



Effect of varying levels of dietary ochratoxin A on the performance of broiler chickens

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ABSTRACT

In the present study, the effect of varying levels of ochratoxin A (OTA) in the diet of broiler chickens was investigated. Day-old broiler chicks (200) were divided into 5 treatment groups (T₁- control; T₂ – T₁ + 100 ppb OTA; T₃ – T₁ + 200 ppb OTA; T₄ – T₁ + 300 ppb OTA; and T₅ – T₁ + 400 ppb OTA. Each diet was fed to 4 replicated groups of 10 birds each from day-old to 42 days of age. During overall growth period (0–6 weeks), body weight gain (BWG) in T₃, T₄ and T₅ was significantly lower than that of control group, whereas, the BWG of group T₂ was statistically similar to that of control. OTA contamination at 200 ppb or higher resulted in significant reduction in BWG of broiler chicken. The overall feed intake (FI) in T₅ was significantly lower than that of control group (T₁). The FI in groups T₂, T₃ and T₄ was statistically similar to that of control. OTA contamination at 200 ppb or higher resulted in poor FCR and thus deteriorating feed utilization efficiency in broilers. OTA at 200 ppb or at higher level resulted in increased mortality. Addition of 100 ppb OTA in diet did not produce any change in relative weights of liver, kidney and bursa of Fabricius, however, OTA contamination at 200 ppb or higher resulted in increased relative weights of liver and kidney; and reduced relative weight of bursa of Fabricius. Inclusion of 100 ppb OTA in diet did not produce any change in biochemical parameters of broiler chicken also. It was concluded that ochratoxin A up to 100 ppb in diet had no adverse effect on growth performance, organ weight and certain biochemical parameters in broiler chicken.

Key words: Broiler, Chicken, Feed, Ochratoxin, Tolerance level

Mycotoxins are a group of structurally diverse secondary metabolites of fungi that occur as contaminants of grains worldwide. *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium* and *Penicillium* species of fungi are ubiquitous in nature and under ideal conditions often infect economically important crops during storage, shipment and processing. Many secondary metabolites produced by these fungi can cause serious health problems in poultry and their presence in agricultural commodities may result in serious economic losses. Ochratoxin A (OTA) is a mycotoxin produced mainly by *Aspergillus ochraceus* and *Penicillium verrucosum*. The family of ochratoxins consists of 3 members, viz. ochratoxin A, B and C but ochratoxin A (OTA) is the most toxic one (Chang *et al.* 1979). They are the second major group of mycotoxins characterized after the discovery of aflatoxins. OTA is an isocoumarin derivative linked through the carboxy group to a L-β-phenylalanine (Engelhardt *et al.* 1999). Ochratoxin A is a natural contaminant of animal feedstuffs (Binder *et al.* 2007, Schiavone *et al.* 2008, Pozzo *et al.*

2010), and documented field outbreaks of ochratoxicosis, as well as experimental feeding trials with OTA-contaminated feeds, have indicated its detrimental effects on chicks (Santin *et al.* 2002, Stoev *et al.* 2002, Elaroussi *et al.* 2008, Hanif *et al.* 2008) and its potential risk for the poultry industry (Zaghini *et al.* 2007, Birü *et al.* 2002). Ochratoxin is absorbed into the body and is distributed at a high concentration in the kidney. It inhibits the synthesis of proteins, DNA and RNA in the cell. It shows renal toxicity by inhibiting various enzyme activities in the kidney. Also, OTA induces degenerative changes and an increase in the weight of the kidney and liver, as well as a decrease in the weights of the lymphoid organs (Stoev *et al.* 2000, Stoev *et al.* 2002, Elaroussi *et al.* 2006, Elaroussi *et al.* 2008). The toxic effects of ochratoxin are greatly influenced by the nature of the diet, age and species of animals (Santin *et al.* 2002). The objective of this study was to determine the tolerance level of ochratoxin A in the diets of broiler chickens.

