

# Mycotoxicity of *Aspergillus ochraceus* to Chicks<sup>1</sup>

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Five isolates of *Aspergillus ochraceus*, obtained from peanuts, were grown separately on sterile, moist corn for 14 days and fed to 1-day-old Babcock B-300 cockerels to evaluate their toxic effects. Two isolates were highly toxic, causing death of all birds during the 1st week of the experiment. Two isolates were moderately toxic, causing severe growth suppression with some deaths occurring throughout the 3-week test period. One isolate had no apparent effect. When the two most toxic isolates (diets) were diluted, survival time increased but severe growth suppression was evident. Postmortem examinations revealed a few small hemorrhages in the proventriculi of birds which died between the 2nd and 5th days. Emaciation, dehydration, and dry, firm gizzard linings were observed throughout the experiment. Extensive hepatic injury consisting of either fatty changes or necrotic foci was the principal microscopic finding. Suppression of bone marrow activity and depletion of lymphoid elements in the spleen and bursa of Fabricius were also found. The severity of the histopathological changes was directly related to the concentration of ochratoxin A in the diets.

Toxic isolates of *Aspergillus ochraceus* Wilh. were obtained from cereal and legume products in South Africa by Scott (9) in 1965 and have since been found in foodstuffs by other investigators (4, 6, 14). The toxic principle was 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3 R-methylisocoumarin, linked by an amide bond of the 7-carboxy-group to L- $\beta$ -phenylalanine, and was named ochratoxin A (12, 13). A single 100- $\mu$ g oral dose of ochratoxin A to ducklings and rats resulted in acute hepatic injury (11) which varied from mild fatty degeneration to necrosis. Tubular necrosis of the kidney and enteritis have also been reported (8).

Although *A. ochraceus* is widely distributed in nature (5) and is a common fungus in stored feeds and foodstuffs, the natural occurrence of ochratoxin A was not reported until 1969 (10). Two additional species of *Aspergillus*, *A. sulphureus* (Fres.) Thom and Church and *A. mellus* Yukawa (7), and a *Penicillium* species (14) are reported to produce ochratoxin A.

*A. ochraceus* isolates of undetermined toxigenicity have been obtained from peanut kernels (1). Since toxic studies were not carried out, investigations were initiated on the mycotoxicity of *A. ochraceus* isolates obtained from domestic peanuts. The results of experiments in chicks are

reported here. Some of the results have been published (2).

## MATERIALS AND METHODS

**Fungi.** Ten isolates of *A. ochraceus* were initially screened for toxigenicity to 1-day-old chicks as previously described (3). Eight of these isolates, designated by "P" numbers, were obtained from R. T. Hanlin (University of Georgia, Athens). They were isolated from peanuts grown in several states. One isolate (CPES-0414) was obtained from peanuts grown locally in Southern Georgia. A reference isolate (NRRL-3174), known to produce ochratoxin A, was obtained from C. W. Hesselstine (Northern Regional Research Laboratory, Peoria, Ill.).

Initially, all isolates were highly toxic and resulted in the death of all birds within a 2-week test period. After subculturing, several of these isolates lost their toxigenicity. Subsequently, five isolates were selected for further study; two with high toxicity (P-657 and NRRL-3174), two with low toxicity (P-94 and CPES-0414), and one with no apparent toxicity (P-3602).

**Cultures.** Each isolate was grown singly in 2,800-ml Fernbach flasks containing 500 g of moist, autoclaved, cracked corn at room temperature (27 C). The flasks were shaken daily to reduce mycelial matting of the corn. After incubation for 2 weeks, each culture was dried at 50 C for 15 hr, ground, and mixed singly with a 36% protein supplement (6:4, w/w) to form a diet.

**Chicks.** Each experimental group consisted of ten 1-day-old Babcock B-300 cockerels. A diet containing a single isolate of *A. ochraceus* was provided ad libitum for each group. The control group received

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sterile corn similarly treated. Water was provided ad libitum.

Additional birds were added during the experiments to evaluate the effects of dilution of the diet and age of the chicks on the mortality rates caused by two isolates (NRRL-3174 and P-657).

**Observations.** Body weights of surviving chicks were recorded and averaged for each group at 1, 7, 14, and 21 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, eyes, dorsal root ganglia, peripheral nerve, adrenal glands, trachea, lungs, heart, aorta, spleen, bursa of Fabricius, thymus, bone marrow, crop, esophagus, gizzard, small intestine, large intestine, cloaca, pancreas, liver, kidney, gall bladder, testes, skeletal muscle, femur, and vertebrae.

