Aflatoxin Effects in Poultry (34087)

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Several reports have appeared in the literature recently concerning various aspects of the toxicity of aflatoxin in chickens (1–5). Growth retardation, an increased liver/body weight ratio, biochemical alterations and pathological changes were noted by Carnaghan *et al.* (4) when Rhode Island Red chicks were fed a ration containing 1500 parts per-billion (ppb) aflatoxin from hatching to 8 weeks of age. Not established was the maximum level of aflatoxin intake which can be tolerated by chicks without adverse effects. The work to be reported herein was designed (1) to establish the highest level of aflatoxin which can be fed to broiler chicks without effect under simulated practical conditions in the United States, and (2) to determine whether aflatoxin may be transmitted into the meat. Also, since aflatoxin has been detected in the milk of cows fed aflatoxin (6), the excretion of aflatoxin in eggs must be considered. Therefore, data are included in this report confirming other workers' results to the effect that aflatoxin is not found in eggs of hens fed aflatoxin (2, 7).

Materials and Methods. The desired dietary level of aflatoxin was prepared by mixing aflatoxin-fortified cottonseed meal[3] with unfortified cottonseed meal containing <0.04% free gossypol. The fortified cottonseed meal contained a total of approximately 11 ppm aflatoxin consisting predominately of B_1 along with smaller amounts of B_2, G_1, and G_2. The broiler ration consisted of the following ingredients in grams per kilogram: ground yellow corn 520, cottonseed meal 150, soybean meal (44% protein) 65, fish meal 75, meat and bone meal 50, soybean oil 50, corn gluten meal 25, dried whey 20, alfalfa meal 20, blood meal 10, iodized salt 2.5, DL methionine 0.25, manganese sulfate 0.25, zinc oxide 0.12, a coccidiostat (amprolium plus) 0.50, and vitamin premix 0.5. The vitamin premix supplied the following ingredients in milligrams: choline chloride 550, riboflavin 3.3, niacin 11.0, calcium pantothenate 4.4, vitamin B_{12} 0.05, vitamin A (dry concentrate containing 10,000 IU/g) 264, vitamin E (dry concentrate containing 20,000 IU/lb) 130, and vitamin D_3 (dry concentrate containing 15,000 ICU/g) 264.

In each trial 260 day-old male Arbor-Acres hybrid broiler type chicks were used, one-half being fed a ration containing aflatoxin. Each group of 130 chicks was confined under a large electric brooder on a wooden floor with sawdust as litter. Ten chicks per group were sacrificed at weekly intervals during the 8-week trial. The chicks randomly selected for autopsy were anesthetized with ether, and individual blood samples were collected from the severed jugular vein. It was necessary to pool blood samples from three and two chicks, respectively, during the first and second weeks because of the limited supply of blood. After bleeding, the liver from each chick was excised and weighed and sections were preserved in 10% formalin for subsequent histological examination. Biochemical analyses were run on the blood and

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[3] The Northern Regional Research Laboratory, Peoria, Illinois, provided aflatoxin on rice, inoculated with *Aspergillus flavus* and incubated to allow formation of the toxin. The moldy rice was mixed with cottonseed meal at the Southern Regional Research Laboratory, New Orleans, Louisiana. Both are laboratories of the Agricultural Research Service, U.S. Dept. of Agriculture.

the remaining portion of each liver, the results of which will be the subject of a separate report.

At termination of each trial (Week 8) numerous tissues were preserved in 10% formalin from which hematoxylin and eosin–stained slides were prepared for microscopic examination. Also at Week 8 approximately 20 broilers from each group were bled, scalded, defeathered, and portions of breast and thigh meat were combined as were portions of blood and liver. The samples of meat, blood, and liver were lyophilized, grated in a meat grinder, and preserved at −10°F pending chemical analysis for aflatoxin residues.

A modification of the Pons and Goldblatt method was used for chemical analysis of aflatoxins in the meat, blood, liver, and egg samples (8). As little as 3–5 ppb aflatoxin B_1 can be detected by this method.