MATERIALS AND METHODS

Ochratoxin production: The lyophilised preparation of *Aspergillus westerdijkiae* NRRL 3147 was obtained from U.S. Department of Agriculture, Peoria, Illinois (USA). This lyophilised preparation was revived on potato dextrose agar

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medium and used for experimentation. Ochratoxin was produced (Singh *et al.* 2013). Cracked maize (50 g) was taken in 250 ml conical flasks. The moisture content of substrate was adjusted to have a moisture level of 35%. Thus flasks were plugged with non-absorbent cotton and sealed with aluminium foil. The flasks were autoclaved for 20 min at 121°C and inoculated with 1-week old spores of *Aspergillus westerdijkiae* NRRL 3174. The inoculated flasks were incubated in a BOD incubator for 14 days. After removal from the incubator, the flasks were dried at 70°C and the ochratoxin assays were performed as per AOAC (1995).

Experimental design: Experimental design was completely randomized design (CRD). There were five dietary treatments. Each dietary treatment had 4 replicates and each replicate had 10 chicks. The experiment was conducted in broiler chickens from day-old to 6 weeks of age. The various dietary treatments were prepared by mixing the required quantity of mouldy maize to get the desired concentration of OTA in basal diet. Experimental diets included T₁ control (basal diet), T₂ (basal diet + 100 ppb OTA), T₃ (basal diet + 200 ppb OTA), T₄ (basal diet + 300 ppb OTA), and T₅ (basal diet + 400 ppb OTA).

Biological experiment and analysis: Day-old broiler chicks (200) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into 5 groups. All birds were reared under standard managemental conditions from 0–6 weeks. All birds were fed with broiler starter ration from 1–21 days and broiler finisher ration from 22 to 42 days. The basal diet was prepared using maize, soybean meal and rapeseed meal. The starter diet (0–21 days of age) contained 21.5% protein, 2,890 kcal ME/kg, lysine 1.28%, methionine 0.52%, calcium 1.02% and available P 0.45%. The corresponding values in finisher diet were 19.02%, 29,991 kcal/kg, 0.93%, 0.39%, 1.09% and 0.39%. Body weight of individual birds and feed consumption of each replicate were recorded at weekly interval. The protein (AOAC 1995) and calcium (Talapatra *et al.* 1940) contents were estimated, while the concentrations of lysine, methionine, available P and metabolizable energy values were calculated. At the end of the experiment, 8 birds/dietary treatment were sacrificed randomly and their organs and blood samples were collected. The serum was separated and analysed for various biochemical parameters using commercial kits. The statistical analysis was done using SPSS 16.0 version.

RESULTS AND DISCUSSION

Body weight gain: Significant ($P<0.05$) differences in body weight gain (BWG) among various treatments were observed from first week of age (Table 1), wherein the BWG in T₅ was lower ($P<0.05$) than that of control group (T₁), however, the BWG in T₂, T₃ and T₄ was statistically similar to that of control. At second and third weeks of age, the BWG in T₂ was statistically similar to that of control, however the BWG in T₃, T₄ and T₅ was lower ($P<0.05$) than that of control group. Similar trend was observed during fourth, fifth and sixth weeks of the growth period. During overall growth phase (0–6 weeks), the average BWG in T₃, T₄ and T₅ was significantly ($P<0.05$) lower than that of control group, whereas, the BWG of group T₂ was statistically similar to that of control. In the present investigation, OTA contamination at 200 ppb or higher resulted in significant ($P<0.05$) reduction in BWG of broiler chickens. These results were in agreement with earlier workers who reported significant reduction in BWG at 50 to 800 ppb level of dietary ochratoxin (Sakhare *et al.* 2007, El-Barkouky *et al.* 2010, Hanif *et al.* 2008, El-Barkouky and Abu-Taleb 2008, Santin *et al.* 2006, Hatab 2003 and Elaroussii *et al.* 2006). In the present study, addition of 100 ppb OTA did not produce any significant change in BWG of broiler chicks, indicating that broilers can tolerate up to 100 ppb OTA in their diet. Pozzo *et al.* (2013) also reported that feeding broiler chickens, a diet contaminated with 100 ppb OTA did not affect daily weight gain of broiler chickens. However, further study is required to ascertain the safe level between 100 and 200 ppb of OTA.

