Aflatoxin Susceptibility in Various Breeds of Poultry (34860)

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Large differences in species susceptibility to acute and subacute poisoning by aflatoxin have been recognized since early observations on aflatoxicosis in domestic animals (1–4). In poultry, the duckling is reported to be the most sensitive, followed by the turkey poult, pheasant, and chicken (1, 5). Experimental and field observations on chickens revealed the New Hampshire breed to possess greater sensitivity than several other commercial varieties (6). A difference in susceptibility between 2 strains of hybrid chickens has also been reported (7).

The extent of such variation in poultry to aflatoxin poisoning, including the uniqueness of the sensitivity apparent in the New Hampshire chickens, is difficult to assess. Although aflatoxicosis has been investigated in several other poultry breeds, in addition to those already mentioned, the studies lack sufficient uniformity with regard to toxin dosages and general experimental approach to permit comparisons. The present investigation explores further the range of susceptibility of a number of avian species and includes commercially important hybrid broiler chickens, chickens of pure breed, 2 sources of hybrid turkeys, and several breeds of game birds. Reported are the effects on mortality, growth, blood proteins, liver biochemistry, and liver histology upon feeding to young birds a subacute dose of aflatoxin in the diet.

Materials and Methods. The species and strains of fowl used in this survey were obtained from commercial hatcheries and from the Avian Sciences Department, University of California at Davis. The birds, unsexed except for the hybrid broiler chicks which were male, were started on the experimental diets when 1 or 2 days old. The diets consisted of 15% cottonseed meal containing aflatoxin and either modified commercial broiler ration, which was fed to chickens, or modified turkey starter diet, which was fed to game birds and turkeys; the final protein content was 24 and 28%, respectively. The aflatoxin in the cottonseed meal was derived from added rice which had been cultured with *Aspergillus flavus* and consisted predominantly of B_1 with small amounts of B_2, G_1, and G_2 (8). The overall toxicity of the experimental diets was equivalent to 800 ppb pure aflatoxin B_1 based on a comparative bioassay with turkey poults. Control birds were fed the same ration to which cottonseed meal free of aflatoxin had been added. Two feeding experiments were performed with turkeys: trial 1 with Broad Breasted Whites from the University of California, and trial 2 with the commercial Broad Breasted Whites, Rose-A-Linda.[3]

The number of birds placed on experimental diets was such as to provide at least 2 samplings of 10 test and 10 control animals during a 2- to 6-week period. Chickens and turkeys were autopsied for biochemical investigation in most instances after 2 and 3 weeks. Quail and guinea fowl, because of their small size, were analyzed when 4 to 6 weeks old.

Blood samples were collected from the jugular vein in oxalated tubes after the birds had been lightly etherized. After being bled,

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the birds were decapitated and the livers were immediately removed and weighed. Two small pieces were taken from each liver and preserved in buffered 10% neutral formalin; the remaining liver was frozen in Dry Ice until assayed. Histological examination of the hematoxylin and eosin stained slides of liver tissue was performed by Drs. D. L. Dungworth and J. E. Moulton, veterinary pathologists.

In some instances the small amount of blood or liver obtainable made it necessary to pool material from 2 or more animals thereby reducing the number of samples below 10. Also, high mortality occasionally reduced this number below 10 in the second sampling, and feeding trials were repeated for some of the birds in which losses were considerable. Particularly high mortality (20 to 60%) unrelated to aflatoxicosis occurred among the quail. For turkeys (trial 2) and New Hampshire chickens (third week) there were 8 test samples. The average number and range of samples for the various strains of quail were 8 (6 to 10) control and 5 (3 to 9) test for liver; 4 (3 to 5) control and 3 (2 to 5) test for plasma. All other groups consisted of 10 control and 10 test specimens.

Plasma albumin and total plasma protein were determined by the method described by Annino (9). All liver assays were made on 25% aqueous homogenates. Liver succinic dehydrogenase was estimated by the method of Slater and Bonner (10). RNA was extracted with perchloric acid after mild alkaline hydrolysis (11) and determined spectrophotometrically by the 2-wavelength method of Fleck and Begg (12). DNA was determined by the color reaction with diphenylamine (13).

For lipid determination, 25% liver homogenates were lyophilized to a moisture content equal to that of fresh tissue after which lipid was extracted according to the method of Folch *et al.* (14). Liver nitrogen was assayed by the Kjeldahl technique.

