

Nephrotoxicity of Dietary Ochratoxin A in Broiler Chickens¹

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Graded doses of pure ochratoxin A (0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg of toxin per g of feed) were incorporated into a commercial diet which was fed to chicks from 1 day to 3 weeks of age, at which time the experiments were terminated. Growth was inhibited at 2.0, 4.0, and 8.0 $\mu\text{g}/\text{g}$, whereas the kidneys were enlarged at doses of 1.0 $\mu\text{g}/\text{g}$ and above. Renal function as measured by clearance of phenol red was decreased 15 and 31% by doses of 4.0 and 8.0 $\mu\text{g}/\text{g}$, respectively. Uric acid was increased 38 and 48% over the control values by doses of 4.0 and 8.0 $\mu\text{g}/\text{g}$, respectively. The plasma electrolytes Na, Cl, Ca, and K were measured; however, only K was significantly ($P < 0.05$) altered, showing a decrease at doses of 4.0 and 8.0 $\mu\text{g}/\text{g}$. The percentage dry weight of the kidneys decreased significantly at dose levels of 4.0 and 8.0 $\mu\text{g}/\text{g}$, indicative of edema. Histological examination of kidney sections gave the impression of edema and some tubular necrosis. Pathological changes were observed at all dose levels. These data demonstrate that ochratoxin A is a severe nephrotoxin in young broiler chickens.

Ochratoxins include a group of structurally related, secondary metabolites produced by seven species of *Aspergillus* and six species of *Penicillium* (5). *Aspergillus ochraceus*, from which the toxins acquired their name, appears to be the predominant ochratoxin producer (5). Ochratoxin A, which is the most toxic and most prevalent form, is 7-carboxyl-5-chloro-8-hydroxyl-3,4-dihydro-3-R-methylisocoumarin linked through an amide bond to L- β -phenylalanine (25). The widespread occurrence of ochratoxin-producing fungi, their ability to grow on a variety of economically important feed and foodstuffs (5), and the natural occurrence of ochratoxin (8, 17, 18; P. Krogh, 2nd Int. Cong. Plant Pathol., Abstr. 0360, 1973) constitute a threat to both animal and public health. The dimensions of this threat are undefined as yet, but excellent reviews of the literature exist (5, 20).

Only a limited amount of research has been done on ochratoxicosis in poultry. Huff et al. (10) provided a general description of the disease in young broiler chickens and concluded that on the basis of acute mean lethal dose and minimal growth inhibitory concentration ochratoxin A was the most potent mycotoxin yet studied in chickens. They also reported that the most sensitive indicator of ochratoxicosis in young broiler chickens was enlarged kidneys. A

microscopic evaluation of acute ochratoxicosis in young White Leghorn cockerels (6, 12) revealed acute nephrosis, hepatic degeneration, and suppression of hematopoiesis in descending order of frequency. On the other hand, a histopathological study of ochratoxicosis in ducklings (23) revealed primarily hepatic degeneration characterized by an increase in fatty vacuolation, changes in the matrix of the mitochondria, and disorganization of the endoplasmic reticulum of hepatocytes. In laying hens ochratoxin at low concentrations delays sexual maturity and reduces egg production and hatchability (4).

Because of the high toxicity of ochratoxin to chickens and because ochratoxin can be produced in a variety of feedstuffs used in the poultry industry, it was deemed desirable to study in more detail ochratoxicosis in the economically important broiler chicken. In particular, the nephrotoxicity of ochratoxin was investigated.

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MATERIALS AND METHODS

Animal husbandry. Day-old male broiler chicks obtained commercially were used in these studies. The birds were housed in electrically heated batteries under continuous lighting. Feed and water were available ad libitum. The feed was a commercial

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broiler-starter from which all medications were omitted. Ochratoxicosis was induced by incorporating into small portions of the diet weighed amounts of crystalline ochratoxin A dissolved in 95% ethanol. The portions of feed containing the ochratoxin A were dried at 100 C to evaporate the ethanol before being mixed into the remainder of the feed. The experimental diets were fed from hatching until the experiments were terminated at 4 weeks.

