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of commercial broiler chickens fed
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CONTENTS

| | |
|---|----|
| Introduction and Review of Literature | 3 |
| Materials and Methods | |
| Fungal cultures and feed | 5 |
| Ducklings | 5 |
| Chickens | 6 |
| Assays and analyses | 6 |
| Results | |
| Aflatoxin assay | 8 |
| Effect in ducklings | 8 |
| Performance of chickens | 10 |
| Duckling assay for aflatoxin content of residual chicken feed | 11 |
| Discussion | 16 |
| Summary | 17 |
| References | 19 |

The Mycotoxic Effects of Fungi Isolated From Poultry Feed Ingredients:

The Response of Ducklings and Performance of Commercial Broiler Chickens Fed Experimentally Infected Corn Diets¹

Elizabeth S. Barden², H. L. Chute²

D. C. O'Meara² and Hilda T. Wheelwright²

INTRODUCTION AND REVIEW OF LITERATURE

Mycotoxins can occur naturally in feedstuffs and may endanger the health of livestock and man. They develop as toxic metabolites when temperature and humidity are optimum for the growth of certain fungi on grain (fig. 1), whether in the field, in transport or in storage.

Disease syndromes in both animals and man, which have resulted from the ingestion of cereal grains contaminated with fungus, have oc-

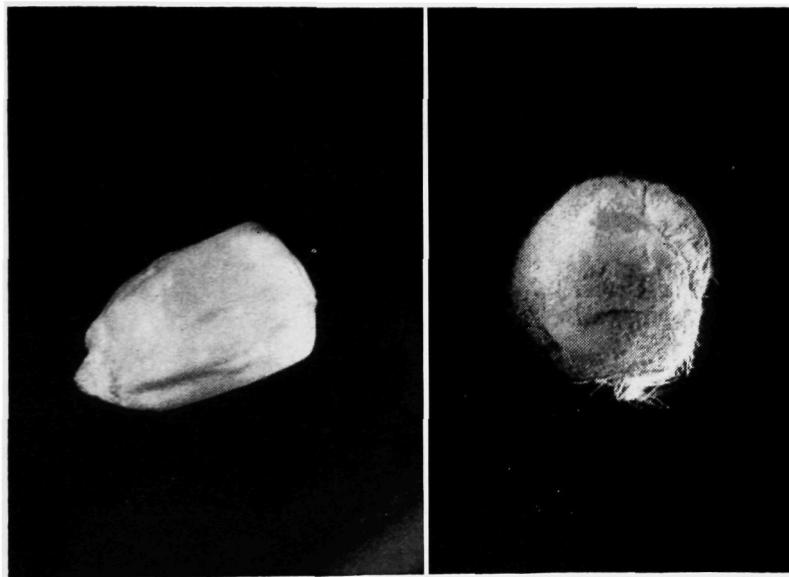


FIGURE 1. Corn kernels. Kernel on left is normal. Kernel on right is overgrown with *Aspergillus flavus*. Kernels with less conspicuous growth may contain higher levels of fungal toxins, as optimum time for production of these metabolites may be before sporulation. Infected kernels can cause contamination of the storage area or foul tons of grain under poor storage conditions,

¹ This paper represents a portion of the first author's thesis submitted for the Ph.D. degree in August, 1969 at the University of Maine.

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curred spasmodically and been reported over the years (Forgacs, 1962; Joffe, 1965). In the last decade several mycotoxins have been defined (Bamburg *et al.*, 1969), and experimental work with them in various animal species has been performed (Newberne *et al.*, 1964, 1966; Halver, 1965; Shank and Wogan, 1966; Cyzewski *et al.*, 1968; Svoboda *et al.*, 1968).

Although not as susceptible a species as other animals to fungal toxicity, chickens have been affected by the hemorrhagic syndrome associated with moldy feed toxicosis, occurring under both natural and experimental conditions. The intensity of the disease is dependent on the amount, type and potency of the toxin ingested. It is characterized by varying degrees of morbidity and mortality with hemorrhage in the tissue and depression of the blood forming elements in the bone marrow (Cover *et al.*, 1955; Forgacs, 1962). After experimentally subjecting chickens to moldy diets, Richardson and Webb (1962), reported a progressively slower growth rate as mold growth in the feed increased with time.

Chickens are apparently less susceptible than ducklings and turkey poults to aflatoxin, a toxic metabolite of *Aspergillus flavus*, first noted when thousands of turkey poults died in Britain in 1960. They had been fed Brazilian peanut meal, processed from carelessly harvested peanuts which were heavily contaminated with *Aspergillus flavus* (Blount, 1961). Chickens did not figure conspicuously in these losses, but in a similar outbreak in South Africa in 1963, ducklings and New Hampshire chickens were seriously affected. However other commercial breeds of chickens fed the same feeds (Cornish Game, White Rock, White Leghorn and Rhode Island Red) grew normally with no impairment of weight gain, lesions or mortality (Abrams, 1965).

