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# Effect of dietary ochratoxin on body weight and biochemical changes in broiler chicks

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**Abstract**
Ochratoxin A (OTA) is the second most mycotoxin contamination after aflatoxin in poultry industry. It is due to ingestion of OTA contaminated feed causes acute ochratoxicosis in poultry. It poses a great threat to the lives of poultry, animals and humans. It has hepatotoxic, nephrotoxic, immune suppression and teratogenicity effects in birds. The present study was designed to evaluate the OTA effects on FCR, body weight gains and blood biochemical parameters of broilers. For this day old broilers were divided into two equal groups and were given OTA at 2 ppm and without OTA for six weeks. Results indicated that feeding OTA alone caused reduction in body weight gain, poor FCR, increased gross lesions, altered relative organ weights and serum biochemical values.

**Keywords:** Broilerchicks, Ochratoxin A, Serum biochemical parameters, body weight gains and feed conversion ratio

## 1. Introduction
Ochratoxin is a term that can include three toxins Ochratoxin A, B and C (Vander Merwe *et al.*, 1965a; Steyn and Holzapfel, 1967). Among three variants Ochratoxin A which is the most common is 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3R-methylisocoumarin linked by an amide bond of the 7-carboxyl group to L-p-phenylalanine.The name OTA is derived from its source by Aspergillus ochraceus (Vander Merwe *et al.*, 1965b). Comparatively OTA is easily grown on feed stuffs of birds and posses a great threat to the human and poultry industry by increased mortality and reduced body weight gain. Several toxigenic fungal strains of *Aspergillus* (Ghosh *et al*. 2015) [16] and *Penicillium*, the most important being *Aspergillus ochraceus*, are being involved in the production of ochratoxin.Ochratoxin A displays a multiple toxicity, including immunotoxicity (Daniela Marin and Ionelia Tarenu, 2014). Ochratoxins are considered to be the main reason for causing a serious anomaly namely Balkan Endemic Nephropathy (BEN) in humans exposed to its dietary exposure(Zahoor-ul-Hassan *et al.*2012;Solcan *et al.*2015;Iftikar *et al*.2015;Ben Salah-Abbes *et al.*2015) [46, 39, 22, 4].
Ochratoxin A (OTA) is produced mainly by *Aspergillus ochraceus*in tropical and warmer region and *Penicillium verrucosum* in temperate and cold areas. The family of ochratoxins consists of three members, viz.Ochratoxin A, B and C but OTA is the most toxic one.OTA is an isocumarin derivative linked through the carboxyl group to a L-β-Phenylalanine (Engelhard *et al*. 1999).Ochratoxin is absorbed into the body and is distributed at a high concentration in the kidney. It shows renal toxicity by inhibiting various enzyme activities in the kidney (Stoev *et al.* 2000; Stoev *et al*. 2002; Elaroussi *et al*. 2006; Elaroussi *et al*. 2008) [24, 41, 42, 10].
Live yeast addition to animal feed has been known to improve the nutrient quality of feed and performance of animals (Santin *et al*.2002; Brake, 1991; Moore *et al*.1994; Pagan, 1990; Day, 1997;Onifade and Babatunde, 1996) [37, 6, 29, 32]. Whole yeast products or yeast cell wall components have been used to improve growth and affect the physiology ,morphology and microbiology of the intestinal tract of turkey (Badleyetal., 1994; Hooge, 2004b; Sisetal., 2004; Zdunczyketal., 2004; 2005; Huff *et al*., 2007; Rosen, 2007b; Soils De Los Santos *et al*. 2007; Huff *et al* .2010.) [5, 18, 40, 36]. Therefore, yeast culture in dried form is used in the present work to evaluate its effects on the performance, biochemical parameters and the internal organ weights of broiler chicks when fed on OTA contaminated diets.

## 2. Materials and Methods
**2.1** Production, extraction and quantification of OTA: The culture used for production of OTA *A. ochraceus* was obtained from MMTC (Institute of Microbial Technology), Chandigarh.

The OTA was grown on oat meal agar slants at 28˚C for 2 weekson large scale as per Trenk *et al*.(1971).OTA was extracted and quantified using column chromatography and TLC, respectively as per AOAC (1995).

