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Aflatoxicosis in Poultry

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AFLATOXICOSIS IN POULTRY

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Head, Animal Science Dept. Date

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INTRODUCTION

The term "aflatoxicosis" is applied to the toxicosis which is produced by "aflatoxin", a poisonous metabolite produced by certain strains of Aspergillus flavus. This toxin is a part of a series of toxins produced by fungi, which may be ingested by livestock, poultry and humans to produce toxigenic effects--the so-called mycotoxicoses. The occurrence of mycotoxicoses are frequent enough considering the prevalence of fungi and their proliferation in nature. They develop under favorable environmental conditions in foods and feeds of plant origin before and after harvest.

The investigation of "mycotoxicoses" and the isolation of mycotoxins began a few decades ago. Scientists were tending to approach the causes of animal diseases through a process of elimination. If the causative agent was not found to be bacterial, viral or nutritional, it was concluded to be chemical in nature. Even though this may be true, the possibility that such toxic chemicals may be fungal in origin was usually ignored.

Failure to appreciate the potential significance of feed-borne mycotoxins in problems of animal and human health appears to have persisted despite extensive knowledge concerning ergotism, which has been recognized for several centuries and probably represents the most documented example of mycotoxicosis.

In parallel with the toxic effect of the metabolites produced by fungi, one should not neglect the fact that there may be a beneficial function. Several also possess antimicrobial activity.

Some investigators described a disease in horses called "Leucoencephalomalacia" which they attributed to the feeding of moldy fodder. Biester and co-workers (1940) produced a similar toxicosis in horses by feeding moldy ear corn.

Eckles (1924) reviewed the work of other investigators on the death of animals caused by ingestion of moldy silage. He stated that in no case was there evidence that fungi could be incriminated. They also fed moldy silage to cattle, sheep and horses without ill effects. Several fungi including strains of Aspergillus glaucus, A. niger, Penicillium expansum, P. glaucum, Phizopus nigricans and others had been cultured from this silage.

A substantial literature has, in fact, been developed attesting to the lack of deleterious and, on occasion, to the beneficial effects of feeding moldy diets to a variety of domestic animal species.

Schofield (1924) in Canada was the first investigator to isolate a fungus from a naturally occuring outbreak of "mycotoxicosis" and to reproduce the toxicosis experimentally. He investigated "sweet clover poisoning" in cattle and although he was not a mycologist he made some excellent mycological observations. He observed that the toxicity of moldy sweet clover hay varied markedly with the type of fungus prevailing on the substratum. He also observed that although some lots of hay did not appear to be grossly contaminated with fungi on the surface, nevertheless the fungus was frequently found within the hollow space of the plant stems. By feeding sweet clover stalks, on which an Aspergillus (isolated by him) had proliferated, he was able to induce, in rabbits, signs of sweet clover poisoning which

closely resembled mild cases among cattle.

Some of these findings are compatible with the currently available evidence which, though limited, indicates that probably a relatively small proportion of molds commonly found on foodstuffs and feedstuffs are capable of producing toxic metabolites.

In addition occasional reports have appeared during the past three decades, in which ingestion of mold-contaminated foodstuffs and feedstuffs has been clearly associated with a variety of toxicity syndromes in domestic animals and in humans.

REVIEW OF LITERATURE

Ronk and Carrick (1931) investigated moldy corn, naturally infected with species of Aspergillus, Diplodia, Fusarium, Mucor, Penicillium, Rhizopus and unidentified fungi. They found the corn to be nontoxic to chickens maintained on litter changed at frequent intervals.

A systematic study of mycotoxicosis was initiated in the U.S.S.R. in 1938. This followed the devastating equine "stachybotryotoxicosis" in horses that had first occurred in the Ukranian USSR in 1931 and had been characterized as a "disease of unknown etiology". Subsequently several mycotoxicoses have been described in both the Soviet Union and the Western World.

The reports of Carll et al (1954) and those of Forgacs and co-workers (1954) have stimulated a renewed interest in mold intoxication in the United States.

Forgacs and Carll (1955) isolated toxic fungi from feed and litter collected from areas where the poultry hemorrhagic syndrome was enzootic.

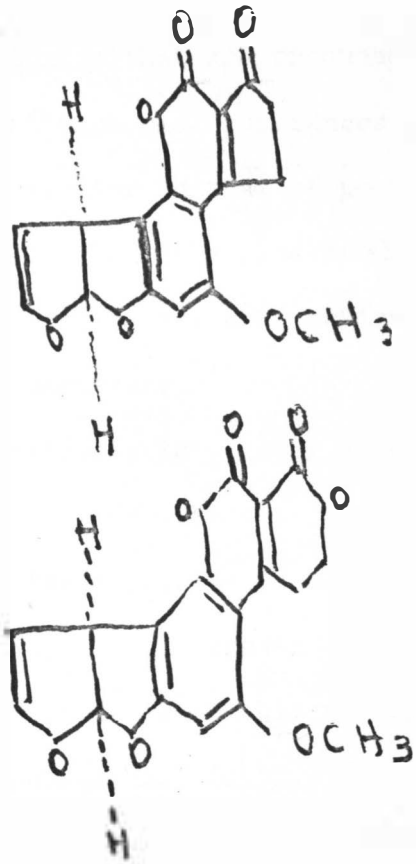
During 1960, a unique disease with high mortality occurred in turkey poults in England that was named turkey X-disease. Aspergillus flavus was found to be the causative agent and had been isolated from a lot of Brazilian ground-nut meal which had been used as a protein supplement for the turkey poults.

The term "Aflatoxicosis" indicates the toxicosis from the toxic antimetobolites produced by certain strains of Aspergillus flavus.

There are four major fractions of metabolites produced. They are designated B_1, B_2, G_1, and G_2 according to their characteristic fluorescence and RF values measured by their thin layer chromatography.

The chemical structures of the various fractions have been identified. The crude complex as well as the primary fraction B_1 are known to be carcinogenic to several species of animals.

The more important aflatoxins, B_1 and G_1, are produced by some strains of Aspergillus flavus growing on peanuts and cottonseed cake under special conditions. Asao T. et al. (1965) proposed two slightly different chemical structures for Aflatoxins B_1 and G_1 as shown below:



B_1

G_1

Several strains of Aspergillus flavus have been reported to be pathogenic to animals and human beings. Kirschstein and Sidransky (1956) described gross clinical, and histopathologic findings in a man who had been infected with a strain of A. flavus. From their description of the clinical and hematologic alterations, the infection appeared to have produced a toxin in various tissues. Furthermore, microscopic evidence of fungal proliferation was found in the heart and visceral pleura of the lower labe of each lung.

As is common with many fungi, both toxic and nontoxic strains occur among the A. flavus group. Thus, of nine strains of A. flavus isolated from moldy corn, Burnside et al. (1957) found only one to be toxic to animals.

Besides the toxins that are produced by Aspergillus flavus, some strains produce antibacterial substances such as "flavicin" which exhibits activity similar to that of penicillin (Bush and Goth, 1943; McKee et al., 1944). They may also produce aspergillic acid (White, 1940; Jones et al., 1943) which is active against both Gram-negative and Gram-positive bacteria.

Moldy feed toxicosis in poultry appeared about 1950 among broilers and was regarded as a new disease. Baker and Jacquette (1953) published one of the first reports of this disease and named it "Hemorrhagic syndrome" because of the presence of hemorrhages in many tissues of the broilers afflicted with this malady. Subsequently, Gray et al. (1954), Cover and co-workers (1955), Washko and Mushett (1955), and other workers described the clinical, hematologic, gross, and histopathologic manifestations of the syndrome in growing chickens raised

under field conditions. Although the disease appears to be most prevalent in broilers from 4 to 7 weeks old, it can occur in birds at any age. The paramount features of the malady, which incidentally is neither contagious nor infectious, are the variability of its clinical sign, its course, mortality and its gross and histopathological manifestations among birds.

Washko and Mushett (1955) observed that separated flocks on the same premises, subjected to identical conditions of feeding and management, were not uniformly affected. Among afflicted flocks, mortality ranged from below 1 to 30%. Similarly, Cover and coworkers (1955) observed that in several instances where two or more broiler houses were filled with chickens at the same time and given the same source of water, litter and feed and managed identically, the syndrome would appear only in one group. In addition, the disease reoccurred among some flocks, whereas, in others it would appear only once. Mortality varied up to 40%, usually reaching a peak and then subsiding within a period of three weeks.

It had been observed that the disease varied from year to year. Although various major broiler-producing areas reported the toxicosis from the late fall through the spring, Camp (1957) noted that in the east Texas area the disease had no seasonal pattern. The condition was very prevalent in 1953 and 1954, somewhat restricted in 1955 and very scarce during the early part of 1956. By the latter part of 1956, however, it was estimated that at least 80% of the broilers between the ages of 2 and 6 weeks were then affected by the syndrome to

some degree. Camp observed the malady in birds as young as 10 days of age, as well as in flocks 16 weeks of age, although the majority of the affected birds were in the 4 to 6 week age group.

The first clinical manifestations of the syndrome are depression, diarrhea (frequently tinged with blood), anorexia, reduced feed efficiency, pale combs, and a variable morbidity and mortality.

Results of hematologic examination of peripheral blood vary, but reveal, in general, a pronounced decrease in hemoglobin and hematocrit values, and a decline in the formed elements corresponding to changes in the bone marrow.

Blood clotting and prothrombin times, reported by Cover et al. (1955) and by Washko and Mushet (1955), were within normal limits, whereas Gray and co-workers (1954), using the capillary tube method, found an increase in coagulation time. Although capillary fragility tests were negative, thrombocytopenia and leucopenia occurred. Not all affected birds within a given flock showed the same degree of disturbance of the blood picture. For instance, within the same flock, could be observed leucocytosis and leucopenia, lymphocytosis, and a relative decrease in other white cell elements, an increase in granulocytes and granulocytopenia, and erythrocyte counts from normal to as few as 340,000 per cu. mm.

Gross necropsy findings include petechial and larger hemorrhages in many tissues, primarily noticeable in the subcutaneous tissue and musculature of the legs, thighs and breast. This is accompanied by erosion in the gizzard, the mucosa and serosa of the small intestine, liver, spleen, heart and kidneys. Occasionally, hemorrhages are

visible in the anterior chamber of the eye. Color changes from pink to a buffy-yellow are observed in the bone marrow.

Histopathological examination of tissues from afflicted birds reveals cloudy swelling and vacuolation, bile staining, early fibroblastic proliferation, and areas of necrosis in the liver, and also the frequent presence of septic thrombi in the blood vessels. The changes in the kidneys range from a cloudy swelling and mild congestion of the tubular epithelium to acute glomerulonephritis and extensive tubular necrosis, and occasionally, lymphocytic infiltration. Splenic changes vary greatly but, in general, are represented by congestion, necrosis, lymphoid exhaustion, hemosiderosis and, increased fibroblastic activity. Occasionally, necrosis, hyalinization of the adenoid sheaths and the lymphoid follicles are noted. Hemorrhages and necrosis are observed in the intestine, being extensive in the villous and glandular epithelium. Vascular lymphocytic infiltration is occasionally observed in all regions of the brain; small areas of hemorrhage are found in the matrix and piamater. The bone marrow changes are striking and indicate supression of hematopoiesis. In the bone marrow of less severely affected birds, decreased hematopoietic activity with moderate increase of fat cells is observed. In severely affected broilers, there is a pronounced hypoplasia with predominance of endothelial, intersitial and fat cells. Birds severely afflicted with this toxicosis may have various types of bacteria in certain tissues. However, such bacteria have no direct causative relation to the syndrome, but are rather related to the leucopenic state of the host.