Feed consumption: At first, fourth, fifth and sixth weeks of age, the feed intake (FI) did not differ significantly ($P<0.05$) among various dietary treatments (Table 2). At second and third weeks, the FI of group T₂ was statistically similar to that of control (T₁), however, the feed consumption of groups T₃, T₄ and T₅ was lower ($P<0.05$) than that of control group. During overall growth phase (0–6 weeks), the FI in T₅ was significantly ($P<0.05$) lower than that of control group (T₁). The FI in groups T₂, T₃ and T₄ was statistically similar to that of control. In the present study, inclusion of 400 ppb OTA in diet of broilers resulted in significant reduction in feed consumption. These results were in agreement with earlier workers who reported significant reduction in FI at 200 to 800 ppb level of dietary ochratoxin (El-Barkouky *et al.* 2010, Hanif *et al.* 2008, Santin *et al.* 2006, Hatab 2003 and Elaroussii *et al.* 2006).

Table 1. Effect of ochratoxin on body weight gain (g/bird) of broiler chicks between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0–6
T ₁	111.9±0.62 ^b	205.2±2.96 ^b	363.0±3.88 ^c	316.8±24.66 ^b	361.8±24.66 ^b	479.6±29.44 ^b	1738.5±77.88 ^b
T ₂	112.3±0.79 ^b	204.7±2.47 ^b	260.6±4.36 ^c	351.3±24.81 ^b	351.3±24.81 ^b	475.3±29.44 ^b	1713.4±77.90 ^b
T ₃	112.5±1.39 ^b	159.0±3.34 ^a	213.2±3.79 ^b	282.6±20.61 ^a	282.6±20.61 ^a	352.6±25.03 ^a	1356.9±62.13 ^a
T ₄	108.9±1.38 ^{ab}	153.0±3.33 ^a	203.5±3.79 ^{ab}	268.4±20.47 ^a	268.4±20.47 ^a	334.7±25.14 ^a	1293.6±62.16 ^a
T ₅	107.6±1.36 ^a	150.6±3.34 ^a	195.2±3.80 ^a	252.6±20.62 ^a	252.6±20.62 ^a	316.6±25.02 ^a	1235.4±62.18 ^a

Table 2. Effect of ochratoxin on feed consumption (g/bird) of broiler chicks between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0-6
T ₁	172.8±0.97	328.7±3.06 ^b	454.4±2.48 ^c	628.7±34.58	758.0±45.45	1019.9±74.17	3362.7±161.18 ^b
T ₂	174.0±1.52	332.6±4.47 ^b	456.8±4.48 ^c	624.5±35.28	725.1±45.42	1024.6±74.19	3364.8±161.17 ^b
T ₃	175.9±1.20	303.7±2.44 ^a	435.0±4.65 ^b	544.4±34.75	681.7±39.99	867.7±50.36	3020.4±114.42 ^{ab}
T ₄	171.4±0.78	296.5±2.44 ^a	423.7±4.83 ^{ab}	529.9±34.71	663.8±39.99	841.3±50.34	2926.8±112.98 ^{ab}
T ₅	171.1±1.20	295.2±2.47 ^a	417.0±4.61 ^a	520.4±34.74	652.5±40.00	839.5±58.89	2896.0±121.45 ^a

Table 3. Effect of ochratoxin on FCR of broiler chicks between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0-6
T ₁	1.54±0.01	1.59±0.00 ^a	1.72±0.01 ^a	1.98±0.01 ^a	2.09±0.02 ^a	2.11±0.02 ^a	1.92±0.01 ^a
T ₂	1.54±0.01	1.62±0.01 ^a	1.75±0.02 ^a	2.02±0.03 ^a	2.14±0.04 ^a	2.15±0.03 ^a	1.96±0.02 ^a
T ₃	1.53±0.01	1.91±0.04 ^b	2.03±0.04 ^b	2.29±0.05 ^b	2.42±0.03 ^b	2.46±0.04 ^b	2.21±0.02 ^b
T ₄	1.57±0.02	1.93±0.04 ^b	2.07±0.04 ^b	2.35±0.06 ^{bc}	2.47±0.05 ^{bc}	2.51±0.05 ^b	2.26±0.03 ^b
T ₅	1.58±0.03	1.95±0.04 ^b	2.13±0.05 ^b	2.44±0.07 ^c	2.59±0.06 ^c	2.65±0.05 ^c	2.34±0.03 ^c

