Microscopic changes due to fumonisin B_1 and ochratoxin A induced nephropathy in Japanese quail

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Received: 28-02-2012, Accepted: 25-03-2012, Published Online: 11-06-2012
doi:10.5455/vetworld.2012.535-540

Abstract

Aim: The present study was designed to evaluate the toxic interaction of fumonisin B_1 and ochratoxin A, alone and in combination for pathology of the kidneys in day old Japanese quail.

Materials and Methods: Day old Japanese quail chicks were divided into 4 groups and fed with basal quail chick mash containing fumonisin B_1 @ 200 ppm (Group FX), Ochratoxin A @ 2 ppm (Group OX), and fumonisin B_1 @ 200 ppm + Ochratoxin A @ 2 ppm (Group FO) and standard toxin free feed (Group CX, control) for 28 days.

Results: The quail chicks were assessed for various microscopic changes in the kidneys. Mean microscopic score values for congestion was found to be significantly higher ($P \leq 0.05$) in FO group when compared to that of other groups FX and OX at 7 and 21 DPF. Similarly, mean microscopic score values for degeneration was found to be significantly higher ($P \leq 0.05$) in FO group as compared to that of groups FX and OX at 7 and 14 DPF. Mean microscopic score values for necrosis and luminal hyaline bodies were significantly higher ($P \leq 0.05$) in FO group when compared to that of groups OX and FX at 7 DPF.

Conclusion: Total score card for microscopic lesions observed in the kidneys when compared between different treatment groups showed that the lesions appeared to be more pronounced in the OTA fed group at 14 DPF as compared to other treatment groups. The overall lesion score values after the conclusion of the experiment were found to be higher in the combination group followed by the groups OX and FX, respectively.

Keywords: Fumonisin B_1, ochratoxin A, Japanese quail, total lesion score and mean lesion score.

To cite this article:
Khan MA, Iqbal A, Asrani RK (2012) Microscopic changes due to fumonisin B_1 and ochratoxin A induced nephropathy in Japanese quail, *Vet World*, 5(9):535-540, doi: 10.5455/vetworld.2012.535-540

Introduction

The fumonisins were isolated for the first time from the fungus *Fusarium moniliforme* (now renamed *Fusarium verticillioides)* in South Africa. A number of fumonisins have since been isolated and characterized, but FB_1 remains the most toxic compound [1]. FB_1 either in naturally contaminated maize or maize-based feeds or in purified form, has been reported to cause equine leukoencephalomalacia [2], porcine pulmonary edema and hydrothorax syndrome [3]. The majority of reports on the toxic effects of FB_1 in avian species pertain to chickens, ducklings and turkeys, in which primary pathological changes have been reported in the liver characterized by multifocal hepatic necrosis and hepatocellular and biliary hyperplasia [4]. Unlike aflatoxins and ochratoxins, which have been studied extensively with respect to the changes induced by them in lymphoid and other organs of poultry [5, 6] limited information is available on the pathologic effects of fumonisins on organs other than the liver.

Ochratoxins, produced mainly by *Aspergillus ochraceus* (now called *A. alutaceus*) and *Penicillum verrucosum*, are chemically characterized as 3, 4 dihydromethylisocoumarin derivatives linked with an amide bond to the amino group of L-D phenylalanine. Ochratoxin-A (OTA) is more toxic than other ochratoxins (B and C) and has been reported to be more toxic than even aflatoxin B_1 [7]. An OTA causes significant loss to the poultry industry due to its effects on performance and health. It causes a reduction in growth rate and feed consumption, poorer feed conversion and increased mortality [8]. OTA induces degenerative changes and an increase in weight of the kidneys and liver, as well as decrease in the weight of

Table-1. Table representing dietary treatments starting from day one until the end of the experiment.

Group	Treatment	Total level of culture material (s) used	Level of mycotoxin (s) supplied (ppm)
CX	Chick mash alone	0 %	0
FX	FB1 alone	3.25 %	200
OX	OTA alone	2.5%	2
FO	FB1 and OTA	3.25% and 2.5%	200 FB1 + 2 OTA

lymphoid organs [9].

The effects of FB_1 and OTA in combination have not been investigated in Japanese quail. The purpose of this study was to investigate the toxicity and describe the major effects of feeding quail chicks diets containing FB_1 and ochratoxin A alone and in combination from day 1 to 4 weeks of age.