Results. The effects of aflatoxin in the diet equivalent to 800 ppb B_1 on body weight, liver weight, and biochemical constituents of liver and plasma are presented as a percentage of control means in Figs. 1, 2, and 3. From Figs. 1–3, which provide profiles of susceptibility to subacute aflatoxin poisoning, a considerable range in sensitivity is evident among the various breeds of avian species. Growth was generally depressed for all, except Austrolop and Barred Rock chickens, Japanese quail and guinea fowl. While Austrolops were unique, in that body weight significantly increased in test animals relative to controls at 3 weeks, the Barred Rock chicks appeared least affected of all breeds tested (Fig. 1).

Most adversely affected were New Hampshire chicks and turkey poults of both trials (Fig. 1). In these birds inhibition of growth was greatest and was accompanied by severely reduced total plasma protein and plasma albumin. Liver constituents were also generally reduced with the exception of lipid concentration which was elevated at 2 weeks in New Hampshire chicks and turkeys of trial 1. At 3 weeks liver lipid remained elevated in New Hampshire chicks but had decreased significantly below control levels in the turkeys. Mortality for New Hampshire chickens resulting from aflatoxicosis was 30% at 1 week and 40% at 2 weeks. For turkeys it was 50% and 20% in trials 1 and 2, respectively. No other increases in mortality related to aflatoxin were observed.

Hybrid chicks showed intermediate sensitivity between the purebred New Hampshire and Barred Rock strains (Fig. 2). Of these, Foster Farms appeared most sensitive with growth reduced to between 85 and 90% of controls. As in the Broad Breasted White turkey, liver lipid was significantly decreased at 3 weeks. The two reciprocal crosses of New Hampshire with White Leghorn possessed low susceptibility to aflatoxin, especially when compared to the parent New Hampshire strain.

Moderate liver involvement occurred in the other two pure strains of chickens studied, namely Rhode Island Red and Pilch White Rock (Fig. 1). The latter showed considerable signs of aflatoxin poisoning at 2 weeks but at 4 weeks, with the exception of lowered total plasma protein, there was little difference between test and control birds.

Among the game birds (Fig. 3), the

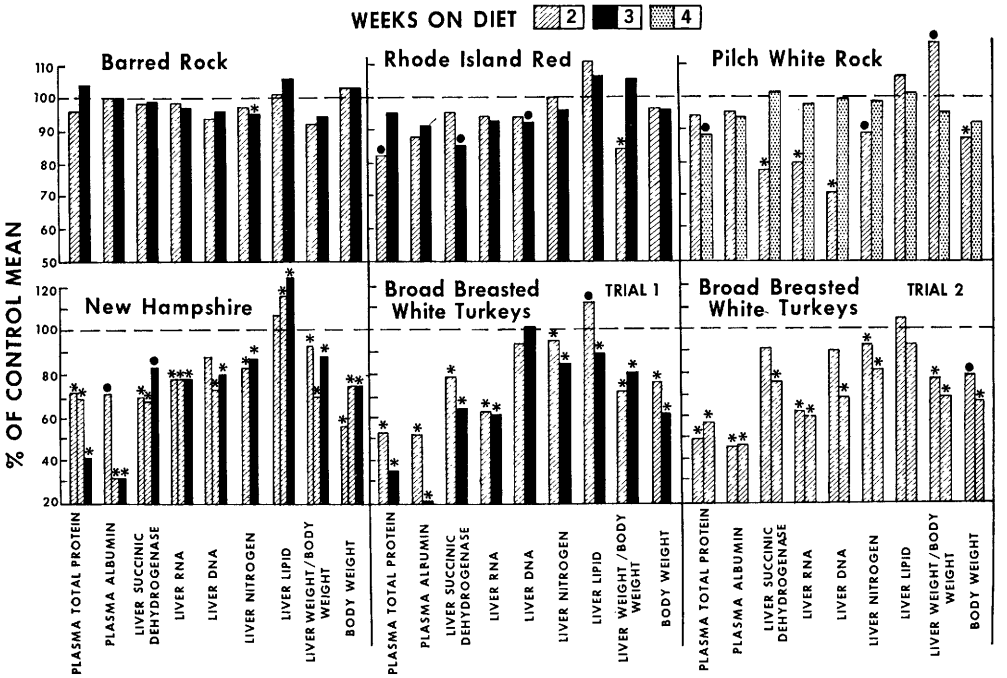

FIG. 1. Effect of aflatoxin (≅ 800 ppb B_1) in the diet on pure strain chickens and on turkeys. Significance of difference between test and control means: (●), $p < .05$; (*), $p < .01$. Two mean values for similar time periods indicate repeated feeding trials.

