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Acute Toxicity of Ochratoxins A and B in Chicks[1]

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Ochratoxins A and B were given to 1-day-old Babcock B-300 cockerels to evaluate acute toxic effects. Two trials with ochratoxin A gave 7-day oral median lethal dose estimates of 116 μg (3.3 mg/kg) and 135 μg (3.9 mg/kg) per chick. Chicks given daily oral doses of 100 μg of ochratoxin A died on the second day. Single subcutaneous doses of 400 μg of ochratoxin A were also lethal. The 7-day oral median lethal dose of B was estimated at 1,890 μg (54 mg/kg) per chick. Chicks given oral doses of 100 μg of ochratoxin B daily for 10 days survived. Sublethal doses of both ochratoxins A and B resulted in growth suppression which was proportional to the amount of ochratoxin given. Visceral gout was the principal gross finding. Microscopic examinations revealed acute nephrosis, hepatic degeneration or focal necrosis, and enteritis. Suppression of hematopoiesis in the bone marrow and depletion of lymphoid elements from the spleen and bursa of Fabricius were frequently seen. Both ochratoxins appeared to have similar pathological effects. This is the first report on the toxicity of ochratoxin B.

The toxic effects of ochratoxin A, a mycotoxin produced by *Aspergillus ochraceus* Wilh., have been established (3-6, 10). The natural occurrence of ochratoxin A in wheat (9) and corn (11) has focused attention on the hazards of contamination in human foods and animal feeds.

Initial reports of ochratoxin A gave a median lethal dose (LD_{50}) of 25 μg per 1-day-old duckling (16) which was revised to 150 μg per duckling (6). The oral LD_{50} in 150-g rats was approximately 20 mg/kg (7). Acute nephrosis, hepatic degeneration, and enteritis were observed as toxic effects (6, 7, 14) of ochratoxin A. Initially, both ochratoxins B and C were considered nontoxic (15); however, ochratoxin C, the ethyl ester of ochratoxin A, was later reported to be toxic (13). Ochratoxin B, the dechloro derivative of A, has not been reported to be toxic (13, 15).

In an earlier investigation in our laboratory, several isolates of *A. ochraceus* isolated from peanuts and grown on corn proved highly toxic to 1-day-old chicks (2). The present investigation was, therefore, made to determine the acute toxicity of ochratoxins A and B in 1-day-old chicks and to compare the lesions caused by infested corn (2) to the lesions caused by purified ochratoxins.

MATERIALS AND METHODS

Ochratoxins. Crystalline ochratoxins A and B were supplied by A. D. Campbell and S. Nesheim (Food and Drug Administration, Washington, D.C.). They were isolated and purified as reported by Nesheim (5). A small amount of ochratoxin A was also supplied by I. F. H. Purchase (National Nutritional Research Institute, Pretoria, South Africa).

The ochratoxins were dissolved in 0.1 M aqueous sodium bicarbonate solution. Ochratoxin A dissolved rapidly, whereas B required approximately 16 hr to dissolve. The ochratoxins were given orally by a plastic tube attached to a tuberculin syringe in trials 1, 2 and 3. Ochratoxin A was injected subcutaneously in trial 4. In all trials, chicks of control groups received the aqueous sodium bicarbonate solution.

Chicks. The chicks were 1-day-old Babcock B-300 cockerels. The average body weight at the beginning of the trials was 35 g (minimum of 32 to maximum of 40 g). The diet consisted of a commercial chick starter. Water was provided ad libitum. Chicks were dosed before receiving feed and water except in trial 3 when they had access to feed and water before dosing.

Observations. Body weights of surviving chicks were recorded and averaged for each group at 7 days of age. Birds which died were examined for gross lesions as soon as possible after death. Chicks that survived the test period were killed and examined for gross lesions. All abnormalities were recorded. Tissues were collected in 10% neutral buffered Formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. The tissues examined included brain (three levels), spinal cord, dorsal root ganglia, peripheral nerve, adrenal glands, trachea, lungs, heart, aorta, spleen, bursa of Fabricius, bone marrow, crop, esophagus, gizzard, proventriculus, small intestine, large intestine, cloaca, pancreas, liver, kidney, gall bladder, testes, skeletal muscle, femur, and vertebrae.