A critical review of the literature reveals that various etiologic theories have been advanced for the hemorrhagic syndrome. Unfortunately alleged causes of field cases of the syndrome have been ascertained on the basis of laboratory experiments in which chickens were maintained in batteries. Camp (1957), in an attempt to find a therapeutic agent for this disease, submitted afflicted birds from field cases to various dietary regimes. Such birds maintained in batteries received the same diets as in the field but showed no difference in response when compared to control birds. He, therefore, concluded that the hemorrhagic disease was aggravated by some factor present in the litter or perhaps by a "stress" factor. Since there was also no response when litter from "enzootic" broiler houses was added to the battery brooders, he concluded that stress was the major factor responsible for the field syndrome. If Camp initially assumed a microbiological cause of the field syndrome, he apparently ignored the fact that conditions requisite to microbiological activity in broiler house litter may be quite at variance with that of a battery brooder. Indeed some of the observations taken by Forgacs and Carll were strikingly similar to those of Camp. They observed that, whereas under field conditions mold growth in litter was profuse, fungal growth was often absent in the confined brooder house even though the litter was dampened daily. Under these conditions, the combined effects of heat from the brooder, constant activity of the chickens, and excellent aeration caused the moisture in the litter to evaporate rapidly and, therefore, the fungi could not grow.

In other cases, certain factors were considered responsible for

the syndrome in the field, yet another flock subjected to similar factors remained free of the malady. One consistent finding in the poultry hemorrhagic syndrome, according to the literature, is the inconsistency of suspected etiologic factors (Washko and Mushet, 1955; Cover et al., 1955; Couch et al., 1954; Goldhaft, 1955).

Among the assumed causes is the addition of various medications to the diet--including antibiotics--with a resultant depression of intestinal bacterial growth considered to be necessary for synthesis of vitamin K (Anderson et al., 1955). This would include the addition of coccidiostats to the diet, particularly sulfaquinoxaline, which causes either real drug toxicity or anti-vitamin K effect (Chrisman, 1955; Anderson et al., 1955). Use of diets, marginal in vitamin K requirements (Griminger et al., 1953), causing K avitaminosis after unusual stress, has also been advanced as a causal factor in the syndrome (Chrisman, 1955). Reduced absorption of vitamin K owing to a low fat content of the diet, or because of the intestinal lesions, has been suggested (Prebluda, 1955). Liver damage from indefinite causes resulting in a decreased flow of bile necessary for optimum absorption of vitamin K has been suggested. Inhibition of prothrombin production required for proper functioning of the blood clotting mechanism, has been mentioned (Prebluda, 1955; Anderson et al., 1955). Presence of toxic compounds in stored soybeans arising spontaneously or from some unknown source has also been suggested (Hare et al., 1953). Still another theory suggested the presence of toxic substances as secondary factors (Henderson et al., 1957). Anderson and co-workers (1955) using a freshly prepared

simplified ration containing neither coccidiostats nor antibiotics, found that, in one trial approximately 10% of the chickens died from a hemorrhagic syndrome. In a second similar trial, the hemorrhagic syndrome and concomitant death did not occur. Although they offered no explanation for the differences observed in these two trials, the source of the corn and soybean oil meals (unextracted with trichloroethylene) varied. It would appear that the toxin(s) responsible for the hemorrhagic syndrome came from either the corn or soybean meals used, and that the microbiologic and toxigenic activity within the two sources of meal could have played a part. Toxins of microbiologic origin present in dietary ingredients used by some workers and not in those used by others may explain some of the conflicting results reported by various workers who have used apparently identical diets.

Various workers have observed that, in some field cases, either oral or parenteral administration of vitamin K elicits a rapid positive response, while others report no effect. Therefore, it has been assumed that there exists two distinct types of poultry hemorrhagic syndrome, one that responds to vitamin K, and another that does not (Caskey, 1953).

Obviously the etiologic picture of poultry hemorrhagic syndrome is confusing, but the most popular theory centers on avitaminosis K induced by sulfaquinoxaline toxicity, or by antagonism of this coccidiostat to vitamin K. The fact that the syndrome has occurred in the complete absence of sulfaquinoxaline or other drugs regarded as antimetabolic to vitamin K precludes this theory.

Careful studies of the pathology of the syndrome, as reported by Gray et al. (1954), Washko and Mushet (1955), Cover and co-workers (1955) indicate that the condition is the end result of a toxicosis. Henderson and co-workers (1957) stated that the fundamental pathologic nature of the poultry hemorrhagic syndrome is an aplastic anemia. Feed obtained from enzootic areas, when fed to chickens in the laboratory, caused a thrombocytopenia, a leucopenia, and other evidence of aplastic anemia. The feed contained medication and components of the feed were not the cause of the toxicosis.

Although there appear various manifestations of this toxicosis in a given flock, these signs merely indicate stages of a particular pathological process which may occur in both an acute and a chronic form. Varying degrees of damage have been observed in the two major excretory organs, namely, liver and kidneys. Although the toxin per se can cause toxic manifestations (including hemorrhages) it is conceivable that inclusion in the diet of a subtoxic substance normally excreted through the kidneys, could act as a stress factor and accentuate the toxicity under conditions of impaired renal function. In some instances, toxic substances depress the blood clotting mechanism, causing a hypoprothrombinemia and liver damage; in such cases vitamin K therapy has no therapeutic effect (Mushet and Seeler, 1947). In other cases, as for example in dicoumarol poisoning, administration of vitamin K does have a beneficial effect on the host. The possible relation of fungal toxins to avitaminosis K is discussed below.

A consideration of clinical, hematologic, and pathologic

deviations of the poultry hemorrhagic syndrome in the field reveals striking similarities to the mycotoxicoses, particularly stachybotryotoxicosis and alimentary toxic aleukia. Forgacs and Carll (1955) recognized these similarities and made a preliminary study of the relationship of certain toxic fungi to the syndrome. Using fungal isolates from feed scattered in the damp litter of broiler houses where the poultry hemorrhagic syndrome was enzootic, they produced acute toxic signs in chickens similar to those reported by other workers. Later, Forgacs et al. (1955, 1961a) confirmed these findings in young chickens and produced a condition of chronic toxicosis. Forgacs and co-workers (1958a) reproduced these toxic signs with diets infected with two selected fungal isolates of varying toxicity. The toxic diets were amply fortified with a protein-mineral-vitamin supplement, including vitamin K (menadione sodium bisulfite). These workers also presented data on the time required for certain fungi to grow in moist feedstuffs and produce toxins under laboratory conditions. The considered those factors associated with the production of the toxicosis in chickens under simulated field conditions.

Forgacs et al. (1961a) effectively produced mycotoxicosis under field conditions by application of toxin-producing fungi to feed and litter. They were able to prevent the toxicosis by suppression of the fungi through the addition of antifungal compounds to the broiler mash, (Forgacs et al., 1961 b,c,d).

Surprisingly little had been published on the role of fungi in poultry prior to 1955. Indeed the scant data published suggested

that fungi were not a source of danger to poultry health. Ronk and Carrick (1931) cited the work of Waite from Maryland, who observed that hens consuming moldy wheat gained more weight and laid more eggs than control birds fed sound wheat. In addition, they cited unpublished observations of the Indiana Experiment Station at Purdue University, where it was found that moldy grain fed to laying hens for 30 days had no deleterious effect. Gorcica and co-workers (1935) reported that the mycelium of Aspergillus sydowi fed at the 1% level was a good source of thiamin for chicks. Petty and Quigley (1947) studying the influence of the microflora of wheat on incidence of blue comb disease indicated beneficial rather than detrimental effects of fungi. Borgers and Peltier (1947) fed chickens mixtures composed of wheat bran, soybean oil meal and cracked corn on which four species of aspergilli and two of fusaria had been cultured for 4 days. They observed that the molded substrata improved the growth of chickens and concluded that the particular molds they used could be fed without deleterious effect. It is perhaps unfortunate that Petty and Quigley did not extend the time of incubation beyond 4 days because, if any of the fungi had been toxin-producers, this period of incubation would hardly have been sufficient for an adequate level of toxin formation. Cover et al. (1955) fed chickens soybean oil meal, corn meal, and regular mash on which had been cultured a strain of Fusarium graminearum and found no ill effects. Although these workers attempted to reproduce the poultry hemorrhagic syndrome using fungi, it is regrettable that they did not try fungi with a greater potentiality for toxin production of feed substrata

than F. graminearum, the toxin of which is primarily phytotoxic. Previous experiences with mycotoxicosis in other farm animals prompted Forgacs and co-workers (1954) to approach their moldy feed toxicosis studies in poultry with tenacity.

Among the many fungi isolated by Forgacs and Carll (1955) and Forgacs et al. (1961a) from feed and litter collected from the major broiler areas in the United States where poultry hemorrhagic syndrome was enzootic were strains of the following fungi: Aspergillus clavatus, A. flavus, A. fumigatus, A. glaucus, Paecilomyces varioti, Penicillium citricum, P. purpurogenum, P. rubrum, a species of Alternaria and several other as yet unidentified species of Penicillium, all of which were shown to induce mycotoxicosis in chickens. Undoubtedly there exist other toxin-producing fungi not yet isolated and identified.

Since these fungi were isolated from broiler mash and from substances toxic to poultry, this toxicosis has been tentatively designated as "moldy feed toxicosis".

a) Microbiology of Aflatoxin (Moody D. P. et al., 1964)

Following the original isolation of a toxin-producing strain of Aspergillus flavus from a toxic sample of ground nuts, other strains of the species have been selected and studied. By far the greater proportion of these have not exhibited any ability to produce aflatoxin, but several--a dozen or more--toxin-producers have been identified.

The mold does not produce a single toxin, but rather a mixture of closely related toxins. The toxin-producing potential of these

strains varies as to total production and the proportion in which the active components are synthesized.

Much work has been done on the artificial culture of the aflatoxin-producing strains. Initially, growth was induced on sterilized non-toxic groundnut kernels, a method that is still used. Later, synthetic media were studied and formulated to induce toxin production, particularly the Czapek-Dox medium with added yeast extracts, and Raulins' medium. Addition of traces of zinc to the former medium increased toxin yield, particularly of one of the aflatoxin components. These methods have been used for the large scale production of aflatoxins for chemical and toxicological research.

A. flavus is a common mold; it can be isolated from many stored dried foodstuffs and from tropical soils. It grows rapidly, but requires somewhat more moisture than most other molds. At tropical temperatures (30°C), it will grow at 80-85% relative humidity and above; this corresponds to moisture contents in groundnut kernels and defatted meals of about 9% and 16% or more respectively. This knowledge is of great practical importance in considering control measures.

Of considerable practical significance regarding a means of control was the discovery that in any "naturally" toxic batch of groundnuts, mold development and the toxic factor occur in a small proportion of kernels only. These toxic kernels are generally distinguished by a discolored appearance.

b) Chemistry of Aflatoxin (Asao T. and et al., 1965)

The amount of aflatoxin is only a few parts per million in even the most toxic samples of groundnuts, and concentration of the active

material is laborious. This particular difficulty has been much reduced, however, by the artificial culture of the aflatoxin-producing organism on sterile ground nuts and synthetic media.

The prolonged efforts to purify and characterize the toxic factor have shown that aflatoxin is, in fact, a complex of compounds apparently very closely related structurally. It is not yet certain just how many compounds there are; four seem quite well established and two of these--now named aflatoxin B_1 and aflatoxin G_1--have been isolated in sufficient quantity to be examined physically and chemically. Formulae of $C_{17}H_{12}O_6$ and $C_{17}H_{12}O_7$ have been suggested for these two components as shown on p. 5. This was determined by microanalysis and mass-spectrometric determination of molecular weight. Lactone and methoxyl groupings appear to be present but free hydroxyls are absent. Other features of the structure are proving hard to elucidate, but it is hoped that current X-ray studies will be informative. Aflatoxin B_1 which in thin layer chromatography is the faster-running blue fluorescent component, is highly toxic. The LD50--that is the dose required to kill 50% of treated animals--is less than 20 mcg. for day-old Khaki-Campbell ducklings. This compound may be responsible for most of the toxicity in naturally toxic ground nuts. Aflatoxin G_1--a compound with a greenish-blue fluorescence--seems to be less toxic, the LD50 for day-old ducklings being about 60 mcg.