Ochratoxin A. *A. ochraceus* NRRL 3174 was grown on wheat by the method of Trenk et al. (24). Ochratoxin A was extracted from the wheat by the method of Steyn and van der Merwe (21) and purified by thick-layer preparatory chromatography on Silica Gel G using benzene-acetic acid (9:1, vol/vol) as the solvent (7). Ochratoxin A was eluted from the silica gel by making a slurry of the silica gel in hot benzene-acetic acid (9:1, vol/vol) solution. Approximately 100 ml of solution per g of Silica Gel G was used. The slurry was then filtered through paper. This procedure was repeated twice to insure that all of the ochratoxin A was removed from the silica gel. The combined filtrates were evaporated to 1% of the total extract volume. Cold benzene was then added to precipitate the ochratoxin A. The ochratoxin A crystals were then recrystallized twice from benzene and washed repeatedly with cold benzene before allowing to dry in the air.

Assays. The growth rate of the chickens was determined by weighing the chickens weekly. A renal function test was conducted by the method of Pitts (14). Plasma uric acid levels were measured by the method of Caraway (3). Serum potassium, sodium, calcium, and chloride ion concentrations were measured by an independent laboratory (Rex Hospital, Raleigh, N.C.) using Technicon S.M.A. methodology. Tissue taken from the right kidney was fixed in neutral buffered formalin and prepared for histopathological examination by standard procedures. The relative kidney weight and percentage dry weight of the kidney were also measured.

Experimental design. There were four replicates of 10 birds at each dose level. The dose levels were 0, 0.5, 1, 2, 4, and 8 μg of ochratoxin A per g of diet. The treatments and birds were completely randomized. The replicate means were evaluated statistically by analysis of variance in which an F ratio was calculated. If the F ratio were significant ($P < 0.05$), the treatment means were compared by the method of least significant differences (2).

RESULTS

The effect of graded doses of ochratoxin A on the parameters measured in this study are given in Table 1. The growth rate of broiler chickens was inhibited significantly ($P < 0.05$) at doses of 2.0 $\mu\text{g}/\text{g}$ and above, and the degree of inhibition was dose related.

The weight of the left kidney relative to the total body weight was increased by doses of 1 $\mu\text{g}/\text{g}$ and above, which agrees with the previous report (10). At the highest dose the relative kidney weight was more than doubled. The

enlargement of the kidney could be the result of edema, a general increase of protoplasm, or an increase of a specific constituent(s). The possible occurrence of edema in the kidney was tested by determining the dry weight ratios at the different dose levels. This ratio was decreased significantly at doses of 4 and 8 $\mu\text{g}/\text{g}$. Thus, the enlargement of the kidney during ochratoxicosis results in part from edema.

The effect of dietary ochratoxin on the excretory function of the kidney was determined by measuring the rate of clearance of phenol red from the blood by the kidney. The rate of clearance was decreased significantly ($P < 0.05$) at 4 and 8 $\mu\text{g}/\text{g}$. This impairment of renal excretory function should be reflected by alteration in the levels of blood constituents which are excreted by the kidney. The primary product of nitrogen catabolism in the chicken is uric acid (22). The plasma uric acid levels were increased significantly ($P < 0.05$) at dose levels of 4 and 8 $\mu\text{g}/\text{g}$, with an approximate 50% increase at the highest dose. During kidney impairment an electrolyte imbalance is often seen. Serum potassium levels were decreased, and this hypokalemia also was significant ($P < 0.05$) at 4 and 8 $\mu\text{g}/\text{g}$. Sodium, calcium, and chloride concentrations in the serum were measured, but they were not altered from control values.

The most striking histopathological changes seen in the kidney during ochratoxicosis were swelling of the tubular epithelial cells, tubular dilation, and proteinaceous material in the lumen. The severity of these changes appeared to parallel the dose of ochratoxin A administered. There was also a differential effect on the convoluted tubules in that the proximal portion was more severely affected than the distal portion to the extent that some generalized necrosis of the epithelial cells of the proximal tubules was observed.