Further work, reporting on the biochemical mode of action of aflatoxin in ducklings and chickens confirmed the existence of this breed difference in chickens' susceptibility to 0.5 ppm of aflatoxin B₁ in the rations (Brown and Abrams, 1965). The altered metabolism for affected birds was reflected in a retarded growth curve. Biochemical changes included: a decrease in activity of some of the mitochondrial enzymes and dehydrogenases, coupled with a lowering of the rate of ATP synthesis, and in turn a suppression of protein synthesis—noted especially in the reduction of blood serum albumin; and an increase of certain plasma enzymes associated with severe liver lesions.

The high tolerance of the Rhode Island Red breed to rations containing a highly toxic meal was also indicated by Carnaghan *et al.*, (1966). However hepatic tissue was affected and there occurred an overall retardation of growth.

Results of recent experiments with Pilch strain White Rocks at Auburn, Alabama, however indicated that serious problems may result from low levels of aflatoxin, whereas depressed body weight, delayed maturity and high mortality are caused by high levels (Cottier *et al.*, 1968).

The characteristic aflatoxin liver lesion found in ducklings, used as a test animal for aflatoxin potency because of its extreme susceptibility, consists of marked parenchymal cell damage with fatty infiltration and necrosis. Most prominent is the extensive and rapid proliferation of cords of bile ductular cells. These radiate from the portal tracts and are arranged in tubular formation as a bile duct hyperplasia (Allcroft *et al.*, 1961; Newberne *et al.*, 1964).

The present work, planned to investigate the possibility of mycotoxins occurring in feed ingredients fed to poultry in Maine, was designed with the following objectives: (1) to isolate fungi from poultry feed ingredients; (2) to grow them separately on corn (the carbohydrate source of poultry rations) for later mixing into the diets; (3) to test the variously infected lots of this corn substrate for mycotoxicity by feeding ducklings, a bioindicator for toxins; (4) to determine the effects of aflatoxin and other mycotoxins from feed ingredients, on the performance of commercial broiler chickens; and (5) to appraise this response as a measure of toxicity of the fungi found in feed ingredients.

MATERIALS AND METHODS

Fungal cultures and feed

Fungi isolated from poultry feed ingredients, and a peanut meal strain of *Aspergillus flavus*, found to be toxic to ducklings in a previous experiment (Chute *et al.*, 1965), were cultured on corn in wide mouth gallon jars for mycotoxin production. The fungus-infected corn was combined with corn meal and mixed with the other elements of broiler rations prescribed by the N.E.C.C.³

Ducklings

Forty-five White Pekin ducklings were used as a biological index to screen seven species of fungi for toxicity. They were divided into nine diet groups: **A**—Control; **B**—Processed corn; **C**—Toxic meal *A. flavus*, previously known to affect ducklings (Chute *et al.*, 1965); **D**—

³ N.E.C.C.—New England College Conference Board of Poultry Nutritionists, associated with the Cooperative Extension Service of the University of Maine and the other five New England Land Grant Universities.

A. flavus; **E**—*A. fumigatus*; **F**—*Penicillium cyclopium*; **G**—*P.* sp.; **H**—*A. oryzae*; **I**—*Cephalosporium* sp. The ducks were kept on wood shavings in plywood pens (24 x 18 x 12 inches), in a temperature regulated house of between 80 and 85°F, with extra heat provided by a 150 W Amplex reflector lamp suspended over the center partition of each pair of pens.

After administering diets to the chickens, ducklings were again used as an indicator, in this instance for testing the potency of the aflatoxin in diet C, the toxic peanut meal *Aspergillus flavus*-infected feed, given the chickens. Toxic meal *A. flavus* starter and finisher diets, residual from the chicken experiment, were fed respectively to two lots of six ducklings each. Plywood pens with wood shavings for litter were used for the ducklings on the starter diet and the controls. The ducklings fed the finisher diet were housed in a wire cage with a wire bottom. Birds were examined at autopsy, and tissues were removed for histopathological observation.

Chickens

The chickens used to study the effect of aflatoxin and of other possible mycotoxins, in relation to growth, were all of one breed, Line 50 x Vantress cross. As it was of interest to know whether both sexes would be similarly affected, 120 birds of each sex were assigned to the first six diet groups that were used for ducklings: **A**, **B**, **C**, **D**, **E**, and **F**. There were five birds to a sex group and four replications. Birds were individually weighed weekly, and autopsied after cervical dislocation at the end of the eighth week. Macroscopic lesions of birds were noted and tissues (brain, heart, kidney, liver, lung and spleen) were removed for later microscopic examination.

The diet groups were randomly assigned to tiers in the batteries of heat-controlled, wire-floor, brooder cages (1st 4 wk), and growing cages (2nd 4 wk), with replications represented by the four tiers. The male and female groups of each diet replication (5 birds to each sex) were separated by a solid partition on a battery tier.

Assays and analyses

Aspergillus flavus cultures on corn were tested by a crude thin layer chromatography (TLC) assay (Shotwell *et al.*, 1966) for aflatoxin B₁ potency, before animal experimentation began (fig. 2). After feeding the diets to chickens, the serial supply of toxic meal *Aspergillus flavus* cultures on agar slants was examined for aflatoxin B₁ production in corn, by TLC assay of corn extract.