Asao et al. claim the resolution of extracts from toxic strains of A. flavus into at least twelve fluorescent components, five of which have been shown to be toxic to ducklings. In this extraction and purification work the possibility of artifact production has to be

considered. It is known that at least one of the aflatoxin components is photosensitive and gives rise to other fluorescent substances. Alkali also produces changes which may, or may not, be reversible, according to the conditions of treatment.

c) Toxicology of Aflatoxin (Forgacs et al., 1962)

Cattle and pigs are susceptible to toxic groundnut meal as well as turkeys and ducks. Feeding trials, however, revealed that sensitivity to aflatoxin varies considerably from one species to another, although, in all cases, the young animal is much more susceptible than the corresponding adult. Among poultry, ducklings are highly sensitive, young turkeys somewhat less so, and chickens comparatively resistant. Of the farm mammals, baby pigs appear to be the most sensitive; young calves also were highly susceptible, but lambs much less so. In all species the principal site of toxic action is the liver.

In order to devise appropriate control measures, it is important to ascertain the dietary levels of aflatoxin below which no adverse effects will be produced in farm animals of various species and ages. Extensive long-term feeding experiments necessary for this are now being conducted in many countries.

The whole picture is somewhat complicated, however, by the fact already mentioned that aflatoxin is a mixture of several components of varying toxicity. These toxins may not always be present in the same proportion. The possibility of synergism between toxic components also needs to be examined.

In the course of laboratory studies, rats have not shown the

acute toxicity signs seen in ducklings and young turkeys, however, when fed small amounts of toxic ground nut meal for periods of six months, rats have developed a primary carcinoma of the liver.

Naturally, the possible consequences to man have to be carefully examined. To get the best possible idea of what man's reactions would be if exposed to aflatoxin, a study of both acute and chronic effects in monkeys is necessary. Some preliminary work of this kind is in progress in Britain, and an extensive investigation has already been started in India.

EXPERIMENTAL

The experiments were conducted in the Poultry Department facilities at South Dakota State University. Two thermostatically heated battery brooders were used for each experiment. The brooders were comprised of twenty four compartments each and equipped with feeder troughs, waterers and wire floors. Each brooder contained 24 pens, six decks with 4 pens per deck. Continuous artificial illumination was provided and the battery room was heated as needed to maintain temperatures above 21°C. The brooder temperature was regulated for the first week of each experiment at 28°C and was lowered 2.4°C per week for the next three weeks. Day-old S.C.W.L. chicks were used in all experiments. Forty eight groups of 5 or 6 chicks per group (per pen) were randomly selected and individually weighed and wing-banded. The chicks were fed ad libitum an all mash diet on a 24 hour feeding and watering cycle per day.

Sterilized, mold-inoculated and incubated wheat or soybeans, as prepared by the Plant Pathology Department, were mixed with the respective basal diets on a 1:1 or 1:3 basis. Nearly 115 strains of Aspergillus flavus were tested in these experiments. Up to 23 molded samples could be tested in each experiment with at least one control group receiving non-inoculated soybeans or wheat. The natural inhibition of soybean inhibitors were assumed to be destroyed by the sterilization process, 20 minutes at 123°C. Diets No. 117 and No. 118 were used for the 1:3 mixes in the first two experiments while diets No. 119 and No. 120 were used for the 1:1 mixes for the

rest of the experiments. The products cultured on soybeans were mixed with the basal diets No. 117 or No. 119, and those on wheat were mixed with basal diets No. 118 or No. 120. A protein level of 20% was provided and in all cases contained the necessary levels of vitamins, minerals and energy. The final diets (mixes) were identified with the suffix Sb (soybean or W (wheat) referring to the feedstuff on which the molds were cultured.

During each experiment, the duration of which was four weeks, the chicks were weighed two times (at 2 and 4 weeks of age) in addition to the weights taken initially. All chicks dying during the experimental period were taken to the Veterinary Department for necropsy. Following the termination of each study two live chicks from each pen were taken to the Veterinary Department for post-mortem examination while those remaining were examined at the Poultry Laboratory by the author. Data on the chicks' behavior, death loss and feed consumption were also recorded for each experiment.

Any modifications of the above set-up are mentioned with the results for the individual experiments.

TABLE 1

Chick Starter Diets Used in 1:3 Dilution with Molded Soybeans or Wheat

Experiments 1 and 2

Ingredients	Diet 117* %	Diet 118** %
Yellow corn	59.5	39.0
Soybean meal	10.0	26.0
Alfalfa meal	2.0	2.0
Dicalcium Phosphate	2.0	2.0
Limestone	1.0	1.0
Vitamin mix[1]	0.5	0.5
Salt[2]	0.5	0.5
Methionine	0.1	0.1
Yellow grease	0.0	4.5
Total	75.6	75.6

* Basal diet to which 25% of ground inoculated soybeans are added.
** Basal diet to which 25% of ground inoculated wheat is added.

[1]

Ingredients	Amt. in 1 lb.	Will Supply Per Kg. of feed
Vitamin A	480,000 I.U.	5,280 I.U.
Vitamin D_3	125,000 I.C.U.	1,375 I.C.U.
Vitamin E	1,000 I.U.	11 I.U.
Menadione (sodium bisulfate)	0.1 gm	1.1 mg
Riboflavin	0.4 gm	11.0 mg
Pantothenic acid	0.8 gm	8.8 mg
Choline	10.0 gm	110.0 mg
Niacin	2.4 gm	26.4 mg
Cobalamine (B_{12})	0.8 mg	8.8 mcg
Santoquin	10.0 gm	110.0 mg

[2]Containing 0.455% Mn, 0.011% I, 0.01% Co, 0.165% Fe, 0.048% Cu, 0.30% S, and 97.0% NaCl.

TABLE 2

Chick Starter Diets Used in 1:1 Dilution with Molded Soybeans or Wheat

Experiments 3 to 6

Ingredients	Diet 119* %	Diet 120** %
Corn	0.0	12.5
Soybean meal	0.0	25.0
Alfalfa	2.0	2.0
Cerelose	40.0	0.0
Dicalcium Phosphate	2.0	2.0
Limestone	1.0	1.0
Vitamin mix[1]	0.5	0.5
Salt[2]	0.5	0.5
Methionine	0.1	0.1
Yellow grease	0.0	7.0
Cellulose	4.5	0.0
Total	50.1	50.1

* Basal diet to which 50% of ground inoculated soybeans are added.
** Basal diet to which 50% of ground inoculated wheat is added.

Diet 119 was fortified with the ingredients below to supply per kg.
1) Thiamine HCL 2 mg.
2) Pyridoxine HCL 2 mg.
3) Biotin .10 mg.
4) Methionine .50 gm.

1

Ingredients	Amt. in 1 lb.	Will Supply Per Kg. of feed
Vitamin A	480,000 I. U.	5,280 I. U.
Vitamin D_3	125,000 I. C. U.	1,375 I. C. U.
Vitamin E	1,000 I. U.	11 I. U.
Menadione (sodium bisulfate)	0.1 gm	1.1 mg
Riboflavin	0.4 gm	11.0 mg
Pantothenic acid	0.8 gm	8.8 mg
Choline	10.0 gm	110.0 mg
Niacin	2.4 gm	8.8 mcg
Cobalamine (B_{12})	0.8 mg	110.0 mg
Santoquin	10.0 gm	

[2]Containing 0.455% Mn, 0.011% I, 0.01% Co, 0.165% Fe, 0.048% Cu, 0.30% S, and 97.0% NaCl.

RESULTS AND DISCUSSION

Experiment Number 1

Six chicks were used per pen. Moldy inoculated feedstuffs were used on a 1:3 basis with the respective diets, No. 117 for molded soybeans and No. 118 for molded wheat. Twenty two strains of Aspergillus were tested here, cultured on soybeans and wheat. Two control groups were used with each series. Tables 3 and 4 indicate the strains tested along with the culture and pen number on which they were used. Tables 5 and 6 indicate the average weights on the chicks along with the respective culture number used for each pen. Feed consumption per bird and feed conversion were also reported. Table 7 indicates mortality data.

At two weeks of age (9-25-66) the groups fed Sb.3, Sb.5, Sb.11, Sb.19, Sb.20, Sb.27, and Sb.31 showed very low average weights (below 100 gm.). At four weeks of age (10-9-66) all the groups showed average weights higher than 200 gm., ranging from 213 gm. (Sb.30) to 292 gm. (Sb.2). Control group average bird weight reached 227 gm. Feed consumption ranged between 447 gm. per chick for the pen fed Sb.30, which coincided with the low final average weight, and 670 gm. for the pen fed Sb.4 which also coincided with the high of final average weights. Mean feed consumption for the two control groups was 528 gm. Feed conversion was best for the pen fed Sb.1 (2.0) while the mean for the control groups was 2.7.

The groups fed W.3, W.6, W.11, W.29, W.30 and W.31 showed average weights below 100 gm. at two weeks of age. At four weeks

of age these same groups still showed poor performance, though W.6 and W.31 had improved somewhat. The control group for the wheat series showed a final average weight of 284 gm. Feed consumption was surprisingly low for pens fed W.29, W.30, W.3, and W.11, averaging from 325 gm. to 369 gm. per chick. This coincided with the poor average weight for these respective pens. The highest rates of feed conversion were obtained by the pens fed W.3, W.29, and W.30, while the average control group feed conversion was 2.1.

Chicks fed W.11 showed distress and poor growth typical of aflatoxin injury similarly to Sb.30. Post mortem examination revealed typical liver and kidney damage as indicated in Table 8.

TABLE 3

Molds Treated on Soybeans and Mixed with Diet No. 117

Experiment 1

Pen No.	Culture No.	Strain No.	Name of Fungus
1	Sb.1	10	Aspergillus giganteus
2	Sb.2	1780	" "
3	Sb.3	A 14,106	" clavatus
4	Sb.4	4	" "
5	Sb.5	6	" "
6	Sb.6	A 14,542	" "
7	Sb.11	2999	" flavus
8	Sb.18	447	" oryzae
9	Sb.19	451	" "
10	Sb.20	458	" "
11	Sb.21	468	" "
12	Sb.22	506	" "
13	Sb.23	692	" "
14	Sb.24	696	" "
15	Sb.25	699	" "
16	Sb.26	1808	" "
17	Sb.27	2219	" "
18	Sb.28	2220	" "
19	Sb.29	1958	A. Oryzae V. Effesus
20	Sb.30	465	A. parasiticus
21	Sb.31	504	A. "
22	Sb.32	1731	A. "
23	Sb. Control 1	----	---- ----
24	Sb. Control 2	----	---- ----

TABLE 4

Molds Treated on Wheat and Mixed with Diet No. 118.

Experiment 1

Pen No.	Culture No.	Strain No.	Name of Fungus
1	W.1	10	Aspergillus giganteus
2	W.2	1780	" "
3	W.3	A 14,106	" clavatus
4	W.4	4	" "
5	W.5	6	" "
6	W.6	A 14,542	" "
7	W.11	2999	" flavus
8	W.18	447	" oryzae
9	W.19	451	" "
10	W.20	458	" "
11	W.21	468	" "
12	W.22	506	" "
13	W.23	692	" "
14	W.24	696	" "
15	W.25	699	" "
16	W.26	1808	" "
17	W.27	2219	" "
18	W.28	2220	" "
19	W.29	1958	A. oryzae v. effesus
20	W.30	465	A. parasiticus
21	W.31	504	A. "
22	W.32	1731	A. "
23	W. Control 1	----	---- ----
24	W. Control 2	----	---- ----

TABLE 5
Experiment 1
Average Weights and Feed Efficiency

Pen Number	Culture Number	Average Weights (gms) 9-11-66	9-25-66	10-9-66	Feed Consumption (per chick)	Feed Conversion*
1	Sb.1	32	106	249	473	2.0
2	Sb.2	35	124	292	585	2.2
3	Sb.3	35	89	223	535	2.8
4	Sb.4	34	123	290	670	2.6
5	Sb.5	33	99	262	587	2.6
6	Sb.6	33	122	266	663	2.8
7	Sb.11	34	99	224	467	2.4
8	Sb.18	34	111	265	577	2.5
9	Sb.19	34	91	233	495	2.4
10	Sb.20	34	97	221	487	2.6
11	Sb.21	31	105	257	617	2.7
12	Sb.22	31	107	256	602	2.6
13	Sb.23	31	101	243	537	2.4
14	Sb.24	31	121	268	588	2.4
15	Sb.25	32	108	250	523	2.3
16	Sb.26	33	109	260	630	2.7
17	Sb.27	34	96	231	505	2.5
18	Sb.28	33	116	248	518	2.4
19	Sb.29	32	103	250	569	2.6
20	Sb.30	33	106	213	447	2.4
21	Sb.31	34	94	217	483	2.6
22	Sb.32	33	114	265	580	2.5
23	Sb control 1	32	85	226	530	2.7
24	Sb control 2	33	104	229	526	2.6
Control Groups Average		32	94	227	528	2.7

* Unit of feed per unit of body weight increase.