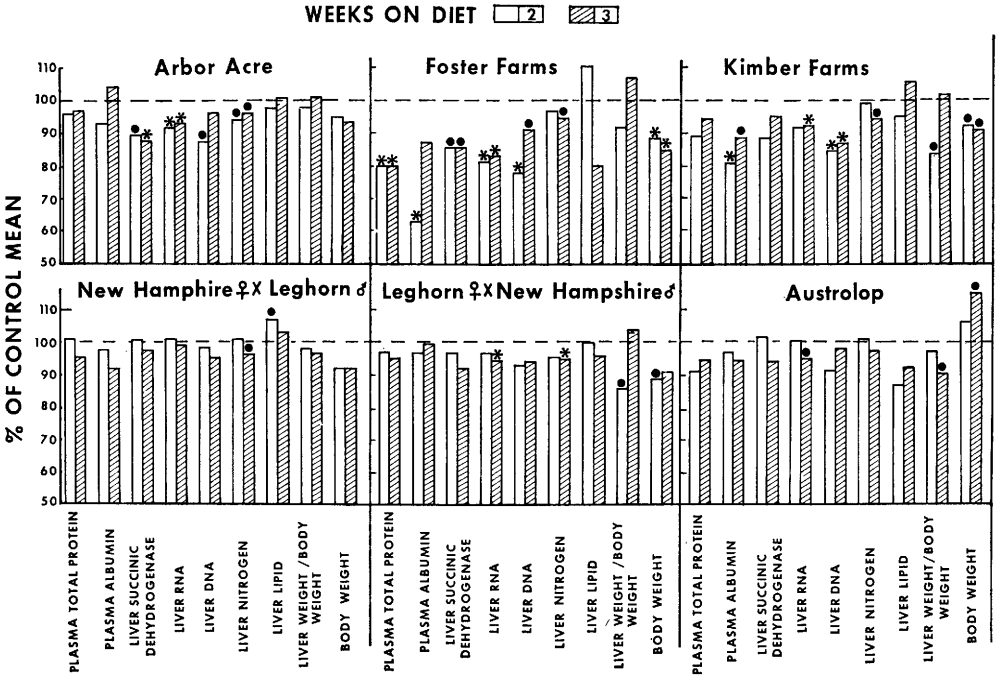

FIG. 2. Effect of aflatoxin (≅ 800 ppb B_1) in the diet on hybrid cross chickens. Significance of difference between test and control means: (●), $p < .05$; (*), $p < .01$.