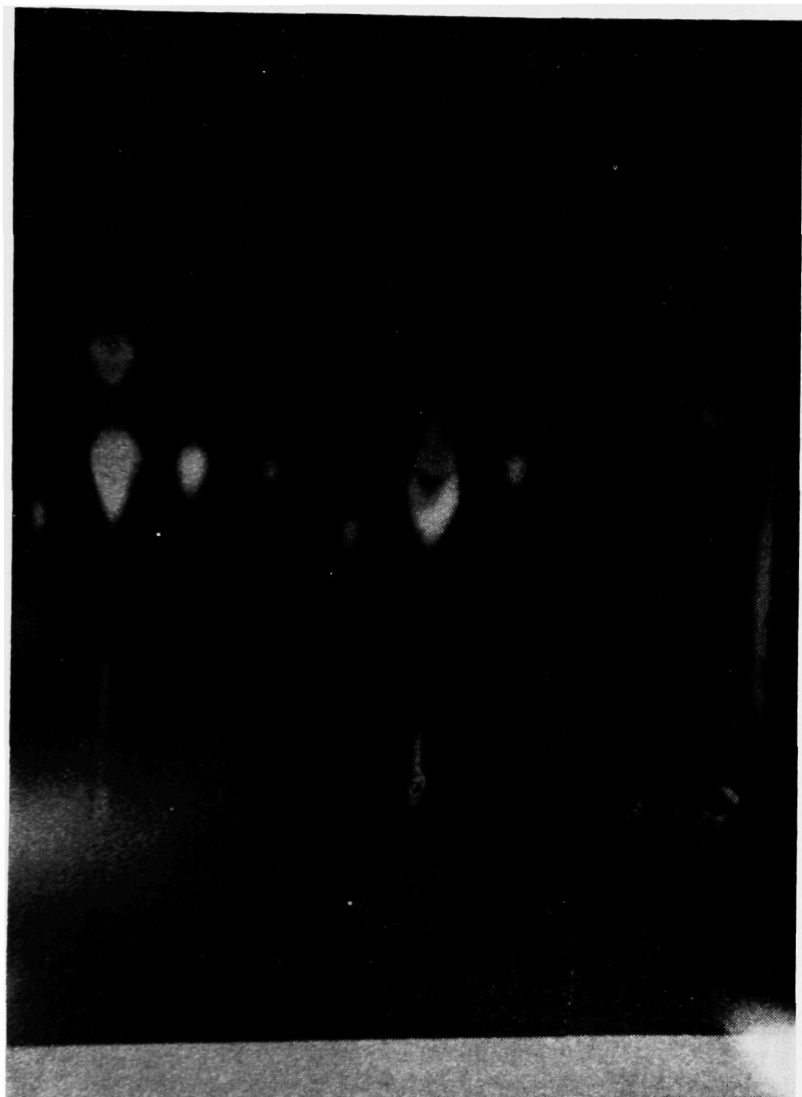


FIGURE 2. Crude thin layer chromatogram of samples of extracts from *Aspergillus flavus* cultures on rice and corn. Spotting was made with 20 microliter Drummond microcaps. They are, left to right, 1 and 5 - aflatoxin standard; 2, 3 and 4 - toxic meal *A. flavus* on rice, 10⁻¹, 10⁻²; 7 and 8 - toxic meal *A. flavus* on corn, 10⁻¹; 9 - another strain of *A. flavus*.

Performance data of average consumption, average weight gain and feed conversion for the eight week period were calculated and statistically analyzed.

All tissues removed from the ducks and chickens were processed by an alcohol paraffin method and stained with hematoxylin and eosin. Liver tissues were also stained with Masson's trichrome stain as an aid to distinguish the fibrous connective tissue.

RESULTS

Aflatoxin assay

The aflatoxin B₁ content of a culture of toxic meal *Aspergillus flavus* in corn assayed by crude TLC before experimentation was approximately 1.3 mg/kg feed. The amount consumed per duck in the two weeks of the toxicity trial was estimated to be about 1.9 mg/kg. A negligible amount of aflatoxin was produced by the other *A. flavus* strain isolated from poultry feed ingredients which was used in both the duck and chicken experiments in diet D.

At the conclusion of the chicken experiment, when all groups had adequately gained and showed no signs of malaise or retardation from mycotoxins, the further TLC assay for aflatoxin indicated negligible amounts produced in corn cultures. These toxic meal *Aspergillus flavus* cultures in corn were inoculated from a serial supply of agar slant sub-cultures remaining in stock after those used for preparing the infected feed.

Effect in ducklings

The ducklings in the toxic meal *A. flavus* diet group C were severely poisoned with aflatoxin (fig. 3). No other fungus-infected diet group was noticeably affected at the end of two weeks. Variation in weight gains among the other groups was not significant since there was so much weight variation within the groups.