TABLE 6
Experiment 1
Average Weights and Feed Efficiency

Pen Number	Culture Number	Average Weights (gms) 9-11-66	9-25-66	10-9-66	Feed Consumption (per chick)	Feed Conversion*
1	W.1	33	111	255	583	2.6
2	W.2	33	108	274	603	2.4
3	W.3	33	63	144	335	3.0
4	W.4	33	131	303	490	1.8
5	W.5	33	121	275	686	2.8
6	W.6	34	96	234	492	2.4
7	W.11	33	65	105	369	2.1
8	W.18	32	108	296	584	2.2
9	W.19	30	124	296	580	2.1
10	W.20	31	113	282	570	2.2
11	W.21	33	128	293	558	2.1
12	W.22	31	121	296	589	2.3
13	W.23	31	102	227	473	2.4
14	W.24	31	115	257	542	2.3
15	W.25	31	126	276	523	2.1
16	W.26	33	117	296	640	2.4
17	W.27	31	120	269	565	2.3
18	W.28	31	124	273	580	2.4
19	W.29	33	85	138	325	3.0
20	W.30	32	61	105	355	4.8
21	W.31	30	99	237	487	2.3
22	W.32	30	112	267	529	2.2
23	W.Control 1	32	106	270	510	2.1
24	W.Control 2	30	124	297	580	2.1
Control Group Average		31	115	284	545	2.1

* Unit of feed per unit of body weight increase.

TABLE 7

Mortality During Experiment 1

Pen Number	Cumulative Mortality	Percent Mortality
Sb.28	1	16.6
W.4	1	16.6
W.11	2	33.3
W.26	1	16.6
W.29	1	16.6
W.30	1	16.6
Total	7	

TABLE 8

Results of Post-mortem Examination-Experiment 1

Band No.	Pen No.	Symptoms: Dark Colored	Swollen	Discolored	Marble-Like Discoloration
9543	Sb.5	Kidneys			
9596	Sb.6	Kidneys	Kidneys		
9578	Sb.6	Kidneys	Kidneys		
9600	Sb.11	Left Kidney		Liver	
9651	Sb.19				Liver
9731	Sb.20				Liver
9718	Sb.20				Liver
9756	Sb.22	Kidneys			
9747	Sb.22				Liver
9800	Sb.24		Kidneys, Heart		
9875	Sb.25				Liver
9703	Sb.28		Right Kidney		
9712	Sb.29		Kidneys, Heart		
9721	Sb.31		Kidneys		
9756	W.2	Liver			
9763	W.4	Kidneys			
9765	W.4	Kidneys			
9776	W.6	Liver, Kidneys	Kidneys		
9780	W.6	Liver, Kidneys	Kidneys		
9786	W.11			Liver	
9791	W.18		Kidneys	Grayish Liver	
9792	W.18		Kidneys	Grayish Liver	
9799	W.20	Kidneys	Kidneys	Grayish Liver	
9713	W.20	Kidneys	Kidneys	Grayish Liver	
9961	W.27			Grayish Liver	
9980	W.30			Liver	
9983	W.30			Liver	
9994	W.32	Kidneys			
9995	W.32	Kidneys			

Experiment 2

Six chicks were used per pen. The molded feedstuffs were again mixed with the basal diets on a 1:3 basis using diets No. 117 and No. 118. Twenty two strains of Aspergillus were again cultured on soybeans and/or wheat, with two control groups per treated feedstuff. The strains tested are indicated in Tables 9 and 10 along with the culture and pen number on which they were used. Tables 11 and 12 indicate the average weights of the chicks, along with the respective culture number used for each pen, feed consumption and feed conversion. Mortality data is indicated on Table 13. Table 14 indicates the findings of post-mortem examination.

For the first six days nothing abnormal was noticed in any of the forty eight pens. At the begining of the second week chicks fed Sb.11, Sb.11(5), W.11 and W.11(5) showed a distress condition which continued throughout the experimental period, while the chicks in the rest of the pens didn't show any abnormality.

The chicks fed Sb.11(5), Sb.11 and Sb.34 showed average weights below 100 gm. at two weeks of age (11-14-66). At four weeks of age (11-28-66), the same groups again showed the lowest average weights ranging from 131 gm., for the pen fed Sb.11, to 227 gm., for the pen fed Sb.34, compared to the control group's final average weight of 281 gm. As before, the groups fed W.11(5) and W.11 showed average weights at 2 weeks of age below 100 gms. i.e. 79 gm. and 95 gm. respectively where the average weight of the control groups was 120 gm. The pen fed W.50 showed the highest average weight of 137 gm.

The same two pens at four weeks of age showed the lowest average weights of 134 and 159 gm while the control average weight was 290 gm.

Feed consumption was lowest for the pen fed W.11(5) while feed conversion ratio was highest for the pen fed W.11(5).

TABLE 9

Molds Treated on Soybeans and Mixed with Diet No. 117 Experiment 2

Pen No.	Culture No.	Strain No.	Name of Fungus
1	Sb.11 (5)	2999	A. flavus
2	Sb.11	2999	A. flavus
3	Sb.33	242	A. sydowi
4	Sb.34	243	A. "
5	Sb.35	244	A. "
6	Sb.36	250	A. "
7	Sb.37	1732	A. "
8	Sb.38	A 12.373	A. "
9	Sb.39	A 12.469	A. "
10	Sb.40	A 12.477	A. "
11	Sb.41	A 12.807	A. "
12	Sb.42	A 12.981	A. "
13	Sb.46	237	A. versicolor
14	Sb.47	239	A. "
15	Sb.48	573	A. "
16	Sb.49	661	A. "
17	Sb.50	A 12.331	A. "
18	Sb.51	A 12.473	A. "
19	Sb.52	A 12.810	A. "
20	Sb.53	A 12.814	A. "
21	Sb.54	1934	A. "
22	Sb.55	A 12.290	A. "
23	Sb Control (5)	--	--
24	Sb Control	--	--

(5) Culture obtained from previous experiment.

Sb Control (5): Noninoculated soybean obtained from previous experiment.

TABLE 10

Molds Treated on Wheat and Mixed with Diet No. 118

Experiment 2

Pen Number	Culture Number	Strain Number	Name of Fungus
1	W.11 (5)*	2999	A. flavus
2	W.11	2999	A. flavus
3	W.33	242	A. sydowi
4	W.34	243	A. "
5	W.35	244	A. "
6	W.36	250	A. "
7	W.37	1732	A. "
8	W.38	A 12.373	A. "
9	W.39	A 12.469	A. "
10	W.40	A 12.477	A. "
11	W.41	A 12.807	A. "
12	W.42	A 12.981	A. "
13	W.46	237	A. versicolor
14	W.47	239	A. "
15	W.48	573	A. "
16	W.49	661	A. "
17	W.50	A 12.331	A. "
18	W.51	A 12.473	A. "
19	W.52	A 12.810	A. "
20	W.53	A 12.814	A. "
21	W.54	1934	A. "
22	W.55	A 12.290	A. "
23	W Control (5)**	--	--
24	W Control	--	--

*(5) Culture obtained from previous experiment.

** W Control (5): noninoculated wheat obtained from previous experiment.

TABLE 11

Average Weights and Feed Efficiency

Experiment 2

Pen Number	Culture Number	Average Weights (gms) 10-31-66	11-14-66	11-28-66	Feed Consumption (per chick)	Feed** Conversion
1	Sb.11(5)*	26	88	169	483	3.3
2	Sb.11	29	87	131	331	3.2
3	Sb.33	29	125	293	540	2.0
4	Sb.34	25	88	227	462	2.3
5	Sb.35	28	116	248	568	2.5
6	Sb.36	26	112	247	551	2.5
7	Sb.37	28	119	286	551	2.1
8	Sb.38	28	128	286	590	2.2
9	Sb.39	28	122	277	625	2.4
10	Sb.40	28	130	301	565	2.0
11	Sb.41	28	127	288	563	2.1
12	Sb.42	28	105	276	532	2.2
13	Sb.46	28	133	278	523	2.1
14	Sb.47	32	133	293	582	2.2
15	Sb.48	31	113	278	637	2.5
16	Sb.49	33	110	258	490	2.1
17	Sb.50	32	123	265	517	2.2
18	Sb.51	28	110	284	515	2.0
19	Sb.52	28	120	255	587	2.5
20	Sb.53	31	138	322	568	1.7
21	Sb.54	28	115	278	520	2.0
22	Sb.55	31	117	273	550	2.2
23	Sb Control(5)*	29	116	278	552	2.2
24	Sb Control	28	112	284	575	2.2
Control Group Average		29	114	281	563	2.2

* Sb.11(5): Culture 11 on soybean and Sb Control (5): untreated soybean from previous experiment.

** Unit of feed per unit of body weight increase.

TABLE 12

Average Weights and Feed Efficiency

Experiment 2

Pen Number	Culture Number	Average Weights (gms) 10-31-66	11-14-66	11-28-66	Feed Consumption (per chick)	Feed Conversion**
1	W.11(5)*	27	79	134	397	3.6
2	W.11	29	95	159	480	3.7
3	W.33	26	123	299	622	2.3
4	W.34	26	123	281	497	1.9
5	W.35	25	126	308	605	2.1
6	W.36	31	128	320	568	1.9
7	W.37	27	127	290	600	2.3
8	W.38	27	128	300	532	1.9
9	W.39	26	125	294	575	2.1
10	W.40	26	109	254	490	2.1
11	W.41	25	132	331	627	2.0
12	W.42	27	134	331	575	1.8
13	W.46	25	121	279	573	2.2
14	W.47	27	132	318	550	1.8
15	W.48	25	131	283	512	1.9
16	W.49	25	127	278	512	2.0
17	W.50	27	137	316	553	1.9
18	W.51	27	113	290	538	2.0
19	W.52	26	125	294	525	1.9
20	W.53	29	111	285	512	1.9
21	W.54	25	117	282	570	2.2
22	W.55	26	121	306	573	2.0
23	W Control(5)*	24	123	293	527	1.9
24	W Control	26	116	286	467	1.7
Control Group Average		25	120	290	497	1.8

* W.11(5): Culture 11 on wheat and W. Control (5): untreated wheat from previous experiment.

** Unit of feed per unit of body weight increase.

TABLE 13

Mortality During Experiment 2

Pen Number	Mortality Cumulative	Percent
Sb.42	1	33.3
W.11	1	33.3
Total	2	

TABLE 14

Results of Post-mortem Examination-Experiment 2

Symptoms

Band No.	Pen No.	Discolored	Swollen	Marble-like Discoloration	Congestion
6142	Sb.11 (5)			Liver	
6145	Sb.11 (5)			Liver	
6150	Sb.11	Liver			
6173	Sb.11	Liver			
6161	Sb.34	Liver			
5897	Sb.34	Liver			
6169	Sb.36		Right Kidney		
6184	Sb.37		Right Kidney		
6181	Sb.37		Right Kidney		
6190	Sb.38		Right Kidney		
6188	Sb.38	Liver			
6194	Sb.39		Heart, Spleen		
6219	Sb.40	Liver			
6226	Sb.41	Liver			
6235	Sb.46	Liver			Left Kidney
6247	Sb.48	Liver			
6246	Sb.48			Liver	
6251	Sb.49	Liver			
6277	Sb.53	Liver			
6280	Sb.53	Liver			
6308	W.11 (5)	Liver, Kidney			
6307	W.11 (5)	Liver, Kidney			
6305	W.11 (5)	Liver, Kidney			
6313	W.11	Liver, Kidney			
6315	W.11	Liver, Kidney			
6328	W.34		Kidney		Liver
6323	W.34		Left Kidney		Liver
6364	W.40	Liver			
6359	W.40				Liver, Kidney
6372	W.42	Liver	Spleen		
6371	W.42				Kidney
6387	W.47				Liver
6386	W.47				Liver,Kidney
6403	W.50	Liver			
6431	W.55				Liver

Experiment 3

Six chicks were used per pen. The molded feedstuffs were mixed with the basal diets on a 1:1 basis using diets No. 119 and No. 120. It was decided that some of the possible toxic samples could be missed with 25% mixes, therefore the diets were formulated so as to allow for 50% mixes. Twenty-two strains of Aspergillus were tested as indicated in Tables 15 and 16. The average weights of the chicks are given in Tables 17 and 18 along with feed consumption, feed conversion and culture number of the molds used per pen. Mortality data are given in Table 19 while Table 20 shows the results of the post-mortem examination.