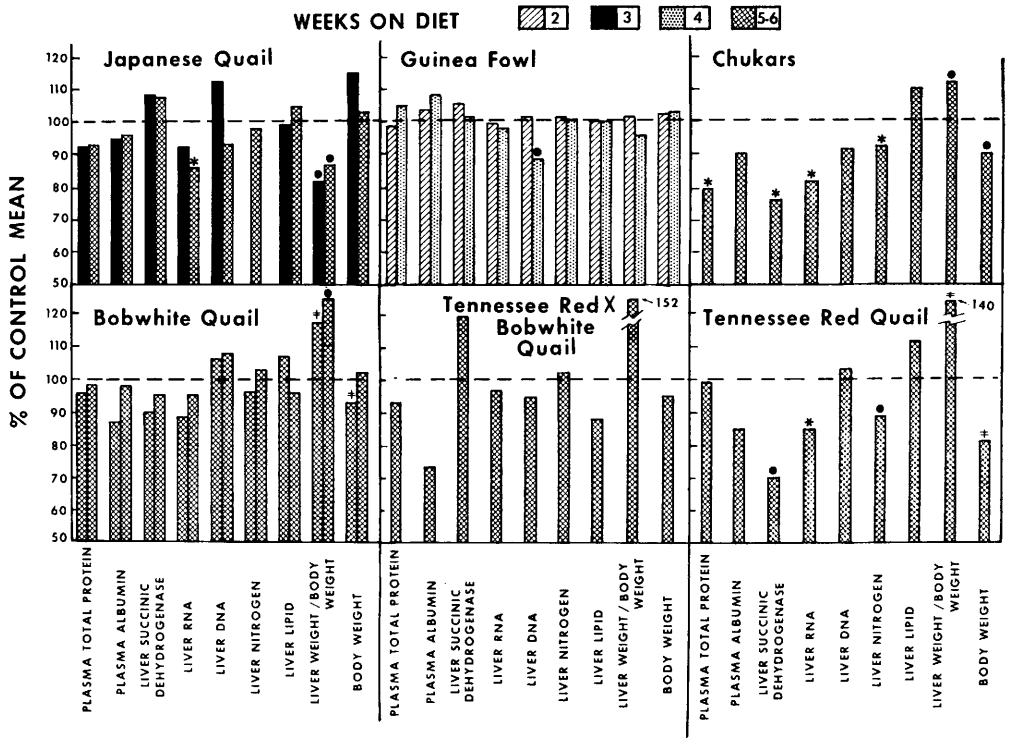

FIG. 3. Effect of aflatoxin (≅ 800 ppb B_1) in the diet on quail and guinea fowl. Significance of difference between test and control means: (●), $p < .05$; (*), $p < .01$. Individual body weights not obtained, ‡. Two mean values for similar time periods indicate repeated feeding trials.

greatest signs of toxicity were evident in the chukar (a partridge) followed possibly by Tennessee Reds. The sensitivity of chukars was less, however, than New Hampshire chickens with regard to effects on growth and plasma proteins. The cross between Tennessee Red and Bobwhite quail appeared to be more resistant to aflatoxin than the Tennessee Red strain alone based on liver nitrogen, RNA, succinic dehydrogenase, and growth.

Japanese quail showed signis of toxic effects with significantly lowered liver RNA concentration and liver weight, although growth was not inhibited. The guinea fowl appeared to have high resistance to aflatoxin comparable to that of Barred Rock chickens. The only significant indication of aflatoxicosis in guinea fowl was reduced liver DNA concentration at 3 weeks.

A good correlation was observed between the degree of biochemical effects produced and the histological abnormalities seen in the livers of chicks fed aflatoxin. Thus, in both New Hampshire chicks and turkey poults the pathological lesions detected were hepatic cell and bile duct cell proliferation after 3 weeks of the feeding trial. Some degenerative changes were also noted in the liver cells of the most severely involved livers. In contrast, no detectable liver lesions were seen in hybrid chicks from reciprocal crosses of New Hampshire and White Leghorn breeds. In both Foster Farms and Rhode Island Red chicks 2 of 10 showed beginning hepatic cell hyperplasia at this time. No microscopic lesions were detected in the liver sections of Austrolop, Barred Rock, and Kimber Farms chicks fed the aflatoxin diet for 3 weeks or in Pilch White Rock chicks and guinea fowl after 4 weeks. Microscopic lesions due to aflatoxin including proliferation of the bile duct epithelium and hepatic cells were detectable in the livers at 5 weeks of game birds including chukars, Bobwhite quail, and Tennessee Red quail. A recovery in the de-

gree of aflatoxicosis was noted in Japanese quail in that 5 of 9 livers were positive after 3 weeks, but after 5 weeks only 1 of 10 was abnormal.

Discussion. Experience with newly hatched chicks in our laboratory has shown biochemical changes resulting from aflatoxicosis to be generally maximal during the first 3 weeks. After this period, in cases of moderate or slight toxicosis, a lessening of effects can be observed which is consistent with the increase in resistance to aflatoxin known to occur with maturity in many species (1, 2). This period of maximal biochemical change coincides with or perhaps precedes slightly the optimum time (between 17 and 31 days) for detecting histopathologic changes in chick liver such as bile ductule and hepatocytic lesions (8).

Hypoproteinemia, particularly involving plasma albumin was one of the most sensitive biochemical responses to the degree of aflatoxin intoxication as revealed by growth inhibition, mortality, and histopathology. A similar observation with New Hampshire chicks and ducklings was reported by Brown and Abrams (6). In liver, reduction in succinic dehydrogenase and nucleic acid concentration were also sensitive indicators of intoxication. Changes in liver nitrogen levels often paralleled changes in nucleic acid concentration and may be regarded as reflecting alterations in protein metabolism.