Ducks in the diet group C ate only sparingly of what was apparently a very distasteful, pungent smelling diet. They used only half as much feed as ducks in other groups. Four out of five ducks were dead by the end of two weeks, and the one remaining live duck weighed 95.8 g less than the control. It had weighed approximately the same as the control at the start of the experiment. The livers of the ducklings in this group were all critically damaged. They were putty colored with reticulation of blood vessels and fibrous tissue visible below the surface, in the birds which died; some lobes were characterized by a white lacey

pattern of abnormal tissue. The liver of the one surviving duck was dark and compact with an indented pattern from the reticulated texture of fibrosis (fig. 4). Microscopically the whole pattern of liver cell

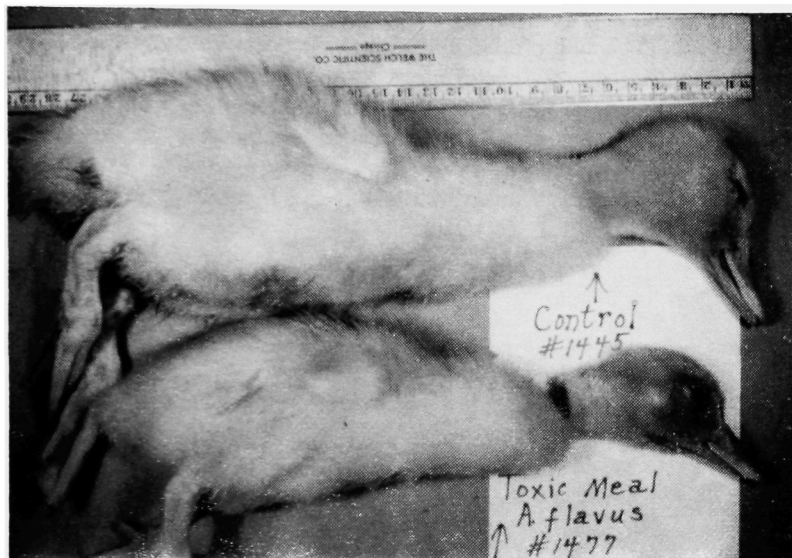


FIGURE 3. White Pekin ducklings at two weeks of age. Duckling weights: control = 303.6 g; toxic meal *Aspergillus flavus* = 210.5 g. The control weighed 0.9 g more than the T.M. *A. flavus* duckling at hatch.

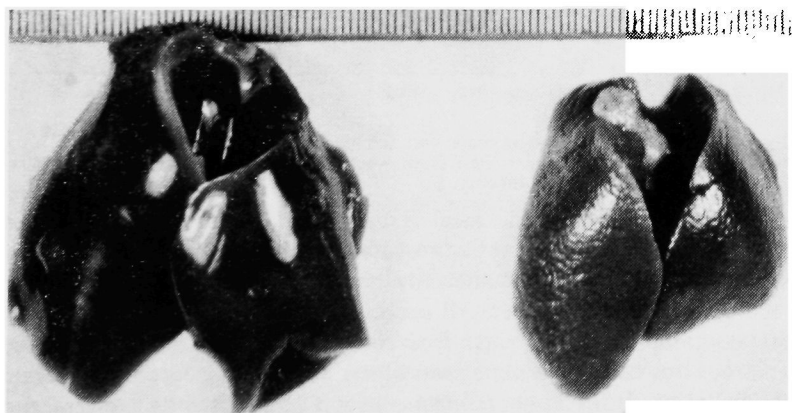


FIGURE 4. Livers of White Pekin ducklings at two weeks of age. Liver of control on left is smooth and shiny. Liver of duckling fed toxic meal *Aspergillus flavus* diet (1.3 mg aflatoxin B₁/kg feed) is more compact and has reticulated appearance due to fibrosis.

arrangement and cell size was altered. There were hepatic cell degeneration, necrosis, bile duct hyperplasia and proliferation of bile ductular cells extending in a chain-like arrangement towards centro-lobular regions. Dying liver cells were replaced by fibrous connective tissue.

Performance of chickens

The commercial broiler chickens, Line 50 x Vantress cross, responded to the several fungus-infected diets with no extreme adverse effects. Variation in the growth response among the groups was so slight that the pattern of growth, regardless of treatment, was essentially the same (fig. 5).

There were significant variations in feed conversion efficiencies (table 1).

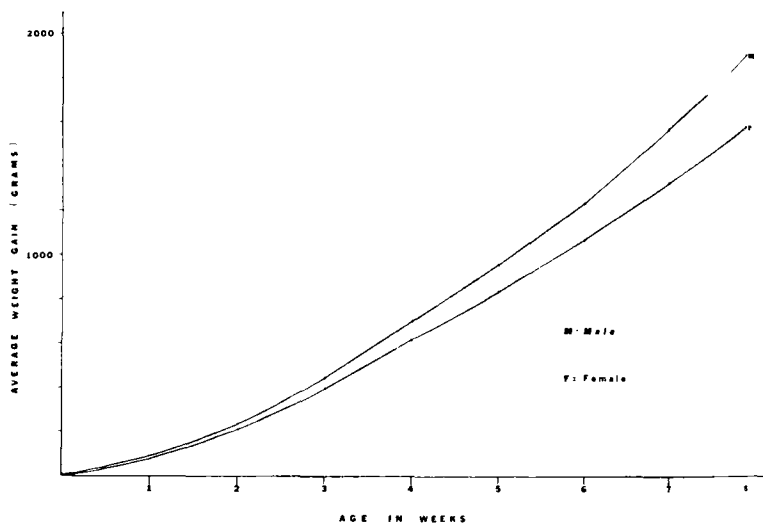


FIGURE 5. Growth curves of male and female broiler chickens, Line 50 x Vantress cross, constructed from weekly average weight gains, on experimental diets A through F.

