increase in serum ALT with OTA feeding in poultry. Serum total protein and albumin were decreased in OTA-treated quails which were supported by the findings of Shahzad et al. (2014) in quails. Nonetheless, blood creatinine and urea levels were augmented in the OTA treated group in the present study. Hassan et al. (2012) and Shahzad

et al. (2014) also reported the same results.

The hematological values of quails fed with OTA contaminated diet decreased with an increase in the levels of OTA in diet. Specifically, TEC, PCV and hemoglobin concentrations were significantly decreased with increased OTA in diet than control group (OTA-0). The results obtained were similar to findings of Hassan et al. (2012) who reported that OTA feeding to breeders decreased the hematological values in a dose dependent manner. Similar results of hematological alterations by feeding of OTA to birds have been reported by Stoev et al. (2011). Similarly, Shahzad et al. (2014) reported similar results in quails by feeding OTA in combination with aflatoxin B1.

Microscopically, the liver of OTA-treated group showed degenerative changes consisting of swelling and vacuolar changes in hepatocytes. This has been reported in quails by Vikram et al. (2015) and in poultry by Stoev et al. (2002) and Hassan et al. (2012). However, in contrast Elaroussi et al. (2006) findings were different who fed 0.4- 0.8 mg/kg OTA to broilers. This dissimilarity might be due to the low concentration and period of OTA feeding. The hepato-toxic effect of OTA at 0.354 mg/kg basal diet was reported by Biro et al. (2002) to broiler chickens. Likewise, Elaroussi et al. (2008) reported that pathological alteration resulted at 0.4 and 0.8 mg OTA/kg with more sever effects of increase dose of OTA in feed.

Microscopically, the kidneys of the quails fed with 1mg and 2 mg/kg OTA showed degenerative changes in proximal convoluted tubules. The epithelial cells showed swelling, pyknotic nuclei with increase in OTA concentration in diet. Similar changes in quails were reported in literature by (Hassan et al. 2010; Patial et al. (2013). The same histo-pathological alteration caused by OTA contaminated diet particularly in liver and kidney with 0.5, 1, 2 and 4 mg OTA/kg to the broiler chicken has been documented by many scientist elsewhere (Hanif et al. 2008; Patil et al. 2014).

## CONCLUSION

The this study develops the first dataset on the toxico-pathological effects on liver, kidney and haematobiochemical profile of quails fed on ochratoxin (OTA) at 1 and 2 mg/kg basal diet. The study reveals that OTA at the dose rate of 1 mg/kg basal diet has nephrotoxic, hepatotoxic impact on quails. Also, it resulted in hemato-biochemical disorders in quails which ultimately lead to reduction in production



performances and eventually lead to the economic losses of the farmer around the globe. Furthermore, screening of OTA contamination in quails feed is important to ensure maximum production. Therefore, further research should be conducted to determine the minimum level of OTA which cause toxicity in quails

and other birds that helps for planning of control strategy of ochratoxicosis.

## CONFLICT OF INTEREST

The authors do not have any conflict of interest.