DISCUSSION

The primary effect of ochratoxin A in chickens appears to be on the kidneys. The most sensitive visible indicator of ochratoxicosis is kidney enlargement (10). This enlargement, which occurred at doses as low as 1 $\mu\text{g}/\text{g}$, was not accompanied by changes indicative of altered excretory function, such as increased serum uric acid, hypokalemia, decreased dry weight, and decreased clearance of injected phenol red, until doses of 4 and 8 $\mu\text{g}/\text{g}$ were reached. However, minor histopathological changes could be seen at doses of 1 and 2 $\mu\text{g}/\text{g}$, which did cause enlargement. Presumably, the enlargement without an accompanying impair-

TABLE 1. Effect of graded doses of dietary ochratoxin A on parameters related to nephrotoxicity the broiler chicken

Parameter	Ochratoxin ($\mu\text{g/g}$)					
	0	0.5	1.0	2.0	4.0	8.0
Body weight (g)	415 \pm 65	413 \pm 40	405 \pm 60	355 \pm 20 ^a	250 \pm 40 ^a	107 \pm 25 ^a
Kidney size (g/100 g of body weight)	0.55 \pm 0.02	0.55 \pm 0.02	0.64 \pm 0.02 ^a	0.66 \pm 0.02 ^a	0.92 \pm 0.05 ^a	1.17 \pm 0.05 ^a
Kidney dry weight (% of wet weight)	21.0 \pm 0.5	20.7 \pm 0.4	20.3 \pm 0.7	20.2 \pm 0.2	18.8 \pm 0.2 ^a	15.2 \pm 0.4 ^a
Plasma uric acid (mg/100 ml)	1.9 \pm 0.3	2.1 \pm 0.3	2.2 \pm 0.3	2.4 \pm 0.1	3.1 \pm 0.4 ^a	3.7 \pm 0.2 ^a
Serum potassium (meq/liter)	5.5 \pm 0.2	5.5 \pm 0.1	5.3 \pm 0.2	5.2 \pm 0.1	4.8 \pm 0.2 ^a	4.6 \pm 0.1 ^a
Phenol red clearance (%)	63.8 \pm 0.8	59.7 \pm 1.7	66.0 \pm 1.7	63.4 \pm 1.5	54.3 \pm 1.7 ^a	43.9 \pm 8.9 ^a

^a These values differ significantly ($P < 0.05$) from control values.

ment of function represents a compensation by the chicken to the nephrotoxicity of ochratoxin. Once the compensating ability of the kidney is overmatched, alteration in function would be expressed. The suggestion that ochratoxin is primarily a nephrotoxin in broiler chickens agrees with prior observations on acute ochratoxicosis in White Leghorns (6, 12), where the kidney was the organ most frequently affected. It differs from the observations with ducklings, where the basic lesion of ochratoxicosis is fatty infiltration of the liver, and from the rat, where both the liver and kidney appear equally affected (13) and the liver exhibits a hyaline degeneration and focal necrosis (23). Indeed, the nephrotoxicity of ochratoxin in broilers offers an easy differential diagnosis of ochratoxicosis from aflatoxicosis, which is primarily a hepatotoxin in broilers (19).

The exact mechanism whereby ochratoxin exerts its nephrotoxicity cannot be stated on the basis of the present study, but some comments can be made. Extensive kidney damage is indicated by the renal function test, by the accumulation of uric acid in the blood, and by the severe histopathological changes observed. However, the differential effect on serum potassium and serum sodium suggests a differential effect on the kidney tubules. Sodium and chloride presumably are reabsorbed uniformly along the proximal and distal tubules (1). Potassium, on the other hand, is reabsorbed in the proximal and secreted in the distal tubules (11, 15). Ochratoxin in rats differentially damages the proximal tubules (16) as it does in chickens. The lowered blood potassium can then be explained on the basis that its reabsorption is impaired during ochratoxicosis.

It should not be assumed that ochratoxin is only a nephrotoxin in chickens. For example,

growth is inhibited at 2 $\mu\text{g/g}$, whereas a dose of 4 $\mu\text{g/g}$ is required to depress the kidney excretory function, and at 8 $\mu\text{g/g}$ the renal clearance of phenol red is decreased only 31%. A loss of 75% of the kidney causes only a slight decrease in an otherwise healthy chicken (9). This suggests a more direct effect on the growth process. It is interesting that ochratoxin A can cause an inhibition of carbohydrate and protein metabolism and oxidative phosphorylation in rats (5). In addition, ochratoxin appears to have an affinity for the gastrointestinal tract. In broilers treated the same as in the present study, an enlargement of the crop, proventriculus, and gizzard was noted (10). In acute lethal ochratoxicosis, chickens exhibit a severe enteritis (10, 12). The neural system is also affected during acute lethal ochratoxicosis, although no neural disturbances were noted in the present study. It seems obvious that more detailed studies of ochratoxicosis are needed before this disease can be understood.

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