Surprisingly the toxic meal *Aspergillus flavus* group of chickens made the highest gains with an overall smaller amount of feed consumed—the result of a significantly better conversion efficiency of the feed than by any other diet. All processed corn diets, of both male and female groups, effected better feed conversion than the control, as the control birds consumed more than others yet maintained only moderate weight gains. Birds in *A. fumigatus* group E consistently ate less and gained less.

The only significant difference in response by males and females was in the order of decreasing values for conversion efficiency of

TABLE 1

Treatment Means for Male and Female Chickens, Line 50 x Vantress Cross, in Feed Conversion Analysis.

| Male | | Female | |
|----------------------------------|------------------------|----------------------------------|--------------------|
| Diet Group | Feed/Gain ¹ | Diet Group | Feed/Gain |
| A - Control | 2.04 ^{a2} | A - Control | 2.11 ^a |
| B - Processed corn | 1.93 ^{bc} | B - Processed corn | 2.11 ^a |
| C - Toxic meal | | C - Toxic meal | |
| <i>Aspergillus flavus</i> | 1.90 ^c | <i>Aspergillus flavus</i> | 2.03 ^b |
| D - <i>Aspergillus flavus</i> | 1.93 ^{bc} | D - <i>Aspergillus flavus</i> | 2.05 ^b |
| E - <i>Aspergillus fumigatus</i> | 1.96 ^b | E - <i>Aspergillus fumigatus</i> | 2.08 ^{ab} |
| F - <i>Penicillium cyclopium</i> | 1.96 ^b | F - <i>Penicillium cyclopium</i> | 2.03 ^b |

¹ Grams feed consumed/grams weight gained.

² Numbers in the same column followed by the same superscript are not significantly different ($P < .05$). Calculations were based on conversion figures taken to the third decimal place.

feeds among the diet groups (table 1). The *Penicillium cyclopium* group of females showed the best feed conversion of all diet groups whereas the efficiency of the diet by males in this group was third poorest. Males of diet group C excelled with the lowest conversion efficiency of feed by this sex.

In general, the graphs of weekly consumption, gain and feed conversion for the females followed much the same pattern as those for the males (figs. 6-9). The females ate less and gained less but consumed proportionately more for their gain as evidenced in the higher values for feed conversion efficiency by the females.

The average eight-week weights of male and female chickens in each diet group are listed in table 2.

Duckling assay for aflatoxin content of residual chicken feed

Although birds in the chicken experiment had suffered no apparent ill effects from toxins in any of the four fungus-infected feeds there was evidence from the second duckling response, the biological aflatoxin indicator, that the diet C administered to the chickens did contain aflatoxin. However there was a smaller amount than in the same diet prepared for the first lot of ducklings, and the amount was apparently even less in the last prepared feed of the finisher diet C.

Ducklings on the toxic meal *Aspergillus flavus* starter diet gained 74 g less than the controls in a two-week period. However none died as

TABLE 2

Average Eight-week Weights of Male and Female Chickens, Line 50 x Vantress Cross, on Experimental Diets A through F.

| Diet Group | Male g | Female g |
|---|-----------|-------------|
| A - Control | 1971 | 1642 |
| B - Processed corn | 2051 | 1611 |
| C - Toxic meal <i>Aspergillus flavus</i> | 2074 | 1647 |
| D - <i>Aspergillus flavus</i> | 1959 | 1608 |
| E - <i>Aspergillus fumigatus</i> | 1924 | 1603 |
| F - <i>Penicillium cyclopium</i> | 1948 | 1619 |
| Total | 11927 | 9730 |
| Average | 1987.8 | 1621.7 |

