SYMPOSIUM: OILSEED PROCESSORS CHALLENGED BY WORLD PROTEIN NEED

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Aflatoxin Effects in Livestock[1]

A.C. KEYL and **A.N. BOOTH**, Western Regional Research Laboratory,[2] Albany, California 94710

ABSTRACT

Feeding trials were conducted with swine, beef cattle, dairy cattle and poultry to determine adverse effects, if any, of graded levels of aflatoxins in rations. In addition, samples of meat, eggs and milk from these animals were analyzed chemically to determine if aflatoxin was transmitted into these products. In growing-fattening swine, no evidence of toxic effects was observed when the aflatoxin level fed was 233 ppb or less. In a swine reproduction experiment, no adverse effects were detected in pigs produced from sows fed 450 ppb aflatoxin. No toxic effects were observed at levels of 300 ppb or lower in cross-bred beef steers fed aflatoxin rations for 4.5 months. Using recognized chemical methods, we detected no aflatoxin in meat from swine and cattle fed rations containing 800 and 1000 ppb of aflatoxin, respectively. In dairy cows, weekly intakes of 67 to 200 mg of aflatoxin B_1 per cow produced 70 to 154 ppb aflatoxin M_1 in lyophilized milk. Rapid disappearance of aflatoxin M_1 in the milk took place after withdrawal of aflatoxin from the ration. No adverse effects were discernible in broilers fed from one day to eight weeks of age a ration containing 400 ppb aflatoxin. Lyophilized meat from broilers fed 1600 ppb aflatoxin for eight weeks contained no detectable aflatoxin. Striking differences in aflatoxin susceptibility were observed in 17 different breeds and strains of poultry and game birds fed from two to six weeks of age a ration of 800 ppb aflatoxin B_1. New Hampshire chicks and turkey poults were highly susceptible to aflatoxin in contrast to the resistance of Barred Rock and Australop chickens and guinea fowl. Hybrid chicks from a New Hampshire-White Leghorn cross were highly resistant to aflatoxin. Eggs and meat from White Leghorn hens fed a ration containing 2700 ppb aflatoxin contained no detectable aflatoxin.

INTRODUCTION

With the recognition of the mycotoxin aflatoxin as a serious threat to the world feed and food supplies, the need for information on the possible transmission of aflatoxin into meat, milk and eggs was given a high priority by the Agricultural Research Service of USDA. Summarized in this

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[2]W. Utiliz. Res. Dev. Div., ARS, USDA.

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TABLE I

Effect of Aflatoxin on Rate of Gain and Feed Conversion in Swine

Meal	Aflatoxin content of ration, ppb B_1	B_2	G_1	Avg. daily gain,[a] kg	Gain/feed, kg
Trial 1					
Soybean	ND	ND[b]	ND[b]	0.58	.24
Peanut	<2	ND	ND	0.67	.24
Peanut	<8	ND	ND	0.69	.25
Peanut	51	ND	ND	0.65	.25
Peanut	105	52	15	0.62	.26
Peanut	233	ND	70	0.62	.24
Trial 2					
Soybean	ND	ND	ND	0.75[c]	.26
Peanut	<6	ND	ND	0.71[c,d]	.26
Peanut	450	ND	30	0.68[c,d]	.27
Peanut	615	105	45	0.60[d]	.27
Peanut	810	135	60	0.47[e]	.23

[a]Values with unlike superscript letters are significantly different ($P < .05$).

[b]ND, none detected.

report are the results of investigations carried out over a four year period on the effects of aflatoxin in growing, fattening and reproduction of swine, in fattening of beef steers, in dairy cattle, in poultry broilers and laying hens. Under simulated practical conditions, these animals were fed graded levels of aflatoxin (a) to establish "no-effect" levels where appropriate, and (b) to obtain samples of meat, milk and eggs to analyze for evidence of transmission of aflatoxin into these products from the rations fed. To achieve these objectives the cooperative efforts of several groups of specialists were required. Experts at the Northern Regional Research Laboratory in Peoria, Illinois, produced the large quantities of aflatoxin needed, by growing the *Aspergillus flavus* mold on a rice substrate. Peanut and cottonseed meals were then fortified and blended with the rice substrate containing aflatoxin at the Southern Regional Research Laboratory in New Orleans. The meals were then formulated into rations and fed to the animals on the Davis campus of the University of California. Lastly, all blood and tissue analyses were carried out by personnel at the Western Regional Research Laboratory, Albany, California.