**RESULTS**

**Ochratoxin assays.** The ochratoxin A content of undiluted NRRL-3174-infested corn was 7,925 parts per billion (ppb), whereas that of P-657 was 1,050 ppb as determined by thin-layer chromatography. No detectable ochratoxin A was found in either the CPES-0414-, P-94-, or P-3602-infested corn. These analyses were made by N. D. Davis (Auburn University, Auburn, Ala.).

**Mortalities.** The mortalities of 1-day-old chicks are presented in Table 1. Diets containing NRRL-3174-, P-657-, and P-94-infested corn caused 100% mortality; however, those birds on P-94-infested corn died at a much slower rate. CPES-0414-infested corn caused two deaths during the experiment, whereas none occurred in chicks fed the P-3602-infested corn.

TABLE 1. Mortalities and body-weight changes in 1-day-old chicks fed corn infested with five isolates of *Aspergillus ochraceus*

Isolate <sup>a</sup>	Mortalities <sup>b</sup>			Weight of survivors (per cent of control)	
	1st Week	2nd Week	3rd Week	2nd Week	3rd Week
NRRL-3174	10/10				
P-657	10/10				
P-94	1/10	5/10	4/10	44	
CPES-0414	0/10	1/10	1/10	56	
P-3602	0/10	0/10	— <sup>c</sup>	112	— <sup>c</sup>

<sup>a</sup> Diets for the toxicity tests were prepared by mixing infested corn (6:4, w/w) with a 36% protein supplement.

<sup>b</sup> Ratio of deaths to the number birds in the test.

<sup>c</sup> Diet exhausted.

TABLE 2. Mortalities in chicks of different ages fed several concentrations of corn infested with two isolates of *Aspergillus ochraceus*

Iso ate	Diet <sup>a</sup>	Age of birds (days)	Mortalities <sup>b</sup>		
			1st Week	2nd Week	3rd Week
NRRL-3174	%				
	60	1	12/12		
	60	4	12/12		
	60	10	3/4	1/4	
	60	25	0/4	4/4	
	30	4	8/12	4/12	
	30	10	1/4	3/4	
	15	4	9/12	3/12	
P-657	60	1	12/12		
	60	10	0/4	4/4	
	30	1	6/12	6/12	
					1/4

<sup>a</sup> All test diets were prepared by mixing infested corn (6:4, w/w) with a 36% protein supplement. Infested corn was mixed with control corn in appropriate ratios to obtain the 30 and 15% diets.

<sup>b</sup> Ratio of deaths to the number of birds in the test.

TABLE 3. Gross postmortem findings in chicks fed corn infested with four isolates of *Aspergillus ochraceus*

Finding	Isolates <sup>a</sup>			
	NRRL-3174	P-657	P-94	CPES-0414
Dehydration.....	10	10	0	0
Emaciation.....	10	10	7	8
Gizzard				
Dry, firm mucosal lining.....	10	5	0	0
Ulceration.....	1	0	0	0
Proventriculus				
Mucosal hemorrhage.....	6	5	0	0
Pale and enlarged..	0	0	0	4
Hearts				
Pale and enlarged..	0	0	0	4
Ascites, hydropericardium and edema...	0	0	0	4
No. of chicks examined.....	10	10	7	8

<sup>a</sup> Diets were prepared by mixing infested corn (6:4, w/w) with a 36% protein supplement.

TABLE 4. *Microscopic lesions in chicks fed corn infested with four isolates of Aspergillus ochraceus*

Lesion	Isolates					
	NRRL-3174			P-657 60%	P-94 60%	CPES-0414 60%
	60% <sup>a</sup>	30%	15%			
Liver						
Bile duct proliferation...	1 <sup>b</sup>	0	0	2	0	1
Foci of fatty changes...	2	6	3	7	1	2
Necrotic foci.....	0	2	6	1	1	0
Lung						
Congestion and edema.	1	0	0	0	0	0
Mycotic pneumonia.....	0	3	1	0	3	0
Spleen						
Lymphocytic depletion...	0	3	3	4	1	1
Bursa of Fabricius						
Lymphocytic depletion...	0	3	6	2	1	0
Bone marrow						
Hematopoietic suppression.....	2	9	6	8	0	1
Crop or esophagus						
Focal fibrinosuppurative inflammation.....	0	2	1	2	0	1
Gizzard						
Focal suppurative inflammation.....	0	0	0	0	3	0
Proventriculus						
Hypertrophy and edema.....	0	0	0	0	0	2
No significant lesions....	8	1	0	2	1	3
No. of chicks examined....	12	12	9	12	7	9

<sup>a</sup> Percentage of infested corn in diet.<sup>b</sup> Birds affected.

