The effects of soil on retention and distribution of aflatoxins in tissues of chickens fed aflatoxin-contaminated rations amended with soil

by

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DEDICATION

Dedication of this thesis goes to the people who have been instrumental in my life and have contributed to my development to this stage of achievement. Special dedication goes to my father and mother Mr. and Mrs. Obediah Augustus Madden and family in Jamaica, West Indies, for their love, sacrifices, discipline and continuous support have made it possible for me to progress. I also would like to extend warmest appreciations to my Poisonous Plants professor and his dear wife, Dr. and Mrs. Robert L. Judkins and to Mr. and Mrs. Wilson Richburg in Tuskegee Alabama, as both were my guardian parents who encouraged and supported me as I progressed throughout my educational career.

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GENERAL INTRODUCTION

Aflatoxin B_1 (AFB_1) belongs to a group of secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and is highly toxic and a potent carcinogen (1,2,3,4,5,6). The incidence of contamination of agricultural commodities by aflatoxin depends on several factors such as growing regions and seasonal conditions under which crops are grown, harvested and stored (4,5). Molds producing mycotoxins are capable of growth on a variety of substrates (2,7) and under diverse conditions of moisture, pH and temperature (2). The occurrence and toxicity of AFB_1 in agricultural products consumed by animals and man has received considerable attention during the past 20 years (1,2,3) due to their biochemical and biological effects on living organisms (7). Aflatoxin B_1 was found to be associated mostly with grain crops. Contaminated crops are unfit for consumption by animals and are usually disposed of by plowing into the soil (2,5,6).

Random routine testing of commercially available on-the-shelf brands of peanut butter during the past eight years showed that 74% of the samples tested were positive for aflatoxin. It was found that 8.6% of these samples were contaminated between 20 and 50 ppb, 37% were between 50 and 100 ppb and 2.2 % were above 100 ppb. When all samples were

considered, 9.8% of 2510 samples exceeded the US Food and Drug Administration (FDA) action level for total aflatoxin of 20 ppb (8). Current aflatoxin action levels of the FDA are shown in Table 1 (9).

The economic impact from aflatoxin contamination may be observed in production, marketing and utilization of agricultural products. The exposure of animals to aflatoxin due to the consumption of contaminated feeds can present a difference between profit and loss in the poultry industry (4,10). The effects of aflatoxins on animals are shown in Tables 2 and 3 (9). The effects that aflatoxins have on animals may be passed on to humans (2,3), if animals which are exposed to high levels of aflatoxin retain these residues in their tissues. Acute toxicity of aflatoxin in animals is shown in Table 4 (9).

Food consumed from exposed animals can result in serious health problems to consumers (2) because aflatoxin B_1 is highly toxic and carcinogenic (1,2,3,4,5,6). Comparative LD_{50} toxicities and dietary aflatoxin B_1 concentrations causing toxicosis in animals are shown in Table 5 (9,11) and Table 6 (9,12).

There is need for applicable methods to detoxify aflatoxin-contaminated feedstuffs to reduce or prevent the detrimental effects aflatoxins can have on the livestock industry, and at the same time, reducing the possibility of serious health

problems from aflatoxin residues passed to consumers of animals and animal products. This has led to the evaluation of several substances for application in the detoxification of aflatoxins.

Table 1. Aflatoxin action levels of the Food and Drug Administration (9)

Human foods Peanuts, corn, other nuts	20.0 ng/g
Milk[a]	0.5 ng/g
Corn (interstate commerce)	20.0 ng/g
Corn (intrastate)[a] To be fed to mature non-lactating animals	20.0-100 ng/g

[a]Action level for fluid milk products (0.5 ppb) established only for a particular condition that existed in Southeastern United States during 1978 (Federal Register 42, No.234, 861630, December 6, 1977 and FDA Administrative Guidelines 7406.06 Transmittal 77-13 dated December 6, 1977). FDA recommends that contaminated corn (20-100 ppb) could be safely used as feed for mature non-lactating animals. Current action level for aflatoxin in animal feed is 20 ppb.

Table 2. Adverse effects of aflatoxins on animals (9)

	Swine	Chickens	Dairy cattle	Feedlot
	ng/g	ng/g	ng/g	ng/g
Poor performance Stunting	200-400	200-1000+	200+	700+
Hemorrhage	400+	500	700+	700+

Table 3. Effects of aflatoxicosis[a] in animals (9)

Species	Stunting	BDP	VAC	Icterus	Depression	Death
Quail	+	++	+	+	+	+
Turkeys	+	++	++	+	+	-
Ducklings	+	++	++	++	+	+
New Hampshire Chicks	+	++	++	+	+	+
Broilers	+	+	+	+	+	-
Leghorns	-	+	+	-	-	-
Dogs	-	++	++	+	+	-
Pigs	++	++	++	+	+	+
Holstein calves (Rough hair-coat, arched backs, severe straining, edema and hemorrhage)	++	++	++	+	+	-

[a]Observations made at the University of Florida, College of Veterinary Medicine, Gainesville, Florida during the years 1967 through 1975
BPD = Bile duct proliferation
VAC = Parenchymal cell vacuolation
+ = Mild response
++ = Severe response
- = No response

Table 4. Acute toxicity of aflatoxins in animals (9)

Animal species	Feed levels	Chronic exposure
Broiler chicks	200.0 ug/kg	10 weeks
Layers	610.0 ug/kg	33 weeks
Swine	400.0 ug/kg	9 weeks
Cattle, Feedlot	800.0 ug/kg	12 weeks
Trout	0.5 ug/kg	1 week
Man	6.0 ug/kg	1-12 weeks

Table 5. Comparative LD_{50} of aflatoxin B_1 in animals (9)

Trout	0.5-1.0 mg/kg in 10 days
Ducks	0.4-0.6 mg/kg in 5 days
Turkeys	0.5-1.0 mg/kg
Rabbits	0.3 mg/kg
Cattle, young calves	0.5-1.0 mg/kg
Cats	0.3-0.6 mg/kg
Pigs, 6-7 kg	0.62 mg/kg
Puppies	0.5-1.0 mg/kg
Rats, day-old 21 days old	1.0 mg/kg 5.5-7.2 mg/kg
Guinea pigs	1.4 mg/kg
Swine and young foals	2.0 mg/kg
Sheep	2.0 mg/kg
Chickens, New Hampshire	2.0 mg/kg
Chickens, Rhode Island Red	6.3 mg/kg
Monkeys	7.8 mg/kg
Mice	9.0 mg/kg
Hamsters	10.2 mg/kg
Catfish, channel	10.0-15.0 mg/kg

Wogan, G. N., 1966 and 1969

Table 6. Dietary Aflatoxin B_1 concentrations causing toxicosis in animals (9)

Species	Age	Feed levels ug/g	Duration of feeding	Signs
Calves	Weanling	0.2-2.2	16 weeks	Stunting Liver damage
Steers	1-2 years	0.5-0.7	20 weeks	Liver damage
Cows	2 years	2.4	7 months	Liver damage
Pigs	Newborn	0.23	4 days	Stunting
Pigs	4-6 weeks	0.4-0.7	3-6 weeks	Stunting Liver damage
Chickens	1-7 days	0.2-0.8	10 weeks	Stunting Liver damage
Ducks	1-7 days	0.3-0.6	7-14 days	Liver damage Death
Rainbow trout	Fingerling	0.4	12 months	Hepatoma-40%

Allcroft, R., Mycotoxin in Foodstuffs by G. N. Wogen MIT Press, 1965, 154-160

Structures of Aflatoxins

The structure of aflatoxins consist of a coumarin nucleus fused to a bifuran ring to either a pentanone or a six-membered lactone. Four distinct fluorescent compounds can be chromatographically separated, including aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2(AFG_2). AFB_2 and AFG_2 are dihydroderivatives of AFB_1 and AFG_1, respectively (Fig. 1). Other aflatoxins which have been isolated from peanuts and corn are 4-hydroxylated derivatives of AFB_1 and AFB_2, aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2), respectively (Fig. 2) (13).

Metabolism and Distribution of Aflatoxins

AFM_1 was first isolated from milk of cows fed toxic rations and later from lactating rats, rat liver and urine, liver and kidney of sheep. Animals produce various metabolites after ingestion of aflatoxin-contaminated ration. AFB_1 is metabolized to aflatoxins M_1, Q_1, P_1, and AFB_1 epoxide (Fig. 3) (1,13). Aflatoxin M_1 is relatively stable and is both mutagenic and carcinogenic. It is mostly present in excreta of exposed animals and its secretion in milk has been a serious food safety concern (13).

The distribution of AFB_1 in tissues of farm animals became

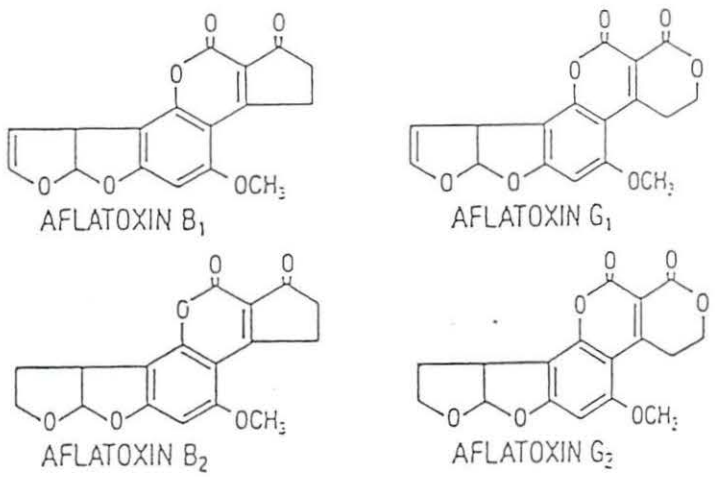

Figure 1. Chemical structures of aflatoxins B_1, B_2, G_1 and G_2

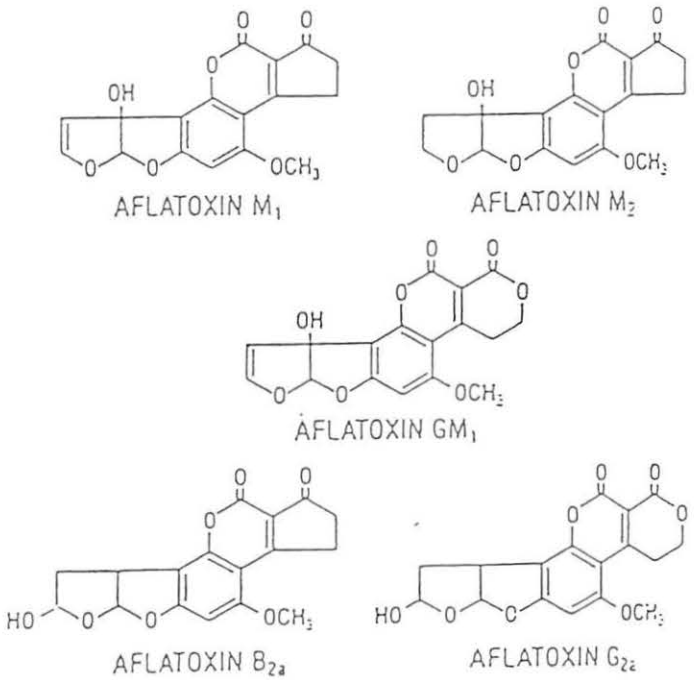

Figure 2. Chemical structures of hydroxylated aflatoxins

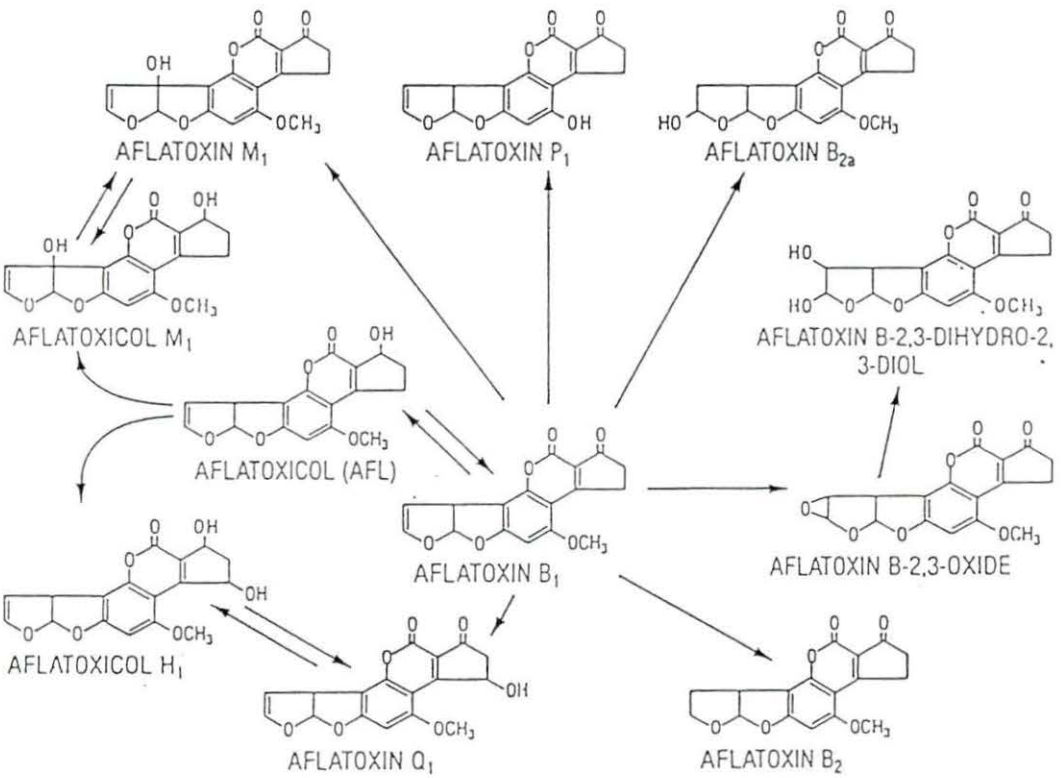

Figure 3. Major aflatoxin B_1 metabolites in animals

important when extracts from cow's milk induced hepatic lesions in ducklings resembling those caused by AFB_1. Forty-eight hours after oral administration of a single dose, 90% of the administered aflatoxin was excreted in milk and urine; AFM_1 was in milk. Liver was found to retain the highest concentration of toxin or its metabolites, and the toxin persisted for a long period (1,13). In chickens, results after oral administration of a single dose of radiolabeled ^{14}C-aflatoxin indicated that most aflatoxin was excreted in feces and most of the retained aflatoxin was found in the liver (14).

Methods of Detoxification of Aflatoxins

Detoxification of aflatoxin-contaminated food has become a challenge to the food industry because of the insensitivity of aflatoxin to physical and chemical treatments. Aflatoxin levels are affected by several factors including moisture content, location of toxin in food, form of food and interaction of toxins with food components (15). Physical properties of aflatoxins are shown in Table 7 (9). The efficiency of detoxification by ammoniation of aflatoxin-contaminated groundnut oil cakes in a long-term feeding study in rats showed that aflatoxin was reduced considerably from 1000 to 140 ppb by pressurized application of ammonia at 2

bars and to 6 ppb at 3 bars. Hepatic tumors observed in rats fed treated groundnut cakes were reduced at 2 bars and was zero at 3 bars (16). It was found that cell growth and metabolic products of *Lactobacillus* species were effective in reducing the amount of aflatoxin produced in liquid medium (17).

In inhibition and mutagenesis studies using *S. Typhimurium* TA 100 and TA 98 strains, results showed that carotenoids were effective in reducing aflatoxin B_1 induced mutagenesis in both tester strains (18). Published reports have shown that growth of several mycotoxigenic fungi was completely inhibited by ammonium bicarbonate. It was also reported that sodium bicarbonate inhibited growth and production of aflatoxin by *Aspergillus* and *Penicillium* species. Buffered ammonium sulfate at pH 9 completely inhibited all mycotoxigenic fungi (19,20). Investigation of mycelia and growth of mycotoxin on Sabouraud Dextrose Agar was carried out with media containing pyro-, poly- or methyl phosphate, tetrasodium phosphate (TSPP), and sodium poly phosphate, glassy (SPG) and combinations of various proportions of sodium acid pyrophosphate (SAPP). The results showed that inhibition of *Aspergillus* was observed in media containing 2.0% TSPP, 1.0% and 2.0% SPG and 2.0% of various combinations of SAPP. Production of aflatoxin B_1 and G_1 was totally inhibited in single or blended concentrations (21,22).

Table 7. Physical properties of aflatoxin (9)

Resistant to heat
Cooking does not destroy
Retorting does not destroy completely
Sunlight degrades somewhat
Detoxification by Ammoniation
Prevention of mold growth but no effect on aflatoxin; Propionates and Sorbates

In a study conducted using gamma radiation and hydrogen peroxide for inactivation of aflatoxin B_1, it was found that 100 krad dose of gamma rays was sufficient to inactivate 50 micrograms of aflatoxin B_1 in 5.0% hydrogen peroxide and 400 krad was required for total degradation of 100 micrograms of aflatoxin B_1 in the same system (23).

There have been reports of evaluation of materials such as alumina, silicas and aluminosilacates for application in the detoxification of aflatoxin which resulted in the selection of hydrated sodium calcium aluminosilicate (HSCAS) for in vivo testing due to its high affinity for aflatoxin B_1. HSCAS is generally recognized as safe (GRAS) and when added to Leghorn and Broiler diets at 5.0% level was found to reduce the detrimental effects of aflatoxin B_1 at 7.5 mg per kg of feed (10). Published reports of the addition of zeolite, bentonite or spent bleaching clay from canola oil refining to the diets of rats and swine reduced the effects of T-2 toxin and

Zearalenone (4,10). In experiments conducted to evaluate the ability of sodium bentonite, hydrated sodium calcium aluminosilicate and ethacal to antagonize aflatoxicosis in broiler chickens with the addition of each compound at 1.0% to diet containing 5 ppm aflatoxin, the results showed reduced feed intake by 19%, bodyweight by 27%, and increased liver weights by 51% and lipids by 71%. The addition of sodium bentonite and hydrated sodium calcium aluminosilicate at 0.5% to diet without aflatoxin B_1 did not affect birds' performance, but ethacal reduced feed intake and body weight gain and increased water consumption. Ethacal had no effect on the reduction of aflatoxicosis. When hydrated sodium calcium aluminosilicate and sodium bentonite were added to the chicken diets, both reduced the toxic effects of aflatoxin on feed intake, bodyweight gains, liver weights and liver lipids. The results suggested that sodium bentonite may be more effective in the reduction of aflatoxicosis than hydrated sodium calcium aluminosilicate when used at 0.5% of diet (24).

In male turkey poults, it was found that when hydrated sodium calcium aluminosilicate was added at 0.5% to feed contaminated with 1 or 5 mg per kg aflatoxin, mortality rate was reduced from 88% to 28%, during a three-week experimental period. Hydrated sodium calcium aluminosilicate also reduced the adverse effects of aflatoxin on body weight gains, most relative organ weights, hematological values, serum

biochemical values and enzyme activities with 0.5 mg aflatoxin per kg feed, but not with 1 mg aflatoxin per kg feed (25). The amelioration of aflatoxicosis in Broilers and Leghorn chickens was performed by feeding hydrated sodium calcium aluminosilicate or activated charcoal at concentration of 0.5% of total diet with aflatoxin at 7.5 mg or 5 mg per kg feed. The results suggested that aflatoxin B_1 reduced body weight gains in Broilers by 21% to 38% between 0-3 weeks of age and reduced body weight gains in Leghorns from 0-4 weeks of age by 20%. Hydrated sodium calcium aluminosilicate seemed to reduce the growth inhibitory effects of aflatoxin B_1 or aflatoxin by 50% to 67% in growing chickens. The relative weights of livers, kidneys, proventriculus and gizzards were significantly increased in Leghorn chickens when fed 5 mg aflatoxin per kg diet with or without charcoal. Serum gamma glutamyl transferase activity was also increased. There was significant decrease in the concentration of serum protein and albumin. The toxic effect of aflatoxin was reduced or prevented in all organs except the bursa of Fabricius with the addition of hydrated sodium calcium aluminosilicate to the aflatoxin diets (4). Studies on the reversal of aflatoxicosis by activated charcoal, phenobarbital and reduced glutathione after the induction of chronic aflatoxicosis showed a trend toward improvement in feed consumption and weight gain in birds fed 10 ppm aflatoxin B_1 and 1.0% activated charcoal in

feed or 0.05% reduced glutathione concurrently compared to birds fed 10 ppm aflatoxin feed alone. The presence of the reversal agents seemed to reduce the inhibitory effects of aflatoxin B_1 on microsomal cytochrome P-450 enzyme and benzphetamine N-demethylase activity. The elevation of SGOT enzyme activity suggests that these agents were able to provide moderate protection against aflatoxin B_1-induced liver injury (26). An investigation of the safety of feeding aflatoxin-inactivated corn to White Leghorn layer-breeders showed that aflatoxin-ammoniated corn resulting in an aflatoxin level of 3.5 ppb had no deleterious effects on production, egg quality, reproduction, feed consumption per dozen eggs or mortality rates. However, there was a trend which showed that birds fed aflatoxin-ammoniated corn consumed less feed and gain less body weight compared to control (27).