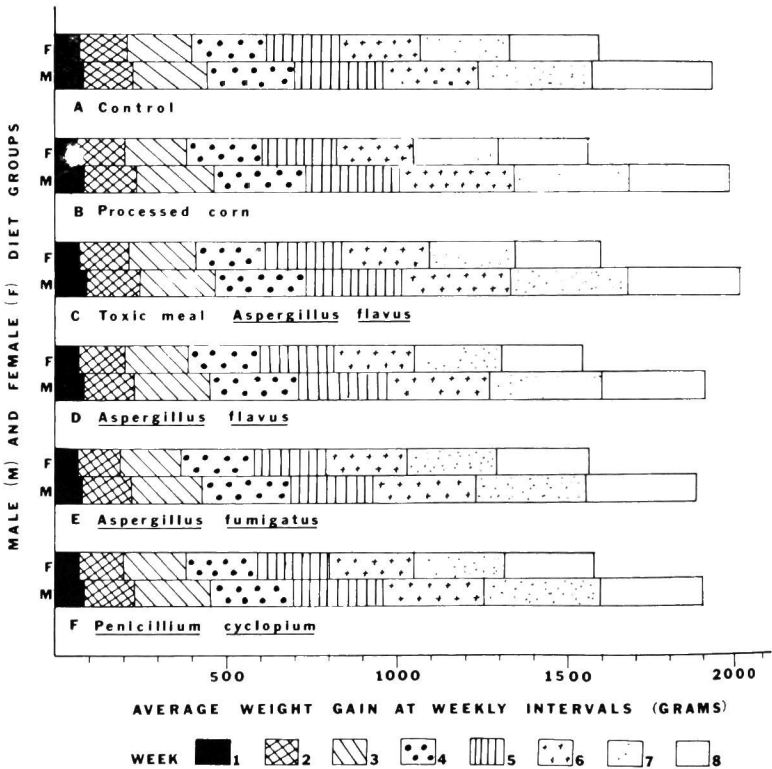


FIGURE 6. Average weight gains, calculated at weekly intervals, of male and female chickens, Line 50 x Vantress cross, on experimental diets A through F.

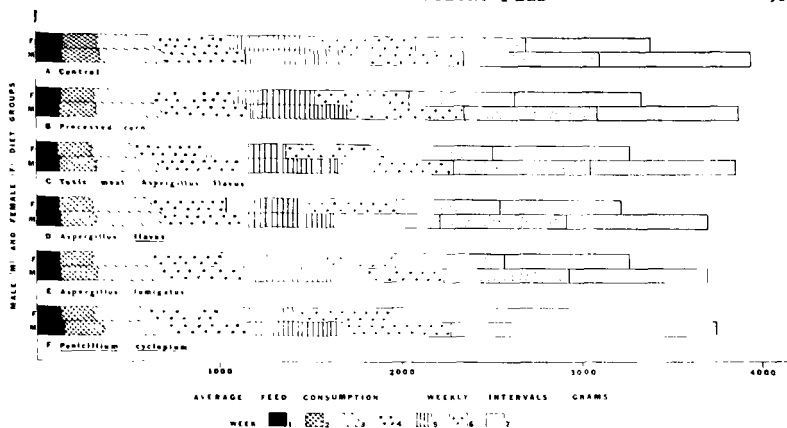


FIGURE 7. Average consumption, calculated at weekly intervals, of male and female chickens, Line 50 x Vantress cross, on experimental diets A through F.

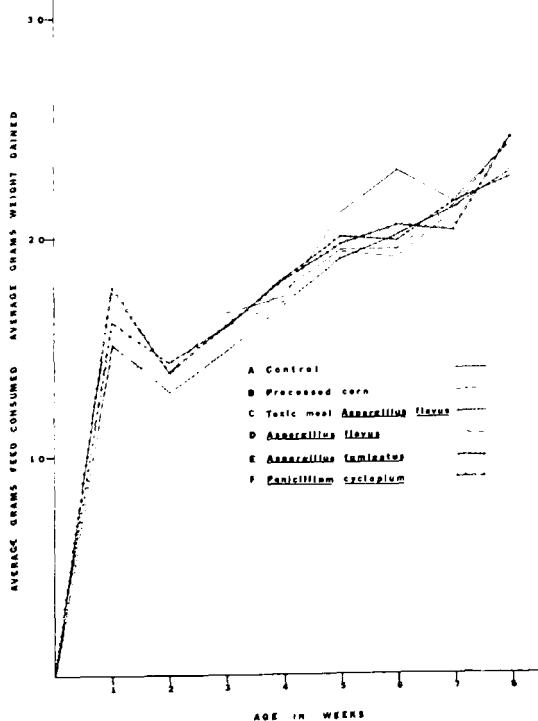


FIGURE 8. Average feed conversion efficiency, calculated at weekly intervals, by male chickens, Line 50 x Vantress cross, on experimental diets A through F.