Feed conversion ratio: Significant ($P<0.05$) differences in feed conversion ratio (FCR) among various dietary treatments were recorded from second week of age onward (Table 3). From second to sixth week of age, the FCR of group T₂ was statistically similar to that of control (T₁) however the FCR in groups T₃, T₄ and T₅ was higher ($P<0.05$) than that of control. In overall FCR (0–6 weeks), the FCR of control group (T₁) was 1.92 which significantly ($P<0.05$) increased to 2.21, 2.26 and 2.34 in T₃, T₄ and T₅, respectively, due to ochratoxin contamination. The FCR of group T₂ (1.96) was statistically similar to that of control. OTA contamination at 200 ppb or higher resulted in increased FCR, thus deteriorating feed utilization efficiency of broilers. Similar observations of reduced feed efficiency were also made by earlier workers (Gibson *et al.* 1989, El-Barkouky *et al.* 2010, Hanif *et al.* 2008). In the present study, addition of 100 ppb OTA in the diet did not bring out any significant change in FCR of broiler chickens.

Livability: The livability percentage was not affected in basal diet and basal diet with 100 ppb OTA. However, the livability percentage decreased at OTA levels above 100 ppb level (93.5%, 91.6% and 88% livability in diets with OTA level of 200, 300 and 400 ppb, respectively). Moreover, mortality started from second week of age. However, El-Barkouky and Abu-Taleb (2008) reported that contamination of broiler diet with OTA at 50 and 100 µg/kg diet resulted in a significant increase in mortality rate of broilers. El-Barkouky *et al.* (2010) observed significant increase in mortality in broiler chicks fed on OTA contaminated diet at a level of 200 ppb for 5 weeks of age. Prakash *et al.* (2000) observed significant increase in mortality percentage in broiler chicks fed dietary supplementation with 2 ppm OTA for 6 weeks. Increased mortality due to ochratoxicosis in poultry was also reported by Hamilton *et al.* (1982), and Bodnarchuk and Kaspruk

Table 4. Effect of ochratoxin on relative weights of organs (% of live weight) fed different dietary treatments

Treatments	Liver	Kidney	Spleen	Bursa
T ₁	2.53±0.05 ^a	0.69±0.04 ^a	0.31±0.02	0.29±0.02 ^b
T ₂	2.50±0.04 ^a	0.71±0.05 ^a	0.29±0.02	0.30±0.01 ^b
T ₃	2.91±0.05 ^b	1.12±0.06 ^b	0.33±0.03	0.20±0.03 ^a
T ₄	2.90±0.06 ^b	1.15±0.05 ^b	0.31±0.03	0.21±0.02 ^a
T ₅	3.02±0.08 ^b	1.19±0.07 ^b	0.33±0.02	0.20±0.02 ^a

(1984). The results of the present study showed that broiler chicken can tolerate 100 ppb of OTA in their diet without any adverse effect on survivability.

Organ weights: The relative weights of liver and kidney (Table 4) in groups T₃, T₄ and T₅ was higher ($P<0.05$) than that of control (T₁), however, the relative weights of liver and kidney in group T₂ was statistically similar to that of control. In the present study, OTA contamination at 200 ppb or higher resulted in increased liver and kidney weights. Stoev *et al.* (2004) observed a significant increase in relative weight of liver in broiler chicks fed a diet contaminated with gradual concentration of OTA at 130, 300 or 800 ppb. Gibson *et al.* (1989), Huff *et al.* (1992) and Elkady (1993) also reported significant increase in the relative liver weight at OTA levels ranging from 2 to 4 mg/kg. The effects of OA were dose and time-dependent. Verma *et al.* (2004) and Hanif *et al.* (2008) reported a significant increase in the relative weight of kidney when broilers were fed with OTA at a dietary levels of 0.5, 1, 2 and 4 ppm over 42 day period. El-Barkouky *et al.* (2010) also reported significant increase in relative weight of kidney at OTA level of 200 µg/kg feed from one-day old to 5 weeks of age in broiler chicks.