In a recent study, addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diet significantly reduced the amount of aflatoxin M_1 in liver, kidney and muscle tissues of growing pigs. Aflatoxin B_1 was not reduced in liver or kidney, but was decreased in muscle (28). It has been reported that hydrated sodium calcium aluminosilicate (HSCAS) added at 2.0% to diets of lambs reduced the toxic effects of 2.6 mg aflatoxin/kg feed (29).

In barrows fed 3 mg of aflatoxin/kg of feed plus 2.0% HSCAS, body weight gains did not differ from that in control

barrows. However, body weight gains decreased significantly in barrows consuming 3 mg of aflatoxin/kg of feed (30). In cross-bred barrows fed dietary aflatoxin and Deoxynivalenol (DON), the results showed that no lesions were detected in DON-treated pigs and controls, whereas lesions seen in pigs fed aflatoxin alone were compatible with a diagnosis of aflatoxicosis (31).

Concentrations of serum total protein and albumin were found to be reduced in chicks fed diets containing 5 mg aflatoxin per gram of diet. Chicks fed aflatoxin diet plus 0.5% HSCAS per kg feed showed substantial protection as protein and albumin values were much higher than those of chicks fed aflatoxin feed alone (4,25). It was observed that body weight and aflatoxin in diet had a dose response relationship to dietary protein levels and that low protein diet enhanced the effects of aflatoxin on growth (32). Proteins are the most common receptors because they possess an abundance of nucleophilic N, O and S heteratoms in their functional groups. Mycotoxins can display non-specific-irreversible covalent and specific reversible non-covalent binding which alter the structures and activities of proteins. Proteins may represent a means of sequestering and deactivating toxins (13). Most aflatoxin retained in the liver was found to be associated with hepatic proteins (2).

Leaching and adsorption studies conducted with four

different soil types utilizing columns showed that aflatoxin B_1 was retained in all soil types. It also showed that 80-90% of the total amount of aflatoxin was retained in the upper 25 cm of the soil column (5). In studies conducted on aflatoxin decomposition in various soils, the results showed that aflatoxin was degraded relatively quickly, but the decomposition of aflatoxin in silty clay loam occurred at a reduced rate when compared with the other soils. The reduction in the decomposition of aflatoxin in silty clay loam may be due to the adsorption to clay (6). In another study, it was observed that aflatoxin B_1 had little or no effect on soil microorganisms at concentrations of 1,000 and 10,000 ng/ml (3). Results from degradation studies of radiolabeled ^{14}C pesticides in soil have indicated that soil microorganisms were responsible for enhanced degradation of some pesticides (33,34).

Pathology of Aflatoxicosis

The principal lesions resulting from aflatoxicosis occur in the liver and may be classified as toxic hepatitis. One of the most consistent responses to aflatoxin B_1 is proliferation of small bile ductules at the periphery of hepatic lobules. Changes observed in hepatocytes; vacuolation, fatty change, loss of parenchyma and pyknosis lead to necrosis

in different parts of the hepatic lobule in different species. Observation of effects in the periportal area were seen in ducklings, cats, adult rats, turkeys, chickens and rhesus monkeys (35,1). Microscopic examination of chicken livers showed yellowish, mottled appearance, indicative of extensive fatty change (27,1).

In birds fed aflatoxin-ammoniated corn pathological changes were not observed in the crops. Multifocal necrosis, hemorrhage, sinusoidal congestion, hemosiderosis, hepatocyte regeneration and lymphoid nodular hyperplasia were observed in livers of some birds (28). Gross pathology results have shown that livers from chicks fed aflatoxin diet with or without charcoal were enlarged and pale in appearance when compared to controls. Microscopic hepatic lesions caused by 5 mg of aflatoxin per kg feed alone were mild in severity and were not markedly altered by the addition of hydrated sodium calcium aluminosilicate to the diet (4). Hepatic lesions observed in barrows fed aflatoxin feed alone were characterized by peripheral lipidosis accompanied by periportal and interlobular fibrosis and bile duct hyperplasia (30).

The objectives of this study were:

1) To evaluate the effectiveness of silty clay loam soil in the detoxification aflatoxin B_1 in chickens fed aflatoxin-contaminated rations amended with soil,
2) To determine the residual concentrations of aflatoxin B_1

and its metabolites retained in tissues of chickens (crop, liver and muscle) fed the soil amended aflatoxin-contaminated rations,

3) To evaluate gross and histopathological changes observed in crops and livers of chickens fed the soil amended aflatoxin-contaminated rations and assess how silty clay loam soil influenced observed changes,
4) To determine total protein levels in sera collected from chickens fed the soil amended aflatoxin-contaminated rations and assess how protein levels are influenced by silty clay loam soil,
5) To assess how silty clay loam soil would influence the detrimental effects of aflatoxicosis on animal productivity and the retained residues in tissues, and
6) To assess the risk of exposure of humans to the aflatoxin residues.

Explanation of Thesis Format

The thesis is composed of four papers, three have been submitted for publication. The thesis begins with some preliminary studies which were conducted to investigate the feasibility of using silty clay loam soil as a feed additive to detoxify aflatoxin B_1. Studies to determine aflatoxin binding to soil when leaching through soil with water and

degradation of aflatoxin in soil were conducted to investigate the interactions of soil and aflatoxin. The decision to use soil in a feeding study was based on the results gained from these studies in association with information obtained from reports in current literature. The sequence in which the information in this thesis is presented was a logical approach to investigate the problem and to help us better understand the results of the various studies. The sequence of presentation of the information gathered from the investigations should assist other scientists and readers to understand the problem and interpret the results. Also, it was thought that the results and experience gained from the preliminary studies would enhance the ability to address problems that were encountered during the experimental period. Following the last paper is a general summary. References cited in the general introduction follows the general summary.

PAPER I. DETERMINATION OF AFLATOXIN B_1 BINDING TO SOIL WHEN LEACHING THROUGH SOIL WITH WATER

DETERMINATION OF AFLATOXIN B_1 BINDING TO SOIL WHEN LEACHING THROUGH SOIL WITH WATER

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ABSTRACT

Contaminated crops that are left in the field are potential contaminants of groundwater. Aflatoxin B_1 (AFB_1) distribution in soil water systems and the comparative response of contaminated corn and pure aflatoxin when leached through soil were investigated using columns. Each experiment was repeated once. AFB_1 was detected in water samples from columns containing 10% and 20% silty clay loam soil and aflatoxin-contaminated corn mixtures and in the upper (top) 2.5 cm of soil from the 10 cm soil column.

Aflatoxin B_2 (AFB_2) was detected in eluates from the column containing 10% soil and aflatoxin-contaminated corn mixture and from the column containing aflatoxin-contaminated corn alone. No AFB_2 was detected in eluates from the column containing 20% soil and aflatoxin-contaminated corn mixture. No detectable amount of aflatoxin was observed in eluates from the column containing 50% silty clay loam soil and aflatoxin-contaminated corn.

INTRODUCTION

Aflatoxins which are associated with several crops are becoming more important to the public and environmental health workers because of their potential for causing serious health problems to consumers of aflatoxin-contaminated foods. Crops contaminated with high levels of aflatoxin are unfit for consumption and are usually disposed of by plowing under soil (Angle, J.S. 1986; Goldberg, B.S. et al, 1985) or left in the field thereby creating the potential for contamination of groundwater. Leaching and adsorption studies carried out in 1985 showed that aflatoxin B_1 or its derivatives were retained in different types of soil (Angle, J.S., 1986; Angle, J.S. et al, 1981).

Soil microorganisms affect the concentration and movement of aflatoxin B_1 in the soils. Aflatoxin B_1 contamination of water supply could be detrimental to the health and welfare of the public, since it is both toxic and carcinogenic (Angle, J.S., 1986; Goldberg, B.S et al, 1985). The present study was undertaken to determine the distribution of aflatoxin in soil-water systems and the comparative response of aflatoxin-contaminated corn from pure aflatoxin when leached through soil columns. The specific purpose was to evaluate the leaching of aflatoxin through silty clay loam and to determine

which portion of soil contained aflatoxin B_1 or its metabolites.

MATERIALS AND METHODS

Experiment I

The experiment was conducted to determine if soil will prevent AFB_1 from leaching into water supply. Four similar columns were set up on a stand. Glass wool was placed in the bottom of each column. About 2.5 cm of silty clay loam soil was added to each column on top of the glass wool. A mixture of 50 g of 30 ppm aflatoxin B_1-contaminated corn and 10% silty clay loam (5.0 g) soil was added to column one. A mixture of 50 g of 30 ppm aflatoxin B_1-contaminated corn and 20% silty clay loam (10 g) soil was added to column two. A mixture of 50 g of 30 ppm aflatoxin B_1-contaminated corn and 50% silty clay loam (25 g) soil was added to column three. To column four, 50 g of 30 ppm aflatoxin B_1-contaminated corn was added with no soil. Millipore^R water (H_2O) was added to each column and collection of water eluted from columns was done over a week in increments of 100 ml. Extraction of aflatoxin was performed using methylene chloride ($MeCl_2$) in a 1:1 ratio to water (H_2O) samples. Detection and identification of aflatoxin B_1 was performed by thin-layer chromatography (TLC) using long-wave UV light at Rf values corresponding to aflatoxin standards (Stahr, H.M., 1991).

Experiment II

Part I

The experiment was conducted to determine if aflatoxin B_1 (AFB_1) in water solution will leach through soil, and to identify which form of aflatoxin was present in leachates and soil extracts. Two similar columns were set up on a stand and glass wool placed in the bottom of each column. Approximately 10 cm of silty clay loam soil were added to each column on top of the glass wool. A mixture of 100 ml of Millipore^R H_20 and 50 ng of AFB_1 (5.4 ul/9.3 ng/ul) was added to each column. Elution was performed by adding Millipore^R H_20 to each column. Water samples were collected in increasing increments of 20 ml, 20 ml, 40 ml, 60 ml etc. Collection was done over one week. Extraction of aflatoxin was performed by using methylene chloride ($MeCl_2$) in 1:1 ratio to water (H_20). Detection and identification of AFB_1 was done by thin-layer chromatography using long-wave UV light at Rf values corresponding to aflatoxin standards and by UV spectrofluorophotometry (Stahr, H.M., 1991).

Part II

The experiment was conducted to determine which portion of soil from Part I contained AFB_1 and to identify AFB_1 or its metabolites. The soil samples were removed from the above columns and each 2.5 cm of soil was collected separately and

analyzed for the presence of aflatoxin B_1 and its metabolites. Extraction of aflatoxin was done by adding 200 ml of 90:10 acetonitrile:H_2O to each 2.5 cm of soil in a Waring blender and blending for 4 minutes, then 100 ml of each sample was collected. Methylene chloride ($MeCl_2$) was used to clean up each extract sample in a 1:1 ratio to water (H_2O). Detection and identification of AFB_1 was done by thin-layer chromatography and UV spectrofluorophotometry (Stahr, H.M., 1991).

RESULTS

The results from the first study in Experiment I showed that the transit time, (time for the appearance of the first drop) for water through the columns varied as follows: column 4 took three minutes, column 1 took four minutes and column 3 took twenty minutes. The first 100 ml was collected from column 4 which took one hour and forty minutes, while column 1 took one hour and forty-five minutes. Columns 2 and 3 took much longer. There was a steady decline in the flow rate of eluates from all columns as several samples were collected during the week.

Detection and identification of aflatoxin B_1 was done by thin-layer chromatography (TLC) which showed fluorescent bands for AFB_1 in eluates from columns 1 and 2 with mixtures of 10% and 20% soil and 30 ppm aflatoxin-contaminated corn and from column 4 with 30 ppm aflatoxin-contaminated corn alone. No aflatoxin was detected in eluates from column 3 with 50% soil and 30 ppm aflatoxin-contaminated corn mixture. When TLC plates were scraped and UV Spectrofluorophotometric determination performed, no detectable amount of AFB_1 was seen in any sample (Table 1).

In Experiment II, part I, no detectable amount of aflatoxin was observed in any eluates from the 10 cm soil columns by thin-layer chromatography. In part II, aflatoxin B_1 was

detected in the first (top) 2.50 cm of soil (Table 2). The experiments were repeated in a second study. Results from Experiment I showed that AFB_1 was detected in eluates from column 1 and 2 with 10% and 20% soil and 30 ppm aflatoxin-contaminated corn mixtures and from column 4 with 30 ppm aflatoxin-contaminated corn with no soil (Table 3).

Fluorescence spectrophotometric determination revealed that the amount of aflatoxin B_1 detected in eluates from column 1 with 30 ppm aflatoxin-contaminated corn plus 10% soil was 58.6 ng, column 2 with 30 ppm aflatoxin-contaminated corn plus 20% soil had 54.0 ng, and column 4 with 30 ppm with no soil added was 58 ng. There was no detectable amount of AFB_1 observed in eluates from column 3 with 30 ppm aflatoxin-contaminated corn and 50% soil. Aflatoxin B_2 detected in eluates from column 1 with 30 ppm aflatoxin-contaminated soil plus 10% soil was 4 ng, and column 4 with 30 ppm aflatoxin-contaminated corn with no soil had 3.5 ng. No detectable amounts of aflatoxin B_2 were observed in eluates from columns 2 and 3 containing 30 ppm aflatoxin-contaminated corn plus 20% and 50% soil respectively (Table 3).

In Experiment II, part II, no aflatoxin was detected in any eluates from 10 cm soil columns. When extracts of soil samples from Part I in Experiment II fluorescent spots were scraped from TLC plates and eluted with 90:10 HPLC methanol:water,

there was much background interference during quantitation which resulted in poor estimate of aflatoxin present.

Table 1. Thin layer chromatographic and UV spectrofluorophotometric detection of aflatoxin B_1 in eluates from columns in first study

Experiment I: 50 g 30 ppm AFB_1-contaminated corn and silty clay loam soil

Test Columns	Samples	TLC	UV
1	30 ppm corn + 10% soil	+ (ng)	-
2	30 ppm corn + 20% soil	+ (ng)	-
3	30 ppm corn + 50% soil	- (ng)	-
4	30 ppm corn - no soil	+ (ng)	-

+ = Positive AFB_1
- = Negative AFB_1

ng = Nanogram amount of AFB_1 standard used on TLC plates for detection and identification

Table 2. Thin layer chromatographic and UV spectrofluorophotometric detection of aflatoxin B_1 in eluates and soil extracts from columns in first study

Experiment II: - 100 ml Millipore^R H_2O with 50 ng pure AFB_1			
Part I	Water Samples[a]	TLC	UV
	1 - 20 ml	-	-
	2 - 40 ml	-	-
	3 - 60 ml	-	-
	4 - 80 ml	-	-
	5 - 100 ml	-	-
Part II	Soil Samples[a]	TLC	UV
	1 - 1st 2.5 cm	+	-
	2 - 2nd 2.5 cm	-	-
	3 - 3rd 2.5 cm	-	-
	4 - 4th 2.5 cm	-	-

+ = Positive AFB_1
- = Negative AFB_1

[a]Water and soil samples were collected from two columns

Table 3. Thin-layer chromatographic detection and fluorescence spectrophotometric determination of aflatoxins in eluates from columns in repeated experiment 1-30 ppm aflatoxin-contaminated corn

Test Columns	Samples[b]	TLC	Fluorescence Spectrophotometer Determination[a]	
			B_1 (ng)	B_2 (ng)
1	30 ppm corn + 10% soil	+	58.6	4.0
2	30 ppm corn + 20% soil	+	54.0	NDA
3	30 ppm corn + 50% soil	-	NDA	NDA
4	30 ppm corn + no soil	+	58.0	3.5

\+ = Positive aflatoxin
\- = Negative aflatoxin

[a]Quantitation of aflatoxins was done from standard curves using linear regression equations

[b]Three samples were evaluated from column one, two from column two, one from column three and three from column four

NDA = No detectable amount

DISCUSSION

The results from these experiments have shown that aflatoxin B_1 was detected in eluates from columns containing 30 ppm aflatoxin-contaminated corn and 10% and 20% silty clay loam soil and 30 ppm aflatoxin-contaminated corn alone. No aflatoxin was detected in eluates from the column containing 30 ppm aflatoxin-contaminated corn with 50% silty clay loam soil (Table 1). These results suggest that 50% silty clay loam soil was effective in preventing aflatoxin B_1 from leaching through soil. The amount of aflatoxin B_1 detected in eluates from column 1 with 30 ppm aflatoxin-contaminated corn and 10% soil (58.6 ng) and column 4 containing 30 ppm aflatoxin-contaminated corn with no soil (58 ng) were higher than that which was detected in column 2 with 30 ppm aflatoxin-contaminated corn plus 20% silty clay loam soil (54 ng) (Table 3). This result suggests that 20% silty clay loam soil retained some aflatoxin B_1 in the column. Aflatoxin B_2 was detected in eluates from column 1 with 30 ppm aflatoxin-contaminated corn plus 10% silty clay loam soil (4 ng) and column 4 containing aflatoxin-contaminated corn with no soil (3.5 ng) whereas no aflatoxin B_2 was detected in eluates from column 2 and 3 containing 30 ppm aflatoxin-contaminated corn with 20% and 50% silty clay loam soil, respectively.

These results suggest that 20% silty clay loam soil and greater will prevent aflatoxin B_2 from leaching through soil into water.

CONCLUSION

Aflatoxin B_1 was detected in eluates from columns 1, 2, and 4 with 30 ppm aflatoxin-contaminated corn plus 10% and 20% silty clay loam soil and no soil whereas no aflatoxin was detected in eluates from column 3 containing 30 ppm aflatoxin-contaminated corn with 50% silty clay loam soil. Aflatoxin B_2 was detected in columns 1 and 4 whereas no aflatoxin B_2 was detected in column 2 and 3. These results indicate that no aflatoxin leached through column 3 with 30 ppm aflatoxin-contaminated corn plus 50% silty clay loam soil whereas only aflatoxin B_1 leached through column 2 with 30 ppm aflatoxin-contaminated corn plus 20% silty clay loam soil. Both aflatoxin B_1 and B_2 leached through columns 1 and 4 containing 30 ppm aflatoxin-contaminated corn with 10% silty clay loam soil and no soil, respectively.

The recommendation that could be given concerning aflatoxin-contaminated commodities buried in soil is that 20% silty clay loam soil will reduce aflatoxin B_1 and prevent aflatoxin B_2 from leaching into water, whereas 50% silty clay loam soil will prevent both aflatoxin B_1 and B_2 from leaching into water. Present farm practices of discing aflatoxin-contaminated corn into the soil should prevent aflatoxin from gaining access to groundwater if aflatoxin-contaminated

commodities are covered with 50% silty clay loam soil or greater. This should result in the reduction of public concern about the safety of potable water.