On the second day of the experiment chicks in certain pens started showing symptoms of distress and uneasiness. Death loss started on the third day and continued throughout the experiment so that more than one-fifth of the number of the chicks started died, that is 62 chicks out of 288. Most of the deaths occurred in the pens fed basal diet 119 mixed with samples of moldy inoculated soybean, i.e. 50 chicks out of 62. The average weights were less than 100 gm at two weeks of age (12-31-66) for all groups fed inoculated soybeans.

At four weeks of age (1-15-67) all the groups showed poor growth. Feed consumption was poor for the pens fed Sb.30, Sb.64 and Sb.65. Feed conversion values were very high for the pens fed Sb.30 and Sb.65.

Feed consumption and rates of growth were about normal for chicks on the wheat diets. The best feed conversion was shown by

the pen fed W.60. Some mortality due to omphalitis was encountered during the first week among all groups after which mortality was restricted to chicks on the soybean diets. Exploratory work at this point showed that the basal diet could be markedly improved by adding extra methionine, thiamine, pyridoxine and biotin so that future studies would include these supplements as indicated in subsequent tables.

TABLE 15

Molds Treated on Soybeans and Mixed with Diet No. 119 - Experiment 3

Pen Number	Culture Number	Strain No.	Name of Fungus
1	Sb.11	2999	Aspergillus flavus
2	Sb.30	465	A. parasiticus
3	Sb.33	242	A. sydowi
4	Sb.34	243	A. "
5	Sb.35	244	A. "
6	Sb.36	250	A. "
7	Sb.37	1732	A. "
8	Sb.38	A 12.373	A. "
9	Sb.39	A 12.469	A. "
10	Sb.40	A 12.477	A. "
11	Sb.41	A 12.807	A. "
12	Sb.42	A 12.981	A. "
13	Sb.56	303	A. candidus
14	Sb.57	308	A. "
15	Sb.58	309	A. "
16	Sb.59	310	A. "
17	Sb.60	A 12.327	A. "
18	Sb.61	A 12.395	A. "
19	Sb.62	A 12.250	A. "
20	Sb.63	A 12.328	A. "
21	Sb.64	A 12.329	A. "
22	Sb.65	1720	A. "
23	Sb Control 1	--	--
24	Sb Control 2	--	--

TABLE 16

Molds Treated on Wheat and Mixed with Diet No. 120

Experiment 3

Pen Number	Culture Number	Strain Number	Name of Fungus
1	W.11	2999	Aspergillus flavus
2	W.30	465	A. parasiticus
3	W.33	242	A. sydowi
4	W.34	243	A. "
5	W.35	244	A. "
6	W.36	250	A. "
7	W.37	1732	A. "
8	W.38	A 12.373	A. "
9	W.39	A 12.469	A. "
10	W.40	A 12.477	A. "
11	W.41	A 12.807	A. "
12	W.42	A 12.981	A. "
13	W.56	303	A. candidus
14	W.57	308	A. "
15	W.58	309	A. "
16	W.59	310	A. "
17	W.60	A 12.327	A. "
18	W.61	A 12.395	A. "
19	W.62	A 12.250	A. "
20	W.63	A 12.328	A. "
21	W.64	A 12.329	A. "
22	W.65	1720	A. "
23	W Control 1	--	----
24	W Control 2	--	----

TABLE 17

Average Weights and Feed Efficiency

Experiment 3

Pen Number	Culture Number	Average Weights (gms) 12-17-66	12-31-66	1-15-67	Feed Consumption (per chick)	Feed Conversion*
1	Sb.11	33	73	123	316	3.4
2	Sb.30	33	41	68	165	4.6
3	Sb.33	32	63	173	488	3.2
4	Sb.34	33	89	191	420	2.6
5	Sb.35	30	85	152	425	3.4
6	Sb.36	33	81	210	460	2.5
7	Sb.37	33	79	185	440	2.8
8	Sb.38	34	75	176	470	3.3
9	Sb.39	35	77	178	538	3.7
10	Sb.40	33	80	143	347	3.1
11	Sb.41	31	70	186	431	2.7
12	Sb.42	33	72	190	528	3.3
13	Sb.56	32	89	169	280	2.0
14	Sb.57	32	78	152	362	3.0
15	Sb.58	31	68	180	453	3.1
16	Sb.59	30	30	120	242	2.6
17	Sb.60	31	69	180	477	3.2
18	Sb.61	31	79	160	324	2.5
19	Sb.62	32	88	168	415	3.0
20	Sb.63	33	59	153	344	2.9
21	Sb.64	31	65	93	200	3.0
22	Sb.65	32	57	73	200	4.8
23	Sb Control 1	30	48	70	397	9.9
24	Sb Control 2	31	47	---	289	---
Control Group Average		30	48	70	343	9.9

* Unit of feed per unit of body weight increase.

TABLE 18

Average Weights and Feed Efficiency

Experiment 3

Pen Number	Culture Number	Average Weights (gms) 12-17-66	12-31-66	1-15-67	Feed Consumption (per chick)	Feed Conversion**
1	W.11	32	97	251	--*	--
2	W.30	32	40	--	--	--
3	W.33	30	88	211	--	--
4	W.34	32	109	220	--	--
5	W.35	32	101	198	--	--
6	W.36	32	103	267	--	--
7	W.37	31	84	292	--	--
8	W.38	32	112	231	--	--
9	W.39	32	58	240	--	--
10	W.40	31	98	252	--	--
11	W.41	32	80	265	--	--
12	W.42	32	60	193	--	--
13	W.56	31	81	263	515	2.2
14	W.57	30	100	298	602	2.2
15	W.58	33	94	218	595	3.2
16	W.59	32	125	260	600	2.6
17	W.60	32	105	303	562	2.0
18	W.61	33	101	277	633	2.5
19	W.62	32	98	238	569	2.7
20	W.63	30	98	238	540	2.5
21	W.64	31	100	247	545	2.5
22	W.65	31	98	258	562	2.4
23	W Control 1	32	98	288	744	2.9
24	W Control 2	30	92	238	590	2.8
Control Group Average		31	95	263	667	2.8

* Feed consumption data not obtained.
** Unit of feed per unit of body weight increase.

TABLE 19

Mortality During Experiment 3

Pen Number	Mortality Cumulative	Percent
Sb.11	4	66.6
Sb.30	1	16.6
Sb.33	1	16.6
Sb.35	1	16.6
Sb.36	2	33.3
Sb.38	1	16.6
Sb.39	1	16.6
Sb.40	3	50.0
Sb.41	2	33.3
Sb.42	1	16.6
Sb.56	2	33.3
Sb.58	3	50.0
Sb.59	5	83.3
Sb.61	5	83.3
Sb.62	1	16.6
Sb.63	1	16.6
Sb.64	4	66.6
Sb.65	2	33.3
Sb Control 1	6	100.0
Sb Control 2	6	100.0
W.11	1	16.6
W.30	6	100.0
W.42	2	33.3
W.60	2	33.3
W Control 1	1	16.6
Total	60	

TABLE 20

Results of Post-Mortem Examination

Experiment 3

Band No.	Pen No.	Symptoms				
		Discolored	Swollen	Congestion	Marble-Like Discoloration	Dehydration
6484	Sb.30	Liver	Ceca Kidney Pancreas			
6486	Sb.30	Liver	Kidney Pancreas			
6491	Sb.33		Kidney	Kidney		
6492	Sb.33		Kidney	Kidney		
6496	Sb.34		Ceca			
6498	Sb.34		Pancreas			
6494	Sb.34	Liver Spleen				
6497	Sb.34		Kidney			
1910	Sb.35			Kidney		Carcass
1940	Sb.35			Kidney		Carcass
1980	Sb.36					Carcass
1330	Sb.37		Pancreas			
8130	Sb.37		Kidney Pancreas			
8138	Sb.38	Liver				
8136	Sb.38		Kidney			
8404	Sb.41		Gall Bladder			
8408	Sb.42	Liver				
8411	Sb.42		Pancreas			
8416	Sb.40	Liver				Carcass
8421	Sb.57	Liver				Carcass
8423	Sb.57	Liver				
8439	Sb.60		Pancreas			Carcass
8427	Sb.58					Carcass
8464	Sb.64					Carcass
8458	Sb.63					

TABLE 20 continued

Results of Post-Mortem Examination

Band No.	Pen No.	Symptoms				
		Discolored	Swollen	Congestion	Marble-Like Discoloration	Dehydration
8489	W.11	Liver				
8488	W.11	Liver				
8487	W.11	Liver				
8501	W.33	Liver				
8505	W.34			Liver		
8507	W.34	Liver				
8510	W.35	Liver				
8514	W.35	Liver				Carcass
8521	W.36	Liver				
8517	W.36	Liver				
8524	W.37				Liver	
8526	W.37				Liver	
8528	W.38	Liver				
8532	W.38	Liver				
8544	W.40	Liver				
8552	W.41	Liver	Kidney			
8549	W.41	Liver				
8554	W.42	Liver				
8553	W.42	Liver				Carcass
8563	W.56		R. Kidney	R. Kidney		
8600	W.62		L. Kidney			

Experiment 4

Six chicks were used per pen. The moldy feedstuffs were mixed on a 1:1 basis with the basal diets No. 119 (supplemented as indicated in the previous experiment) and No. 120. The twenty-two strains tested are indicated in Tables 21 and 22 along with the name of the fungus, pen number and culture number. The average weights of the chicks per pen are indicated in Tables 23 and 24 along with feed consumption, feed conversion and culture number of the mold. Mortality data are given in Table 25 and post-mortem examination findings in Table 26.

Deathloss started on the fourth day and continued through the fourteenth day with the highest incidence occurring among chicks fed the soybean diet. Average weights were less than 100 gm. at two weeks of age (3-8-67) for the groups fed the Sb.11, Sb.19, Sb.24, Sb.27 and Sb.40 diets. These same groups also grew poorly through four weeks of age. Feed consumption was also least for these groups which coincides with the smaller average weights. Feed conversion was poorest for the pen fed the Sb.11 diet while an optimum value was obtained for the pen fed Sb.35

For the wheat inoculated feed, the groups fed W.11, W.21, W.22, W.24 and W.28 showed poor performance. The least average weight at two weeks of age was shown for pens fed W.24 (50 gm) and the highest for the pen fed W.19 (149 gm). At four weeks of age the pen fed W.22 showed the least average weight gain while the pen fed W.19 showed the best performance. The lowest feed consumption was shown by the groups fed the W.24 feed (429 gm) whereas the highest

consumption was observed for the chicks fed the W.35 feed (713 gm). The best performance in feed conversion was for the W.41 feed.