Materials and Methods

Experimental birds: The present studies were conducted on three hundred one-day-old Japanese quail chicks procured from the Central Poultry Development Organization, Chandigarh. The birds were kept under strict hygienic conditions throughout the period of the experiment. The animal care and experimental protocol were approved by the University and by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Feeding schedule: The quail chicks were maintained on chick mash (Quail mash procured from the Department of Animal Nutrition, COVAS, CSK HPKV, Palampur) from day one until the end of the experiment. Feed was autoclaved for 15 minutes at 15 pounds pressure before feeding or mixing with *Fusarium* culture material (s). Boiled (for 15 minutes) and subsequently cooled water was given to the birds throughout the experiment. Feed and water were given *ad libitum*, and no medication was given during the period of the experiment. Before feeding, the representative samples of chick mash were submitted to the Animal Feed Analytical and Quality Control Laboratory, Veterinary Hospital Campus, Trichy Road, Namakkal, Tamil Nadu, India for the analysis of common mycotoxins, and to the Department of Veterinary Preventive Medicine and Epidemiology, CCS HAU, Hisar for analysis of fumonisins contents. The feed samples were found to contain 12 ppb of aflatoxin- B_1 and were free from ochratoxin-A, citrinin, zearalenone, aflatoxin-B_2, aflatoxin-G_1, aflatoxin-G_2 and T-2 toxin. The mycotoxins for the present studies i.e. fumonisin-B_1 and ochratoxin-A were supplied by *Fusarium verticillioides* M-1325 culture material (FCM) and *Aspergillus ochraceous* NRRL-3174 (Courtesy: Dr. G. E. Rottinghaus, University of Missouri, Columbia, USA). Fusarium culture material containing 6200 mg FB_1 per kg and ochratoxin culture material containing 80 mg OTA per kg was incorporated at the rate of 3.25 per cent and 2.5 per cent in the chick mash to supply 200 ppm FB_1 and 2 ppm OTA, respectively. The fumonisin culture material and ochratoxin culture material were not incorporated in the diet of control group (CX).

Experimental design: Three hundred, one-day-old Japanese quail chicks were randomly divided into four groups i.e. FX (fumonisin B_1), OX (ochratoxin-A), FO (fumonisin B_1+ochratoxin A), and CX (control) with 75 birds in each of the above mentioned four groups. The present study was conducted using three pen replicates of 25 quail per pen in each of the four groups for a period of 28 days. Various dietary treatments starting from day one until the end of the experiment are presented in the tabulated form in Table-1.

Total and mean lesion scores: Total microscopic lesion scores for the sacrificed birds were determined at various time intervals by sum total of all the lesion scores obtained by multiplying the intensity score by the number of birds showing that particular intensity of lesion. The lesion score for each of the microscopic lesions in different groups were subjected to statistical test for calculation and comparison of mean lesion scores among different groups at 95% confidence level ($P \leq 0.05$).

Statistical analysis of data: Data were subjected to statistical analysis for drawing inferences using a standard procedure [10]. Treatment means were compared using Duncan's Multiple Range Test (2-way ANOVA) to determine the effect of treatment, age and their interactions [11]. All levels of significance were based on the 95 per cent level of probability.

Results

Microscopic pathology: When total score card for microscopic lesions observed in the kidneys was compared between different treatment groups, the lesions appeared to be more pronounced in the OTA fed group at 14 DPF as compared to other groups. The

Table-2. Total microscopic lesion scores in the kidneys of Japanese quail at different intervals

Time interval	Total microscopic lesion scores in different groups Kidneys		
	FX	OX	FO
7 DPF	47	38	88
14 DPF	73	107	102
21 DPF	43	30	76
28 DPF	44	52	55
Overall score	207	227	321

FX = fumonisin B_1 only, OX = ochratoxin A only, FO = fumonisin B_1 + ochratoxin A, DPF = days post- feeding

Table-3. Interstitial congestion indicated by mean microscopic lesion score in different groups of Japanese quail

Group	Days post-feeding 7	14	21	28	Mean treatment effect
CX	0.88 ± 0.30^{c}	0.77 ± 0.22^{c}	1.22 ± 0.27^{ab}	0.22 ± 0.14^{b}	0.77 ± 0.13^{Z}
FX	1.66 ± 0.16^{ab}	1.00 ± 0.16^{c}	0.88 ± 0.20^{b}	0.66 ± 0.23^{ab}	1.05 ± 0.11^{yZ}
OX	1.00 ± 0.23^{bc}	2.44 ± 0.17^{a}	0.88 ± 0.30^{b}	1.00 ± 0.23^{a}	1.33 ± 0.15^{xy}
FO	2.00 ± 0.23^{a}	1.77 ± 0.22^{b}	2.00 ± 0.28^{a}	0.55 ± 0.24^{ab}	1.58 ± 0.15^{x}
Mean age Effect	1.38 ± 0.14^{A}	1.50 ± 0.14^{A}	1.25 ± 0.15^{A}	0.61 ± 0.11^{B}	
Age x treatment effect					4.59^{HS}

a-c: Values within columns (between groups CX, FX, OX and FO) with different superscripts are significantly different by ANOVA ($P \leq 0.05$). x-z: Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P \leq 0.05$) HSF-value indicating interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P \leq 0.01$). A-BValues within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P < 0.05$). 1. Data are means ± SE of three replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B1; OX = birds fed ochratoxin A; and FO = birds fed fumonisin B1 and ochratoxin A.