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PAPER II. THE EFFECTS OF SOIL AND POPLAR TREES ON AFLATOXIN B_1

THE EFFECTS OF SOIL AND POPLAR TREES ON AFLATOXIN B_1

by

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INTRODUCTION

Mycotoxins which are associated with several crops are becoming more important to the public and environmental health workers because of their potential for causing health problems. Contaminated crops with high levels of aflatoxin are unfit for consumption by humans and animals and are usually disposed of by plowing under soil (1,2,3), thereby creating the potential for contamination of groundwater. Aflatoxin contamination of the water could be detrimental to the health and welfare of the population because aflatoxin B_1 is both toxic and carcinogenic (1,3).

Leaching and adsorption studies have shown that aflatoxin B_1 and its metabolites were retained in different types of soil (1,2). Other studies have also shown that soil microorganisms affect the concentration and the movement of aflatoxin in the soil (1,3). Poplar trees were found to be effective in removing nitrate-nitrogen from the soil, thereby reducing the amount of nitrates in groundwater. Trees were planted in the area, providing rooting systems which bind the soil, preventing soil erosion and removing excess nitrogen from the water-table (4).

The purpose of this study was to investigate the effectiveness of soil and poplar trees in the detoxification

of aflatoxin B_1 in soil. The objectives of this study were to determine the fate of aflatoxin in Iowa soil, to determine procedures for the disposition of mycotoxin-contaminated commodities and to utilize this control system to successfully prevent the contamination of municipal and private wells. Trees will prevent erosion of the soil, which will prevent the transport of aflatoxins and other contaminants in the soil from gaining access to the groundwater from which a portion of the potable water that the public consumes is derived.

MATERIALS AND METHODS

Three tubs of soil were used in this study. One tub was filled with soil taken from a soybean field while the other two were filled with soil taken from a corn field. Mini-wells were made from a one and one half inch PVC pipe tubing with aluminum cloth screen covering the bases of the wells. The deep wells were placed at a depth of about 30 cm into the soil in the tubs while the shallow wells were at a depth of about 15 cm In one tub, containing soybean soil and the second containing corn soil, 150 g of aflatoxin-contaminated corn was buried into the soil equidistant from each well, which was about 7.5 cm. In the third tub containing corn soil, four cuttings of propagated poplar trees were planted approximately 7.5 cm apart and 10 cm away from the deep well; then 150 g of aflatoxin-contaminated corn was buried into the soil approximately 5 cm from the trees and 10 cm away from the 15 cm well.

Water samples were collected from the mini-wells after rainfall during the first, second, fourth, seventh and ninth week and analyzed for the presence of aflatoxin. After twelve weeks samples of buried aflatoxin-contaminated corn and soil surrounding the aflatoxin-contaminated in tubs were taken and analyzed, along with samples from the original soil and

original aflatoxin-contaminated corn for the presence of aflatoxin. Extraction of aflatoxin from water samples was performed by using methylene chloride ($MeCl_2$) in a 1:1 ratio to water (H_2O), and soil samples were extracted using 90:10 acetonitrile:water, then with $MeCl_2$:H_2O (1:1). The corn samples were extracted according to Analytical Methods in Toxicology procedure (5). All extracts were cleaned up by C-18 Sep-Pak^R (Waters, Division of Millipore, Milford, MA) and dried under nitrogen gas, then redissolved in 100 ul of $MeCl_2$.

Detection and identification of aflatoxin was by thin-layer chromatography (TLC). Ten ul of each sample were spotted on TLC plates along with appropriate amounts of standards. The plates were developed in 90:10 chloroform:acetone, 1-2 cm from the top of each plate and examination of plates was done under long-wave UV light for positive identification of aflatoxins. Each fluorescent spot was scraped from the plates and placed into a two dram vial; 3 ml of 90:10 high performance liquid chromatography grade methanol:water was added to each vial in order to elute the aflatoxin. Quantitation of aflatoxins was performed by spectrophotofluorometry with excitation set at 360 nm and emission set at 430 nm, with sensitivity set at .1% scale (5).

RESULTS

The total aflatoxins present in the corn sample buried in the soil in each tub was 208 ng/g B_1 and 12 ng/g B_2. No aflatoxin was detected in water samples collected from the mini-wells after rainfall during week 1, 2, 4, 7 and 9 nor from original soil samples. After three months, no aflatoxin B_1 was detected in soil surrounding the aflatoxin-contaminated corn, but aflatoxin B_2 was present. Both aflatoxin B_1 and B_2 were present in the aflatoxin-corn samples that were buried into the soil (Table 1). Aflatoxin B_1 was decreased in all aflatoxin-contaminated corn samples buried in soil whereas aflatoxin B_2 was increased (Table 2).

DISCUSSION

The results from this study have shown that no aflatoxin was detected in any water sample taken from the mini-wells, which suggests that no aflatoxin from aflatoxin-contaminated corn leached through soil with water. These results agreed with previous reports that aflatoxin B_1 and its metabolites were retained in soil. Concentrations of aflatoxin determined by spectrophotofluorometry revealed that aflatoxin B_1 was present in all samples from the aflatoxin-contaminated corn buried in soil in the tubs (Table 1). Corn soil with trees contained 37 ng/g, soybean soil had 3 ng/g, and corn soil had 13 ng/g, were substantially lower than the original aflatoxin-contaminated corn (208 ng/g). Aflatoxin B_2 was higher in all samples from the aflatoxin-contaminated corn buried in soil suggesting that all soils were effective in reducing the amount of aflatoxin B_1 present (Table 2).

The concentration of aflatoxin B_1 detected in aflatoxin-contaminated corn buried in soybean soil (3 ng/g) was substantially less than the amount present in the aflatoxin-contaminated corn buried in corn soil, corn soil with trees and the original aflatoxin-contaminated corn sample. The amount of aflatoxin B_2 present in the aflatoxin-contaminated corn buried in soybean soil (25 ng/g) was greater than the amount in the tubs with the corn soil (corn soil with trees 21

ng/g, corn soil 17 ng/g) and also the original aflatoxin-contaminated corn (12 ng/g) (Table 2). Therefore, soybean soil was more effective than corn soil with poplar trees and corn soil in reducing aflatoxin B_1 present in the aflatoxin-contaminated corn and converting it to aflatoxin B_2 (Figure 1). No aflatoxin B_1 was detected in any soil samples surrounding the aflatoxin-contaminated corn which suggests that aflatoxin B_1 in soil is readily degraded to aflatoxin B_2.

Table 1. Detection of aflatoxin B_1 and B_2 in original aflatoxin-contaminated corn, original soil, buried aflatoxin-contaminated corn and soil surrounding aflatoxin-contaminated corn after three months in corn soil with poplar trees, soybean soil and corn soil[a]

Samples	Orig. AFB_1-Corn		Orig. Soil		Buried AFB_1-Corn		Soil/Corn	
	B_1	B_2	B_1		B_1	B_2	B_1	B_2
1. Corn Soil Trees	+	+	-	-	+	+	-	+
2. Soy Soil	+	+	-	-	+	+	-	+
3. Corn Soil	+	+	-	-	+	+	-	+

+ = Positive detection of aflatoxin

- = Negative detection of aflatoxin

[a]Positive detection and identification of aflatoxins were done by thin layer chromatography with long-wave UV light at Rf values corresponding to that of aflatoxin standards

The concentrations of aflatoxin B_2 present in all soil samples surrounding the aflatoxin-contaminated corn in tubs (corn soil/trees 10 ng/g, soybean soil 7 ng/g and corn soil 11 ng/g) were lower than the amount present in the original aflatoxin-contaminated corn sample (12 ng/g) and the buried aflatoxin-contaminated corn samples (Figure 2). This suggests that aflatoxin B_2, like aflatoxin B_1 in soil is readily degraded to other compounds. The concentration of aflatoxin B_2 present in soybean soil sample surrounding the aflatoxin-contaminated corn was lower than the samples from the other tubs. These results show that soybean soil was more effective in reducing both aflatoxin B_1 and B_2 than the corn soil (Table 2).

Table 2. Concentrations aflatoxin B_1 and B_2 in original aflatoxin-contaminated corn, original soil, buried aflatoxin-contaminated corn and soil surrounding aflatoxin-contaminated corn after three months in corn soil with poplar trees, soybean soil and corn soil[a]

Samples	Orig. AFB_1 Corn ng/g		Orig. Soil ng/g		Buried AFB_1-Corn ng/g		Soil/Corn ng/g	
	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2
1. Corn Soil Trees	208	12	-	-	37	21	-	10
2. Soy Soil	208	12	-	-	3	25	-	7
3. Corn Soil	208	12	-	-	13	17	-	11

[a]Concentrations of aflatoxins was determined by spectrophotofluorometry

Quantitation of aflatoxins was done by linear regression from aflatoxin standard curves

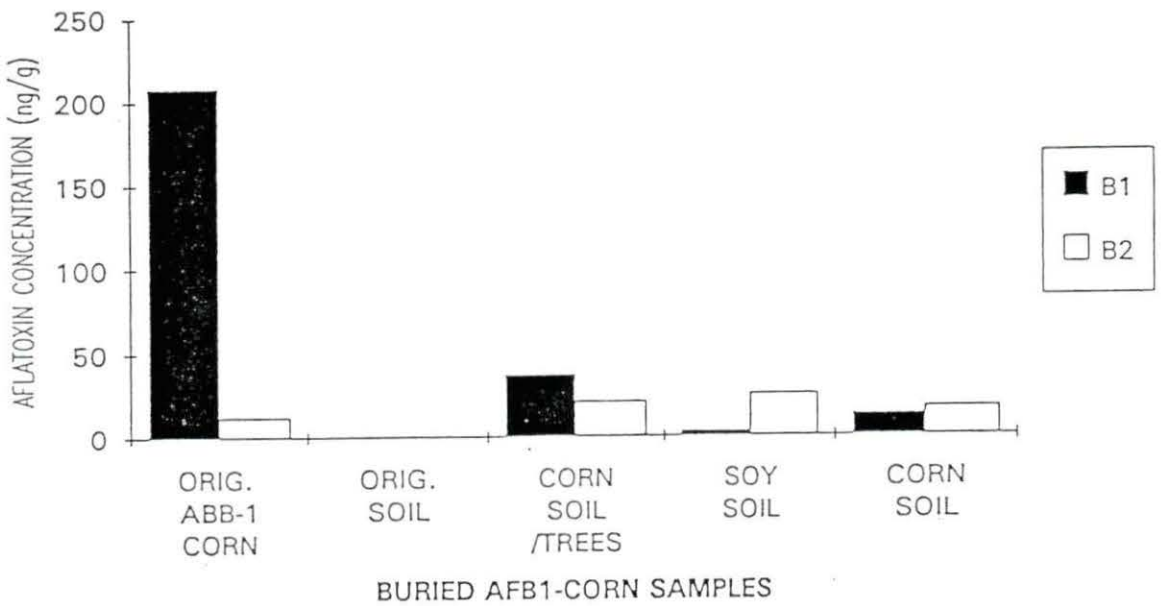

Figure 1. Concentrations of aflatoxin B_1 and B_2 in original aflatoxin-contaminated corn, original soil, buried aflatoxin-contaminated corn in corn soil with trees, soybean soil and corn soil after three months

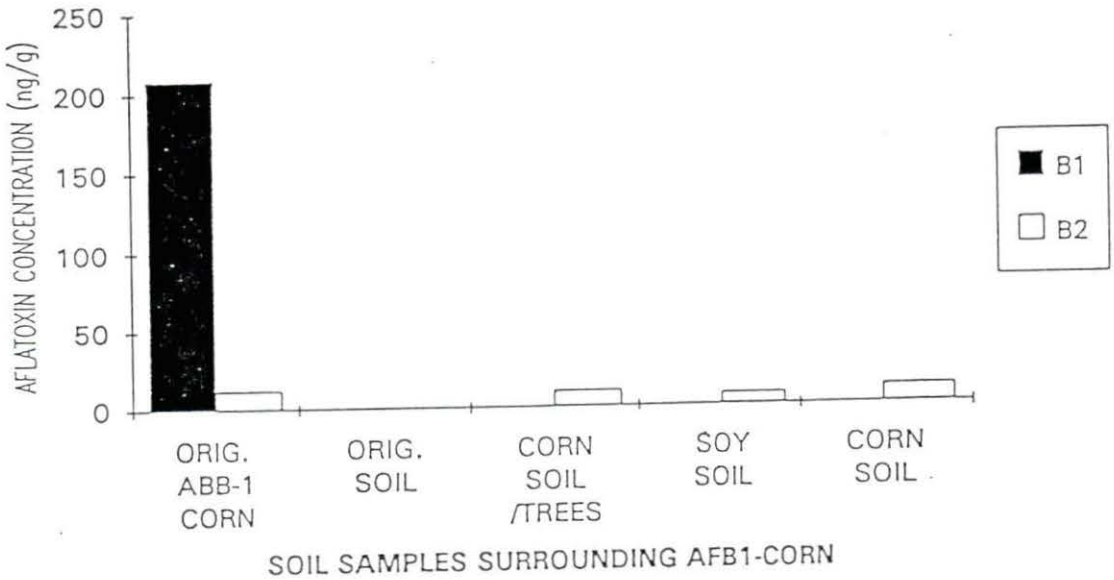

Figure 2. Concentrations of aflatoxin B_1 and B_2 in original aflatoxin-contaminated corn, original soil, and soil surrounding aflatoxin-contaminated corn buried in corn soil with trees, soybean soil and corn soil after three months

SUMMARY

Aflatoxin B_1 in corn samples was reduced substantially when buried in soil for a period of three months. Aflatoxin B_1 did not remain in the soil and was converted to B_2, since only aflatoxin B_2 was detected in the soil surrounding the buried aflatoxin-contaminated corn. The amount of aflatoxin B_2 present in the aflatoxin-contaminated corn buried the soil was substantially higher than that which was present in the original aflatoxin-contaminated corn and the soil samples surrounding the aflatoxin-contaminated corn. This result suggests that if aflatoxin B_1-contaminated commodities are buried in the soil, aflatoxin B_1 is readily converted to aflatoxin B_2 which is a less toxic compound and non mutagenic.

The amount of aflatoxin B_2 detected in soil samples surrounding the aflatoxin-contaminated corn was lower that the amount present in the original aflatoxin-contaminated corn and the buried aflatoxin-contaminated corn which suggests that aflatoxin B_2 in soil is readily degraded to other compounds.

There was no detectable amount of aflatoxin present in water samples taken from mini-wells around buried aflatoxin-contaminated corn which indicates that no aflatoxin leached into the mini-wells after rainfall. This result further indicates that aflatoxin B_1 is degraded to aflatoxin B_2 which when further degraded is not detected as a leachate in the

water samples taken from the mini-wells.

The recommendation that could be given concerning aflatoxin-contaminated commodities is that plowing aflatoxin-contaminated commodities under soil will allow for successful detoxification of aflatoxin B_1 without potential contamination of groundwater. Present farm practices of discing aflatoxin-contaminated corn into the soil should result in successful detoxification of aflatoxin-contaminated land if the land is allowed enough time for reduction of aflatoxin to occur. If proper management is instituted and practiced, then public concerns about the safety of potable water will be eliminated or reduced.

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PAPER III. THE EFFECTS OF SOIL ON AFLATOXIN TISSUE RETENTION IN CHICKENS WHEN ADDED TO AFLATOXIN-CONTAMINATED POULTRY RATIONS

Effect of Soil on Aflatoxin Tissue Retention in Chickens When Added to Aflatoxin-Contaminated Poultry Rations

by

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ABSTRACT

The effects of silty clay loam soil on aflatoxin B_1 (AFB_1) were investigated when added to the diets of chickens fed aflatoxin-contaminated rations. Sixty 1-day-old White Leghorn chickens were fed a control ration (< 5 ng/g AFB_1), a low aflatoxin- contaminated ration (55 ng/g AFB_1), a high aflatoxin-contaminated ration (4,488 ng/g AFB_1) and a high aflatoxin-contaminated ration plus 50% and 25% soil. The livers in each group were pooled and analyzed for the presence of aflatoxin B_1 and its metabolites. The addition of silty clay loam soil significantly reduced the levels of aflatoxin B_1 in livers from the chickens in the groups fed high aflatoxin-contaminated ration amended with soil when compared to livers from the group fed high aflatoxin-contaminated ration with no soil added. The concentrations of aflatoxin B_1 and M_1 were lower in the livers of chickens from the group fed the high aflatoxin-contaminated ration amended with 25% soil when compared to the group fed high aflatoxin-contaminated ration amended with 50% soil.

INTRODUCTION

Aflatoxin B_1 belongs to a group of secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and is highly toxic and a potent carcinogen (1,2,3). The occurrence and toxicity of aflatoxin B_1 in agricultural products consumed by animals and man has received considerable attention during the past 20 years (1,2,3). Aflatoxin B_1 is found to be mostly associated with grain crops. Contaminated crops are unfit for consumption by animals and are usually disposed of by plowing into the soil (1,4,5)

Leaching and adsorption studies conducted with four different soil types utilizing columns showed that aflatoxin was retained in all soil types (4). Aflatoxin decomposition studies have shown that aflatoxin was degraded relatively quickly in all soils except for silty clay loam soil. Adsorption of aflatoxin to clay may be responsible for a reduced rate of decomposition (5).

Recent publications have reported that the addition of hydrated sodium calcium aluminosilicate (HSCAS) to aflatoxin-contaminated diet significantly reduced the amount of aflatoxin M_1 in liver, kidney and muscle tissues in growing pigs. Aflatoxin B_1 was not reduced in liver or kidney, but was decreased in muscle (6). In chickens, results after oral

administration of a single dose of radiolabeled ^{14}C-aflatoxin indicated that most aflatoxin was excreted in feces and most of the retained aflatoxin was found in the liver (7).

In male turkey poults, it was found that when hydrated sodium calcium aluminosilicate was added at 0.5% to 1 or 5 mg per kg aflatoxin feed, mortality rate was reduced by 68%, from 88% to 28%, during a three-week experimental period. Hydrated sodium calcium aluminosilicate also reduced the adverse effects of aflatoxin on bodyweight gains, most relative organ weights, hematological values, serum biochemical values and enzyme activities with 0.5 mg per kg aflatoxin, but not with 1 mg per kg aflatoxin (8). The examination of amelioration of aflatoxicosis in Broilers and Leghorn chickens was performed by feeding hydrated sodium calcium aluminosilicate or activated charcoal at concentration of 0.5% of total diet with aflatoxin at 7.5 mg or 5 mg per kg feed. The results suggested that aflatoxin B_1 reduced bodyweight gains in Broiler by 21% to 38% between 0-3 weeks of age and reduced bodyweight gains in Leghorns from 0-4 weeks of age by 20%. Hydrated sodium calcium aluminosilicate seemed to reduce the growth inhibitory effects of aflatoxin B_1 or aflatoxin by 50% to 67% in growing chickens (9). Studies on the reversal of aflatoxicosis by activated charcoal, phenobarbital, and reduced glutathione after the induction of chronic aflatoxicosis showed a trend in the improvement in feed consumption and weight gain in birds fed

10 ppm aflatoxin B_1 and 1% activated charcoal in feed or .05% reduced glutathione concurrently than in birds fed 10 ppm aflatoxin feed alone (10).

An investigation of the safety of feeding aflatoxin-inactivated corn to White Leghorn layer-breeders, showed that aflatoxin-ammoniated corn resulting in an aflatoxin level of 3.5 ppb had no deleterious effects on production, egg quality, reproduction, feed consumption per dozen egg or mortality rates (11). It has been reported that hydrated sodium calcium aluminosilicate added at 2.0% to diets of lambs reduced the toxic effects of 2.6 mg aflatoxin/kg feed (12).

In barrows fed 3 mg of aflatoxin/kg of feed plus 2.0% HSCAS, body weight gains did not differ from that in control barrows. However, body weight gains decreased significantly in barrows consuming 3 mg of aflatoxin/kg of feed (13). In cross-bred barrows fed dietary aflatoxin and Deoxynivalenol (DON), the results showed that no lesions were detected in DON treated pigs and control, while lesions seen in pigs fed aflatoxin alone were compatible with a diagnosis of aflatoxicosis (14).