Thirty-four White Leghorn hens were divided into two equal groups and housed in individual wire-bottomed cages designed to facilitate egg collection. One group received a practical laying mash ration containing the same amount of unfortified cottonseed meal as was added to the ration containing 2700 ppb aflatoxin for the treated group. The ration consisted of the following ingredients in grams per kilogram: ground milo 380, ground corn 200, cottonseed meal 250, alfalfa meal 50, dried whey 25, fish meal 20, limestone 55, dicalcium phosphate 17, iodized salt 2.5, zinc oxide 0.05, and vitamin premix 0.5. The hens were equilibrated for a period of 2 weeks on the experimental rations, and eggs collected for the succeeding 34 days were studied for hatchability and aflatoxin residue.

Results. In trial 1 a relatively high dietary level of aflatoxin was fed (1600 ppb) with the expectation of toxic effects. The body weights and mortality with respect to time are shown in Table I. Mortality was unaffected, but growth retardation was evident although not significant, during the entire 8 weeks of the trial. There was no reduction in feed intake by the group of chicks fed aflatoxin. The histological findings revealed that lesions were present in most livers, the optimum time for detection of bile ductule and hepatocyte lesions being between 17 and 31 days after initiation of the feeding trial. At 8 weeks, other tissues examined including kidneys, spleen, heart, adrenals, gut, and pancreas showed no pathological effects related to aflatoxin ingestion. Biochemical changes in the blood and liver indicated that 1600 ppb aflatoxin was a positive effect level.

The aflatoxin level fed in trial 2 was 800 ppb; otherwise the same format as trial 1 was used. The growth and mortality data (Table II) gave no evidence of toxicity; in fact, the mean body weight of the aflatoxin-fed chicks exceeded that of the control group. Histological evidence, however, of hepatocyte changes and bile ductule proliferation was detected in the livers, although to a lesser degree of severity than in trial 1. Therefore, a third

TABLE I. Chronological Effects on Body Weight and Mortality of Chicks Fed a Ration Containing 1600 ppb Aflatoxin.

	Days on test								
Treatment	0	11	18	25	32	39	46	53	60
Control group[a]									
Number chicks	130	129	97	77	67	57	46	36	26
Mean wt (g)	38	149	278	487	662	909	1078	1341	1604
Number deaths	0	1	2	0	0	0	1	0	0
Aflatoxin (1600 ppb) group[a]									
Number chicks	130	129	99	79	69	58	48	38	28
Mean wt (g)	38	135	234	424	605	865	1017	1226	1485
Number deaths	0	1	0	0	0	1	0	0	0

[a] Duration of trial 1 from Oct. 10 to Dec. 5, 1967. Total feed consumption, control group 177 kg; aflatoxin group 186 kg.

TABLE II. Chronological Effects on Body Weight and Mortality of Chicks Fed a Ration Containing 800 ppb Aflatoxin.

Treatment	Days on test								
	0	11	18	25	32	39	46	53	60
Control group[a]									
Number chicks	130	128	97	77	66	55	45	35	23
Mean wt (g)	37	146	276	493	683	796	1078	1370	1624
Number deaths	0	2	0	1	1	1	0	0	2
Aflatoxin (800 ppb) group[a]									
Number chicks	130	128	97	75	65	55	45	35	25
Mean wt (g)	38	144	260	497	683	988	1211	1539	1744
Number deaths	0	2	1	2	0	0	0	0	0

[a] Duration of trial 2 from Jan. 5 to Mar. 5, 1968. Total feed consumption: control group 168 kg; aflatoxin group 150 kg.

trial was run at the reduced level of 400 ppb aflatoxin. No adverse effects were noted in body weight and mortality data (Table III). Lower final body weights of both the control and treated birds of trial 3 compared to those of trials 1 and 2, are primarily due to a shorter trial period of 56 days. The histological findings were also negative for liver sections of aflatoxin-fed chicks in trial 3. Therefore, it was concluded that 400 ppb should be regarded as a "no effect" level for chicks subjected to the experimental conditions as described.