Calculations. The LD_{50} was determined by the

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TABLE 1. *Mortalities in 1-day-old chicks given daily oral doses of ochratoxins: trial 1*

Ochratoxin	Daily dose[a] (μg/chick)	Total dose (μg/chick)	Mortalities[b]
A	0.1	1	0/5
	1	10	0/5
	10	100	0/5
	100	200	5/5
B	0.1	1	0/5
	1	10	0/5
	10	100	0/5
	100	1,000	0/5

[a] All groups, except ochratoxin A 100, were dosed for 10 days. Chicks given 100 μg of ochratoxin A died on the 2nd day.

[b] Ratio of deaths to number of birds in the test.

Reed and Muench method (8) with semilogarithmic paper.

RESULTS

Mortalities. The mortalities are presented in Tables 1 to 3. No deaths occurred in the control groups. In trial 1 (Table 1), chicks given daily oral doses of 100 μg of ochratoxin A died on the second day, whereas those given 10 μg or less daily for 10 days survived. With single oral doses of ochratoxin A, the 7-day LD_{50} in trial 2 (Table 2) was 116 μg per chick (3.3 mg/kg of body weight) and in trial 3 (Table 3) was 135 μg per chick (3.9 mg/kg). The small sample of ochratoxin A from South Africa was divided into two doses of 150 μg each and given orally to two chicks. Both chicks died, one within 24 hr and the second within 1 week. When two chicks in trial 4 (*data not shown*) were each given a single dose of 400 μg of ochratoxin A subcutaneously, both died; chicks receiving 200, 100, and 50 μg of ochratoxin A subcutaneously survived.

Ochratoxin B was much less toxic than A. No mortalities occurred in trial 1 (Table 1) or in trial 2 (*data not shown*) when single oral doses of 100, 200, 400, and 800 μg per chick were given. By using the remaining ochratoxin B in trial 3 (Table 3), the 7-day LD_{50} was estimated at 1,890 μg per chick (54 mg/kg).

Growth. Sublethal doses of both ochratoxins A and B resulted in growth suppression (Table 2 and 3). The degree of growth suppression was proportional to the amount of ochratoxin given. The control chicks had normal body weight gains.

Gross findings. The principal postmortem finding was visceral gout (12) with white, flakelike deposits (uric acid crystals) in the kidneys and ureters, as well as on the heart, pericardium, liver, and spleen of affected chicks. The condition was found in 10 of 32 chicks which died in groups given ochratoxin A. It also occurred in three of the four chicks which died after receiving ochratoxin B. Emaciation, dehydration, and firm mucosal linings in the gizzards were also observed in birds which died after the second day. The gizzards were empty in these chicks. No gross lesions were observed in chicks of the control groups.

Histopathological findings. Acute nephrosis was a consistent finding in chicks which died in groups given either ochratoxin. These renal lesions oc-

TABLE 2. *Mortalities and body-weight changes in 1-day-old chicks given single oral doses of ochratoxin A: trial 2*

Dose of ochratoxin A[a] (μg/chick)	Accumulated mortalities on day[b]				Weight gain of survivors at 1 week (per cent of control group)[c]
	1	2	3	7	
500	4/4	4/4	4/4	4/4	
250	3/5	3/5	3/5	3/5	70
100	1/5	1/5	1/5	2/5	81
50	1/5	1/5	2/5	2/5	95

[a] Seven-day LD_{50} estimated at 116 μg per chick (3.3 mg/kg body weight).

[b] Ratio of deaths to the number of birds in the group.

[c] Mean weight gain of chicks in control group was 27.2 g.