This study was undertaken to determine the potential of soil in the detoxification of aflatoxin B_1. The specific objective of this research was to evaluate the concentration of the aflatoxin B_1 and its metabolites retained in chicken livers after feeding aflatoxin-contaminated rations with the

addition of soil when compared with livers from chickens fed aflatoxin-contaminated ration with no soil added.

MATERIALS AND METHODS

The animals of choice for this experiment were chickens because chicken meat constitutes a large proportion of food consumed by humans, and the major source of chicken feed is corn. Sixty 1-day-old White Leghorn chickens were obtained and randomly assigned to five groups of twelve each. They were weighed, wing-banded, housed and fed separately. The rations were formulated by the addition of Biotin Stress Pak^{R}, a vitamin and mineral supplement at 1 g/kg feed to ground clean corn, low aflatoxin-contaminated corn, and high aflatoxin-contaminated corn. Silty clay loam soil was crushed with pestle and mortar, homogenized and sieved, then used to formulate two rations at 50% and 25% concentration, respectively.

The type of ration each group of chickens received is as follows: Group 1 = clean corn; Group 2 = low aflatoxin-contaminated corn; Group 3 = High aflatoxin-contaminated corn plus 50% soil; Group 4 = High aflatoxin-contaminated corn plus 25% soil; Group 5 = High aflatoxin-contaminated corn with no soil added. The rations and water were fed ad libitum for nine days. The chickens were observed daily for clinical signs, and a daily log was kept on mortality. At the end of the period, they were weighed, euthanized with CO_2, and necropsied. Liver

samples were collected and frozen for chemical analysis of residual levels of aflatoxin B_1 and its metabolites. The livers were pooled due to the small size of each liver. Aflatoxins were extracted from the livers according to the Analytical Methods in Toxicology procedure (7).

Following silica gel column clean-up, extracts were dried under nitrogen gas. Extracts were then dissolved in 100 ul of methylene chloride ($MeCl_2$), and 50 ul of each sample was spotted on thin-layer chromatography (TLC) plates along with 10 ng of aflatoxin B_1, B_2, G_2 and M_1 standards. Development of the TLC plates was done in 90:10 chloroform:acetone, 1-2 cm from top of plates. The TLC plates were examined under long-wave ultra violet light for detection and positive identification of aflatoxins.

The remaining 50 ul of extract of each sample was cleaned up by C-18 Sep-Pak^R cartridge (Waters, Millipore Corporation, Milford, MA) and dried under nitrogen gas. The extracts were redissolved in 100 ul of $MeCl_2$; 50 ul of each sample was spotted on TLC plates along with the appropriate aflatoxin standards. Plates were developed by the previously mentioned procedure for positive identification of aflatoxins.

The remaining 50 ul of extract from each sample was used for chemical analysis by high performance liquid chromatography (HPLC). The extracts were transferred to vials, derivatized with 100 ul trifluoroacetic acid (to each sample)

and vortexed for 30 seconds (7). The mixtures were evaporated to dryness under nitrogen gas. The samples were redissolved in HPLC elution solvent (80:12:8) water: isopropanol: acetonitrile. Twenty ul of each sample was injected into the mobile phase of water: isopropanol: acetonitrile (80:12:8). Separation of aflatoxin B_1, and its metabolites occurred on a Brownlee Labs C-18, Spheri-6 ODS, 100 x 4.6 mm, 5 um column. Detection was performed by a McPherson SF-749 Fluorescence Spectrophotometer with a xenon-mercury lamp. Excitation was set at 365 nm and emission was 430 nm. The detector was a high sensitivity accessory filter-CF400, range sensitivity 0.1, lamp current 8 amperes, time constant-5 and gain-370, detection limit 0.1 ng.

Quantitation of aflatoxins was performed by measuring the peak height of each sample at the corresponding retention time of each standard, and comparing the ratio of peak height of each sample of aflatoxin B_1 and M_1 to the peak height of the respective standard. The chart recorder was set at 0.2 volt, and paper speed was 20 cm/hr with a flow rate of 1 ml per min. (7,8).

RESULTS AND DISCUSSION

The concentrations of aflatoxin present in rations fed to chickens was as follows: control ration = < 5 ng/g AFB_1, low aflatoxin-contaminated ration = 55 ng/g AFB_1, high aflatoxin-contaminated ration plus 50% soil = 2244 ng/g, high aflatoxin-contaminated ration plus 25% soil = 3366 ng/g and high aflatoxin-contaminated rations = 4,488 ng/g AFB_1. A total of eight chickens died during the experimental period. Most mortality (6 chickens) was seen in the group fed high aflatoxin-contaminated ration plus 50% soil. At necropsy examination, some chickens crops were empty, whereas others had fluid accumulation in their abdominal cavities.

Thin-layer chromatography results showed that no positive bands were detected for any of the aflatoxins in liver samples from chickens in group 1 (control ration), but positive bands for aflatoxin were detected in the samples from the other groups. The highest concentration of aflatoxins detected in chicken livers was in group 5 which received high aflatoxin-contaminated ration with no soil (1.52 ng/g). The lowest amount of aflatoxin was detected in the group fed low aflatoxin-contaminated ration (0.28 ng/g) (Table 13). The concentration of aflatoxin B_1 present in livers from chickens fed rations containing high aflatoxin-contaminated corn plus

50% and 25% silty clay loam soil (0.69 ng/g and 0.41 ng/g) were lower than that of the group fed high aflatoxin-contaminated corn without soil (1.52 ng/g).

The concentrations of aflatoxin (0.41 ng/g B_1 and 0.026 ng/g M_1) were lower in livers from the groups fed high aflatoxin- contaminated corn ration plus 25% soil than the group that received high aflatoxin-contaminated corn ration plus 50% soil (0.69 ng/g and 0.071 ng/g) (Table 1). The results suggest that silty clay loam soil was effective in reducing the residual levels of aflatoxin in chicken livers. The results further indicate that 25% silty clay loam soil was more effective in reducing the residual level of aflatoxin B_1 and M_1 in chicken livers than 50% soil which is indicative of lack of dose response to increased soil in diet. No detectable amount of aflatoxin B_2 or G_2 was found in any of the liver samples.

Table 1. Effects of soil on concentrations of aflatoxins in livers from White Leghorn chickens fed aflatoxin B_1 contaminated rations amended with different levels of soil for 9 days feeding trial

		Liver Aflatoxins Concentrations[a](ng/g)			
Rations	Number of Chickens	B_1	G_1	M_1	Total
Clean corn	9	NDA	NDA	NDA	NDA
Low aflatoxin -contaminated corn	11	0.26	NDA	0.019	0.28
High aflatoxin corn + 50% soil	12	0.69	NDA	0.071	0.76
High aflatoxin corn + 25% soil	12	0.41	NDA	0.026	0.44
High aflatoxin corn	7	1.52	NDA	NDA	1.52

[a]Determination of aflatoxin concentrations was done by high performance liquid chromatography

NDA = No detectable amount of aflatoxin

CONCLUSION

Silty clay loam soil addition to aflatoxin-contaminated rations reduced residual concentrations of aflatoxin B_1 and M_1 observed in the chicken livers. Livers of chickens fed high aflatoxin- contaminated ration amended with 25% soil had lower aflatoxin concentrations (AFB_1 and AFM_1) than chickens fed high aflatoxin contaminated ration with 50% soil and no soil. Therefore, 25% silty clay loam soil was more effective in reducing residual concentrations of aflatoxin in chicken livers than 50% soil. The results further indicated that silty clay loam soil may be a useful feed additive for reduction of aflatoxicosis in chickens if a feasible dose lower than 25% soil can be achieved. These results may have important implications in the feeding of contaminated feeds to animals since humans are the ultimate consumers of animals and animal products.

The reduction of aflatoxins in livers of chickens fed aflatoxin-contaminated rations with silty clay loam soil added may have some impact on agriculture, economics and human health. Aflatoxin in contaminated feeds and feedstuffs consumed by animals can affect both productivity and reproductivity of animals resulting in loss of profit to the livestock industry. Residues of aflatoxin in meat and animal products can result in detrimental effects on human health

because aflatoxins are both toxic and carcinogenic. Reduction of aflatoxicosis in animals will reduce effects on reproductivity and productivity, and at the same time, the possibility of detrimental effects being passed to humans should be reduced.

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PAPER IV. THE EFFECTS OF SOIL ON RETENTION AND DISTRIBUTION OF AFLATOXINS IN TISSUES OF CHICKENS FED AFLATOXIN-CONTAMINATED RATIONS AMENDED WITH SOIL

INTRODUCTION

Aflatoxin B_1 (AFB_1) belongs to a group of secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and is highly toxic and a potent carcinogen (1,2,3,4,5,6). The incidence of contamination of agricultural commodities by aflatoxin depends on several factors such as growing regions, season conditions under which crops are grown, harvested and stored (4,5). Molds producing mycotoxins are capable of growth on a variety of substrates and under diverse conditions of moisture, pH and temperature (2). The occurrence and toxicity of AFB_1 in agricultural products consumed by animals and man has received considerable attention during the past 20 years (1,2,3). Aflatoxin B_1 was found to be associated mostly with grain crops. Contaminated crops are unfit for consumption by animals and are usually disposed of by plowing into the soil (2,5,6).

The resulting economic impact from aflatoxin contamination may be observed in production, marketing and utilization of agricultural products. The exposure of animals to aflatoxin due to the consumption of contaminated feeds can present a difference between profit and loss in the poultry industry (4,7). Animals produce various metabolites after ingestion of aflatoxin-contaminated ration. AFB_1 is metabolized

to aflatoxins M_1, Q_1, P_1 and AFB_1 epoxide (Fig. 3) (1, 8). Aflatoxin M_1 is relatively stable and is both mutagenic and carcinogenic. It is mostly present in excreta of exposed animals and its secretion in milk which has been a serious food safety concern (8). The effects that aflatoxin have on animals can be passed on to humans (2,3), if animals which are exposed to high levels of aflatoxin retain these residues in their tissues. Food consumed from these animals can result in serious health problems to consumers (2) because aflatoxin B_1 is highly toxic and carcinogenic (1,2,3,4,5,6).

There is need for applicable methods to detoxify aflatoxin-contaminated feedstuffs in order to reduce or prevent the detrimental effects aflatoxins can have on the livestock industry, and at the same time, reducing the possibility of serious health problems from aflatoxin residues passed to consumers of animals and animal products. This has led to the evaluation of several substances for the application in the detoxification of aflatoxins.

The objectives of this study were; 1) To evaluate the effectiveness of silty clay loam soil in the detoxification aflatoxin B_1 in chickens fed aflatoxin-contaminated rations amended with soil; 2) to determine the residual concentrations of aflatoxin B_1 and its metabolites retained in tissues of chickens (crop, liver and muscle) fed the soil amended aflatoxin-contaminated rations; 3) to evaluate gross and

histopathological changes observed in crops and livers of chickens fed the soil amended aflatoxin-contaminated rations and assess how silty clay loam soil influenced the observed changes; 4) to determine the total protein levels in sera collected from chickens fed the soil amended aflatoxin-contaminated rations and assess how protein levels are influenced by silty clay loam soil; 5) to assess how silty clay loam soil would influence the detrimental effects of aflatoxicosis on animal productivity and; 6) to assess the risk of exposure of humans to the aflatoxin residues.

MATERIAL AND METHODS

The animal species of choice for this study was avian, because chicken meat constitutes a large proportion of human diet and the major portion of chicken feed is corn. One hundred one-day-old White Leghorn chickens were obtained through the Department of Laboratory Resources for this study. The chickens were randomly assigned to five groups of twenty each. They were weighed, wing-banded and housed and fed separately. The cages were heated with heat lamps and the thermostatically control room temperature was set at about 85° F. The rations used in the study were formulated by the addition of Biotin Stress Pak^{R}, a vitamin-mineral supplement, at 1 g/kg feed to ground clean corn, low aflatoxin-contaminated corn (120 ppb B_1) and high aflatoxin-contaminated corn (700 ppb B_1). Silty clay loam soil was obtained from an area outside the diagnostic lab, crushed with mortar and pestle, homogenized and sieved, then incorporated into two rations at 10% and 25% concentrations, respectively.

The chickens received their feed as follows: group one = clean corn, group two = low aflatoxin-contaminated corn, group three = high aflatoxin-contaminated corn plus 25% soil, group four = high aflatoxin-contaminated corn plus 10% soil, and group five = high aflatoxin-contaminated corn without soil.

The concentrations of aflatoxin B_1 in the rations fed to chickens during the experiment were 120 ppb for the low aflatoxin-contaminated ration, 700 ppb for the high aflatoxin-contaminated ration, 525 ppb for the high aflatoxin-contaminated ration plus 25% soil and 630 ppb for the high aflatoxin-contaminated ration plus 10% soil. Rations were analyzed for moisture, protein and crude fiber contents (MVLT Laboratories, Nevada, IA) and soil was analyzed for sand, silt, clay, organic matter contents and pH (Agronomy Department, Iowa State University, Ames, IA).

The composition of rations used for the experiment is shown in Table 1, and characteristics of soil used are shown in Table 2.

Table 1. Composition of experimental rations fed to White Leghorn chickens for 28 days

Rations[a]	% Moisture	% Protein	% Crude Fiber
Clean Corn	8.6	9.0	1.8
Low AFB_1-Corn	12.0	13.0	3.0
High AFB_1-Corn + 25% Soil	7.5	8.4	2.7
High AFB_1-Corn + 10% Soil	10.4	10.5	2.8
High AFB_1-Corn	11.4	11.2	3.0

[a]Analysis of rations fed to chickens was performed according to: Official Methods of Analysis of the Association of Official Analytical Chemists (MVLT Laboratories, Nevada,IA).

Table 2. Characteristics of soil used to formulate aflatoxin-contaminated corn rations fed to chickens for 28 days

Sample	Sand	Co.Silt	Fi.Silt	Clay	pH	Organic Matter
Particle size density	2-0.5mm	50-20u	20-2u	<2u		%
%	39.3	20.4	18.8	21.5	7.3	4.34

Methods: PSD by pipette; pH in 1:1 soil/water; organic matter by Leco. The estimated cation exchange capacity of Iowa soil is 22.0 milliequivalents/100 grams soil (Agronomy Department, Iowa State University, Ames, IA).

The chickens were first fed a commercial diet ad libitum and water was provided free choice. The experimental feeding started when the chickens were 14 days old and continued until they were 42 days old. The chickens and feed were weighed and weights were recorded once per week. Approximately 0.5 ml of blood was taken using a 25 gauge 5/8 or a 26 gauge 3/8 needle with 1 ml syringe from the jugular vein of 10 chickens in each group once per week starting at 21 days of age. The blood was collected in 12 X 75 mm polystyrene tubes with caps containing approximately 1 ml of Alsevers' solution at pH 6.1. The blood was centrifuged at 1500 rpm for 10 minutes. The sera were collected in Sarsted^{R} screw-cap micro tubes and stored at -80° C until total protein evaluation was performed.

The chickens were observed twice daily for presence of any clinical signs and a daily log was kept of mortality. At the end of the experimental period, feed and chickens were weighed and blood was taken from all chickens. The chickens were

euthanized with CO_2, and subjected to necropsy. The livers and crops were removed and examined for the presence of gross pathological changes, weighed, and specimens were fixed in 10% buffered formalin for histopathological examination. Pooled samples from each of the five groups were taken from the breast muscles, livers and crops and frozen for later chemical analysis of residual concentrations of aflatoxin B_1 and its metabolites.

Analysis of Aflatoxins

Aflatoxins were extracted from livers, crops and muscles according to the Analytical Methods in Toxicology procedure (9). Aflatoxin G_2 was used as a standard with control tissue samples spiked at 2 ng/g. Following silica gel clean up, the extracts were concentrated to dryness under nitrogen gas. The extracts were then cleaned up by C-18 Sep-Pak^R cartridges (Waters, Division of Millipore, Milford, MA) and dried under nitrogen gas. The extracts were redissolved in 100 ul methylene chloride ($MeCl_2$); 10 ul of each sample was spotted on normal phase thin layer chromatography (TLC) plates along with the appropriate aflatoxin standards, 4.0 ng B_1, 4.0 ng B_2, 3.0 ng G_2 and 1.0 ng M_1. The TLC plates were developed in 90:10 chloroform:acetone, 1-2 cm from the top of each plate and examination of plates was done under long-wave ultra violet

(UV) light for the detection and positive identification of aflatoxins B_1, B_2, G_2 and M_1 at Rf values corresponding to aflatoxin standards. The TLC plates were redeveloped in 85:10:5 chloroform:acetone:2-propanol and re-examined under long-wave UV light for further resolution of aflatoxin M_1.

The remaining 90 ul of each extract was used for chemical analysis by high performance liquid chromatography (HPLC). The extracts were concentrated to dryness under a stream of nitrogen and low heat steam bath, then 200 ul of $MeCl_2$ was added to each vial and vortexed for 30 seconds. Twenty ul of each extract were transferred to individual 2 dram vials and concentrated to dryness under a stream of nitrogen and low heat steam bath. Each sample was derivatized with 100 ul of trifluoroacetic acid and vortexed for 30 seconds. The mixtures were evaporated to dryness under nitrogen gas and low heat steam bath.

The control liver and muscle sample extracts were redissolved in 2 ml of HPLC elution solvent water:2-propanol: acetonitrile (80:12:8) and vortexed for 30 seconds. All other sample extracts were redissolved in 100 ul of HPLC elution solvent water:2-propanol:acetonitrile (80:12:8) and vortexed for 30 seconds. Twenty ul of each sample were injected into the mobile phase water:2-propanol: acetonitrile (80:12:8). Separation of aflatoxin B_1 and its metabolites occurred on a Brownlee Labs C-18, Spheri-6 ODS, 100 x 4.6 mm, 5 um column.

Detection was performed by a McPherson SF-749 Fluorescence Spectrophotometer with xenon-mercury lamp. Excitation was set at 376 nm, emission was 493 nm, range sensitivity 0.1, lamp current 8 amperes, time constant 5, gain 370 and detection limit 0.1. Quantitation of aflatoxins was performed by measuring the peak height of each sample at the corresponding retention time of each aflatoxin standard, and comparing the ratio of peak height of each sample of aflatoxin B_1, B_2, G_2 and M_1 to the peak height of the respective aflatoxin standard. The chart recorder was set at 0.1 volt, and paper speed was 30 cm/hr with flow rate of 1 ml per min (9,10).

Analysis of Total Protein

Determination of total protein present in sera from chickens was performed by refractometer, TS meter (American Optical Corporation). Protein concentrations have been determined as differences between non-protein solids and total solids. The protein concentration were consistent with protein/nitrogen ratio of 6.54 rather than concentration of 6.25. Micro hematocrit capillary Monoject tubes (Division of Sherwood Medical, St. Louis, Missouri) were used for application of sera to the TS meter. Percent total solids (TS%) by weight was read directly from the scale of the TS meter and recorded. Conversion of TS% to total protein

concentration in sera was done by reading the value of protein concentration in g/100 ml corresponding to the TS% value from the conversion table for refractometers, TS meters and concentrimeters (American Optical Corporation).

Statistical Analysis

The data were analyzed using the SAS System software package and utilizing Analysis of Variance Procedure. Variable means showing significant differences were indicated using Duncan's Multiple Range Test. All statements of significance were based on a probability value of 0.05.

RESULTS AND DISCUSSION

During the experimental period chickens receiving rations containing high aflatoxin-contaminated corn appeared unthrifty and had ruffled feathers. However, there was no mortality.