TABLE 21

Molds Treated on Soybeans and Mixed with Diet No. 119

Experiment 4

Pen Number	Culture Number	Strain Number	Name of Fungus
1	Sb.11	2999	A. flavus
2	Sb.18	447	A. oryzae
3	Sb.19	451	A. "
4	Sb.20	458	A. "
5	Sb.21	468	A. "
6	Sb.22	506	A. "
7	Sb.23	692	A. "
8	Sb.24	696	A. "
9	Sb.25	699	A. "
10	Sb.26	1808	A. "
11	Sb.27	2219	A. "
12	Sb.28	2220	A. "
13	Sb.33	242	A. sydowi
14	Sb.34	243	A. "
15	Sb.35	244	A. "
16	Sb.36	250	A. "
17	Sb.37	1732	A. "
18	Sb.38	A 12.373	A. "
19	Sb.39	A12.469	A.
20	Sb.40	A 12.477	A. "
21	Sb.41	A 12.807	A. "
22	Sb.42	A 12.981	A. "
23	Sb Control 1	----	----
24	Sb Control 2	----	----

TABLE 22

Molds Treated on Wheat and Mixed with Diet No. 120

Experiment 4

Pen Number	Culture Number	Strain Number	Name of Fungus
1	W.11	2999	A. flavus
2	W.18	447	A. oryzae
3	W.19	451	A. "
4	W.20	458	A. "
5	W.21	468	A. "
6	W.22	506	A. "
7	W.23	692	A. "
8	W.24	696	A. "
9	W.25	699	A. "
10	W.26	1808	A. "
11	W.27	2219	A. "
12	W.28	2220	A. "
13	W.33	242	A. sydowi
14	W.34	243	A. "
15	W.35	244	A. "
16	W.36	250	A. "
17	W.37	1732	A. "
18	W.38	A 12.373	A. "
19	W.39	A 12.469	A. "
20	W.40	A 12.477	A. "
21	W.41	A 12.807	A. "
22	W.42	A 12.981	A. "
23	W Control 1	--	--
24	W Control 2	--	--

TABLE 23

Average Weights and Feed Efficiency

Experiment 4

Pen Number	Culture Number	Average Weights (gms) 2-22-67	3-8-67	3-22-67	Feed Consumption (per chick)	Feed Conversion*
1	Sb.11	41	64	103	275	4.4
2	Sb.18	43	105	215	475	2.7
3	Sb.19	43	53	117	274	3.7
4	Sb.20	45	108	212	568	3.4
5	Sb.21	45	106	222	493	2.7
6	Sb.22	45	100	235	620	3.2
7	Sb.23	42	115	206	502	3.0
8	Sb.24	44	53	153	338	3.1
9	Sb.25	45	118	254	647	3.0
10	Sb.26	45	113	225	537	2.9
11	Sb.27	43	90	208	482	2.9
12	Sb.28	42	110	218	505	2.8
13	Sb.33	45	132	249	527	2.5
14	Sb.34	43	118	208	537	3.2
15	Sb.35	42	111	238	277	1.4
16	Sb.36	42	129	215	558	3.2
17	Sb.37	42	119	236	537	2.7
18	Sb.38	40	118	226	325	2.8
19	Sb.39	43	130	242	533	2.6
20	Sb.40	42	50	144	247	2.4
21	Sb.41	42	137	257	590	2.7
22	Sb.42	44	118	240	523	2.6
23	Sb Control 1	47	108	228	630	3.3
24	Sb Control 2	45	63	185	296	2.1
Control Group Average		46	85	207	463	2.7

* Unit of feed per unit of body weight increase.

TABLE 24

Average Weights and Feed Efficiency

Experiment 4

Pen Number	Culture Number	Average Weights (gms) 2-22-67	3-8-67	3-22-67	Feed Consumption (per chick)	Feed Conversion*
1	W.11	43	103	193	573	3.8
2	W.18	43	125	181	627	2.6
3	W.19	43	149	325	680	2.4
4	W.20	44	144	301	650	2.5
5	W.21	43	79	197	636	4.1
6	W.22	42	60	164	625	5.1
7	W.23	42	99	228	550	2.9
8	W.24	42	50	169	429	3.4
9	W.25	43	83	207	507	3.0
10	W.26	42	128	275	668	2.8
11	W.27	42	143	280	703	2.9
12	W.28	41	70	182	640	4.5
13	W.33	42	135	292	710	2.8
14	W.34	41	116	237	623	3.1
15	W.35	42	137	299	713	2.7
16	W.36	45	127	301	687	2.6
17	W.37	44	141	298	690	2.7
18	W.38	43	143	302	630	2.4
19	W.39	41	127	253	578	2.7
20	W.40	40	123	265	603	2.6
21	W.41	42	124	278	523	2.2
22	W.42	43	134	289	624	2.5
23	W Control 1	43	132	283	643	2.6
24	W Control 2	40	124	282	712	2.9
Control Group Average		42	128	282	677	2.8

* Unit of feed per unit of body weight increase.

TABLE 25

Mortality During Experiment 4

Pen Number	Cumulative Mortality	Percent
Sb.18	1	16.6
Sb.19	3	50.0
Sb.24	5	83.3
Sb.27	1	16.6
Sb.40	4	66.6
Sb Control 1	2	33.3
Sb Control 2	3	50.0
W.21	2	33.3
W.22	2	33.3
W.24	6	100.0
W.25	2	33.3
W.26	2	33.3
W.27	1	16.6
W.28	6	100.0
Total	40	

TABLE 26

Results of Post-Mortem Examination

Experiment 4

Band Number	Pen Number	Discolored	Swollen	Congestion	Marble-Like Discoloration
8751	Sb.11	Liver, Kidney			
8752	Sb.11	Liver, Kidney			
8753	Sb.11	Liver, Kidney			
8755	Sb.11	Liver, Kidney			
8762	Sb.18	Kidney			
8777	Sb.21	Kidney			
8793	Sb.24	Liver, Kidney			
9001	Sb.25	Liver, Kidney			
9004	Sb.25	Liver, Kidney			
9012	Sb.27	Liver, Kidney			
9252	Sb.27	Liver, Kidney			
9253	Sb.38	Liver, Kidney			
9265	Sb.40	Liver, Kidney			
9099	W.11	Liver, Kidney			
9096	W.11	Liver, Kidney			
9130	W.22	Liver, Kidney			
9190	W.36			Liver	
9193	W.37			Liver	
9200	W.38			Liver	
9197	W.38			Liver	
9209	W.40			Liver	
9213	W.40			Liver	
9219	W.41			Liver	
9220	W.41			Liver	
9224	W.42			Liver	
9226	W.42			Liver	

Experiment 5

In this study only five chicks were used per pen. The moldy feedstuffs were mixed in a 1:1 ratio with the respective basal diets No. 119 and No. 120. Twenty-three strains of Aspergillus were cultured on soybeans and/or wheat for this trial, and only one control group was used for each treated feedstuff. The strains of the molds tested are indicated in Tables 27 and 28 along with the name of the fungus, pen number and culture number. The average weights of the chicks per pen are indicated in Tables 29 and 30 along with feed consumption, feed conversion and the culture number of the mold corresponding to each one of the groups. Mortality data are given in Table 31 while post-mortem examination findings are given in Table 32.

Mortality started on the fourth day and continued through the end of the experiment for the groups adversely affected. A lack of coordination was observed for all chicks fed Sb.72 and two chicks fed W.70. Average weights were less than 100 gm. at two weeks of age (5-25-67) for the groups fed Sb.72 (only 48 gm), Sb.75, Sb.81, Sb.66, Sb.68 and Sb.71. At four weeks of age (6-8-67) the same groups (except that fed Sb.68) showed poor growth with the lower value coinciding again with the group fed Sb.72 (62 gm). The lowest feed consumption was in the pen fed Sb.72 and amounted to 214 gm per chick, while the highest consumption was 608 gm for the group fed Sb.76 in comparison to 482 gm for the control group.

Chicks weighing less than 100 gm average weight at two weeks of age were those given W.73, W.74, W.75, W.81, W.79, W.11-35, W.43,

W.45, W.68, W.69 W.70 and W.71 supplemented feed. At four weeks of age the same pens showed poor growth--below 200 gm average weights. The group in the pen fed W.45 showed poor feed intake (24 gm), whereas the highest intake was with the group fed W.76 (602 gm). Feed conversion was best for the pen fed W.77. Mortality was complete (100%) for the pens fed Sb.72-2, Sb.11-35, Sb.69, Sb.70, W.72-2 and W.11-35.

TABLE 27

Molds Treated on Soybeans and Mixed With Diet No. 119

Experiment 5

Pen Number	Culture Number	Strain Number	Name of Fungus	
1	Sb.73	398	Aspergillus	ochraceus
2	Sb.78	404	"	"
3	Sb.72-2	3174	"	"
4	Sb.75	400	"	"
5	Sb.74	399	"	"
6	Sb.77	403	"	"
7	Sb.81	2864	"	"
8	Sb.104	4369	"	petrekii
9	Sb.79	410	"	ochraceus
10	Sb.76	402	"	"
11	Sb.80	1598	"	"
12	Sb.72-1	393	"	quercinus
13	Sb.11-35	2999*	"	flavus
14	Sb.43	1787	"	janus
15	Sb.44	1935	"	"
16	Sb.45	1936	"	"
17	Sb.66	1932	"	granulosus
18	Sb.67	5078	"	pulvinous
19	Sb.68	392	"	quercinus
20	Sb.69	394	"	"
21	Sb.70	396	"	"
22	Sb.71	A 13.493	"	"
23	Sb.72	A 13.494	"	"
24	Sb Control	--	--	--

*Reisolate No. 35 of culture No. 11 of previous experiments.

TABLE 28

Molds Treated on Wheat and Mixed With Diet No. 120

Experiment 5

Pen Number	Culture Number	Strain Number	Name of Fungus	
1	W.73	398	Aspergillus	ochraceus
2	W.77	403	"	"
3	W.76	402	"	"
4	W.72-2	3174	"	"
5	W.74	399	"	"
6	W.78	404	"	"
7	W.75	400	"	"
8	W.81	2864	"	"
9	W.79	410	"	"
10	W.104	4369	"	petrakii
11	W.80	1598	"	ochraceus
12	W.72-1	393	"	quercinus
13	W.11-35	2999*	"	flavus
14	W.43	1787	"	janus
15	W.66	1932	"	granulosus
16	W.44	1935	"	janus
17	W.45	1936	"	"
18	W.67	5078	"	pulvinous
19	W.68	392	"	quercinus
20	W.69	394	"	"
21	W.70	396	"	"
22	W.71	A 13.493	"	"
23	W.72	A 13.494	"	"
24	W Control	--	--	--

* Reisolate No. 35 of culture No. 11 of previous experiments.

TABLE 29

Average Weights and Feed Efficiency

Experiment 5

Pen Number	Culture Number	Average Weights (gms) 5-11-67	5-25-67	6-8-67	Feed Consumption (per chick)	Feed Conversion*
1	Sb.73	39	115	199	552	3.4
2	Sb.78	36	115	212	522	2.9
3	Sb.72-2	39	--	--	12	--
4	Sb.75	37	70	106	318	4.6
5	Sb.74	33	117	221	598	3.1
6	Sb.77	36	117	207	556	3.2
7	Sb.81	40	80	126	422	4.8
8	Sb.104	40	101	193	560	3.6
9	Sb.79	40	113	217	558	3.1
10	Sb.76	40	113	232	608	3.1
11	Sb.80	38	107	192	462	3.0
12	Sb.72-1	38	108	217	582	3.2
13	Sb.11-35	39	--	--	2	--
14	Sb.43	41	116	200	516	3.2
15	Sb.44	37	100	172	484	3.5
16	Sb.45	41	118	225	560	3.0
17	Sb.66	37	69	102	378	5.8
18	Sb.67	38	122	236	530	2.6
19	Sb.68	34	87	188	527	3.4
20	Sb.69	37	--	--	20	--
21	Sb.70	35	--	--	20	--
22	Sb.71	38	96	105	478	7.0
23	Sb.72	35	48	62	214	7.9
24	Sb Control	34	122	200	482	2.9

* Unit of feed per unit of body weight increase.

TABLE 30

Average Weights and Feed Efficiency

Experiment 5

Pen Number	Culture Number	Average Weights (gms) 5-11-67	5-25-67	6-8-67	Feed Consumption (per chick)	Feed Conversion*
1	W.73	36	67	96	388	6.3
2	W.77	32	116	233	510	2.5
3	W.76	36	120	248	602	2.8
4	W.72-2	35	--	--	55	--
5	W.74	36	96	194	464	2.9
6	W.78	38	113	232	528	2.7
7	W.75	40	64	96	436	7.7
8	W.81	40	82	161	488	4.0
9	W.79	40	96	158	442	3.7
10	W.104	36	108	210	512	2.9
11	W.80	36	101	205	568	3.3
12	W.72-1	35	113	223	528	2.8
13	W.11-35	34	40	--	216	--
14	W.43	36	45	70	258	7.6
15	W.66	34	111	222	634	3.4
16	W.44	34	110	245	580	2.7
17	W.45	33	40	55	24	1.0
18	W.67	34	116	202	462	2.7
19	W.68	34	73	120	518	6.0
20	W.69	33	60	55	300	13.6
21	W.70	33	83	138	468	4.4
22	W.71	35	97	159	589	4.7
23	W.72	34	105	210	466	2.6
24	W Control	35	108	219	571	3.1

* Unit of feed per unit of body weight increase.

