

Study of Toxic Interaction between Fumonisin-B₁ and Ochratoxin-A for Certain Serum Biochemical Parameters in Japanese Quail

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ABSTRACT

A total of 300 one day old quail chicks (*Coturnix coturnix japonica*) were divided into 4 groups (3 replicates per treatment), viz. control (CX), fumonisin B₁ (FX), ochratoxin A (OX) and combination (FO) containing 75 birds in each group. Birds in the control group (CX) were fed quail mash alone, where as birds in group FX were fed 200 ppm of fumonisin B₁ (FB₁) from *Fusarium verticillioides* culture material; group OX was fed 2 ppm of ochratoxin A from *Aspergillus ochraceus* NRRL-3174; and group FO was fed a combination of 200 ppm of FB₁ and 2 ppm of OTA. Diets were fed from day 1 to 28 to study serum biochemistry. Birds in general showed anorexia, dullness, diarrhoea, depression in body weight, poor feathering and incoordinated movements. Significantly increased serum creatinine and uric acid values were observed in our study.

Key words: Serum biochemistry, Japanese quail, *Fusarium verticillioides*, Ochratoxin A.

INTRODUCTION

The impact of fungal toxins on animals and poultry extends far beyond the obvious effect of causing death. The economic impact of lowered productivity, lower weight gain, decreased feed efficiency, increased disease incidence because of immune system suppression, is many times greater than that of immediate morbidity and mortality. Besides injurious effects in animals, these fungal toxins pose food borne hazards to humans as the toxic metabolites are finally excreted through meat and eggs leading to human health hazards. In a recent global disease survey, mycotoxins have been regarded as one of the top ranking health concerns in Asia (Sluis and Hunton, 2000). A

number of fumonisins have since been isolated and characterized, but FB₁ remains the most toxic compound (Gelderblom *et al.* 1992). FB₁ either in naturally contaminated maize or maize-based feeds or in purified form, has been reported to cause equine leukoencephalomalacia (Marasas *et al.* 1988), porcine pulmonary edema and hydrothorax syndrome (Harrison *et al.* 1990). FB₁ also causes liver toxicity and liver cancer in rats, and atherosclerosis in monkeys (Norred, 1993).

Ochratoxins (OTA), produced mainly by *Aspergillus ochraceus* (now called *A. alutaceus*) and *Penicillium verrucosum*, causes significant losses 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 (Pecham *et al.*, 1971). OTA induces degenerative changes and an increase in weight of the kidneys and liver, as well as decrease in weight of lymphoid organs (Stoev *et al.*, 2002).

The majority of reports on the toxic effects of FB₁ in avian species pertain to chickens and turkeys, in which it has been found when fed at high levels to be associated with reduced body weight and feed intake, diarrhea, poor performance, and alterations in hematological and biochemical parameters with increased activity of the enzymes alanine transaminase and aspartate transaminase (Bermudez *et al.* 1996; Espada *et al.* 1997; Henry *et al.* 2000). Unlike aflatoxins and ochratoxins, the susceptibility of quail to FB₁ is not well known. In earlier studies we reported the effects of feeding *Fusarium verticilloides* culture material (FCM) supplying 150 ppm of FB₁ to Japanese quail (Deshmukh *et al.* 2005b, c). The present study was undertaken to determine the effects of feeding FCM containing 200 ppm of FB₁ and *Aspergillus ochraceus* culture material (ACM) containing 2 ppm of OTA in relation to Serum biochemistry in Japanese quail.

MATERIAL AND METHODS

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). The quail chicks were maintained on chick mash (Quail mash procured from 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. The feed

samples were found to contain 12 ppb of aflatoxin-B₁ and were free from ochratoxin-A, citrinin, zearalenone, aflatoxin-B₂, aflatoxin-G₁, aflatoxin-G₂ and T-2 toxin. The mycotoxins for the present studies i.e. fumonisin-B₁ and ochratoxin-A was supplied by *Fusarium verticilloides* M-1325 culture material (FCM) and *Aspergillus ochraceus* NRRL-3174 (Courtesy: Dr. G. E. Rottinghaus, University of Missouri, Columbia, USA). *Fusarium* culture material containing 6200 mg FB₁ 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 FB₁ and 2 ppm OTA, respectively. The fumonisin culture material and ochratoxin culture material were not incorporated in the diet of control group (CX).

Three hundred, one-day-old Japanese quail chicks were randomly divided into four groups i.e. FX (fumonisin B₁), OX (ochratoxin-A), FO (FB₁+ochratoxin A), and CX (control) with 75 birds apiece in each of the 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 Table 1.

After weighing, 2 to 3 ml of blood was collected via cardiac puncture from randomly selected nine birds (three quail per replicate) from each treatment group at 7, 14, 21 and 28 days post-feeding for determination of uric acid and creatinine. All the biochemical determinations were done using diagnostic kits (Bayer Diagnostics India Ltd., Baroda, India) in a fully automated Blood Chemistry Analyzer (RA-50 Auto Chemistry System) according to the manufacturer's recommendations. The birds were euthanized by cervical dislocation after collection of blood samples at each of the aforementioned intervals.