TABLE 3. *Mortalities and body-weight changes in 1-day-old chicks given single oral doses of ochratoxins: trial 3*

Ochratoxin	Dose (μg/chick)	Accumulated mortalities on day[a]				Weight gain of survivors at 1 week (per cent of control group)[b]
		1	2	3	7	
A[c]	300	2/5	4/5	5/5	5/5	
	200	1/5	3/5	4/5	5/5	
	150	0/5	2/5	3/5	4/5	100[d]
	125	0/5	0/5	0/5	1/5	53
	100	0/5	0/5	1/5	1/5	73
B[e]	3,000	1/3	2/3	3/3	3/3	
	1,500	0/4	1/4	1/4	1/4	50
	500	0/3	0/3	0/3	0/3	63

[a] Ratio of deaths to the number of birds in the group.

[b] Mean weight gains of chicks in control groups; ochratoxin A was 34.8 g and ochratoxin B was 39.3 g.

[c] Seven-day LD_{50} for ochratoxin A estimated at 135 μg per chick (3.9 mg/kg of body weight).

[d] This surviving chick probably regurgitated most of the toxin.

[e] Seven-day LD_{50} for ochratoxin B estimated at 1,890 μg per chick (54 mg/kg).

curred in all chicks with gout and a few chicks with no evidence of gout. Proteinaceous casts, urates, scattered heterophils, and localized necrosis occurred in the renal tubules. Urates were also seen in the ureters.

Hepatic lesions occurred less frequently than renal lesions and varied in severity from mild diffuse vacuolation of hepatocytes to necrotic foci. One chick given 100 μg of ochratoxin A had multiple foci of fibrous connective tissue which resembled the healing stage of a necrotic lesion. Scattered, irregularly shaped, necrotic foci were seen in one chick given 1,000 μg of ochratoxin B. This was the only chick given ochratoxin B which had liver lesions. The lesion differed markedly from the regularly shaped necrotic foci seen in birds given ochratoxin A.

Suppression of hematopoiesis in the bone marrow and depletion of lymphoid elements from the spleen and bursa of Fabricius were the predominant findings in chicks which survived and were frequently observed in chicks which died. Catarrhal enteritis with heterophils in dilated intestinal glands were seen in a few birds from both ochratoxin groups. No microscopic lesions were observed in chicks of the control groups.

DISCUSSION

The acute oral toxicity of ochratoxin A in chicks reported here was similar to values reported in chicks given a single oral dose of aflatoxin B_1 (1). From our data, chicks appear as sensitive to ochratoxin A as ducklings (6); however, ducklings are much more sensitive than chicks to aflatoxin B_1 (17).

Ochratoxin B, which was initially reported to be nontoxic at a thousandfold higher dose level than ochratoxin A (15), appeared to be about one-tenth as toxic to day-old chicks as ochratoxin A. These results constitute, to our knowledge, the first observation of toxicity of ochratoxin B.

The amount of food in the digestive tract was shown to influence the survival time. Chicks in trial 2 (Table 2) which had not eaten prior to dosing had greater 24-hr mortalities than those which had eaten before being given ochratoxin A in trial 3 (Table 3).

Nephrosis, hepatic degeneration, and enteritis which have previously been reported as effects of ochratoxin A in ducklings and rats (6) were also seen in the chicks. As in the rat (7), the renal lesions in chicks were the predominant toxic effect. Hepatic lesions of ochratoxin A, which occurred less frequently, were identical to hepatic lesions reported in chicks fed corn infested with *A. ochraceus* (2). As previously reported (2), chicks which died peracutely failed to have liver lesions. Gout and acute nephrosis for an unexplained reason were not observed in the chicks fed infested corn.

The pathological findings in chicks given ochratoxin B suggest that the effects of both mycotoxins are similar. The few chicks examined, however, represent such a limited sample that additional pathological studies of ochratoxin B toxicity will be needed for conclusive data.

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