The use of older birds and dilutions of the NRRL-3174- and P-657-infested corn slightly increased the survival times (Table 2).

**Growth.** Birds fed corn infested with P-94 and CPES-0414 had severe growth suppression, whereas those fed corn infested with P-3602 had normal growth rates (Table 1).

**Gross findings.** The gross postmortem findings are summarized in Table 3. The principal findings in birds fed NRRL-3174- and P-657-infested corn were dehydration, emaciation, dry firm mucosal linings in the gizzards, and a few small hemorrhages in the mucosa of the proventriculi. One bird on the NRRL-3174-infested corn had an ulcerated gizzard.

Emaciation was the only gross finding in the chicks fed P-94-infested corn.

The chicks fed corn infested with CPES-0414 were emaciated and had enlarged, pale-red hearts and enlarged, pale-gray proventriculi. Four of these birds also had ascites, hydropericardium, and edema of the air sacs, mesentery, and pancreas. The small intestine appeared dilated.

**Histopathological findings.** The microscopic findings are summarized in Table 4. Hepatic lesions varying from mild scattered foci of fatty changes to severe diffuse foci of necrosis occurred in 19 of 33 birds fed NRRL-3174-infested corn, 8 of 12 birds fed P-657, and 2 of 7 birds fed P-94. Only fatty changes were seen in the livers of 2 of 9 birds fed CPES-0414-infested corn.

Suppression of hematopoietic activity in the bone marrow and depletion of lymphoid elements from the spleen and bursa of Fabricius were seen in chicks on all four isolates, but occurred most consistently in chicks fed NRRL-3174- and P-657-infested corn.

Two chicks fed CPES-0414 had hypertrophic and edematous proventriculi.

Incidental findings consisted of mild mycotic pneumonia (seven birds), bile duct proliferation in livers (four birds), focal suppurative inflammation of the gizzard (three birds), and focal fibrinosuppurative inflammation of the crop (four birds) and esophagus (one bird).

## DISCUSSION

Corn infested with two isolates (NRRL-3174 and P-657) was highly toxic when fed to 1-day-old chicks. The mortality rate during the first week was 100%, which correlated with the high concentration of ochratoxin A. Dilution of these toxic diets and use of older birds resulted in slightly increased survival times (Table 2).

Deaths also occurred in chicks fed corn infested with two other isolates (P-94 and CPES-0414) which had no detectable ochratoxin A. The majority of these deaths occurred during the 2nd

and 3rd weeks. Birds in these groups which survived the 1st week had severe growth suppression. Chicks fed corn infested with P-3602 and control chicks fed the noninfested corn diet had normal body weight gains and no mortalities.

Emaciation, dehydration, and dry, firm mucosal linings in the gizzards were observed in a majority of the birds fed corn infested with NRRL-3174 and P-657. Emaciation was also apparent in the P-94 and CPES-0414 groups. These findings are probably secondary effects of the toxins since similar effects have been produced in normal chicks after water and feed were withheld for 42 hr.

The hepatic fatty changes and necrotic foci found in the NRRL-3174 and P-657 groups were related to the presence of ochratoxin A. Seven chicks which were fed the highest concentration (60%) of isolate NRRL-3174 died before the 3rd day and had no liver lesions. These birds probably died before morphological changes developed in the liver. Liver lesions were a consistent finding in the chicks fed the diluted diets and in the older chicks. Chicks fed corn infested with P-657 also had consistent liver lesions with fatty changes, which represent mild hepatic injury, occurring more frequently than necrotic foci. These findings correlated closely with the ochratoxin A concentration. The two chicks affected in the P-94 group suggested that ochratoxin A was present in this corn at a level not detected by thin-layer chromatography, or some other undefined factor was involved.

Suppression of bone marrow activity and depletion of lymphoid elements from the spleen and bursa of Fabricius were also frequently observed in the NRRL-3174 and P-657 groups. These changes are nonspecific and can be related to emaciation and growth suppression.

Bile duct proliferation, which is considered a characteristic of aflatoxin injury, occurred in the liver of four birds. This lesion appeared to have a random distribution in the groups and was

considered as an incidental finding. None of the control chicks was affected.

One isolate, CPES-0414, produced effects which were entirely different from those observed with the other toxigenic isolates of *A. ochraceus*. The enlarged proventriculi and hearts and edematous tissues were observed only in this group of chicks. Additional experiments will be required to determine the cause of these effects which appear unrelated to ochratoxin A.

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