RESULTS AND DISCUSSION

Biochemical parameters revealed a significant variation between quail fed chick mash alone and those given culture material amended diets. Mean values of creatinine for each of the experimental groups are presented in (Table 2).

They were significantly higher in groups FX and OX than those in groups CX and FO at 7 DPF. The mean creatinine values for groups OX and FO were more or less comparable at 14 DPF and onwards. At 28 DPF, however, the serum creatinine levels in groups OX and FO were found to be significantly higher ($P \leq 0.05$) than that in group CX. The mean treatment effect across the time period revealed a significantly higher mean creatinine values in group FX as compared with the other groups (CX and FO). The overall treatment effect in relation to the progressing age of birds was found to be highly significant ($P \leq 0.01$). Significantly increased serum creatinine values as observed in our study were also reported in turkey poult after feeding 300 mg/

kg fumonisin B₁ and 3 mg/kg ochratoxin A for 20 days (Kubena *et al.*, 1997). While studying the interaction between fumonisin B₁ and moniliformin, Sharma *et al.* (2008) noted a significantly higher mean serum creatinine values in the combination (fumonisin B₁ + moniliformin) group at 28 DPF.

The mean uric acid concentration was significantly higher ($P \leq 0.05$) in group OX and FX at 7 DPF when compared with groups CX and FO. The mean values in groups OX and FO were higher at subsequent intervals but the difference was found to be statistically significant ($P \leq 0.05$) only in the combination group (FO) from that of group CX at 21 DPF (Table 3). The mean treatment effect at the

Table 1: 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	FB ₁ alone	3.25 %	200
OX	OTA alone	2.5%	2
FO	FB ₁ and OTA	3.25% and 2.5%	200 FB ₁ + 2 OTA

Table 2: Effects of fumonisin B₁ and ochratoxin A on creatinine levels (mg/dl) in the serum of Japanese quail¹

Group	Days post-feeding				Mean treatment effect
	7	14	21	28	
CX	0.57±0.10 ^b	0.63±0.11 ^a	0.60±0.08 ^{ab}	0.60±0.08 ^b	0.60±0.04 ^z
FX	1.97±0.27 ^a	0.49±0.06 ^a	0.69±0.04 ^a	0.71±0.06 ^{ab}	0.97±0.12 ^x
OX	1.54±0.24 ^a	0.77±0.11 ^a	0.40±0.06 ^b	0.83±0.03 ^a	0.88±0.09 ^{xy}
FO	0.66±0.19 ^b	0.77±0.10 ^a	0.56±0.06 ^{ab}	0.84±0.02 ^a	0.71±0.05 ^{yz}
Mean ageEffect	1.18±0.14 ^A	0.66±0.05 ^B	0.56±0.03 ^B	0.74±0.03 ^B	
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 ($P \leq 0.05$).

^{x-z}Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P \leq 0.05$).

^{HS}F-value indicating interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P \leq 0.01$).

^{A-B}Values within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P \leq 0.05$).

¹Data 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 3: Effects of fumonisin B₁ and ochratoxin A on uric acid levels (mg/dl) in the serum of Japanese quail¹

Group	Days post-feeding				Mean treatment effect
	7	14	21	28	
CX	11.28±0.79 ^b	8.27±0.82 ^a	7.23±1.35 ^b	6.12±1.33 ^a	8.23±0.62 ^y
FX	16.28±1.34 ^a	10.23±1.38 ^a	7.81±0.51 ^{ab}	5.64±0.98 ^a	9.99±0.85 ^{xy}
OX	16.51±0.71 ^a	10.64±1.94 ^a	9.68±1.59 ^{ab}	7.18±1.03 ^a	11.00±0.88 ^x
FO	11.82±0.45 ^b	10.31±1.10 ^a	12.23±2.25 ^a	8.65±2.39 ^a	10.75±0.86 ^x
Mean ageeffect	13.97±0.59 ^A	9.86±0.66 ^B	9.24±0.81 ^B	6.90±0.76 ^C	
Age x treatment effect					1.70 ^{NS}

^{a-b}Values within columns (between groups CX, FX, OX and FO) with different superscripts are significantly different by ANOVA ($P \leq 0.05$).

^{x-y}Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P \leq 0.05$).

^{NS}F-value indicating interaction between different treatments and age of quail chicks (NS = non significant) by ANOVA ($P \leq 0.05$).

^{A-C}Values within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P \leq 0.05$).

¹Data are means \pm 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

conclusion of the experiment across the age revealed a significantly higher ($P \leq 0.05$) mean uric acid values in the groups OX and FO as compared to that of control group (CX) (Table 4 and Fig. 8). The mean age effect revealed that uric acid values were maximum at 7 DPF and minimal at 28 DPF. The overall treatment effect in relation to the progressing age of birds was found to be non-significant.

Increased serum uric acid values as observed in our study coincided with the observations made by Kubena *et al.* (1997), who noticed a significant increase in serum uric acid

values in turkey poults after feeding 300 mg/kg fumonisin B₁ and 3 mg/kg ochratoxin A. In a study on interaction between fumonisin B₁ and aflatoxin, Kubena *et al.* (1994) reported significantly higher serum uric acid values in the combination group of turkey poults.

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