TABLE 31

Mortality During Experiment 5

Pen Number	Cumulative Mortality	Percent
Sb.72-2	5	100.0
Sb.81	1	20.0
Sb.11-35	5	100.0
Sb.68	1	20.0
Sb.69	5	100.0
Sb.70	5	100.0
Sb.71	1	20.0
W.76	1	20.0
W.72-2	5	100.0
W.11-35	5	100.0
W.43	4	80.0
W.45	4	80.0
W.68	3	60.0
W.69	3	60.0
W.71	1	20.0
Total	49	

TABLE 32

Results of Post-mortem Examination-Experiment 5

Band Number	Pen Number	Discolored	Complete Discoloration
9441	Sb.81	Liver, Kidney	
9452	Sb.79	Liver	
9454	Sb.76	Liver	
9462	Sb.80	Liver	
9478	Sb.43	Liver	
9479	Sb.43	Liver	
9491	Sb.66	Kidney	Liver
9492	Sb.66	Kidney	Liver
9495	Sb.66	Kidney	Liver
8970	W.81	Liver, Kidney	

Experiment 6

Six chicks were again used for this experiment. The moldy feedstuffs were mixed on a 1:1 basis with the basal diets No. 119 or No. 120 respectively. Twenty three strains of Aspergillus were cultured on soybeans and/or wheat together with one control group in each category. The strains of the molds tested are indicated in Tables 33 and 34 along with the name of the fungus, pen number and culture number. The average weights of the chicks are given in Tables 35 and 36 along with the feed conversion, feed consumption and the culture number of the mold used.

Mortality data and post-mortem findings are given in Tables 37 and 38 respectively. Death loss began on the second day and continued through the twenty-fourth day. The chicks fed Sb.95 showed lack of coordination and poor growth. Average weights were less than 100 gm. at two weeks of age (7-26-67) for the groups fed Sb.89, Sb.95, Sb.87, Sb.90, Sb.97, and Sb.93. At four weeks of age (8-9-67) these same pens (except pen Sb.93) continued to show poor growth with the lowest value coinciding with the groups fed Sb.95 (79 gm.). Poorest feed consumption occurred in the pen fed Sb.90 (33 gm.) and the highest occurred in the pen fed Sb.93 (562 gm.).

Average weights below 100 gm. at two weeks of age were shown by the groups fed W.91, W.45, W.87, W.89, W.90, W.93, W.95, W.96, W.99 and W.84. At four weeks of age these same groups continued to show poor performance with the poorest being the pen fed W.84 (55 gm.). The least feed consumption was shown by the group fed W.91 (97 gm.) and the highest by the group fed W.94 (665 gm.). It was evident that

a large number of the mold species used in this experiment were toxin producers.

TABLE 33

Molds Treated on Soybeans and Mixed with Diet No. 119

Experiment 6

Pen Number	Culture Number	Strain Number	Name of Fungus
1	Sb.82	415	Aspergillus sclerotiorum
2	Sb.83	4901	" "
3	Sb.84-1	386	Aspergillus sulphureus
4	Sb.84	387	" "
5	Sb.85	388	" "
6	Sb.86	389	" "
7	Sb.87	390	" "
8	Sb.88	4077	" "
9	Sb.89	A 829	" "
10	Sb.90	A 830	"
11	Sb.91	A 832	" "
12	Sb.92	A 6924	" "
13	Sb.93	391	Aspergillus auricomus
14	Sb.94	397	" "
15	Sb.95	416	Aspergillus melleus
16	Sb.96	5103	" "
17	Sb.97	A 975	" "
18	Sb.98	A 993	" "
19	Sb.99	A 2305	" "
20	Sb.100	A 2306	" "
21	Sb.101	A 13653	" "
22	Sb.102	420	Aspergillus ostianus
23	Sb.103	4850	Aspergillus elegans
24	Sb Control	--	-- --

TABLE 34

Molds Treated on Wheat and Mixed with Diet No. 120

Experiment 6

Pen Number	Culture Number	Strain Number	Name of Fungus
1	W.82	415	Aspergillus sclerotiorum
2	W.83	4901	" "
3	W.84-1	386	Aspergillus sulphureus
4	W.84	387	" "
5	W.85	388	" "
6	W.86	389	" "
7	W.87	390	" "
8	W.88	4077	" "
9	W.89	A 829	" "
10	W.90	A 830	" "
11	W.91	A 832	" "
12	W.92	A 6924	" "
13	W.93	391	Aspergillus auricomus
14	W.94	397	" "
15	W.95	416	Aspergillus melleus
16	W.96	5103	" "
17	W.97	A 975	" "
18	W.98	A 993	" "
19	W.99	A 2305	" "
20	W.100	A 2306	" "
21	W.101	A 13653	" "
22	W.102	420	Aspergillus ostianus
23	W.103	4850	Aspergillus elegans
24	W Control	--	-- --

TABLE 35

Average Weights and Feed Efficiency

Experiment 6

Pen Number	Culture Number	Average Weight (gms) 7-12-67	7-26-67	8-9-67	Feed Consumption (per chick)	Feed Conversion**
1	Sb.82	36	--*	--	12	--
2	Sb.83	36	--	--	17	--
3	Sb.84-1	35	104	230	514	2.6
4	Sb.84	35	--	--	57	--
5	Sb.85	36	108	207	453	2.6
6	Sb.86	36	106	208	518	3.0
7	Sb.87	34	96	196	490	3.0
8	Sb.88	36	--	--	10	--
9	Sb.89	35	55	--	65	--
10	Sb.90	35	68	180	33	0.2
11	Sb.91	37	--	--	207	--
12	Sb.92	35	--	--	27	--
13	Sb.93	35	98	206	562	3.2
14	Sb.94	36	111	222	515	2.7
15	Sb.95	37	55	79	248	5.9
16	Sb.96	34	--	--	10	--
17	Sb.97	36	89	192	469	3.0
18	Sb.98	36	--	--	23	--
19	Sb.99	34	111	241	528	2.5
20	Sb.100	35	--	--	34	--
21	Sb.101	37	--	--	49	--
22	Sb.102	33	113	212	445	2.4
23	Sb.103	35	100	185	469	3.1
24	Sb Control	34	116	240	513	2.4

* Blank spaces indicate no survivors.
** Unit of feed per unit of body weight increase.

TABLE 36

Average Weights and Feed Efficiency

Experiment 6

Pen Number	Culture Number	Average Weights (gms) 7-12-67	7-26-67	8-9-67	Feed Consumption (per chick)	Feed Conversion **
1	W.82	38	--*	--	3	--
2	W.83	35	45	95	100	1.6
3	W.84-1	35	105	216	500	2.7
4	W.84	33	48	55	155	7.1
5	W.85	35	104	229	500	2.5
6	W.86	35	111	239	532	2.6
7	W.87	36	93	203	438	2.6
8	W.88	38	--	--	23	--
9	W.89	37	70	140	404	3.8
10	W.90	38	60	128	180	2.0
11	W.91	38	42	80	97	2.3
12	W.92	36	--	--	33	--
13	W.93	34	98	223	585	3.1
14	W.94	35	104	240	665	3.2
15	W.95	34	90	170	465	3.4
16	W.96	36	85	189	480	3.1
17	W.97	36	120	239	653	3.2
18	W.98	36	--	--	95	--
19	W.99	34	99	208	523	3.0
20	W.100	34	--	--	28	--
21	W.101	35	--	--	189	--
22	W.102	36	109	231	510	2.6
23	W.103	35	113	233	508	2.5
24	W Control	36	121	238	457	2.2

* Blank spaces indicate no survivors.
** Unit of feed per unit of body increase.

TABLE 37

Mortality During Experiment 6

Pen Number	Cumulative Mortality	Percent
Sb.82	6	100.0
Sb.83	6	100.0
Sb.84	6	100.0
Sb.88	6	100.0
Sb.89	6	100.0
Sb.90	5	83.3
Sb.91	6	100.0
Sb.92	6	100.0
Sb.95	2	33.3
Sb.96	6	100.0
Sb.97	1	16.6
Sb.98	6	100.0
Sb.100	6	100.0
Sb.101	6	100.0
W.82	6	100.0
W.83	5	83.3
W.84	3	50.0
W.88	6	100.0
W.89	4	66.6
W.90	4	66.6
W.91	5	83.3
W.92	6	100.0
W.93	1	16.6
W.96	1	16.6
W.98	6	100.0
W.100	6	100.0
W.101	6	100.0
Total	133	

TABLE 38

Results of Post-Mortem Examination

Experiment 6

Band Number	Pen Number	Congestion	Discolored	Marble-Like Discoloration
6761	Sb.84-1	Liver, Kidney		
6783	Sb.87	Liver, Kidney		
6857	Sb.99	Liver		
6855	Sb.99	Liver		
6624	W.84		Kidney	
6676	W.93			Liver
6680	W.94			Liver
6691	W.96			Liver
6694	W.96			Liver
6711	W.99		Liver, Kidney	

Experiment 7

In this experiment a comparison was made between three avian species (chickens, turkeys, and pheasants) concerning their responses to aflatoxins. Proper basal diets were used to provide the necessary protein, vitamins, minerals and energy for each species of bird. Strain No. 465 of Aspergillus parasiticus was cultured on soybeans and fed to day-old chicks, poults and pheasants. The molded feed was mixed with the basal diet at the rate of 5, 10, 20, and 40%. Ten birds were used in each pen.

Table 39 gives average weights at the beginning of the four week experiment, and at two weeks and four weeks of age. Incidence of mortality is shown in Table 40. Chicks, it was noticed, were less responsive to the toxic feedstuffs, showing a higher resistance than poults and pheasants did. With each increase in amount of toxic feedstuff used, chick growth was reduced. The only mortality among chicks occurred in pens fed 5% moldy feed (1 chick) and 40% moldy feed (1 chick) during the tenth and fourth day respectively.

Post-mortem examination revealed liver discoloration for all the chicks fed 20% and 40% moldy feed, two of which (one from 20% and one from 40%) showed a yellow liver. Poults were extremely sensitive to the toxic feedstuffs, in that mortality was 100% for any increment of moldy feed. This occurred within 13 days, making it impossible to obtain average weights at two weeks of age. Post-mortem examination revealed liver discoloration for all the birds fed the 20% and 40% toxic feedstuffs. Nearly half of them fed 5 and 10% moldy feed showed the same condition. Three poults fed the 40% diet showed

yellow colored livers.

Pheasants were even more responsive to the moldy feeds than turkey poults. All pheasants receiving toxic feedstuffs at any level died within eleven days. Post-mortem examination revealed liver discoloration for all birds.

TABLE 39

The Comparative Response of Chicks, Poults and Pheasants to Toxic Feedstuffs

Experiment 7

	Percent Moldy Feed*	Chicks Grams	Poults Grams	Pheasants Grams
Initial Weight	Control (0%)	35	41	22
	5% Moldy Feed	33	43	24
	10% " "	36	42	24
	20% " "	34	37	22
	40% " "	34	40	23
Two Weeks	Control (0%)	114	119	87
	5% Moldy Feed	80	--	--
	10% " "	79	--	--
	20% " "	72	--	--
	40% " "	72	--	--
Four Weeks	Control (0%)	290	355	251
	5% Moldy Feed	197	--	--
	10% " "	187	--	--
	20% " "	127	--	--
	40% " "	113	--	--

* Culture No. 465 which supplied 9 micrograms of aflatoxin B and 40 ug of aflatoxin G per gram of moldy soybeans.