## REFERENCES

- Alshannaq A, Yu JH (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environmental Research and Public Health* 14(6): 632.
- Bancroft JD, Gamble M (2007). Connective tissue stains. In: Bancroft JD, Gamble M (eds) *Theory and practice of histological techniques*, 6th edn. Churchill Livingstone, Philadelphia, 150.
- Bayman P, Baker JL, Doster MA, Michailides TJ, Mahoney NE (2002). Ochratoxin production by *Aspergillus ochraceus* group and *Aspergillus Alliaceus*. *Appl. Environ. Microbiol* 68: 2326-2329.
- Biro K, Solti L, Barna-Vetró I, Bagó G, Glavits R, Szabó E, Fink-Gremmels J (2002). Tissue distribution of ochratoxin A as determined by HPLC and ELISA and histopathological effects in chickens. *Avian Pathology* 31: 141-148.
- Cetin Y (2020). Microbial toxins. In: Demirci, A., Feng, H., Krishnamurthy, K. (eds) *Food Safety Engineering*. Food Engineering Series. Springer, Cham.
- Commission Regulation EC, European Commission. Commission Regulation 123/2005 of 26 January 2005 amending Regulation (EC) No. 466/2001 as regards ochratoxin A. Official J. The European Communities. 25:3-5.
- Duarte SC, Pena A, Lino CM (2010). A review on ochratoxin A occurrence and effects of processing of cereal and cereal derived food products. *Food Microbiol* 27(2): 187-198.
- Elaroussi MA, Mohamed FR, Elgendy MS, El Barkouky M, Abdou AM, Hatab MH (2008). Ochratoxicosis in broiler chickens: functional and histological changes in target organs. *International Journal of Poultry Science* 7: 414-422.
- Elaroussi MA, Mohamed FR, El Barkouky EM, Atta AM, Abdo AM, Hatab MH (2006). Experimental ochratoxicosis in broiler chickens. *Avian Pathology* 35(4): 263-269.
- Eskola M, Gregor K, Christopher E, Hajslova J, Mayar S, Kriska R (2019). Worldwide contamination of food crops with mycotoxins: Validity of the widely cited FAO estimate of 25%. *Critical Reviews in Food Science and Nutrition* 60: 1-17.
- Frazzoli C, Gherardi P, Saxena N, Belluzzi G, Mantovani A (2017). The hotspot for (global) one health in primary food production: Aflatoxin M1 in dairy products. *Frontiers in Public Health* 4: 294.
- Galtier, P, Alvinerie M, Charpentreau, JL (1981). The pharmacokinetic profiles of ochratoxin A in pigs, rabbits and chickens. *Food and Cosmetic Toxicology* 19: 735-738.
- Hanif NQ, Muhammad G, Siddique M, Khanum A, Ahmed T, Gadaihai JA, Kaukab G (2008). Clinico-pathomorphological, serum biochemical and histological studies in broilers fed ochratoxin A and a toxin deactivator (Mycofix Plus). *British Poultry Science* 49: 632-642.
- Hassan ZU, Khan MZ, Khan A, Javed I (2010). Pathological responses of white leghorn breeder hens kept on ochratoxin A contaminated feed. *Pak. Vet. Journal* 30: 118-123.
- Hassan ZU, Khan MZ, Khan A, Javed I, Sadique U, Hameed MR (2012). Effect of ochratoxin A (OTA)-contaminated feed on several health and economic parameters in white leghorn cockerels. *Pak. Vet. Journal* 32: 35-40.
- Jalees MM, Khan MZ, Saleemi MK, Khan A (2011). Effects of cottonseed meal on hematological, biochemical and behavioral alterations in male Japanese quail (*Coturnix japonica*). *Pakistan Veterinary Journal* 31: 211-214.
- Kumar A, Jindal N, Shukla CL, Asrani RK, Ledoux DR and Rottinghaus GE (2004). Pathological changes in broiler chickens fed ochratoxin A and inoculated with *Escherichia coli*. *Avian Pathology*, 33(4): 413-417.
- Marin S, Ramos AJ, Cano-Sancho G, Sanchis V (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chemistry and Toxicology* 60: 218-237.
- Mitchell, NJ, Bowers E, Hurburgh C, Wu F (2016). Potential economic losses to the US corn industry from aflatoxin contamination. *Food Addit. Contam. Part A* 33: 540-550.
- NRC (1994). *Nutrient Requirements of Poultry*. 9th ed. Washington, DC: Natl.
- Ostry V, Malir F, Toman J, Grosse Y (2017). Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Res* 33: 65-73.
- Patil V, Asrani RK, Patil RD, Kumar N, Sharma R (2015). Protective effect of sea buckthorn (*Hippophae rhamnoides* L.) leaves on ochratoxin-A induced hepatic injury in Japanese quail. *Vet Res Int* 3: 98-108.
- Patil RD, Rinku, Sharma, Rajesh KA (2014). Mycotoxicosis and its control in poultry: A review *Journal of Poultry Science and Technology*. 2 (1): 1-10.
- Peles F, Sipos P, Gyori Z, Pfliegler WP, Giacometti F, Serraino, A, Poci I (2019). Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock. *Frontiers in Microbiology* 10: 2861.
- Pitt JJ, Miller JD (2017). A concise history of mycotoxin research. *Journal of Agricultural and Food Chemistry* 65 (33): 7021-33.
- Richard JL, Payne GA, Desjardins AE, Maragos C, Norred WP, Pestka JJ (2003). Mycotoxins: risks in plant, animal and human systems. *CAST Task Force Report* 139: 101-103.
- Sawale GK, Gosh RC, Ravikanth K, Maini S, Rekhe DS (2009). Experimental mycotoxicosis in layer induced by ochratoxin A and its amelioration with herbomineral toxin binder 'Toxiroak'. *International Journal of Poultry Science* 8(8): 798-803.
- Schrickx J, Lektarau Y, Fink-Gremmels J (2006). Ochratoxin A secretion by ATP-dependent membrane transporters in Caco-2 cells. *Archives of Toxicology* 80: 243-249.
- Shahzad M, Khan MUR, Aslam A, Younus M, Khan HM, Anjum AA, Masood S (2014). Diminution of ochratoxin and aflatoxinb1 toxicity on hematology and serum biochemistry of Japanese quail (*Coturnix japonica*). *The Journal of Animal and Plant Sciences* 24(4): 1032-1038.
- Shipton WA (2014). *The biology of fungi impacting human health*. Partridge India.
- Stoev DS, Gundasheva D, Zarkov I, Mircheva T, Zapryanova D, Mitev Y, Daskalov H, Dutton M, Mwanza M, Schneider YJ (2011). Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and fumonisin B1. *Exp. Toxicol. Pathol* 64(7-8): 733-741.
- Stoev SD (2013). Food safety and increasing hazard of mycotoxin occurrence in foods and feeds. *Critical Reviews in Food Science and Nutrition* 53: 887-901.
- Stoev SD, Daskalov H, Radic B, Domijan AM, Peraica M (2002). Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology. *Veterinary Research* 33(1): 83-93.
- Trenk HL, Butz ME, Chu FS (1971). Production of ochratoxins in different cereal products by *Aspergillus ochraceus*. *Applied Microbiology* 21(6): 1032-1035.
- Verma J, Johri TS, Swain BK (2003). Effect of varying levels of aflatoxin, ochratoxin and their combinations on the performance and egg quality characteristics in laying hens. *Asian-Aust. J. Anim. Science* 16:1015-1019.
- Wu F, Mitchell NJ (2016). How climate change and regulations can affect the economics of mycotoxins. *World Mycotoxin Journal* 9(5): 653-63.
- Zaghini A, Simioli M, Roncada P, Rizzi L (2007). Effect of *Saccharomyces cerevisiae* and esterified glucomannan on residues of ochratoxin A in kidney, muscle and blood of laying hens. *Italian Journal of Animal Science* 6: 737-739.