### 2.2 Experimental Design

A total of 100 male day old commercial broiler chicks, were divided at random into 2 groups. In each group 5 replications each of 10 birds was maintained. All the chicks were fed with basal diet from 0-3 weeks and finisher diet from 4-6 weeks.The two groups of chicks were fed the following 2 diets at random

1. Control
2. Ochratoxin A 2ppm

### 2.3 Housing and Management

All the chicks were housed in battery brooders with feeding and watering arrangements. Each replicate group of chicks were housed in one pen. Chicks in all the replicate groups were reared under uniform standard conditions throughout the experiment. Brooding was done till three weeks of age using incandescent bulbs. All the chicks were weighed at the end of every week using digital electronic balance. Weekly feed consumption was recorded replicate wise in all the experimental groups. The number of dead birds in each replicate was recorded. Thorough post mortem examination was conducted on dead birds and noted the lesions due to different experimental feeds.

**Table 1:** Feed composition

| S. No | Ingredients | Basal diet | Finisher diet |
|---|---|---|---|
| 1 | Maize | 58 kg | 63 kg |
| 2 | Soya bean | 34 kg | 39kg |
| 3 | D.C.P | 1.5 kg | 1.5kg |
| 4 | L.S.P | 0.3 kg | 1.5kg |
| 5 | Salt | 0.1 kg | 0.3kg |
| 6 | Lysine | 0.25 kg | 0.1kg |
| 7 | Methionine | 0.10 kg | 0.25kg |
| 8 | T.M.M | 10g | 0.10kg |
| 9 | MnSo4 | 10g | 10g |
| 10 | Zno | 50g | 10g |
| 11 | C.C.50% | 50g | 50g |
| 12 | Cygrow | 10g | 50g |
| 13 | Mineral mix | 10g | 10g |
| 14 | Indomix | 10g | 10g |
| 15 | AB2D3K | 10g | 10g |

### 2.4 Serum Biochemical Profile

Blood was collected in non-heparinized tubes from the birds in each treatment during 0, 21 and 42 days of age. Serum was collected after 8-10 hours and was stored at -20˚C for further analysis. The serum was used to estimate the following parameters.

Total proteins and Albumin (Biuret and BCG dye binding method), Cholesterol (Wybenga and Pileggi method), Creatinine (Alkaline Picrate method), Uric acid (Phospho Tungestic Acid Method), Glucose (O-Toluidine method), ALKP (King and Kings method), AST (Reitmann and Frankle method), ALT (Reitmann and Frankle method), GGT (Kinetic colorimetric method), Triglycerides (GPO method), Calcium (O-Cresolthalein comlexone method) and Phosphorus (Kinetic colorimetric method).

## 3. Results and Discussion

**3.1** Production of Ochratoxin A (OTA)

The colony characteristics of the *A.ochraceus* on oat meal agar slants confirmed to those described for a pure culture and estimated by HPTLC (AOAC, 1995).The OTA content in the culture material was found to be 1200ppmcomparable to the results of Raju (1998).

### 3.2 Performance of broiler chicks

The effective concentration was incorporated into the poultry diet and its effect on serum biochemical profile and vital organs was studied. The mitogen, ochratoxin A (OTA) was used at the rates of 2 ppm 2.0mg/kg in this study for a period of 42 days and the selection of this level was made on the basis of different available studies (Santin *et al*, 2002; Elaroussi *et al*, 2006; Khatoon *et al*, 2013; Abidin *et al*, 2013; Marin and Taranu. 2015).

#### 3.2.1 Body Weight Gains

The data on the Feed consumption and body weight of broilers in different treatments from 1-6 weeks of age is presented in Table 2.The weight gains of broiler chicks from 1-6 weeks were gradually increased on control diet than diets on Yeast culture alone and OTA + Yeast culture. The weight gains were significantly ($P \leq 0.01$) affected by different diets as well as different periods. Feeding OTA to birds caused an adverse effect on the growth of the birds which could be well attributed by a decreased body weight and poor FCR as observed in this study in OTA treated birds. Decreased body weights and poor FCR in OTA intoxicated birds have been reported bymany scientists (Kubena *et al*. 1988; Ramadevi 1993; Verma *et al*. 1995; Raju 1998; Stoev *et al*, 2000; Rajeev *et al*, 2003; Verma *et al*, 2004; Koynarski *et al*, 2007) [40]. The reduction in feed intake along with poor FCR could be a possible reason for reduced weight gain in birds (Elaroussi *et al*, 2006).

