

## OCHRATOXIN-A RESIDUES IN TISSUES OF BROILER CHICKEN UNDER EXPERIMENTAL CONDITIONS

S. GUPTA, N. JINDAL<sup>1</sup> and R. S. KHOKHAR

Department of Veterinary Epidemiology and Preventive Medicine  
College of Veterinary Sciences, CCS Haryana Agricultural University, Hisar -125 004

### SUMMARY

An experimental study was carried out in broiler chicks to estimate ochratoxin-A residues in kidneys and liver tissues by feeding 2.0 ppm ochratoxin-A from *Aspergillus ochraceus* culture material for 35 days. Ochratoxin-A residues of 4.85, 11.87 and 29.5 ppb were detected in kidneys at 21, 28 and 35 days post-feeding, respectively. Ochratoxin-A residues (5.34 ppb) could be detected in liver at 35<sup>th</sup> day post-feeding.

**Key words:** Ochratoxin - A, kidney, liver, broiler chicken

Ochratoxins, the mycotoxins produced by *Aspergillus ochraceus* and *Penicillium viridicatum* are of four major types (A, B, C and D), and ochratoxin - A (OA) is considered to be the most biologically active, toxic and heat stable toxin. It is responsible for causing various ill-effects like decreased body weight and feed intake, increased feed conversion ratio, emaciation, decreased egg production, increased mortality, nephrotoxicity and immunosuppression in poultry (Burns and Dwivedi, 1986, Huff *et al.* 1988). In addition, the presence of OA residues had been reported in various tissues of poultry (Micco *et al.*, 1987, Stoev *et al.*, 2002). The present study was undertaken to estimate the OA residues in kidney and liver of broiler chicks fed ochratoxin contaminated diet under experimental conditions. The present study is a continuation of our earlier work, in which the effects of 2.0 ppm OA were evaluated in relation to growth response, mortality pattern, and certain biochemical changes in the presence of *Salmonella Gallinarum* infection in broiler chickens (Gupta *et al.*, 2005). The interaction between OA and *S. Gallinarum* was studied from day 14 of age through day 35 of age. During this study, OA residues were estimated in tissues of broiler chicks of groups fed broiler mash alone or along with OA.

Twenty four, day-old broiler chicks were randomly divided into group CX and OX having 12 chicks in each group. Birds of group CX served as control and were fed broiler mash alone, whereas birds of group OX were fed OA in the broiler mash for 35 days from day one. *Aspergillus ochraceus* culture material containing 80.0 ppm OA was used in the present study to induce ochratoxicosis in chicks. The culture material containing known levels of OA was incorporated in feed of group OX so as to achieve a level of 2.0 ppm. Liver and kidneys tissues were collected from four birds from each group (groups CX and OX) at 21, 28 and 35 days post-feeding (DPF). The OA contents in these tissue samples were estimated by thin layer chromatography (TLC) following the protocol of Scott (1995). The standard OA solution from crystalline ochratoxin-A (Sigma, U.S.A.) was prepared and primary stock solution contained 21.370 µg/ml OA. Working standard solution was prepared by mixing 1 ml of the primary stock solution with 9 ml of acetic acid: benzene (1:99) solution. The working standard solution containing 2.137 µg/ml OA was used for TLC. Ochratoxin-A concentration in tissue samples was calculated by the following formula:

$$\text{Concentration of OA (}\mu\text{g/kg)} = \frac{S \times Y \times V}{X \times W}$$

<sup>1</sup>Corresponding author

Where, S=il OA standard equal to unknown, Y= concentration of OA standard (ig/ml), V=il of final dilution of sample extract, X=il sample extract spotted giving fluorescent intensity equal to standard, W=sample applied in grams

Ochratoxin-A residue could not be detected in liver and kidneys of broiler chicks fed basal diet alone (group CX). However, OA residues were detected in chicks of group OX. At 21 DPF, OA residues of 4.85 ppb were recorded in kidneys. The amount of residues increased with OA feeding and residues of 11.87 and 29.5 ppb were detected at 28 and 35 DPF, respectively. Ochratoxin-A residues could not be detected in liver tissue of birds upto 28 DPF, however, the residues (5.34 ppb) were detected at 35 DPF. Ochratoxin-A residues were found more in kidneys than liver in the present study. Continuous feeding of OA increased the residues in tissues indicating cumulative nature of the toxin. Ochratoxin residues in tissues of poultry under natural and experimental conditions have been reported by various workers (Prior *et al.*, 1980, Micco *et al.*, 1987, Kaur *et al.*, 1995). Prior *et al.* (1980) on feeding 2.0 ppm OA to broiler chicks for 8 weeks detected OA residues in kidneys (41.0 ppb) and liver (24.0 ppb). More residues in kidneys than liver correlates well with the pathological findings in these tissues of broiler chicks due to OA. More gross and microscopic lesion were observed in kidneys than liver in OA fed birds when compared with birds of control group. Ochratoxin-A seems to produce its toxic effects by one of three major mechanisms (a) inhibition of phenylalanine metabolizing enzymes, thereby affecting DNA, RNA and protein synthesis, (b) promotion of lipid peroxidation by complexing iron, facilitating reduction of iron or (c) inhibition of mitochondrial ATP production by acting as a competitive inhibitor of carrier proteins located in the inner mitochondrial membrane. Ochratoxin-A binds to serum proteins on absorption from gastrointestinal tract. Reabsorption of OA from intestines back to circulation as a consequence of biliary excretion helps in its distribution to various tissues and to its toxicity. Both liver and kidneys are involved in detoxification and elimination of OA from

body. Glomerular filtration might be retarded as OA has high plasma protein binding capacity. Instead, the toxin is excreted through tubules using organic anion transporter proteins (OAT) and is also reabsorbed in all nephron segments using OAT or other transporters. The reabsorption process reduces OA excretion leading to its accumulation in renal tissue and thus contributing to renal toxicity (Dahlmann *et al.*, 1998, Pfohl-Leszkowicz and Manderville, 2007). Ochratoxin-A also affects carbohydrate metabolism, particularly gluconeogenesis in kidneys by reducing the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK). Interference with this rate-limiting step plays a key role in the development of functional damage to the kidneys (Ueno, 1991).

In human beings, OA exposure has been often associated with Balkan endemic nephropathy. Patients with this disease also have a high risk of developing urinary system tumors. Though the disease in human beings is primarily due to consumption of cereal grains contaminated with OA, however, the consumption of poultry meat containing OA might also be one of the contributing factors. Therefore, human beings may also be exposed to ill-effects of ochratoxins through ingestion of OA contaminated meat. To minimize the risk of human exposure through meat, regular screening of poultry feed samples for ochratoxins should be carried out. As a preventive measure, Stoev *et al.* (2002) suggested toxicological analysis of a few blood samples from chickens suspected of spontaneous mycotoxic nephropathy a week before slaughter and a change in the feed source for a week. Also, the period of feed deprivation of chickens before slaughter could be prolonged. Because of the short half life of OA, its concentration in chickens in blood and various tissues decreases quickly after change of feed source or after prolonging the period of feed deprivation of chickens.

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