Body Weight

The highest mean body weight was seen in chickens fed clean corn (control group) (219.5 g) whereas the lowest mean body weight was observed in chickens fed high aflatoxin-contaminated corn plus 25% soil (146.8 g). Mean body weight in chickens fed low aflatoxin-contaminated corn (173.5) was higher than that seen in chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil (147.3 g and 146.8 g) and high aflatoxin-contaminated corn without soil (152.4 g). Chickens fed high aflatoxin-contaminated corn without soil had a slightly higher mean body weight than the chickens in groups fed high aflatoxin-contaminated corn plus 10% and 25% soil. Mean body weight in chickens fed clean corn (control group) was substantially and significantly higher than that in the other groups. However, mean body weight of chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil were not statistically indistinguishable. Mean body weight was

significantly different for days of age, however, mean body weight for days 21 and 28 were statistically indistinguishable (Table 3). The mean body weight in the control group increased continuously during the entire experimental period. There was a continuous increase in mean body weight in all groups of from day 1 to day 21, but mean body weight declined from day 21 to 28 in all groups of chickens fed aflatoxin contaminated corn rations before increasing continuously to the end of the experimental period (Figure 1). The greatest mean body weight gain was seen in chickens in the control group (184.6 g) whereas the lowest body weight was observed in chickens fed high aflatoxin-contaminated corn plus 25% soil (112.3 g). The chickens fed low aflatoxin-contaminated corn had a greater body weight gain (138.8 g) than chickens in groups fed high aflatoxin-contaminated corn plus 10% and 25% soil (112.7 g and 112.3 g) and high aflatoxin-contaminated corn ration without soil (117.4 g) (Table 3).

During the experimental period mean body weight gain was greatest in chickens fed clean corn (control) and the lowest mean body weight gain was in chickens fed high aflatoxin-contaminated corn plus 25% soil (23.7 g). Mean body weight gained during the experimental period in chickens fed high aflatoxin-contaminated corn plus 10% soil (34.6 g) was greater than that gained in chickens fed high aflatoxin-contaminated

Table 3. Mean body weight in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Body Weight[a](g) (N=20)						
	Days of Age						
Rations	1[g]	7[f]	14[e]	21[d]	28[d]	35[c]	42[b]
Clean Corn[h]	34.9	68.8	117.5	157.4	175.0	190.9	219.5
Low AFB_1-Corn[i]	34.7	75.3	127.2	156.3	147.4	161.9	173.5
High AFB_1-Corn[k] + 25% Soil	34.5	75.4	123.1	142.1	133.2	135.4	146.8
High AFB_1-Corn[k] + 10% Soil	34.6	69.2	112.7	135.8	133.4	138.3	147.3
High AFB_1-Corn[j]	35.0	74.8	122.6	142.8	141.8	146.3	152.4

[a]Values represent means of body weight per group of chickens corresponding to days of age

b,c,d,e,f,g- Days of age with the same superscript are not significantly different

h,i,j,k- Rations with the same superscript are not significantly different

corn plus 25% soil (23.7 g) and high aflatoxin-contaminated corn without soil (29.8 g) (Table 4). There was a trend for the mean body weight gain to increase then decrease before increasing toward the end of the period in all groups (Figure 2). The greatest increase in mean body weight gain was seen between day 1 to day 14 all groups whereas the greatest decline was seen between days 15 and 28. Chickens observed with the greatest decline in mean body weight gain were fed low aflatoxin-contaminated corn and high aflatoxin-contaminated corn plus 25% soil followed high aflatoxin-contaminated corn plus 10% soil and high aflatoxin-contaminated corn without soil (Figure 3). All groups fed aflatoxin-contaminated rations showed negative weight gain between days 22-28. The greatest negative gain was observed in chickens fed low aflatoxin-contaminated corn (-8.9 g) and high aflatoxin-contaminated corn plus 25% soil (-8.9 g), followed by high aflatoxin-contaminated corn plus 25% soil (-2.4 g) and high aflatoxin-contaminated corn without(-1.0 g) (Figure 4).

Feed Consumption

The results showed that all groups of chickens consumed more feed than the group fed high aflatoxin-contaminated corn without soil (11953 g). The group fed high aflatoxin-contaminated corn plus 10% soil consumed the most feed (12137

Table 4. Mean body weight gain in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Body Weight Gain[a] (g) (N=20)							
	Days of Age							
Rations	1-7	8-14	15-21	22-28	29-35	36-42	1-42	15-42
Clean Corn	33.9	48.7	39.9	17.6	15.9	28.6	184.6	102.0
Low AFB_1-Corn	40.6	51.9	29.1	-8.9	14.5	11.6	138.8	46.7
High AFB_1-Corn + 25% Soil	40.9	47.7	19.0	-8.9	2.2	11.4	112.3	23.7
High AFB_1-Corn + 10% Soil	34.6	43.5	23.1	-2.4	4.9	9.0	112.7	34.6
High AFB_1-Corn	39.8	47.8	20.2	-1.0	4.5	6.1	117.4	29.8

[a]Values represent means of actual body weight gained per group of chickens corresponding to days of age. Experimental feeding started at 14 days of age

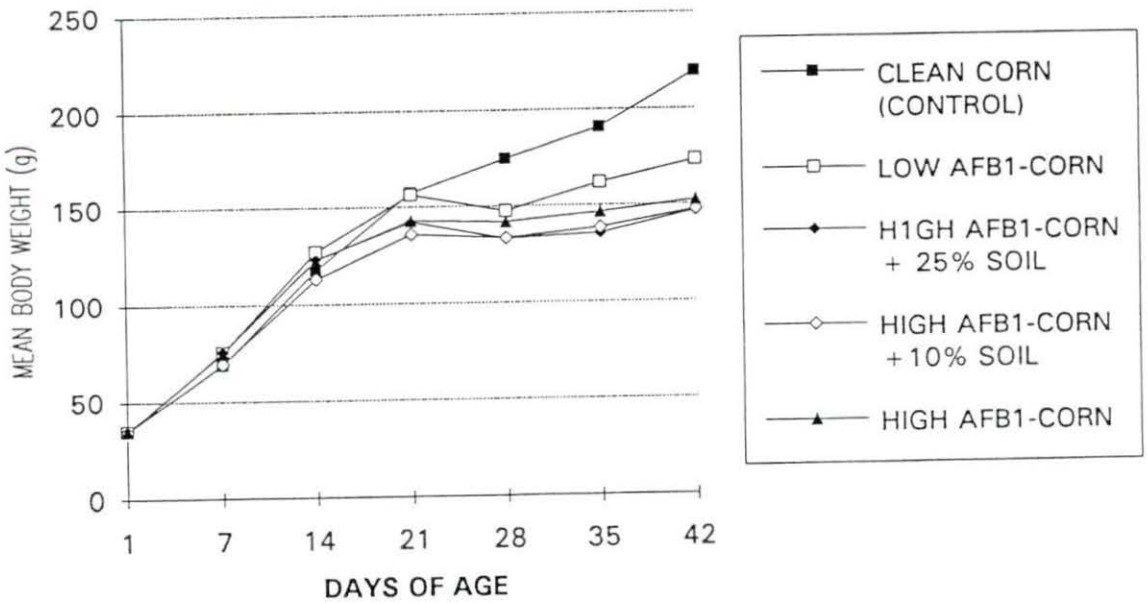

Figure 1. Mean body weight in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil rations for 28 days

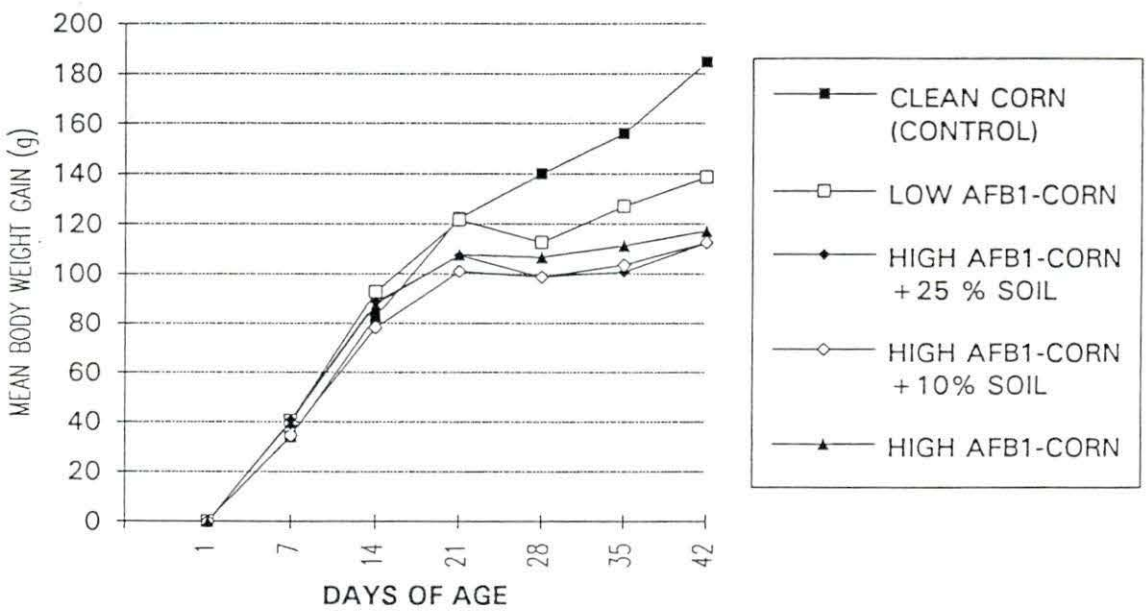

Figure 2. Mean body weight gain in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

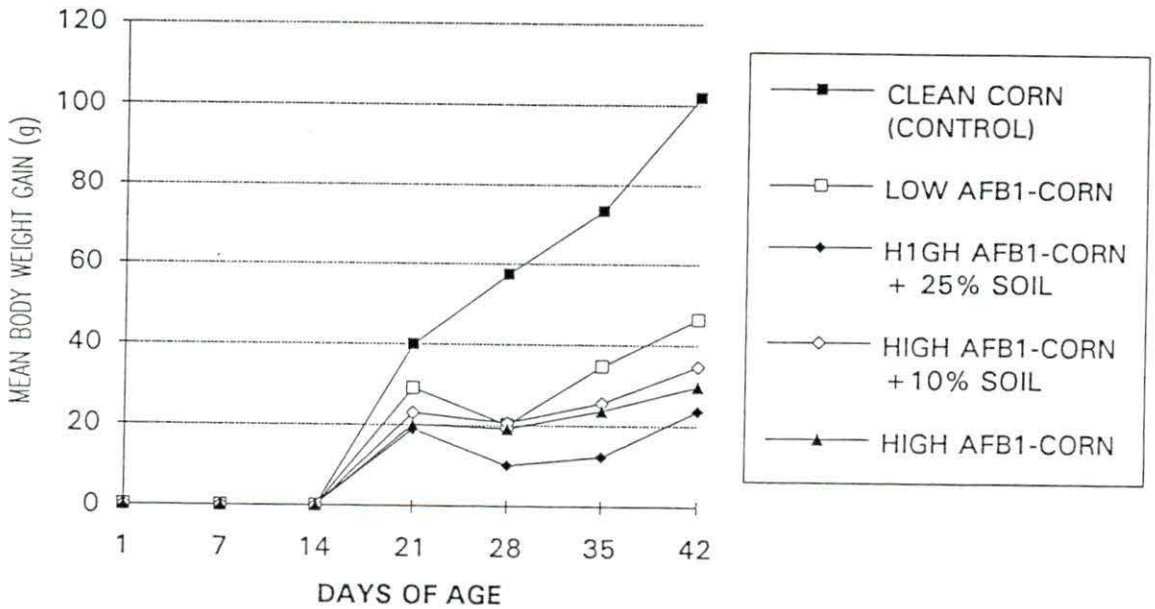

Figure 3. Mean body weight gain in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days- days 14-42

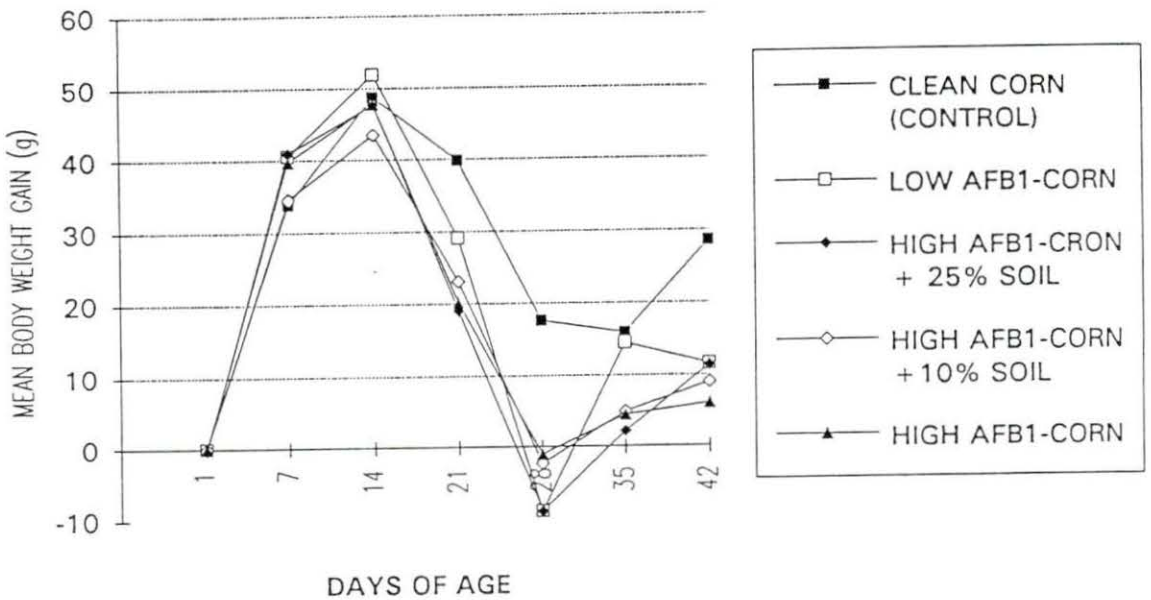

Figure 4. Actual mean body weight gain in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

g), followed by the group fed low aflatoxin-contaminated corn (12657 g), clean corn (control) (12137 g) and high aflatoxin-contaminated corn plus 25% soil (12045 g) (Table 5). There was a trend observed in feed consumption which showed that there was decline in all groups from day 21 to 28 before increasing toward the end of the period (day 29 to 42). The greatest decline in feed consumption was observed in the group fed high aflatoxin-contaminated corn without and the least decline was in the group in the control group fed clean corn.

The greatest increase in feed consumption was seen in the group fed high aflatoxin-contaminated corn plus 10% soil whereas the least increase was seen in the group fed high aflatoxin-contaminated corn without soil (Figure 5). Mean feed consumption in chickens fed high aflatoxin-contaminated ration without soil was lower than all groups between days 1-42 and 15-42 although the chickens had a higher feed consumption than the groups fed clean corn and low aflatoxin-contaminated rations between days 15-21. This lower overall consumption resulted from decline in feed consumption between days 22-35 (Table 6).

Feed Conversion

Feed conversion ratio increase from day 15 to 35 in all groups before decreasing from day 36 to 42. The greatest

increase was observed in the group low high aflatoxin-contaminated corn plus between day 29 and 35, whereas the lowest increase was observed in the group fed clean corn (control). There was negative feed conversion ratio observed in all groups fed aflatoxin-contaminated rations between days 22-28 as negative gain was recorded. Feed conversion ratio declined in all groups from 29 to 42 in all groups. The highest ratio was observed in group fed high aflatoxin-contaminated corn without soil and the lowest was observed in the group fed clean corn (control) (Table 7).

Organ Weight

The results showed that when mean organ weights were expressed as percent of mean body weight chickens receiving high aflatoxin-contaminated corn without soil had the highest percent liver weight (3.93%) and the lowest percent crop weight (0.66%). The lowest percent liver weight was observed in the group fed clean corn (control) (2.55%) whereas the chickens fed high aflatoxin-contaminated corn plus 10% soil had a higher percent liver weight (3.33%) than chickens fed low aflatoxin-contaminated corn (2.88%) and high aflatoxin-contaminated corn plus 25% soil (2.66) rations. The highest percent crop weight was seen in the chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil (0.95%),

Table 5. Total feed consumption in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Feed Consumption[a] (g) (N=20)							
	Days of Age							
Rations	1-7	8-14	15-21	22-28	29-35	36-42	1-42	15-42
Clean corn	1079	1982	2489	1903	2196	2489	12137	9077
Low AFB_1-Corn	1404	1583	2656	1903	2342	2782	12657	9683
High AFB_1-Corn + 25% Soil	1270	1716	2623	1610	2050	2782	12045	9065
High AFB_1-Corn + 10% Soil	1043	1305	2572	1853	2411	3311	12924	10147
High AFB_1-Corn	1154	1423	2553	1569	2063	2700	11953	8884

[a]Values represent total feed consumed by each group of chickens corresponding to days of age

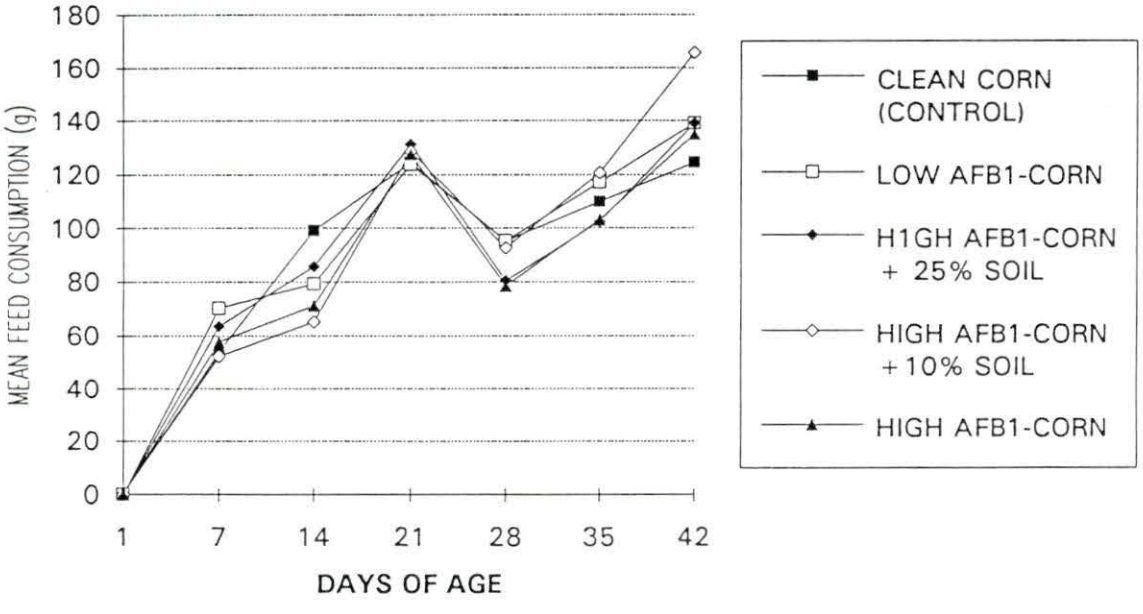

Figure 5. Mean feed consumption in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

Table 6. Mean feed consumption in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Feed Consumption[a] (g) (N=20)							
	Days of Age							
Rations	1-7	8-14	15-21	22-28	29-35	36-42	1-42	15-42
Clean Corn	53.9	99.1	124.5	95.2	109.8	124.5	606.5	454.0
Low AFB_1-Corn	70.2	79.2	123.8	95.2	117.1	139.1	633.6	484.2
High AFB_1-Corn + 25% Soil	63.5	85.8	131.2	80.	102.5	139.1	602.6	453.3
High AFB_1-Corn + 10% Soil	52.2	65.2	128.6	92.7	120.6	165.6	646.4	507.5
High AFB_1-Corn	57.7	71.2	127.6	78.5	103.2	135.0	597.7	444.2

[a]Values represent mean feed consumption per group of chickens corresponding to days of age

Table 7. Feed efficiency in White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Feed Conversion Ratio (Feed Efficiency) g feed consumed: g weight gain[a] (N=20)						
	Days of Age						
Rations	1-14	15-21	22-28	29-35	36-42	1-42	15-42
Clean corn	1.85	3.00	5.95	9.98	3.71	3.3	4.4
Low AFB_1-Corn	1.61	4.56	28.30	50.90	11.99	4.6	10.4
High AFB_1-Corn + 25% Soil	1.65	6.91	---	46.6	20.2	5.4	19.1
High AFB_1-Corn + 10% Soil	1.89	5.60	---	24.6	17.8	5.7	14.7
High AFB_1-Corn	1.76	6.32	---	22.9	22.1	5.1	14.9

[a]Values represent mean feed conversion ratio per group of chickens corresponding to days of age

--- Represents negative feed conversion ratio due to negative weight gain

Table 8. Mean organ weight of White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Organ Weight[a] (%) (N=20)	
Rations	Liver	Crop
Clean Corn	2.55	0.68
Low AFB_1-Corn	2.88	0.81
High AFB_1-Corn + 25% Soil	2.66	0.95
High AFB_1-Corn + 10% Soil	3.33	0.95
High AFB_1-Corn	3.93	0.66

[a]Values represent mean organ weight per group of chickens expressed as percent of mean body weight at the end of experimental period

respectively, followed by low aflatoxin-contaminated corn (0.81%) and clean corn (control) (0.68%) (Table 8).