Comparison of liver lipid concentration between test and control birds was complicated by the rapid depletion during the first 2 or 3 weeks of the normally elevated lipid present in the liver of newly hatched chicks. The rate of depletion differed among individuals with the result that animal variation was large in both test and control groups before stable lipid concentrations, as found in more mature birds, were reached. Carnaghan *et al.* (15) found that liver lipid increased in Rhode Island Red chicks fed 1500 ppb aflatoxin B_1 with a maximum difference between test and control values appearing after 2 to 3 weeks. In the present work in which birds were fed aflatoxin equivalent to 800 ppb B_1, statistically elevated liver lipid occurred only in the New Hampshire chick, one of the New Hampshire crosses, and the turkeys of trial 1. A tendency towards increased liver lipid was apparent in the 3 other pure strain chickens. The significantly decreased liver lipid in turkeys of trial 1 after being elevated 1 week earlier was probably related to the moribund condition of this group.

In contrast to the findings of Carnaghan *et al.* (15) with Rhode Island Red chickens, significant decreases in the liver weight:body weight ratios were encountered which included those of the most sensitive breeds, the New Hampshire chick and Broad Breasted White turkey. For the 3 exceptions where the liver weight:body weight ratio was distinctly elevated, namely in Pilch White Rock chickens at 2 weeks and in chukars and Bobwhite quail at 5 to 6 weeks, the general effects of aflatoxin were slight to moderate.

It is difficult to compare our results on Pilch White Rock chickens with those of Cottier *et al.* (16), who also studied the response of this breed to aflatoxin. Whereas we found growth to be significantly depressed at a dietary level equivalent to 800 ppb aflatoxin B_1 after 2 weeks and still less than that of controls at 3 weeks, Cottier *et al.* reported no effect on growth at 610 ppb after 9 weeks but found considerable growth inhibition at 1834 ppb. Mortality at the latter 2 levels of aflatoxin was 11 and 47% at 9 weeks, compared to no deaths in our work after 3 weeks.

Although chickens are regarded as being more resistant to poisoning by aflatoxin compared to ducklings and turkeys (1), the present work confirms the findings of Brown and Abrams (6) that the New Hampshire chick apparently possesses unique sensitivity. Marked biochemical changes in plasma and liver as well as increased mortality and inhibition of growth place their susceptibility approximately equal to that of turkey poults. Further evidence of sensitivity in New Hampshire chickens to aflatoxin was observed when New Hampshire hens were fed a diet containing 2700 ppb aflatoxin (unpublished experiment). Feed consumption, body weight, and egg production all decreased sharply; whereas, no adverse effects were observed, as reported previously (8), when White Leghorn hens were fed the same aflatoxin diet. The

nature of this susceptibility appears to be genetically recessive as shown by the relative resistance of the crosses between New Hampshire and White Leghorns. In addition to White Leghorns, birds possessing considerable resistance to aflatoxin include Cornish Game and White Rock chickens (6). To these we can now add the Austrolop, Barred Rock, and guinea fowl.

It is apparent that the considerable range in sensitivity among fowl to aflatoxin, which includes differences of breed within a species as well as species differences, precludes generalizations on the adverse response to be expected from specific dose levels of the toxin. Consequently, these differences would have to be considered in any studies attempting to establish dose levels of "no effect."

Summary. The comparative aflatoxin susceptibility in 18 different strains, crosses, or breeds of chickens, turkeys, and quail was evaluated under standardized conditions. One-half of the day-old chicks in each group were fed a diet containing aflatoxin equivalent in toxicity to 800 ppb pure aflatoxin B_1 for periods of 2 to 6 weeks. Chicks were autopsied at weekly intervals to evaluate blood and liver biochemical and liver histological effects. One of the most sensitive biochemical responses to the degree of aflatoxin intoxication was a decrease in plasma albumin. Reduction in liver succinic dehydrogenase and nucleic acid concentration were also sensitive indicators of toxicity. Most adversely affected by aflatoxin were New Hampshire chicks and turkey poults. When New Hampshire hens were crossed with Leghorn males or vice versa, the sensitivity of the chicks to aflatoxin was no longer detectable, suggesting that the susceptibility is a genetically controlled factor. Birds possessing considerable resistance to aflatoxin included Barred Rock and Austrolop chickens and guinea fowl.

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Received Mar. 9, 1970. P.S.E.B.M., 1970, Vol. 134.