No evidence of aflatoxin could be detected by chemical analysis of the meat, liver, and blood of chicks fed the highest dietary level of aflatoxin (1600 ppb).

The results of feeding 2700 ppb aflatoxin to laying hens are presented in Table IV. Feed intake and body weights of the hens were comparable to those of the control group. Further, the aflatoxin ration did not diminish the number of eggs laid, although there was an apparent decrease in the percentage of hatchability. The contents of more than 100 eggs were removed from the shell, combined, lyophilized, and analyzed for aflatoxin residues. No evidence of aflatoxin was detected.

Finally, the hens were bled, scalded, defeathered, and composite samples of blood, meat, and liver were lyophilized and analyzed for aflatoxin. No evidence was found of aflatoxin residues in the tissues of the hens fed at this level for a period of 48 days, although histological examination of the liver

TABLE III. Chronological Effects on Body Weight and Mortality of Chicks Fed a Ration Containing 400 ppb Aflatoxin.

Treatment	Days on test								
	0	7	14	21	28	35	42	49	56
Control group[a]									
Number chicks	130	130	100	80	70	59	49	39	28
Mean wt (g)	38	96	192	350	492	742	963	1133	1435
Number deaths	0	0	0	0	0	1	0	0	1
Aflatoxin (400 ppb) group[a]									
Number chicks	130	130	100	80	70	58	48	37	27
Mean wt (g)	38	91	186	329	452	701	915	1176	1438
Number deaths	0	0	0	0	0	2	0	1	0

[a] Duration of trial 3 from May 21 to July 16, 1968. Total feed consumption: control group 187 kg; aflatoxin group 183 kg.

TABLE IV. Effects of Aflatoxin on Laying Hens.

Parameter measured	Control group	Aflatoxin group[a] (2700 ppb)
Number of hens	16	17
Mean weight (kg)	1.8	1.8
Mean feed intake (kg)	4.49	4.46
Number eggs per hen	16	21
Hatchability (%)	89	74

[a] Total time on aflatoxin regimen = 48 days (includes 14-day equilibration period).

sections revealed minimal to mild lesions.

Discussion. In agreement with reports of other workers (2, 3, 7) no evidence was found of aflatoxin residues in edible meat from broilers fed 1600 ppb aflatoxin in the diet for 8 weeks, or in the meat of hens fed 2700 ppb aflatoxin for a period of 48 days. Similarly, there was no evidence of aflatoxin in the eggs from these hens on the basis of chemical analysis. No evidence of adverse effects on the health and performance of broiler chicks could be detected when the dietary intake was 400 ppb. Minimal lesions were recognized, however, in the livers of chicks fed 800 ppb, and in chicks fed 1600 ppb, the liver lesions were of sufficient severity to impede growth. In contrast to our results with Arbor-Acres hybrid chicks fed 1600 ppb aflatoxin, Rhode Island Red chicks fed 1500 ppb in England (4) were less resistant to aflatoxin. This conclusion is based primarily on the reported 45% decrease in body weight gain. Direct comparisons, however, are admittedly misleading owing to the introduction of other variables such as the overall slower growth rate of the Rhode Island Red control broilers (mean weight of both sexes was 729 g at 8 weeks vs. 1400–1600 g for the Arbor-Acres hybrid males). The dietary protein level also influences aflatoxin toxicity (9). Poultry breed and strain differences in susceptibility to aflatoxicosis have also been reported (2, 5). This will be the subject of a subsequent report from this laboratory.

Summary. A systematic study was made of the effect of graded levels of dietary aflatoxin on the performance of broilers under simulated practical conditions. No adverse effects were detected when a ration containing 400 ppb aflatoxin was fed to Arbor-Acres broiler chicks from 1 day to 8 weeks of age. At higher levels (800 and 1600 ppb) adverse liver effects were detected, based on biochemical and histological studies. By chemical analysis, no evidence of aflatoxin was found in the meat, liver or blood of broilers fed 1600 ppb for 60 days prior to slaughter, nor in the eggs, meat, liver, or blood of White Leghorn hens fed a ration containing 2700 ppb aflatoxin for a period of 48 days.

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