The relative weight of spleen did not vary significantly ($P<0.05$) among different dietary treatments. The relative weights of bursa of Fabricius in groups T₃, T₄ and T₅ was

Table 5. Effect of ochratoxin on serum biochemical parameters fed different dietary treatments

Treatments	Total protein (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Alkaline phosphatase (U/L)
T ₁	6.89±0.18 ^b	195.86±7.55 ^b	8.20±0.27 ^a	201.01±12.16 ^a
T ₂	6.70±0.22 ^b	190.01±6.86 ^b	7.99±0.23 ^a	204.21±10.12 ^a
T ₃	5.36±0.16 ^{ab}	174.02±5.98 ^b	8.39±0.30 ^a	250.24±14.82 ^b
T ₄	5.42±0.13 ^{ab}	131.02±7.15 ^a	10.26±0.15 ^b	274.52±13.91 ^c
T ₅	5.13±0.26 ^a	125.02±6.76 ^a	10.86±0.21 ^b	281.56±10.86 ^c

lower ($P<0.05$) than that of control (T₁), however, the relative weights of bursa of Fabricius in group T₂ was statistically similar to that of control. In the present study, addition of 100 ppb OTA in diet did not produce any change in relative weights of bursa of Fabricius, however, OTA contamination at 200 ppb or higher resulted in increased relative weights of bursa of Fabricius. Stoev *et al.* (2004) observed a significant increase in relative bursal weight in broiler chicks fed a diet contaminated with gradual concentration of OA at 130, 300 or 800 µg/kg feed. Kumar *et al.* (2004), Gupta *et al.* (2008) and Verma *et al.* (2004) also recorded significant reduction in relative weight of bursa of Fabricius due to OTA levels ranging from 2 to 4 mg/kg diet in broiler chickens. In the present study, addition of 100 ppb OTA did not produce any significant change in organ weights of broiler chicks, indicating that broilers can tolerate up to 100 ppb OTA in their diet. Pozzo *et al.* (2013) also reported that feeding broiler chickens, a diet contaminated with 100 ppb OTA did not produce any effect on organ weights of broiler chickens.

Biochemical parameters: The total serum protein in groups T₂, T₃ and T₄ was statistically similar to that of control, however, the total protein in group T₅ was significantly ($P<0.05$) lower than that of control (Table 5). The results showed that inclusion of 400 ppb OTA in the diet of broiler chicks decreased ($P<0.05$) the total serum protein content. The present finding is in agreement with Manning and Wyatt (1984), Ramadevi *et al.* (2000) and Stoev *et al.* (2000), who reported decreased serum proteins during induced ochratoxicosis in broilers. With regard to serum cholesterol content, it did not differ ($P<0.05$) among groups T₁, T₂ and T₃, however, it was significantly ($P<0.05$) lower in groups T₄ and T₅ compared to that of control. In the present study, there was a significant reduction in serum cholesterol level at 300 ppb or more OTA concentration in feed. In respect of serum cholesterol during ochratoxicosis, a similar trend was reported earlier by Manning and Wyatt (1984), Ramadevi *et al.* (2000) and Stoev *et al.* (2000). The serum uric acid level was statistically similar among groups T₁, T₂ and T₃, however, it was significantly ($P<0.05$) higher in groups T₄ and T₅ compared to that of control.

In the present study, there was a significant elevation in serum uric acid level at 300 ppb or more dietary OTA concentration. The present finding is in agreement with those of Manning and Wyatt (1984) and Ramadevi *et al.* (2000) indicating that there was inhibition of protein accretion due to OTA, which might have the reason of lower body weight gains in broilers fed higher level of OTA. The

activities of alkaline phosphatase in groups T₃, T₄ and T₅ was higher ($p<0.05$) than that of control (T₁), however, the activities of alkaline phosphatase in group T₂ was statistically similar to that of control. The addition of 100 ppb OTA in diet did not produce any change in activities of alkaline phosphatase, however, OTA contamination at 200 ppb or higher resulted in increased activities of alkaline phosphatase. Hanif *et al.* (2008) also reported increased alkaline phosphatase due to ochratoxicosis in broiler chicks. In the present study, addition of 100 ppb OTA in diet did not produce any change in biochemical parameters. These results were in agreement with earlier report of Pozzo *et al.* (2013) who reported that 100 ppb dietary OTA did not alter biochemical parameters in broilers.

It was concluded that broiler chicken can tolerate 100 ppb of dietary ochratoxin without any adverse effects on their performance, organ weight and biochemical parameters. However, further study is required to establish its tolerance level by incorporating OTA at different levels in between 100 and 200 ppb.

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