**Table 2:** Body weight gain

| Period (Weeks) | Parameter | Control | Ochratoxin A | OTA+Yeast Culture |
|---|---|---|---|---|
| 1 to 6 Mean±SE | Feed Consumption(g) | 436.75±54.34 | 230±31.15 | 263.83±53.12 |
| 1 to 6 Mean±SE | Weight gain (g) | 268.42±12.56 | 114.46±5.34 | 148.13±7.33 |

### 3.3 Serum Biochemical Profile

Serum biochemical parameters of all the groups fed on OTA alone and control have been presented in Table 3. Regarding serum biochemical alterations, significant increase in creatinine, ALT, AST, ALP, GGT, GST, glucose and uric acid levels in OTA toxicated birds has also been reported by many workers (Kubena *et al*., 1988; Bailey *et al*., 1989; Gentles *et al*., 1999; Stoev *et al*., 2002) [24, 2, 15, 41]. The increase in these enzymes might be due to severe hepatic damage observed in this study that could have altered hepatocyte membrane integrity leading to leakage of enzymes into circulation and biliary hyperplasia. Similarly, reduction in serum total proteins, albumin and globulin, cholesterol, triglycerides, calcium and phosphorus concentrations in OTA treated birds has also been well reported previously (Bailey *et al*, 1989; Gentles *et al*, 1999; Stoev *et al*, 2002; Garcia *et al*,

2003; Koynarski *et al*, 2007) [2, 15, 41]. The decreased values of total protein and albumin might be due to inhibition of t-RNA synthatase or inhibition of amino acyl tRNA formation indirectly by inhibiting ATP formation in the mitochondria (Hohler, 1998). Albumin leakage through damaged tubular epithelial cells noticed in this study in OA fed group in turn resulted in further loss of albumin causing hypo albuminaemia. Alimentary tract lesions with impairment of digestion and absorption, hepato-renal damage and lymphoid cell depletion observed in this study.

**Table 3:** Biochemical Profile

| Biochemical parameter | Mean ±SE | | |
|---|---|---|---|
| | C | OA | OA +YC |
| Total Protein (g %) | 3.83±0.87 | 2.39±0.1 | 2.76±0.18 |
| Albumin (g %) | 1.67±0.12 | 0.99±0.1 | 1.14±0.01 |
| Globulins (g %) | 2.63±0.81 | 1.51±0.2 | 1.65±0.16 |
| A: G ratio (g %) | 0.72±0.14 | 0.66±0.14 | 0.71±0.1 |
| Cholesterol (mg %) | 138.08±13.67 | 85.00±18.7 | 83.75±3.13 |
| Triglycerides (mg %) | 128.58±18.1 | 84.15±13.21 | 98.75±7.22 |
| AST(IU/L) | 119.91±3.07 | 128.75±3.83 | 119.17±5.21 |
| ALT(IU/L) | 26.69±0.82 | 30.16±2.66 | 29.01±2.49 |
| GGT(IU/L) | 9.55±2.57 | 11.87±3.29 | 11.48±3.15 |
| GST?(U/g) | 2.04±0.02 | 4.09±0.01 | 3.89±0.1 |
| ALP(KA units) | 51.08±3.79 | 57.45±5.43 | 58.67±6.01 |
| Creatinine (mg %) | 0.17±0.1 | 0.25±0.04 | 0.23±0.03 |
| Uric acid (mg %) | 12.68±1.2 | 15.32±1.65 | 14.06±1.42 |
| Glucose (mg %) | 98.17±2.7 | 115.54±8.5 | 112.03±9.3 |
| Calcium (mg %) | 12.59±1.05 | 10.42±0.41 | 11.26±0.7 |
| Phosphorus (mg %) | 8.36±0.77 | 6.99±0.42 | 7.02±0.28 |

## 4. Conclusions

It was concluded that feeding of 2 ppm of ochratoin A toxin from 0 day to 6 weeks of age in broiler chicken resulted in liver and kidney dysfunction,hypoglycaemia and increased oxidative stress.

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