Pathology

Gross pathology- Observation of livers from the group of chickens fed clean corn (control) showed that one had yellow discoloration while no abnormality was seen in the others. No abnormalities were observed in livers from chickens fed low aflatoxin-contaminated corn. In each group of chickens fed high aflatoxin-contaminated corn plus 25% and high aflatoxin-contaminated corn plus 10% soil, three livers had yellow discoloration and one was friable. There were seven yellow mottled discolored livers and one friable liver in chickens fed high aflatoxin-contaminated corn without soil. Results suggest that chickens fed high aflatoxin-contaminated corn without soil had more discoloration of livers which is indicative of fatty change when compared to livers from chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil (Table 9).

Observation of crops showed that two were distended with air in chickens fed clean corn ration. One air distended crop was seen in each group fed low aflatoxin-contaminated corn ration and high aflatoxin-contaminated corn plus 25% soil. No abnormalities were observed in groups fed high aflatoxin-

contaminated ration plus 10% soil and high aflatoxin-contaminated ration without soil. Observed distention in chicken crops seemed to be caused by factors other than aflatoxin (Table 10).

Histologic pathology- Microscopic examination of livers revealed no lesions in the group of chickens fed clean corn (control) (Figure 6). In chickens fed low aflatoxin-contaminated corn minute multiple cytoplasmic vacuolations were observed in hepatocytes (Figure 7). Multiple cytoplasmic vacuolations, hepatocellular swelling with possibility of fatty change and hydropic degeneration were observed in groups fed high aflatoxin-contaminated corn plus 10% and 25% soil rations (Figures 8 and 9). The livers in chickens fed high aflatoxin-contaminated corn without soil, extensive vacuolation of hepatocytes, hepatocellular swelling, fatty change and hydropic degeneration were observed (Figure 10). Vacuoles observed in livers from the chickens in the group fed high aflatoxin-contaminated corn without soil stained positively for fat by Oil Red 'O' stain method (Figure 11). There were no abnormalities in any crop samples from chickens in clean corn (control) or chickens fed aflatoxin-contaminated corn rations (Table 10).

Table 9. Gross and histopathologic changes observed in livers from White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

Rations	Gross Appearance[a]	Histologic Appearance[b]
Clean Corn	One yellow colored All others normal	No abnormality observed in any tissue
Low AFB_1-Corn	No abnormalities observed	Multiple minute cytoplasmic vacuolations in hepatocytes
High AFB_1-Corn + 25% Soil	Three showed yellow discoloration, one was friable	Multiple cytoplasmic vacuolations in hepatocytes, hepato-cellular swelling
High AFB_1-Corn + 10% Soil	Three showed yellow discoloration and one was friable	Multiple vacuolations in cytoplasm of hepatocytes hydropic degeneration, hepatocellular swelling
High AFB_1-Corn	Seven showed yellow mottled discoloration and one was friable	Extensive cytoplasmic vacuolation, hepato-swelling, fatty change, hydropic degeneration

[a]Gross examination was performed at necropsy
[b]Microscopic examination of H&E stained slides at 40X

Table 10. Gross and histopathologic changes observed in crops from White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

Rations	Gross Appearance[a]	Histologic Appearance[b]
Clean Corn	Two distended with air	No abnormalities
Low AFB_1-Corn	One distended with air	No abnormalities
High AFB_1-Corn + 25% Soil	One distended with air	No abnormalities
High AFB_1-Corn + 10% Soil	No abnormalities observed	No abnormalities
High AFB_1-Corn	No abnormalities observed	No abnormalities

[a]Gross examination was performed at necropsy
[b]Microscopic examination of H&E stained slides at 40X

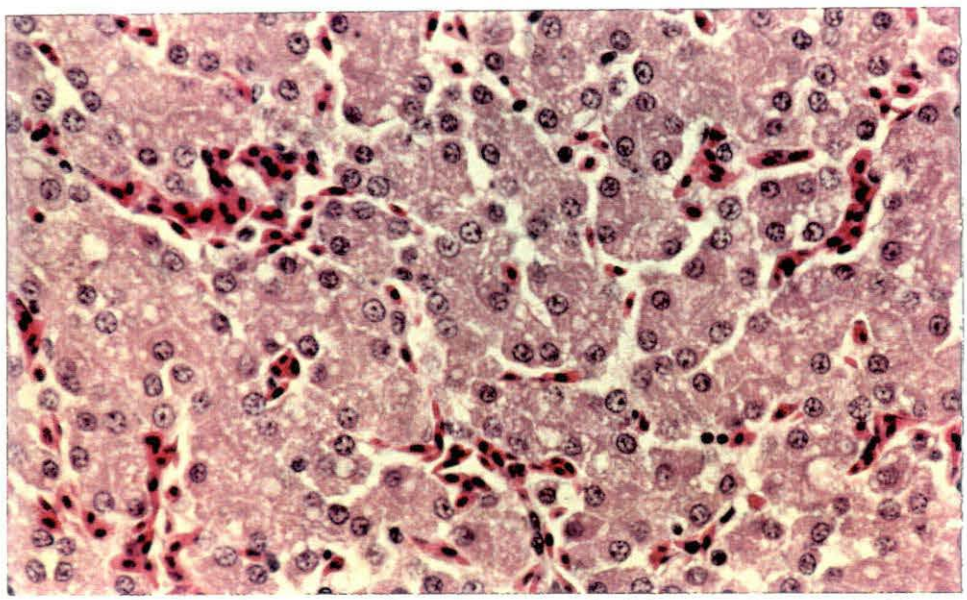

Figure 6. Photomicrograph of normal liver from White Leghorn chickens fed a commercial diet followed by clean corn (control) ration for 28 days. H&E stain at 400X

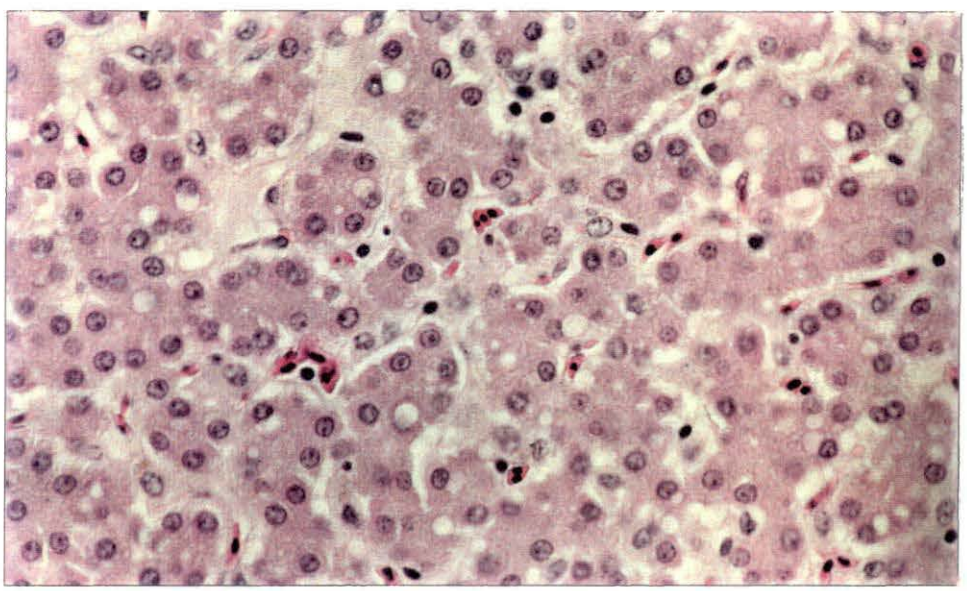

Figure 7. Photomicrograph of liver from White Leghorn chickens fed a commercial diet followed by low aflatoxin-contaminated corn ration for 28 days. Multiple minute cytoplasmic vacuolations within hepatocytes. H&E stain at 400X

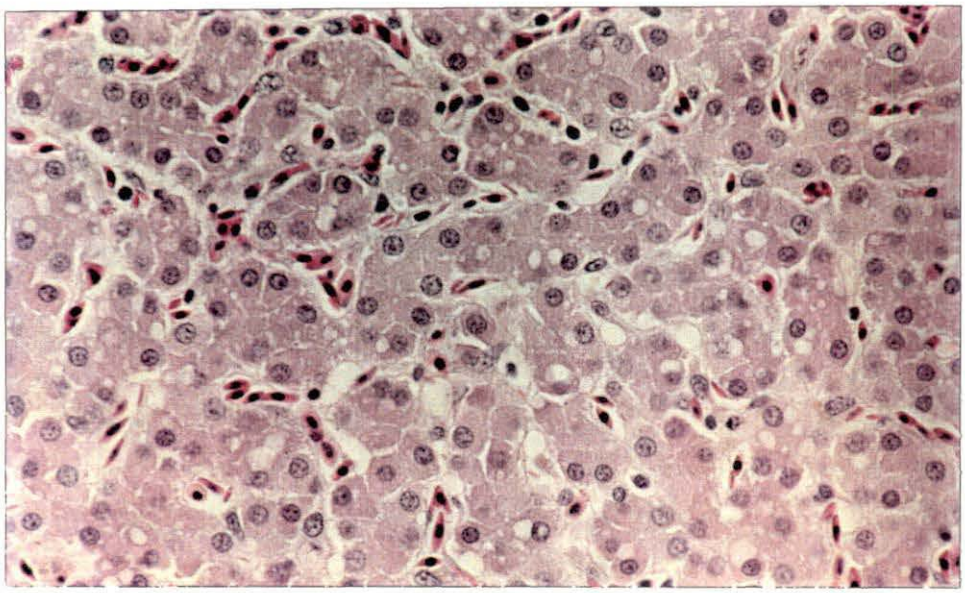

Figure 8. Photomicrograph of liver from White Leghorn chickens fed a commercial diet followed by high aflatoxin-contaminated corn plus 25% soil ration for 28 days. Multiple cytoplasmic vacuolations in hepatocytes and hepatocellular swelling. H&E stain at 400X

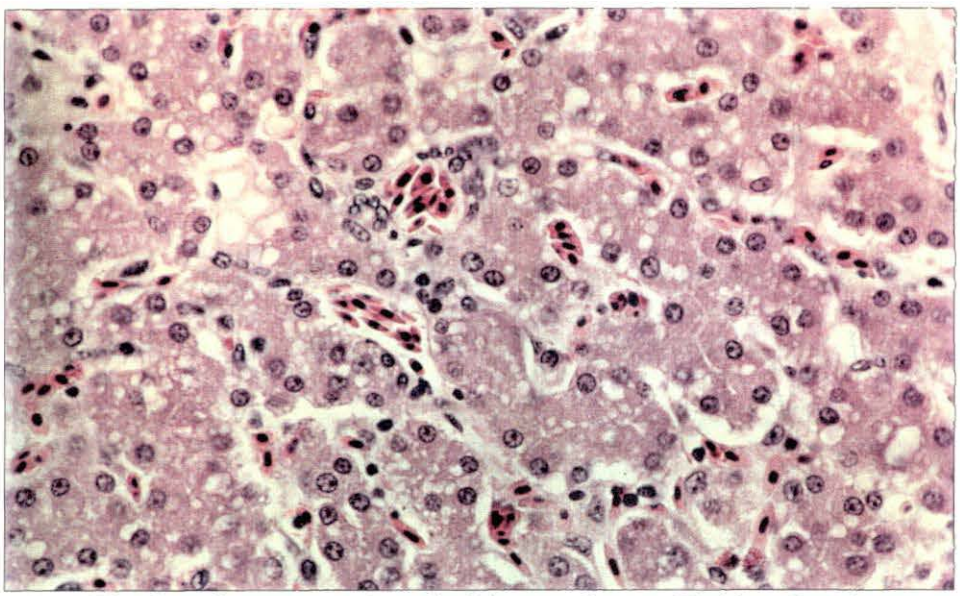

Figure 9. Photomicrograph of liver from White Leghorn chickens fed a commercial diet followed by high aflatoxin-contaminated corn plus 10% soil ration for 28 days. Multiple cytoplasmic vacuolations of hepatocytes, hepatocellular swelling and hydropic degeneration. H&E stain at 400X

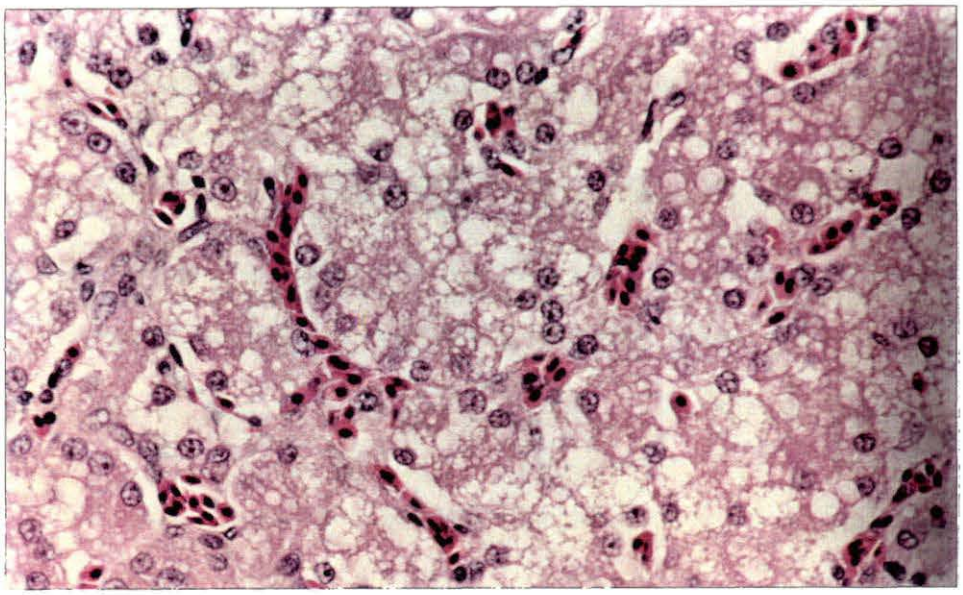

Figure 10. Photomicrograph of liver from White Leghorn chickens fed a commercial diet followed by high aflatoxin-contaminated corn ration without soil for 28 days. Extensive cytoplasmic vacuolation of hepatocytes, hepatocellular swelling, fatty change and hydropic degeneration. H&E stain at 400X

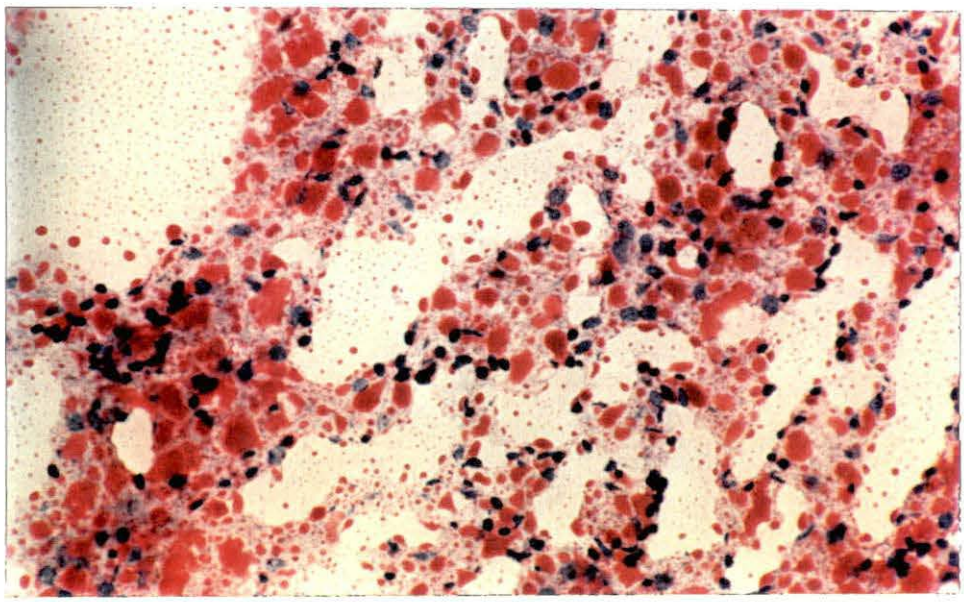

Figure 11. Photomicrograph of liver from White Leghorn chickens fed a commercial diet followed by high aflatoxin-contaminated corn without soil for 28 days. Positive staining for fat accumulation in cytoplasm of hepatocytes.Oil Red 'O' stain at 400X

Analysis of Aflatoxins

Thin-layer chromatography results showed that only aflatoxin G_2 (AFG_2) used as internal standard at 2 ng/g was detected in samples from chickens fed clean corn (control). Aflatoxin B_1 (AFB_1) was detected in liver, crop and muscle in the groups fed high aflatoxin-contaminated corn without soil high aflatoxin-contaminated corn plus 10% soil and high aflatoxin-contaminated corn plus 25% soil rations. AFB_1 was detected only in liver and crop in the chickens fed low aflatoxin-contaminated corn ration. Aflatoxin B_2 was detected in liver, crop and muscle of chickens fed high aflatoxin-contaminated corn without soil, while no AFB_2 was detected in any tissue samples from the other groups. A compound appeared as AFG_2 was detected in liver, crop and muscle in chickens fed high aflatoxin-contaminated corn without soil and high aflatoxin-contaminated corn plus 10% soil ration, but was detected only in liver and muscle in chickens fed low aflatoxin-contaminated corn and high aflatoxin-contaminated corn plus 25% soil rations. This is an unexpected finding because AFG_2 is not regarded as a major animal metabolite of AFB_1.

Aflatoxin M_1 was detected in liver and crop in chickens fed aflatoxin-contaminated corn without soil and only in muscle of

chickens fed low aflatoxin-contaminated corn and high aflatoxin-contaminated corn plus 25% soil.

High-performance liquid chromatography results showed that chickens fed high aflatoxin-contaminated corn without soil retained the highest total aflatoxin concentration in all tissues (2.188 ng/g). The lowest total aflatoxin concentration was detected in the chickens fed low aflatoxin-contaminated corn (0.108 ng/g). Chickens fed high aflatoxin-contaminated corn plus 25% soil had lower total aflatoxin concentration (0.162 ng/g) than chickens fed high aflatoxin-contaminated corn plus 10% soil (0.303 ng/g) (Table 11). The results showed that chickens fed high aflatoxin-contaminated corn rations amended with soil had lower concentrations of total aflatoxin compared to chickens fed high aflatoxin-contaminated corn without soil. The suggest that soil addition to high aflatoxin contaminated rations reduced the residual concentrations of total aflatoxin in chicken tissues.

AFB_1 concentration in the liver of chickens fed high aflatoxin-contaminated corn without soil was substantially and significantly higher (1.294 ng/g) than those in the other groups. However, the AFB_1 concentrations in the liver of the other groups were statistically indistinguishable. AFB_1 concentration in livers of chickens fed high aflatoxin-contaminated corn plus 10% soil (0.007 ng/g) was lower than that in the chickens fed high aflatoxin-contaminated corn plus

25% soil (0.018 ng/g) (Table 11). The results showed that addition of soil to high aflatoxin-contaminated rations reduced the residual concentration of AFB_1 in chicken livers. The results further indicate that the addition of soil at 10% to high aflatoxin-contaminated corn was more effective in reducing AFB_1 concentration in chicken livers than 25% soil.