Table-4. Degenerative changes in the kidneys indicated by mean microscopic lesion score in different groups of Japanese quail

Group	Days post-feeding 7	14	21	28	Mean treatment effect
CX	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{z}
FX	1.11 ± 0.20^{b}	2.22 ± 0.14^{b}	1.66 ± 0.23^{b}	1.55 ± 0.17^{a}	1.63 ± 0.11^{y}
OX	1.44 ± 0.24^{b}	2.22 ± 0.14^{b}	0.88 ± 0.20^{c}	2.11 ± 0.35^{a}	1.66 ± 0.14^{y}
FO	2.22 ± 0.22^{a}	2.77 ± 0.14^{a}	2.55 ± 0.17^{a}	2.00 ± 0.23^{a}	2.38 ± 0.10^{x}
Mean age Effect	1.19 ± 0.16^{B}	1.80 ± 0.19^{A}	1.27 ± 0.18^{B}	1.41 ± 0.18^{B}	
Age x treatment effect					4.10^{HS}

a-d: Values within columns (between groups CX, FX, OX and FO) with different superscripts are significantly different by ANOVA ($P < 0.05$). x-z: Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P < 0.05$) HSF-value indicating interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P < 0.01$). A-B: Values within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P < 0.05$).1. Data are means ± SE of three replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B1; OX = birds fed ochratoxin A; and FO = birds fed fumonisin B1 and ochratoxin A.

overall lesion score values after the conclusion of the experiment were found to be higher in the combination group followed by groups OX and FX, respectively (Table-2).

When mean microscopic score values for congestion observed microscopically were compared between different treatment groups, the difference was found to be significantly higher ($P \leq 0.05$) in FO group when compared to that of other groups FX and OX at 7 and 21 DPF (Table-3). The mean treatment effect at the end of the experiment revealed significantly higher ($P \leq 0.05$) congestion of kidneys in group FO when compared with the other treatment groups FX and OX. The overall treatment effect in relation to progressing age of birds on the occurrence of congestion in the renal interstitial tissue was found to be highly significant ($P \leq 0.01$) (Table-3).

When mean microscopic score values for degeneration were compared between different treatment groups, the difference was found to be

Table-5. Renal tubular epithelial cell necrosis indicated by mean microscopic lesion score in different groups of Japanese quail[1]

Group	Days post-feeding 7	14	21	28	Mean treatment effect
CX	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{z}
FX	0.22±0.14^{b}	0.88±0.11^{a}	0.00±0.00^{b}	0.00±0.00^{a}	0.27±0.07^{y}
OX	0.11±0.11^{b}	1.11±0.20^{a}	0.22±0.14^{ab}	0.00±0.00^{a}	0.36±0.09^{y}
FO	1.44±0.24^{a}	1.00±0.16^{a}	0.55±0.24^{a}	0.00±0.00^{a}	0.75±0.12^{x}
Mean age Effect	0.44±0.12^{B}	0.75±0.10^{A}	0.19±0.07^{C}	0.00±0.00^{D}	
Age x treatment effect					8.35^{HS}

a-b: Values within columns (between groups CX, FX, OX and FO) with different superscripts are significantly different by ANOVA ($P < 0.05$). x-z: Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P < 0.05$) HSF-value indicating interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P < 0.01$). A-DValues within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P < 0.05$). 1. Data are means ± SE of three replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B1; OX = birds fed ochratoxin A; and FO = birds fed fumonisin B1 and ochratoxin A.

Table-6. Occurrence of eosinophilic hyaline bodies in the lumen of renal tubules indicated by mean lesion score in different groups of Japanese quail

Group	Days post-feeding 7	14	21	28	Mean treatment effect
CX	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{z}
FX	0.11±0.11^{b}	1.11±0.20^{a}	0.33±0.23^{a}	0.77±0.22^{a}	0.58±0.11^{y}
OX	0.00±0.00^{b}	1.44±0.17^{a}	0.00±0.00^{a}	0.00±0.00^{b}	0.36±0.11^{z}
FO	1.11±0.30^{a}	1.55±0.24^{a}	0.11±0.11^{a}	0.77±0.14^{a}	0.88±0.13^{x}
Mean age Effect	0.30±0.11^{BC}	1.02±0.13^{A}	0.11±0.06^{C}	0.38±0.09^{B}	
Age x treatment effect					6.54^{HS}

a-d: Values within columns (between groups CX, FX, OX and FO) with different superscripts are significantly different by ANOVA ($P < 0.05$). x-z: Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P < 0.05$) HSF-value indicating interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P < 0.01$). A-B: Values within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P < 0.05$).1. Data are means ± SE of three replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B1; OX = birds fed ochratoxin A; and FO = birds fed fumonisin B1 and ochratoxin A.

significantly higher ($P \leq 0.05$) in FO group as compared to that of groups FX and OX at 7 and 14 DPF (Table-4). At 21 DPF, the comparative values in the group FO were significantly higher than any of the groups fed either FB_1 or OTA alone. The mean treatment effect at the end of the experiment revealed significantly higher ($P \leq 0.05$) degeneration in the kidneys in group FO when compared to those of groups FX and OX.