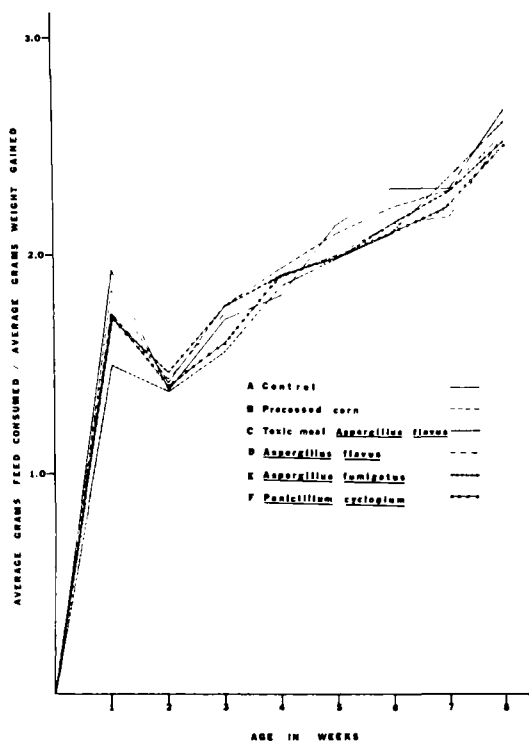
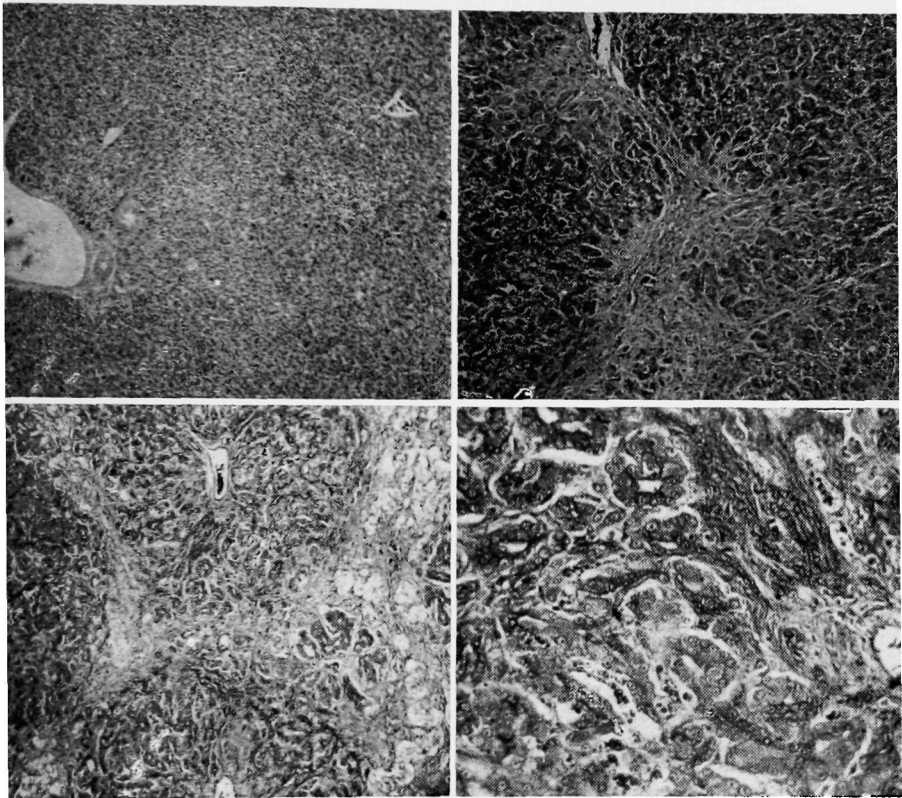


FIGURE 9. Average feed conversion efficiency, calculated at weekly intervals, by female chickens, Line 50 x Vantress cross, on experimental diets A through F.

in the first duckling experiment. At autopsy their livers were putty colored with a reticulated pattern of blood vessels and fibrosis. Histopathologically, there was parenchymal cell damage manifested by vacuolization of cells at the periphery of the lobules (fig. 10c). This area surrounded heavy strands of fibrous tissue intertwined with bile duct epithelium which set apart islands of hepatic tissue. Chains of proliferating bile ductular cells extended towards the area of the central vein (figs. 10c and d).

Ducklings on the finisher diet, which were in an open wire cage, gained 135 g more than the controls. Livers of these ducklings did not appear completely normal. Although they were shiny and smooth without any pattern of reticulation they appeared slightly mottled and yellowish in some areas. Microscopically there seemed to be a small amount of bile ductular cell proliferation. The finisher diet contained approximately

10% less soybean meal and about 8% more corn than the starter diet. Since the finisher diet had a larger percentage of infected feed, it evidently contained less aflatoxin as the ducklings growth was not inhibited. Perhaps the open cage environment may have contributed to greater feed consumption.



10. Sections of livers from White Pekin ducklings, at two weeks of age, fed toxic meal *Aspergillus flavus* diet C. Masson's trichrome stain.

left A: Normal liver from control showing vein, artery and bile duct in left corner of hepatic tissue cells surround central vein at upper right. X90

right B: Liver from living duck with severe aflatoxin poisoning; heavy strands of fibrous tissue intersect each other replacing hepatic cells, which creates the pattern of tation on the liver surface as seen in figure 4; the enlarged liver cells in B give the liver a very different appearance, as shown by comparing photomicrographs A and B, which taken at the same magnification. X90

left C: Liver of duckling fed residual starter diet C given chickens; islands of regenerating cells are surrounded by fibrous connective tissue and degenerated necrotic liver cells; ating bile ductular cells (more darkly shaded cells) extend in chain-like formation to the central vein. X50

right D: Enlarged view of the proliferating bile ductular cells around the lower edge of central vein in C; bile duct hyperplasia is evident in the tubular formation of these cells (left). X180

DISCUSSION

The possibility of mycotoxin presence in poultry rations has been emphasized by the isolation of fungi from feed ingredients. The toxicosis induced in ducklings given feed infected with a toxic *Aspergillus flavus* strain from peanut meal exemplified a drastic poisoning by a mycotoxin, when the delicate balance in environmental conditions was optimum for its production. The level of aflatoxin content in the earlier prepared feed, which killed ducklings, was apparently higher than that in subsequently processed batches which elicited a milder toxicosis in the second lot of ducklings. This circumstance was thought to be due to decreasing aflatoxin-producing potential by serial subcultures of the toxic *A. flavus* strain.