The concentration of AFB_1 in the crop of chickens fed high aflatoxin-contaminated corn without soil was statistically indistinguishable from those in the other groups. AFB_1 concentration in crops of chickens fed high aflatoxin-contaminated corn plus 10% soil (0.211 ng/g) was higher than that of the other groups. AFB_1 in crops of chickens fed high aflatoxin-contaminated corn plus 25% soil (0.019 ng/g) was lower than that detected in groups of chickens fed high aflatoxin-contaminated corn without soil (0.074 ng/g) and in chickens fed low aflatoxin-contaminated corn (0.033 ng/g) (Table 11). The results showed that the addition of soil at 25% to high aflatoxin-contaminated corn was more effective in reducing AFB_1 concentration in the crops of chickens than soil at 10%. The results further indicated that the addition of 10% soil to high aflatoxin-contaminated ration did not reduce the concentration of AFB_1 in crops of chickens as a greater concentration of AFB_1 was detected in crops of chickens fed high aflatoxin-contaminated corn plus 10% soil than in crops of chickens fed high aflatoxin-contaminated corn without soil.

AFB_1 in muscle of chickens fed high aflatoxin-contaminated corn without soil (0.014 ng/g) was significantly higher than that detected in the chickens fed high aflatoxin-contaminated corn plus 10% soil (0.003 ng/g) and 25% soil (0.004 ng/g) rations (Table 11). However, the AFB_1 concentrations detected in chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil were statistically indistinguishable. AFB_1 was not detected in muscle samples from chickens fed low aflatoxin-contaminated corn ration or in any tissue sample from chickens fed clean corn (control) ration. The results showed the addition of soil to high aflatoxin-contaminated corn rations reduced AFB_1 concentration in muscle. The results further indicated that 10% and 25% soil had similar effect in reducing residual concentration of AFB_1 in muscle.

Aflatoxin B_2 concentration was detected only in livers, crops and muscle of chickens fed high aflatoxin contaminated corn without soil. AFB_2 concentrations detected in liver, crop and muscle of chickens fed high aflatoxin-contaminated corn without soil were substantially and significantly higher than the other groups. The concentration of AFB_2 detected in crops (0.029 ng/g) was higher than the AFB_2 concentration detected in livers (0.028 ng/g) and (0.002 ng/g) in muscle (Table 11). The results suggest that addition of soil to high aflatoxin-contaminated corn ration prevented residual concentration of AFB_2 retention in liver, crop and muscle in chickens.

The compound which was detected as aflatoxin G_2 (AFG_2) with thin-layer chromatography appeared at the same retention as AFG_2 in livers of chickens fed high aflatoxin-contaminated corn without soil was higher than that in any other groups. The concentration of the unknown compound detected in livers of chickens fed aflatoxin-contaminated corn plus 10% soil ration was higher than that detected in chickens fed high aflatoxin-contaminated corn plus 25% soil and chickens fed low aflatoxin-contaminated corn rations. The results showed that the addition of soil to high aflatoxin-contaminated corn ration reduced the concentration of the unknown compound in chicken livers. The results further indicated that the addition of 25% soil to high aflatoxin-contaminated corn ration was more effective in reducing the residual concentration of the unknown in chicken livers than 10% soil.

The unknown compound concentration detected in crops of chickens fed high aflatoxin-contaminated corn plus 10% soil was higher than that which was detected in the chickens fed high aflatoxin-contaminated corn ration without soil. No detectable concentration of the unknown compound was in crops of chickens fed high aflatoxin-contaminated corn plus 25% soil ration and low aflatoxin-contaminated corn ration. The results showed that the addition of 25% soil to high aflatoxin-contaminated corn ration prevented the retention of residual concentration of the unknown compound in chicken crops. The

results further suggest that the addition of 10% soil to high aflatoxin-contaminated corn ration had no reducing effect on residual concentration of the unknown compound in chicken crops.

In muscle, no substantial concentration of the unknown compound was detected in chickens fed the high aflatoxin-contaminated corn rations. Additional analytical procedures were conducted in order to confirm AFG_2 presence. Reverse phase thin-layer chromatography and HPLC using a diode array detector did not show any conclusive evidence of AFG_2. Mass spectroscopy results revealed that there was a compound present but it did not produce a spectrum which matches AFG_2

Aflatoxin M_1 concentration was detected only in liver and crop of chickens fed high aflatoxin-contaminated corn without soil and in muscle of chickens fed low aflatoxin-contaminated corn and high aflatoxin-contaminated corn plus 25% soil rations. AFM_1 concentrations detected in liver (0.095 ng/g) and crop (0.569 ng/g) of chickens fed high aflatoxin-contaminated corn without soil were substantially and significantly higher than the other groups. The results suggest that soil was effective in preventing retention of residual concentrations of AFM_1 in liver and crop of chickens fed high aflatoxin contaminated rations. The concentration of AFM_1 in the crops of chickens fed high aflatoxin-contaminated corn without soil (0.569 ng/g) was substantially higher than that detected in

the livers (0.095 ng/g). The results suggest that AFM_1 retention is greater in the crop than the liver.

Greater concentration of AFM_1 (0.569 ng/g) than AFB_1 (0.074 ng/g) was detected in the crop of chickens fed high aflatoxin-contaminated corn without soil (Table 11). The results of higher concentration of AFM_1 than AFB_1 in the crop was unexpected and there are no reports in the present literature which addresses the concentration of AFM_1 in the crop. Previous acute studies with aflatoxin given at 0.2-0.6 mg/kg body weight to steers showed greater concentrations of AFM_1 in feces than AFB_1 (11). The presence of AFM_1 in the crop may be attributed to the fact that crops of gramivorous birds, chickens and other gallinacea are glandular in nature and the length of time the feed remain in the crop (12). Secretion of mucus occurs in the crop of chickens and amylase may be secreted as well. Amylase in the crop or on the crop mucosa may originate from the salivary gland, ingested food, bacteria in the crop, regurgitated duodenal contents or from the crop mucosa itself (13). Although little digestion occurs in the crop, it chief function is a storage organ (14). Bolton (1965) suggested that a significant amount of starch digestion occurred in the crop of chickens as a result of bacterial action. Pritchard (1972) found that sucrose digestion occurred in incubated crop contents treated with chloroform to kill the bacteria. The results suggested that non-bacterial digestion

of carbohydrates can occur in the crop (13). The length of time that the AFB_1 contaminated feed remain in the crop will allow the amylolytic enzymes and other enzymes in the crop to react with AFB_1. Hydroxylation of AFB_1 to AFM_1 in the crop is likely to occur as a result of presence of the enzymes, water consumed by the chickens and microorganisms in the crop and the aflatoxin-contaminated ration.

AFM_1 concentration in muscle of chickens fed low aflatoxin-contaminated corn ration (0.064 ng/g) was significantly lower than that in chickens fed high aflatoxin-contaminated corn plus 25% soil ration (0.113 ng/g) (Table 11). No detectable amount of AFM_1 was found in any muscle samples from the other groups of chickens. The results suggest that soil addition at 25% to high aflatoxin-contaminated corn ration had a negative effect in the reduction of AFM_1 in muscle of chickens.

Analysis of Total Protein

The results of determination of total protein by TS meter showed that total protein concentration in sera increased from day 21-28 in chickens fed clean corn, low aflatoxin-contaminated corn, high aflatoxin-contaminated corn plus 10% soil and high aflatoxin-contaminated corn plus 25% soil rations, but remained the same in chickens fed high aflatoxin-contaminated corn ration without soil. There was an increased

in total protein concentration from day 28-35 in chickens fed high aflatoxin-contaminated corn plus 25% soil ration and high aflatoxin-contaminated corn ration without soil whereas there was a decrease in total protein concentration in chickens fed clean corn, low aflatoxin-contaminated corn and high aflatoxin-contaminated corn plus 10% soil rations. Total protein concentration increased in all chickens from day 35-42. There was no substantial difference observed in the final total protein concentration between chickens fed aflatoxin-contaminated rations with or without soil and those fed clean corn (control) ration (Table 12).

Table 11. Retention and distribution of aflatoxins in liver, crop and muscle of White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

		Tissue Aflatoxin Concentrations[a] (ng/g)			
Rations		Liver	Crop	Muscle	Total
Clean Corn (control)					
	B_1	-	-	-	-
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-
Low AFB_1-Corn					
	B_1	0.005[c]	0.033[b]	-	0.038
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.064[c]	0.064
High AFB_1-Corn + 25% Soil	B_1	0.018[c]	0.019[b]	0.004[c]	0.041
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.113[b]	0.113
High AFB_1-Corn + 10% Soil	B_1	0.007[b]	0.211[b]	0.003[c]	0.221
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-
High AFB_1-Corn					
	B_1	1.294[b]	0.074[b]	0.014[b]	1.382
	B_2	0.028[b]	0.029[b]	0.002[b]	0.059
	G_2	-	-	-	-
	M_1	0.095[b]	0.569	-	0.663

[a]Determination of aflatoxin concentrations was done by high performance liquid chromatography. Values represent means of aflatoxin concentrations per group of five chickens. Tissue samples were pooled into four groups of five for analysis for each ration fed in this experiment

- = No aflatoxin was detected in sample

b,c Means of same toxin in the same column with the same superscript are not significantly different

Table 12. Total protein concentration in sera from White Leghorn chicken fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

	Total Protein Concentrations[a] (g/100 ml)			
	Days of Age			
Rations	21	28	35	42
Clean Corn (Control)	1.55	1.67	1.60	1.90
Low AFB_1-Corn	1.57	1.73	1.68	1.82
High AFB_1-Corn +25% Soil	1.60	1.63	1.69	1.90
High AFB_1-Corn + 10% Soil	1.58	1.65	1.62	2.06
High AFB_1-Corn	1.61	1.61	1.69	1.90

[a]Values represent means of total protein concentration per group of chickens corresponding to days of age. Ten chickens were used for each ration. Determination of protein concentration by TS meter (American Optical Corporation)

CONCLUSION

Silty clay loam soil addition to aflatoxin-contaminated rations reduced residual concentrations of AFB_1 in observed in chicken livers. No residual concentration of AFB_2 and AFM_1 was detected in any liver samples from chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil but was detected in livers from chickens fed high aflatoxin-contaminated corn ration without soil. This suggests that soil was effective in eliminating residual concentrations of AFB_2 and AFM_1 from livers of chickens fed high aflatoxin-contaminated corn rations amended with 10% and 25% soil.

In the crop, AFB_1 was reduced in chickens fed high aflatoxin-contaminated corn plus 25% soil ration but not in chickens fed high aflatoxin-contaminated corn plus 10% soil ration. AFB_2 concentration was not detected in crops of chickens fed high aflatoxin-contaminated corn ration amended with soil but was detected in chickens fed high aflatoxin-contaminated corn ration without soil. This suggests that soil added to high aflatoxin-contaminated corn at 25% was effective in reducing residual concentration AFB_1 and eliminating residual concentration of AFB_2 from crops of chickens fed high aflatoxin-contaminated corn rations amended with soil. AFM_1 was detected in crops and livers from chickens fed high aflatoxin-contaminated ration without soil, whereas no AFM_1 was

detected in crops from chickens fed aflatoxin-contaminated rations amended with soil. This suggests that soil prevented the retention of AFM_1 in the crops and livers of chickens fed high aflatoxin-contaminated rations.

The concentration of AFB_1 was reduced in muscle of chickens fed high aflatoxin-contaminated corn plus 10% and 25% soil, whereas no concentration of AFB_2 was detected. These results suggest that 10% and 25% soil were effective in reducing residual concentration of AFB_1 in muscle and also were effective in eliminating residual concentration of AFB_2 from muscle of chickens fed high aflatoxin-contaminated corn rations.

Soil addition at 25% to high aflatoxin-contaminated corn ration had a negative effect on reduction of AFM_1 concentration in muscle as detectable amount of AFM_1 was observed in chickens fed high aflatoxin-contaminated corn plus 25% soil whereas no detectable concentration of AFM_1 was seen in chickens fed high aflatoxin-contaminated corn ration without soil and high aflatoxin-contaminated corn plus 10% soil rations.

Silty clay loam soil addition to high aflatoxin-contaminated corn at 10% and 25% concentrations were effective in the reduction aflatoxin B_1 in liver and muscle tissue. The reduction of residual concentration of aflatoxins in tissues of chickens fed aflatoxin-contaminated corn rations amended with silty clay loam soil may have some impact on the

livestock industry, economics and human health.

Contaminated grains and feedstuffs which were usually disposed of could now be used as animal feed. Farmers and feed producers could now salvage grains and feedstuffs which should result in reduced economic loss to the livestock industry. The feeding of aflatoxin-contaminated rations amended with soil to animals should result in reduction of the detrimental effects that aflatoxin has on animal growth, production, reproduction and animal health. Animals should not suffer from weight loss, unthriftiness, stunting, poor performance and other secondary insults which can result from exposure to aflatoxin. This should result in reduced operation cost and improvement in profitability of the livestock industry.

Reduction of aflatoxicosis in animals should reduce effects that aflatoxin on animal reproductivity and productivity, and the residual concentration of aflatoxin in animal tissues. This should result in more wholesome meat and animal products entering the food chain, and at the same time, the possibility of exposure of humans to residual concentration of aflatoxin in animal tissue should be reduced. The reduced residual concentration of aflatoxin in animal tissues and the reduced possibility of exposure to aflatoxin residues should result in the reduction in the public concern of the detrimental effects of aflatoxicosis.

FUTURE WORK

1. Determination of the mechanism(s) of aflatoxin binding to silty clay loam soil.

2. Determination of the mechanism(s) of degradation of aflatoxin in silty clay loam soil-microbial vs chemical.

3. Isolation and identification of the microorganisms and enzymes chiefly responsible for aflatoxin degradation in soil.

4. Determination of the major metabolites resulting from aflatoxin degradation.

5. Perform field study to determine the feasibility of feeding aflatoxin-contaminated rations amended with silty clay loam soil to animals (eg. swine reared on soil floor vs swine reared on concrete floor).

6. Perform field study to investigate the effectiveness of using poplar trees and soil to prevent possible aflatoxin-contamination of private and municipal wells and detoxification of aflatoxin.

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GENERAL SUMMARY

The objectives of this study were 1) To evaluate the effectiveness of silty clay loam soil in the detoxification aflatoxin B_1 in chickens fed aflatoxin-contaminated rations amended with soil; 2) to evaluate the residual concentrations of aflatoxin B_1 and its metabolites retained in tissues of chickens (crop, liver and muscle) fed the soil amended aflatoxin-contaminated rations; 3) to evaluate gross and histopathological changes observed in crops and livers of chickens fed the soil amended aflatoxin-contaminated rations and assess how silty clay loam soil influenced observed changes; 4) to evaluate total protein levels in sera collected from chickens fed the soil amended aflatoxin-contaminated rations and assess how protein levels are influenced by silty clay loam soil; 5) to assess how silty clay loam soil would influence the detrimental effects of aflatoxicosis on animal productivity and; 6) to assess the risk of exposure of humans to aflatoxin, because aflatoxin can cause serious health problems to humans.

1. Silty clay loam will retain aflatoxins thereby preventing leaching into water supply.

2. Contaminated commodities covered with 50% silty clay loam or greater will prevent aflatoxins from leaching into groundwater.

3. Aflatoxin B_1 did not remain in soil from corn field or soybean field after three months, it was degraded to less toxic compounds and was not detected in any soil from the tubs or water samples taken from mini-wells.

4. If aflatoxin-contaminated land is allowed enough time, degradation of aflatoxin in soil should occur.

5. Proper management practices of plowing aflatoxin-contaminated commodities under soil should not affect water systems since aflatoxin can be retained and degraded to less toxic compounds in the soil.

6. Silty clay loam addition to high aflatoxin B_1-contaminated rations at 10% and 25% reduced and prevented retention of residual concentrations of aflatoxin B_1 and its metabolites in chicken livers, crop and muscle. This should result in reduction of aflatoxicosis in chickens and at the same time the risk of exposure of humans to high residual aflatoxin concentration in tissues of chickens consuming high aflatoxin-contaminated feeds.

7. Reduction of aflatoxin in chickens should reduce detrimental effects on reproductivity and productivity which should reduce the economic loss which can result from aflatoxicosis.

8. Reduction of residual concentration of aflatoxin in tissues of chickens will reduce the risk of transmission of detrimental effects to humans.

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APPENDIX A

BODY WEIGHTS OF WHITE LEGHORN CHICKENS FED AFLATOXIN-CONTAMINATED RATIONS AMENDED WITH SOIL FOR 9 DAYS

Group 1. < 5 ng/g Aflatoxin-contaminated corn

No. of Birds	Bird No.	Initial Weight(g)	Final Weight(g)
1	670-9	22.0	29.5
2	670-10	27.0	28.5
3	670-11	23.5	26.5
4	670-12	22.0	29.7
5	670-13	23.2	25.7
6	670-14	24.0	22.5
7	670-15	23.5	25.2
8	670-16	25.3	30.0
9	670-17	25.2	23.2
10	670-18	24.2	24.9
11	670-58	23.0	25.8
12	670-20	22.0	25.5
Total		284.9	317.04
Average		23.7	26.4

Group 2. Low aflatoxin-contaminated corn

No. of Birds	Bird No.	Initial Weight(g)	Final Weight(g)
1	670-21	22.0	27.3
2	670-22	26.5	28.5
3	670-23	24.5	29.7
4	670-24	24.0	27.8
5	670-25	24.5	33.0
6	670-26	24.2	28.2
7	670-27	20.0	28.0
8	670-28	23.0	27.5
9	670-29	22.0	D
10	670-30	24.5	27.8
11	670-31	25.5	31.8
12	670-68	24.7	27.0
Total		289.9	317.6
Average		24.2	28.9

D means bird died during experiment

Group 3- High aflatoxin-contaminated corn + 50% soil

No. of Birds	Bird No.	Initial Weight(g)	Final Weight(g)
1	670-33	21.5	D
2	670-34	22.5	22.9
3	670-35	23.5	26.6
4	670-36	22.7	25.5
5	670-37	25.5	D
6	670-38	23.5	D
7	670-39	26.8	26.5
8	670-40	25.8	26.0
9	670-41	28.2	30.3
10	670-42	25.5	D
11	670-43	22.7	D
12	670-44	23.0	D
Total		291.2	157.8
Average		24.3	26.3

D means bird died during experiment

Group 4. High aflatoxin-contaminated corn + 25% soil

No. of Birds	Bird No.	Initial Weight(g)	Final Weight(g)
1	670-45	17.5	25.5
2	670-46	23.0	28.5
3	670-47	23.5	26.0
4	670-48	24.5	32.0
5	670-49	23.5	31.7
6	670-50	25.0	26.2
7	670-51	23.5	22.5
8	670-52	24.0	30.0
9	670-53	24.2	29.5
10	670-54	25.2	32.5
11	670-55	23.5	26.8
12	670-56	23.7	27.2
Total		281.1	338.4
Average		23.4	28.2

Group 5. High aflatoxin-contaminated corn

No. of Birds	Bird No.	Initial Weight(g)	Final Weight(g)
1	670-57	27.5	32.0
2	670-59	23.0	27.0
3	670-60	22.3	26.9
4	670-61	24.5	30.0
5	670-62	21.8	22.8
6	670-63	24.1	27.9
7	670-64	24.0	22.7
8	670-65	20.3	23.3
9	670-66	24.8	D
10	670-67	21.5	22.0
11	670-69	24.0	25.8
12	670-70	22.5	D
Total		280.5	260.4
Average		23.4	26.0

D means bird died during experiment

Body weight gain in White Leghorn chickens fed aflatoxin-contaminated rations amended with soil for 9 days feeding study.