When the mean microscopic score values for necrosis were compared between different treatment groups, the difference was found to be significantly higher ($P \leq 0.05$) in FO group when compared to that of groups OX and FX at 7 DPF (Table-5). The mean treatment effect at the end of the experiment revealed significantly higher ($P \leq 0.05$) values for the occurrence of necrosis in group FO when compared to those of groups OX and FX. The overall treatment effect in relation to progressing age of birds on the occurrence of necrosis in the kidneys was found to be highly significant ($P \leq 0.01$) (Table-5).

Mean score values for luminal hyaline bodies when compared between different treatment groups, the difference was found to be significantly higher ($P \leq 0.05$) in FO group when compared to those of groups OX and FX at 7 DPF (Table 6). The mean treatment effect at the end of the experiment revealed significantly higher ($P \leq 0.05$) occurrence of eosinophilic bodies in the renal tubules in group FO when compared to those of groups FX and OX.

Discussion

The microscopic lesions noticed in the present study in the FB_1 fed group were more or less in conformity with the earlier reports [12] who reported mild focal renal tubular mineralization in broiler chicks fed 100 and 200 ppm FB_1 for 21 days. Similarly [13] revealed progressive degenerative and vacuolar

changes in tubular epithelial cells and occasional infiltration with heterophils in the interstitial tissue near degenerating tubules in quail fed FCM supplying 150 mg FB_1/kg mash for 21 days and demonstrated that FB_1 inhibits enzyme ceramide synthase in the kidneys [14] with a consequent increase in SA concentration and SA/SO ratio. The mechanism of renal toxicity induced by FB_1 has been reported to be quite different from that of OTA. Altered sphingolipid metabolism affecting the cell membrane integrity and functions of the epithelial cells was reported to be the changes preceding even before the development of the lesions evidenced histopathologically.

Swelling with degenerative changes of tubular epithelial cells was a consistent finding in OTA fed group with maximum severity occurring in the birds sacrificed at 14 and 21 DPF in the present study. This period roughly coincided with the time when there was highest mortality in this group. The enlargement and degenerative changes in the epithelial cells of the kidneys were probably due to route of elimination of OTA through the kidneys and partly through the liver (owing to enterohepatic recirculation and the hepatobiliary route of excretion of OTA), and to direct toxic action of OTA on these cells [15]. Similarly, [16] reported cloudy swelling, granular degenerative changes in the cytoplasm and pyknotic nuclei in the epithelial cells of PCTs, when broiler chicks were fed culture material containing 4 and 8 ppm OTA for 1-35 days. In addition to this, a decrease in capsular space of Bowman's capsule, marked degeneration of malphigian corpuscles and obliteration of capsular spaces were also recorded. The severity of these lesions was comparatively higher when a higher dose of OTA (8 ppm) was used in the study. OTA seems to have produced its toxic effects in the present studies by one of three major mechanisms: (a) inhibition of phenylalanine metabolizing enzymes, there by affecting DNA, RNA and protein synthesis [17]. (b) promotion of lipid peroxidation by complexing iron, facilitating reduction of iron, and [18] reported that an iron complex of OTA produced the extremely toxic hydroxyl radical in the presence of NADPH-cytochrome P-450 reductase system and this radical was partly responsible for OTA toxicity. (c) inhibition of mitochondrial ATP production by acting as a competitive inhibitor of carrier proteins located in the inner mitochondrial membrane [19].

Conclusion

The interaction between two mycotoxins as observed in the present study was found to be less than additive in nature for most of the microscopic variables except for interstitial congestion and degenerative changes which yielded an additive interaction at 21 DPF.

Author's contribution

MAK and RKA implemented the study design. MAK and AI done the Statistical analysis of data and collection of research material. RKA had given the guidance during the study. MAK, AI and RKA drafted the manuscript and revised the manuscript. All author read and approved the final version of manuscript.

Acknowledgements

Authors are thankful to Dean, CSK HPKVV Himachal Pradesh Agricultural University, Palampur for their assistance in the completion of this study.

Competing interest

The authors declares that they have no competing interest.

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