TABLE 40

Mortality During Experiment 7

Diets	Chicks		Poults		Pheasants	
	No.	%	No.	%	No.	%
Control	--	0	6	60	2	16.6
5% Moldy Feed	1	10	10	100	10	100
10% Moldy Feed	--	0	10	100	10	100
20% Moldy Feed	--	0	10	100	10	100
40% Moldy Feed	1	10	10	100	10	100

GENERAL DISCUSSION

The chicks were more susceptible to moldy feed during the first and second week of life, showing highest death loss, morbidity and and evidence of stress at this time. After the end of the second week and towards the end of the experiment they appeared to become resistant.

The sensitivity of the chicks was more evident when the feed containing 50% moldy feedstuffs was used compared to that when the 25% cultured feedstuffs was used. Chicks fed cultured soybeans showed generally more detrimental responses than when the cultured wheat was fed. These effects included higher morbidity and mortality with poorer growth and feed consumption.

Chicks on Sb.11 and W.11 appeared to suffer, ate and grew poorly and showed a high rate of morbidity and mortality. Post-mortem examination revealed lesions in the liver and kidney, characteristic of mold toxicosis. These are the two major organs of the bird's body affected by the disease. The Aspergillus flavus culture No. 2999 was used to produce the molded feedstuffs Sb.11 and W.11. The feedstuffs were examined in the Plant Pathology Department by Dr. M. Lai and were found to contain an abundance of aflatoxins B_1 and G_1.

Chicks on Sb.30 and W.30 also showed evidence of stress, poor growth, morbidity and high mortality, while post-mortem examination revealed liver and kidney damage. Aspergillus parasiticus, culture No. 465 was used to produce the moldy feeds labeled Sb.30 and W.30. This product was also tested by Dr. M. Lai for aflatoxin content and

was found to contain 40 to 720 ug/gm., depending on the method of testing and substrate used. Both aflatoxins B_1 and G_1 were found in this product as well.

Further tests revealed that A. janus, A. quercinus, A. ochraceus and A. melleus produced "ochratoxin" a new toxin chemically related to the aflatoxins. These toxic products had been labeled as Sb.45, W.45, Sb.69, W.69, Sb.72-2, W.72-2, Sb.100 W.100 and Sb.101, W.101.

Several other cultured products produced abnormalities and unfavorable side effects. Whereas the average weights under normal conditions at the end of a four week feeding period should approach 300 gm. for S.C.W.L. chicks, few groups attained that average.

Some strains apparently produced beneficial growth factors as was indicated by the final average weights exceeding the 300 gm. figure. Such products were W.4, W.5 and W.19 for the first experiment, Sb.40, Sb.53, W.35, W.36, W.38, W.42, W.47, W.50 and W.55 for the second experiment, W.60 for the third experiment and W.19, W.20, W.36 and W.38 for the fourth experiment. The fifth and sixth experiments showed no evidence of beneficial factors.

Under normal conditions a S.C.W.L. bird up to the age of four weeks will usually attain a feed conversion of about 1.5 units of feed per unit of gain. This degree of performance was reached by the chicks fed Sb.35 and W.83, 1.4 and 1.6 respectively.

Feed consumption under normal conditions would be about 680 grams per bird for a four week feeding period. This amount was approached or exceeded by chicks fed Sb.4 (670 gm), W.5 (686 gm), W.27 (703 gm), W.33 (710 gm), W.35 (713 gm), W.36 (687 gm) and

W.37 (690 gm). A few other groups consumed more than 600 gms. Feed consumption was less for the slowly growing chicks and coincided with higher toxicity.

Day-old chicks were more resistant than day-old poults and pheasants to aflatoxin injury. This was demonstrated in experiment 7. However further work must be done to establish the relative susceptibility and toxicity levels for the three avian species mentioned above. The first two days were also the most difficult for poults and pheasants fed toxic feedstuffs.

SUMMARY

One hundred forty-nine strains of Aspergillus were tested for toxin-producing ability in the seven experiments reported. Day-old S.C.W.L. or Leghorn-type chicks were fed adequate diets containing 25 or 50% of cultured soybeans or wheat, up to the age of four weeks.

Data was obtained on weight gain, feed consumption, feed conversion and mortality. At the end of each experiment the chicks were sacrificed and post-mortem examinations were made.

The following cultures were shown to produce the toxic effect of high mortality and/or liver and kidney damage: Sb.11 and W.11 produced by strain No. 2999 of Aspergillus flavus; Sb.30 and W.30 produced by strain No. 465 of A. parasiticus; Sb.45 and W.45 products of strain No. 1936 of A janus; Sb.69 and W.69, products of strain No. 394 of A. quercinus; Sb.72-2 and W.72-2, products of strain No. 3174 of A. ochraceus; Sb.100, W.100, Sb.101 and W.101, products of strains No. A-2306 and No. A-13653, respectively, of A. melleus.

The first two strains were shown to have produced aflatoxin. The others produced ochratoxin or aflatoxin-like compounds. The majority of the molds tested were not found to be toxic.

LITERATURE CITED

Anderson, G. C., J. K. Blender, N. O. Olsen and D. C. Shelton, 1955. "Research phases of the hemorrhagic condition". Feed Age; 5:37-39.

Asao, T., M. M. Bachi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wagan, 1965. "The structures of Aflatoxin B_1 and G_1". J. Amer. Chem. Soc.; 87:882.

Baker, H. R., and D. S. Jacquette, 1953. "Observations concerning the hemorrhagic syndrome of poultry". Proc. 25th Ann. Conf.; Lab. Workers in Pullorum Disease Control, Amherst, Mass. 1953 (Mimeo).

Borgers, R., and G. L. Peltier, 1946. "Molded Feedstuffs. Supplementary value in chick starting rations". Poultry Sci.; 26: 2:194-197:1947.

Burnside, J. E., W. L. Sippel, J. Forgacs, W. T. Carll, M. B. Atwood and E. R. Doll, 1957. "A disease of swine and cattle caused by eating moldy corn. II. Experimental production with pure cultures of molds". Am. J. Vet. Res., 18:817-824.

Camp, S. A., 1957. "The field hemorrhagic-anemic syndrome. New treatment". Feedstuffs; 29:20,23,24.

Carll, W. T., J. Forgacs, and A. S. Herring, 1954. "The significance of fungi in hyperkeratosis". Military Surgeon, 115:187-193.

Caskey, C. D., 1953. "Hemorrhagic syndrome of poultry". Proc. Semian. Meeting Nutrition Council, Am. Feed Manufacturers Assoc.; November 1953, p. 8.

Chrisman, J., 1955. "Dehydrated alfalfa in hemorrhagic control". Feed Age; 5:42.

Cover, M. S., W. J. Mellen and E. Gill, 1955. "Studies of hemorrhagic syndromes in chickens". Cornell Vet.; 45:366-386.

Couch, J. R., H. T. Cartrite and A. A. Camp, 1954. "Chick hemorrhagic disease". Poultry Comment; 11:1,3.

Forgacs, J., and H. Koch, 1955. "Further mycotoxic studies on poultry hemorrhagic disease". Poultry Science; 34:5:1194:1955.

Forgacs, J. and W. T. Carll, 1955. "Preliminary mycotoxic studies on hemorrhagic disease in poultry". Vet. Med.; 50:172.

Forgacs, J., H. Koch, W. T. Carll and R. H. White-Stevens, 1958a. "Additional studies on relationship of mycotoxicoses to the poultry hemorrhagic syndrome". Am. J. Vet. Research; 19: 744-753.

Forgacs, J. and W. T. Carll, 1962. "Mycotoxicoses". Advances in Veterinary Science; 7:1962. Edited by C. A. Brandly, E. L. Jungherr, pages 275, 314, 316-323.

Forgacs, J., H. Koch, W. T. Carll and R. H. White-Stevens, 1961a. "Mycotoxicoses. I. Relationship of toxic fungi to moldy feed toxicosis in poultry". Unpublished observations.

Forgacs, J., W. T. Carll, A. S. Herring and B. G. Mahlandt, 1954. "A toxic Aspergillus clavatus isolated from feed pellets". Am. J. Hyg. 60:15-26.

Gorcica, H. J., W. H. Peterson and H. Steenbock, 1935. "The nutritive value of fungi. II. The vitamin B, G, and B_4 content of the mycelium of Aspergillus sydowi". J. Nutrition 9:691-714.

Gray, J. E., G. H. Snoeyenbos, and I. M. Reynolds, 1954. "The hemorrhagic syndrome of chickens". J. Amer. Vet. Med. Assoc.; 125:144-151.

Griminger, P., H. Fisher, W. D. Morrison, J. M. Snyder, and H. M. Scott, 1953. "Factors influencing blood clotting time in the chick". Science; 118:379-380.

Hare, J. H., G. C. Anderson, C. E. Weakley, Jr., and J. K. Bletmer, 1953. "Factors contributing to a hemorrhagic condition in experimental chicks fed simplified rations". Poultry Science; 32:904.

Henderson, W., W. R. Pritchard, and D. B. Taylor, 1957. "Observations on aplastic anemia in chickens". Poultry Science; 36:1125.

Huhtanen, C. N. and J. M. Pensack, 1966. "Effect of antifungal compounds on aspergillosis in hatching chick embryos". Applied Microbiology; 15:1:1967:102-109.

Kirschstein, R. L. and H. Sidransky, 1956. "Mycotic endocarditis of the tricuspid valve due to Aspergillus flavus. Report of a case". A. M. A. Arch. Path.; 62:103-106.

Moody, D. P., 1963. "Biogenetic hypotheses for aflatoxin". Nature; 202:188:1964.

Mushett, C. W. and A. O. Seeler, 1947. "Hypoprothrombinemia resulting from the administration of sulfaquinoxaline". J. Pharmacol. Exptl. Therap.; 91:84-91.

Nesbitt, Brenda F., J. O. Kelly, K. Sargeant and Ann Sheridan, 1962. "Toxic metabolites of aspergillus flavus". Nature; 195:1062-63:1962.

Petty, M. A. and G. D. Quigley, 1946. "The microflora of wheat as related to the incidence of blue comb in chickens". Poultry Science; 26:1:7-13:1947.

Pons, W. A. Jr. and L. A. Goldblatt, 1965. "The determination of aflatoxins in cottonseed products". J. Amer. Oil. Ch. Soc.; 42:471-475:June 1965.

Prebluda, J. J., 1955. "Observations on the hemorrhagic condition". Feed Age; 5:40-41.

Prohaszka, L. and Juhasz, 1966. "Persistent anistropy in the livers of ducklings with aflatoxin poisoning". Avian Diseases II; 130-136:1967.

Richardson, L. R., Sue Hays and R. H. Rigson, 1966. "The nutritive value of moldy grains and protein concentrates for growth of poults". Poultry Sci. 46:1:168-176:1967.

Schofield, F. W., 1924. "Damaged sweet clover: The cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg". J. Am. Vet. Med. Assn.; 64:553-575.

Schumaier, G., P. Blagabat, H. M. DeVolt, N. C. Laffer, and R. D. Creek, 1961. "Hemorrhagic lesions in chickens resembling naturally occurring hemorrhagic syndrome produced experimentally by mycotoxins". Poultry Science; 40:4:1132-34:1961.

Schumaier, G., P. Blagabat, H. M. DeVolt, N. C. Laffer and R. D. Creek, 1961. "Mycotoxins as possible causative agents of hemorrhagic syndrome". Poultry Science; 40:5:1953:1961.

Scott, D. B. "Toxigenic fungi isolated from cereal and legume products". Mycopath. et mycologia applicata; IIV:3-4:1965 213-222.

Spensley, P. C., 1963. "Aflatoxine, the active principal in turkey 'X' disease". Endeavour; 22:86:75-79: May 1963.

Tadashi, Arai, Ito Tatsuya and Koyama Yasumasa, 1966. "Anti-microbial activity of aflatoxins". Journal of Bacteriology; 93:1:59-64:1967.

Vernett, Jackelline, Jean-Pierre Marliac and Joseph McLaughlin, Jr., 1964. "Use of the chicken embryo in the assay of aflatoxin toxicity". Journal of the A.O.A.C.; 47:6:1003-1006:1964.

Washko, F. V. and C. W. Mushett, 1955. "Some observations on the pathology of the hemorrhagic condition of chickens". Proc. 92 Ann. Meeting Am. Vet. Med. Assoc. Minn.; 36-363:1955.