Treatment Groups	Mean Final Weight (g)	Mean Initial Weight (g)	Mean Gain Weight(g)
1. Clean corn	26.42	23.74	2.68
2. Low aflatoxin corn	28.87	24.16	4.71
3. High aflatoxin corn + 50% soil	26.30	24.27	2.03
4. High aflatoxin corn + 25% soil	28.20	23.43	4.77
5. High aflatoxin corn	26.04	23.35	2.60

APPENDIX B

BODY WEIGHTS OF WHITE LEGHORN CHICKENS FED A COMMERCIAL DIET FOLLOWED BY AFLATOXIN-CONTAMINATED RATIONS FOR 28 DAYS

Group 1. Clean Corn-Negative Control Body weight (g)

		Days of Age						
No. of Birds	Bird No.	1	7	14	21	28	35	42
1.	1796	28	44	75	102	161	169	160
2.	3625	34	67	94	140	151	167	175
3.	1761	33	61	111	162	163	183	209
4.	1777	39	85	146	182	209	235	265
5.	3650	31	71	123	155	167	187	205
6.	1773	31	67	106	142	159	185	210
7.	3676	40	76	124	173	192	214	230
8.	3627	32	63	115	152	167	181	262
9.	1793	50	76	137	183	193	207	248
10.	1770	33	75	129	169	194	206	222
11.	3666	37	66	117	153	149	152	174
12.	3671	36	71	129	171	192	210	215
13.	1790	32	62	108	143	161	177	217
14.	1781	36	87	150	241	271	269	310
15.	3645	32	80	140	174	186	211	222
16.	3656	33	57	96	130	153	179	212
17.	3609	33	49	86	104	111	117	222
18.	1776	38	73	111	134	145	149	175
19.	3624	37	75	131	172	190	206	235
20.	1791	33	71	122	166	185	214	222
Total		698	1376	2350	3148	3499	3818	4390
Average		34.9	68.8	117.5	157.4	175.0	190.9	219.5

Group 2. Low AFB_1-Corn — Body weight (g)

		Days of Age						
No. of Birds	Bird No.	1	7	14	21	28	35	42
1.	3603	37	70	125	154	144	157	176
2.	1789	32	77	124	142	142	141	152
3.	1775	34	83	132	178	171	188	201
4.	1782	32	77	132	153	144	153	158
5.	1794	32	75	127	165	164	180	200
6.	3644	34	75	127	159	146	171	175
7.	3605	26	49	87	105	106	113	126
8.	3699	35	93	154	196	185	196	188
9.	3668	36	76	137	179	162	176	188
10.	1788	39	89	142	182	170	182	194
11.	3657	37	75	135	160	148	166	184
12.	3633	31	62	94	114	108	126	126
13.	3630	32	72	122	150	140	143	161
14.	3638	40	84	141	161	146	161	182
15.	3694	37	86	141	168	168	196	232
16.	3655	37	74	127	156	153	172	195
17.	3663	37	73	124	162	142	164	171
18.	3620	29	58	101	115	106	112	108
19.	3628	33	74	131	156	141	162	164
20.	1767	43	84	141	170	161	179	189
Total		683	1506	2544	3125	2947	3238	3470
Average		34.7	75.3	127.2	156.3	147.4	161.9	173.5

Group 3. High AFB_1-Corn + 25% Soil Body weight (g)

		Days of Age						
No. of Birds	Bird No.	1	7	14	21	28	35	42
1	1764	36	85	137	151	142	142	150
2	3607	36	57	95	106	99	99	103
3	3651	33	57	97	113	108	111	124
4	3621	37	76	136	139	134	128	132
5	3665	37	85	140	166	152	152	159
6	3677	35	81	125	162	147	156	168
7	3623	29	69	114	135	134	143	146
8	1786	32	82	136	155	144	146	150
9	1787	33	78	127	142	136	135	150
10	3696	36	81	129	144	136	138	132
11	3667	36	79	129	152	139	136	146
12	1799	31	80	133	149	139	143	146
13	3642	34	76	115	129	122	120	125
14	3622	35	77	122	142	138	142	150
15	3639	40	81	135	160	156	166	174
16	1779	35	71	115	133	131	135	148
17	1762	34	70	109	129	111	112	102
18	3697	39	83	131	149	150	149	166
19	3670	35	77	119	155	124	127	137
20	1778	26	62	117	131	122	128	137
Total		689	1507	2461	2842	2664	2708	2935
Average		34.4	75.4	123.1	142.1	133.2	135.4	146.

Group 4. High AFB_1-Corn + 10% Soil Body weight (g)

		Days of Age						
No. of Birds	Bird No.	1	7	14	21	28	35	42
1	3687	30	58	103	130	129	136	146
2	1772	36	66	110	130	124	135	140
3	1783	36	78	95	157	152	158	161
4	3700	33	65	102	117	119	124	129
5	3619	32	67	110	128	128	132	135
6	3649	32	64	120	139	138	147	148
7	3629	39	78	132	156	154	164	166
8	3674	41	86	150	174	168	128	187
9	1763	31	74	129	150	145	148	148
10	3613	42	79	143	169	170	186	199
11	3695	32	73	125	136	133	144	160
12	1798	36	63	105	122	126	135	142
13	3698	28	44	68	84	81	87	91
14	1765	30	65	116	133	130	135	141
15	3662	32	63	103	118	114	124	128
16	3653	35	82	94	155	148	150	156
17	3660	37	73	122	146	140	138	157
18	3654	27	62	98	116	119	126	131
19	3675	44	70	114	124	123	133	145
20	3612	39	74	115	132	126	136	135
Total		692	1384	2254	2716	2667	2766	2945
Average		34.6	69.2	112.7	135.8	133.4	138.3	147.3

Group 5. High AFB_1-Corn Body weight (g)

		Days of Age						
No. of Birds	Bird No.	1	7	14	21	28	35	42
1	1792	33	76	132	154	157	161	180
2	1774	36	76	125	142	131	126	126
3	3616	30	65	112	131	137	138	146
4	3672	40	74	123	131	122	128	136
5	3635	37	73	115	125	128	128	140
6	3652	39	72	105	131	122	117	106
7	3673	45	88	148	170	170	176	189
8	3631	38	77	123	152	152	159	167
9	1769	32	71	113	131	134	142	153
10	1780	38	81	129	147	150	158	163
11	3611	29	58	91	107	105	109	113
12	3615	35	65	117	143	142	149	159
13	3608	41	79	127	153	155	162	167
14	3669	37	78	126	144	146	152	160
15	3658	31	70	118	138	143	152	161
16	3641	35	78	136	159	153	171	177
17	1795	33	76	129	150	152	156	161
18	3610	32	73	122	147	146	146	135
19	3604	39	85	146	164	153	156	160
20	3643	29	80	115	136	138	140	148
Total		699	1495	2452	2855	2836	2926	3047
Average		35.0	74.8	122.6	142.8	141.8	146.3	152.4

APPENDIX C

ORGAN WEIGHTS TAKEN AT NECROPSY FROM WHITE LEGHORN CHICKENS FED A COMMERCIAL DIET FOLLOWED BY AFLATOXIN-CONTAMINATED RATIONS AMENDED WITH SOIL FOR 28 DAYS

Group 1. Clean Corn		Organ Weights (g)	
No. of Birds	Bird No.	Liver	Crop
1.	1796	4.0	0.9
2.	3625	5.4	1.8
3.	1762	7.0	1.8
4.	1777	6.6	1.4
5.	3650	6.2	1.7
6.	1773	4.8	1.1
7.	3676	5.2	2.0
8.	3627	4.3	1.9
9.	1793	5.8	1.5
10.	1770	4.6	1.0
11.	3666	5.0	1.3
12.	3671	5.7	1.6
13.	1790	6.5	1.5
14.	1781	8.5	1.6
15.	3645	6.5	1.8
16.	3656	5.8	2.1
17.	3609	3.6	1.0
18.	1776	4.5	0.8
19.	3624	5.2	1.0
20.	1791	7.0	1.6
Total		122.2	29.4
Average		5.6	1.5

Group 2. Low Aflatoxin B_1-Corn		Organ Weights (g)	
No. of Birds.	Bird No.	Liver	Crop
1.	3603	5.3	1.1
2.	1789	4.6	1.7
3.	1775	5.6	1.5
4.	1782	3.8	1.1
5.	1794	5.4	1.4
6.	3644	5.4	1.8
7.	3605	4.2	0.9
8.	3699	5.6	1.8
9.	3668	4.9	1.3
10.	1788	5.3	1.9
11.	3657	5.8	1.2
12.	3633	4.2	1.2
13.	3630	4.1	1.2
14.	3638	3.7	1.2
15.	3694	7.0	1.5
16.	3655	4.7	1.4
17.	3663	4.3	1.4
18.	3620	4.4	0.8
19.	3628	5.8	1.6
20.	1767	6.0	1.4
Total		100.1	27.4
Average		5.0	1.4

Group 3. High AFB_1-Corn + 25% Soil — Organ Weights (g)

No. of Birds	Bird No.	Liver	Crop
1.	1764	3.9	0.7
2.	3607	2.6	0.7
3.	3651	3.8	0.8
4.	3621	4.2	1.0
5.	3665	3.1	1.0
6.	3677	4.1	1.4
7.	3623	4.1	1.5
8.	1786	4.4	1.1
9.	1787	4.1	1.1
10.	3696	3.9	1.3
11.	3667	5.0	1.5
12.	1799	3.2	1.0
13.	3642	3.0	1.1
14.	3622	3.6	1.1
15.	3639	4.8	1.4
16.	1779	3.4	1.2
17.	1762	3.6	1.5
18.	3697	5.5	1.0
19.	3670	4.2	1.1
20.	1778	3.7	1.0
Total		78.2	28.8
Average		3.9	1.4

Group 4. High AFB_1-Corn + 10% Soil		Organ Weights (g)	
No. of Birds	Bird No.	Liver	Crop
1.	3687	4.7	1.2
2.	1772	4.2	1.0
3.	1783	5.1	1.3
4.	3700	4.3	0.9
5.	3619	3.8	1.0
6.	3649	5.3	1.5
7.	3629	8.8	0.9
8.	3674	6.2	1.1
9.	1763	4.2	0.7
10.	3613	7.4	0.7
11.	3695	4.8	1.2
12.	1798	4.0	1.0
13.	3698	4.3	0.6
14.	1765	4.4	1.3
15.	3662	4.0	1.0
16.	3653	4.3	1.3
17.	3660	5.3	1.0
18.	3654	4.1	1.0
19.	3675	5.0	1.0
20.	3612	4.6	1.2
Total		98.8	27.2
Average		4.9	1.4

Group 5. High AFB_1-Corn-Positive Control Organ Weights (g)

No. of Birds	Bird No.	Liver	Crop
1.	1792	5.8	1.0
2.	1774	8.8	0.9
3.	3616	4.6	1.1
4.	3672	3.5	1.1
5.	3635	5.0	1.0
6.	3652	5.3	1.0
7.	3673	4.7	1.1
8.	3631	8.5	1.4
9.	1769	5.9	1.1
10.	1780	5.0	1.0
11.	3611	4.6	0.8
12.	3615	4.6	1.1
13.	3608	5.2	1.0
14.	3669	6.6	1.0
15.	3658	14.8	0.7
16.	3641	5.7	0.9
17.	1795	7.4	1.2
18.	3610	4.6	0.6
19.	3604	4.7	0.8
20.	3643	4.6	1.0
Total		119.9	19.8
Average		6.0	1.0

APPENDIX D

TOXICOLOGY DATA FROM WHITE LEGHORN CHICKENS FED AFLATOXIN-CONTAMINATED RATIONS FOR 9 DAYS AND WHITE LEGHORN CHICKENS FED A COMMERCIAL DIET FOLLOWED BY AFLATOXIN-CONTAMINATED RATIONS FOR 28 DAYS

Liver weights of White Leghorn chickens fed aflatoxin-contaminated rations amended with soil for 9 days study.

Rations	No. of Livers	Weight(g)
1. Clean corn(control)	9	5.3
2. Low aflatoxin corn	11	8.1
3. High aflatoxin corn + 50% soil	12	6.3
4. High aflatoxin corn +25% soil	12	7.0
5. High aflatoxin corn	7	4.3

Tissue weights of White Leghorn chickens fed commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

Group 1. Clean Corn-Negative Control		Tissue Weights (g)		
No. of Birds	Bird No.	Liver	Crop	Muscle
1.	1796	3.7	0.8	13.6
2.	3625	4.9	1.7	11.9
3.	1761	6.4	1.4	17.2
4.	1777	6.0	1.3	20.7
5.	3650	5.8	1.6	11.4
6.	1773	4.3	1.0	18.9
7.	3676	4.5	1.3	18.5
8.	3627	3.9	1.5	19.6
9.	1793	5.2	1.3	16.0
10.	1770	4.0	0.9	21.2
11.	3666	4.4	1.0	11.8
12.	3671	5.6	1.4	17.1
13.	1790	5.9	1.2	16.7
14.	1781	7.2	1.5	20.2
15.	3645	6.6	1.4	14.4
16.	3656	5.1	1.8	15.8
17.	3609	3.0	0.8	5.8
18.	1776	4.4	0.8	15.9
19.	3624	4.7	1.0	17.3
20.	1791	6.2	1.4	19.0
Total		101.8	25.1	389.7
Average		5.1	1.26	15.6

Group 2. Low Aflatoxin B_1-Corn — Tissue Weights (g)

No. of Birds	Bird No.	Liver	Crop	Muscle
1.	3603	4.6	1.1	9.4
2.	1789	4.0	1.5	12.5
3.	1775	5.2	1.3	12.6
4.	1782	3.5	1.1	10.5
5.	1794	4.9	1.1	12.6
6.	3644	4.2	1.7	9.6
7.	3605	3.6	0.9	10.8
8.	3699	4.7	1.5	11.8
9.	3668	4.2	1.0	14.0
10.	1788	4.6	1.8	15.8
11.	3657	5.0	1.2	10.0
12.	3633	3.8	1.0	8.2
13.	3630	3.3	1.0	10.4
14.	3638	3.3	1.0	11.0
15.	3694	5.6	1.5	13.4
16.	3655	4.5	1.5	12.6
17.	3663	3.8	1.5	11.0
18.	3620	4.0	0.8	5.1
19.	3628	4.8	1.1	10.5
20.	1767	5.6	1.4	13.2
Total		87.2	28.9	260.6
Average		4.4	1.2	10.4

Group 3. High AFB_1-Corn + 25% Soil Tissue Weights (g)

No. of Birds	Bird No.	Liver	Crop	Muscle
1.	1764	3.2	0.6	6.2
2.	3607	2.2	0.6	4.6
3.	3651	3.2	0.7	5.3
4.	3621	3.2	0.9	5.8
5.	3665	2.8	0.8	10.7
6.	3667	3.0	1.2	11.3
7.	3623	3.9	1.1	8.4
8.	1786	4.3	0.8	7.6
9.	1787	3.3	1.0	5.7
10.	3696	3.4	1.1	7.8
11.	3667	4.2	1.2	7.0
12.	1799	3.0	0.9	13.2
13.	3642	2.6	0.8	5.6
14.	3622	3.2	1.0	10.3
15.	3639	4.0	1.3	10.7
16.	1779	3.0	1.0	7.1
17.	1762	2.9	1.4	4.6
18.	3697	4.8	0.8	11.1
19.	3670	3.4	1.0	6.8
20.	1778	3.3	1.0	10.5
Total		69.9	19.2	193.3
Average		3.4	0.96	7.7

Group 4. High AFB_1-Corn + 10% Soil Tissue Weights (g)

No. of Birds.	Bird No.	Liver	Crop	Muscle
1.	3687	4.2	1.3	5.6
2.	1772	3.8	0.9	9.8
3.	1783	4.7	0.9	12.7
4.	3700	3.6	0.8	6.2
5.	3619	3.4	0.7	8.4
6.	3649	4.5	1.5	7.1
7.	3629	8.0	0.7	8.2
8.	3674	5.4	0.9	10.0
9.	1763	3.6	0.5	8.4
10.	3613	6.5	1.8	9.8
11.	3695	3.8	1.2	9.0
12.	1798	3.4	0.9	8.3
13.	3648	2.5	0.8	4.1
14.	3698	3.5	0.6	2.4
15.	3662	3.4	0.7	8.6
16.	3653	3.8	1.1	8.7
17.	3660	4.8	0.9	9.2
18.	3654	3.4	1.1	8.7
19.	3675	4.6	0.9	7.8
20.	3612	4.1	1.1	7.8
Total		85.0	19.3	170.6
Average		4.3	0.97	8.1

Group 5. High AFB_1-Corn-Positive Control Tissue Weights (g)

No. of Birds.	Bird No.	Liver	Crop	Muscle
1.	1792	5.4	0.8	10.0
2.	1774	8.2	0.7	3.2
3.	3616	4.3	0.8	8.8
4.	3672	3.0	1.2	7.8
5.	3635	4.4	0.8	8.3
6.	3652	4.9	1.0	5.8
7.	3673	4.0	1.0	12.7
8.	3631	7.8	1.2	7.9
9.	1769	5.1	0.9	8.2
10.	1780	4.4	0.7	7.9
11.	3611	4.0	0.7	7.2
12.	3615	4.2	0.8	9.4
13.	3608	4.6	0.8	10.1
14.	3669	6.0	0.8	14.2
15.	3658	13.7	0.7	6.4
16.	3641	4.6	0.8	11.2
17.	1795	6.8	1.0	10.1
18.	3610	3.9	0.7	7.4
19.	3604	3.9	0.6	10.2
20.	3643	4.2	0.8	11.7
Total		107.4	16.8	183.8
Average		5.4	0.84	8.8

Concentrations of aflatoxins in liver, crop and muscle from White Leghorn chickens fed a commercial diet followed by aflatoxin-contaminated rations amended with soil for 28 days

Group 2.		Tissue Aflatoxin Concentrations[a]			
Low AFB_1 Corn		Liver ng/g	Crop ng/g	Muscle ng/g	Total
2.1					
	B_1	0.005	0.031	-	0.036
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.047	0.047
2.2					
	B_1	0.006	0.052	-	0.058
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.048	0.048
2.3					
	B_1	0.005	0.026	-	0.031
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.095	0.095
2.4					
	B_1	0.005	0.023	-	0.028
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.064	0.064

[a]Determination of Aflatoxin concentrations was performed by HPLC.

Group 3.		Tissue Aflatoxin Concentrations[a]			
High AFB_1-Corn + 25% Soil		Liver ng/g	Crop ng/g	Muscle ng/g	Total
3.1					
	B_1	0.016	0.042	0.007	0.065
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.153	0.153
3.2					
	B_1	0.044	0.011	0.003	0.058
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.086	0.086
3.3					
	B_1	0.006	0.011	0.003	0.020
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.112	0.112
3.4					
	B_1	0.006	0.013	0.003	0.022
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	0.100	0.100

[a]Determination of Aflatoxin concentrations was performed by HPLC.

Group 4.		Tissue Aflatoxin Concentrations[a]			
High AFB_1-Corn + 10% Soil		Liver ng/g	Crop ng/g	Muscle ng/g	Total
4.1					
	B_1	0.006	0.013	0.003	0.022
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-
4.2					
	B_1	0.009	0.775	0.003	0.787
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-
4.3					
	B_1	0.007	0.027	0.003	0.037
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-
4.4					
	B_1	0.005	0.029	0.003	0.087
	B_2	-	-	-	-
	G_2	-	-	-	-
	M_1	-	-	-	-

[a]Determination of Aflatoxin concentrations was performed by HPLC.

Group 5.		Tissue Aflatoxin Concentrations[a]			
High FB_1-Corn		Liver ng/g	Crop ng/g	Muscle ng/g	Total
5.1					
	B_1	0.054	0.032	0.033	0.119
	B_2	0.002	0.016	-	0.018
	G_2	-	-	-	-
	M_1	0.052	0.825	-	0.877
5.2					
	B_1	1.508	0.142	0.003	1.655
	B_2	0.027	0.053	-	0.080
	G_2	-	-	-	-
	M_1	0.088	0.319	-	0.407
5.3					
	B_1	1.599	0.046	0.013	1.659
	B_2	0.037	0.016	0.005	0.058
	G_2	-	-	-	-
	M_1	0.101	0.953	-	1.054
5.4					
	B_1	2.013	0.077	0.007	2.085
	B_2	0.047	0.030	0.002	0.079
	G_2	-	-	-	-
	M_1	0.140	0.179	-	0.319

[a]Determination of Aflatoxin concentration was performed by HPLC.