The conspicuous enlargement of individual hepatic cells in the aflatoxin-affected duckling liver tissue apparently resulted from a suppression of liver cell division. This would seem to indicate that aflatoxin B₁ attacks a very basic metabolic process such as at the level of DNA synthesis (Legator, 1966).

The difference in species susceptibility to aflatoxin was demonstrated when the chickens performed normally in response to the same diet which had been toxic to ducklings; at least this breed of chicken (Line 50 x Vantress cross) was not adversely affected. On the contrary, performance of the group of chickens on this toxic meal *Aspergillus flavus* diet was better than that of any other diet group including the controls. Perhaps feed, previously partly digested by a fungal organism, was advantageous to the chickens. Müller (1961), in Germany, reported that greater weight gains resulted in experimental animals fed various kinds of fodder which had been subjected to controlled self-heating and microbial treatment. When processing the fungus-infected corn in the present experiment, it was noted that the *A. flavus* species, especially the toxic meal strain, more thoroughly digested the corn than did other fungal species.

The only significant difference found in the research on the chickens was in feed conversion. How much importance should be attached to this, however, is questionable. Feed conversions are only ratios (feed/gain) which in this analysis were carried to the third decimal place. The higher feed conversion by the *Aspergillus fumigatus* group E possibly could have resulted from a mild fungal toxicity. Because of the large number of chickens that are processed commercially in Maine even slight variations in gain performance can result in appreciable economic significance.

The procedural step of autoclaving fungus-digested cultures (5 min. to suppress spores), before processing for feed, precludes the possibility

of any heat labile mycotoxins being present. However so far as is known from present literature reports, mycotoxins are stable within the temperature range in which the fungi producing them are able to grow. For thermophilic fungi this would include temperatures of self-heating or fermentation. According to Joffe (1965) and Bamburg *et al.* (1969), the mold (*Fusarium tricinctum*) implicated in alimentary toxic aleukia (ATA) outbreaks in Russia produces trichothecane toxins at relatively low temperatures (*ca* + 10° C), which are stable for long storage at room temperatures and are not destroyed by cooking. Aflatoxins B₁ and B₂ are stable at temperatures well above 250° C whereas aflatoxin G₁ and G₂ decompose below this temperature (Wogan, 1966).

SUMMARY

1. Fungi isolated from poultry feed ingredients were cultured on corn for mycotoxin production. This infected substrate was mixed with corn meal and combined with the other elements of broiler rations prescribed by the N.E.C.C.

2. A toxic peanut meal strain of *Aspergillus flavus*-infected corn, incorporated into feed, provided the only one of seven fungus-infected diets which affected the ducklings (White Pekin) employed as a biological indicator for mycotoxicity. Of the nine diets given to ducklings, the same first six (A—Control; B—Processed corn; C—Toxic meal *Aspergillus flavus*; D—*A. flavus*; E—*A. fumigatus*; F—*Penicillium cyclopium*) were fed to chickens to determine the effects of aflatoxin (from the known toxic peanut meal *A. flavus* strain) and other possible mycotoxins from feed ingredients, on the performance of commercial broiler chickens (Line 50 x Vantress cross).

3. The two-week short term response in 45 ducklings indicated mycotoxin production only by the peanut meal strain of *A. flavus*. The aflatoxin content was estimated to be 1.3 mg/kg feed by thin layer chromatography. Four out of five ducklings died from severe aflatoxicosis. All of these and the one surviving duck evidenced the typical liver lesion of parenchymal cell degeneration and necrosis, bile duct hyperplasia with bile ductular cell proliferation, and fibrosis.

4. The chickens responded to all fungal diets with no adverse effects and thus demonstrated the non-susceptibility of this breed to the levels of aflatoxin B₁ in the toxic meal *Aspergillus flavus*-infected feed. In this 240-bird experiment, equally divided between the sexes, there was no significant variation in performance data among the six diet groups (for which there were four replications) except for feed conversion. The group of chickens on the toxic meal *A. flavus* diet

performed better than any other diet group including the controls. Their feed, previously partly digested by this fungal species, seemed to be advantageous to these chickens. The only significant sex difference was in the order of decreasing values for feed conversion quotients (feed/gain) among the groups.

5. A second lot of ducklings given toxic meal *A. flavus* feed, residual from the chicken experiment, confirmed the aflatoxin content of the starter diet, although the level of potency was apparently lower than in the feed which was initially fed ducklings. No ducks died from acute poisoning, and histology of their livers showed a pattern of recovery in the islands of regenerating parenchymal cells. Superior weight gains of ducklings on the finisher diet were an indication that the aflatoxin content was below toxicity level.

6. A thin layer chromatography assay for aflatoxin in corn cultures, especially prepared from agar subcultures of the toxic meal *Aspergillus flavus* strain, was negative. The fact that these subcultures were serially successive to the ones used for the feed processing was further indication that aflatoxin B₁ in the feed decreased as the fungus lost its potential to produce aflatoxins in subculturing.

7. Although no fungal diet, with the exception of the toxic meal *A. flavus*-infected feed given to ducklings, produced noticeable pathology or significant variation in performance, the *A. fumigatus* group of chickens showed lower consumption and gain throughout the experiment.

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