EXPERIMENTAL PROCEDURES

The parameters used for the detection of aflatoxin effects in animals were as follows: (a) Growth, mortality and feed consumption. (b) Hematology including hemoglobin, hematocrit, and red and white cell counts. (c) Selected biochemical analyses were run primarily on blood and liver samples. (d) Pathology included both macro and microscopic examinations of appropriate tissues, as well as organ weights. (e) Eggs, meat and milk samples were analyzed chemically for aflatoxin residues by currently accepted methods.

RESULTS AND DISCUSSION

Fattening Swine

Two separate trials were run involving a total of 110 weanling pigs of both sexes. Ten animals per group were fed dietary levels of aflatoxin ranging from 0 to 810 ppb for a period of 120 days (1). For up to and including dietary levels of 233 ppb, the results were negative in that there were no significant differences between control and treated groups with respect to mean weight gains and feed efficiency values (Table I). Three biochemical values were significantly different from those of the control at aflatoxin levels of 51 ppb or higher, namely increased serum alkaline phosphatase and decreased nonprotein and urea nitrogen (2). No microscopic lesions were detected in the livers of the group fed 233 ppb (3).

The virtual absence of positive effects in the first trial dictated a repeat study at higher levels of aflatoxin intake, namely from 450 to 810 ppb. In trial 2 one pig died on the highest level, owing to severe liver degeneration. Decreased weight gains were observed at the two highest levels and a drop in feed efficiency was noted at the 810 ppb level (1). At autopsy, after 117 days of the feeding trial, organ weights were recorded (Table II). Liver weights at the 450 ppb level or higher and kidney weights of the group fed 810 ppb aflatoxin were significantly increased.

Microscopic lesions were detected in only 3 of the 10 livers examined from the group fed 450 ppb aflatoxin, whereas most livers from pigs fed the two highest dosages were abnormal, and the lesions were more severe (3). These included karyomegaly, cytoplasmic degeneration, and proliferation of fibrous tissue and bile ductule epithelium. The biochemical analyses, as expected, were the most sensitive indicators of aflatoxin effects. In the blood, increased alkaline phosphatase and decreased nonprotein and urea nitrogen were detected in the groups fed aflatoxin levels of 51 ppb or higher. Liver alkaline phosphatase and lipid contents were significantly elevated above the control values at the 233 ppb level.

Blood and tissues (lean meat, fat, spleen, liver and kidneys) were collected at slaughter, lyophilized and subjected to chemical assay for aflatoxin. All tissues including blood were found to be free of aflatoxin residues.

Excluding the positive biochemical effects noted, the no-effect level of aflatoxin B_1 intake for swine is tentatively judged to be between 233 and 450 ppb.

Swine Reproduction

Five male and five female pigs were fed a ration containing 450 ppb aflatoxin B_1 continuously during growth, pregnancy and lactation (4). Spermatogenesis was normal in the aflatoxin fed boars, and the five gilts all cast normal litters. Lactation was also normal resulting in piglets

TABLE II

Organ Weights of Swine Fed Aflatoxin for 120 Days

Aflatoxin B_1 ration, ppb	Liver, g[a]	Kidney, g	Spleen, g	Heart, g	Adrenals, g	Thyroid, g
0	714 ± 22	134 ± 4	42	142	1.69	3.1
5.5	749 ± 24	118 ± 5	44	139	1.99	3.06
450	917 ± 41[b]	133 ± 5	51	153	2.0	3.45
615	970 ± 40[b]	136 ± 5	48	153	2.06	3.20
810	1067 ± 39[b]	168 ± 20[b]	58	141	2.26	3.61

[a]Grams per 100 lb. body weight.

[b]$P < 0.01$

TABLE III

Reproductive Performance of Gilts Fed 450 ppb Aflatoxin B_1

Treatment	No. of gilts	Avg. No. of pigs farrowed Alive	Dead	Avg. litter wt. at birth, kg, live pigs	Avg. No. weaned at 42 days	Avg. litter wt. at weaning, kg
Aflatoxin	5	8.6	0.6	10.9	6.8	63.5
Control	9	8.2	0.2	10.8	7.3	68.2

TABLE IV

Weight Gains and Feed Utilization of Cattle Fed Cottonseed Meal Containing Graded Levels of Aflatoxin B_1 for 133 Days

Item	Aflatoxin B_1 in the ration, ppb Control	100	300	700	1000
Nol of animals	10	10	10	10	9[a]
Initial weight, lb	401	427	417	406	433
Daily gain, lb	2.51[b]	2.63[b]	2.40[b]	1.90[c]	1.76[c]
Daily feed consumption,[d] lb/100 lb body weight	2.61[b]	2.66[b]	2.59[b]	2.27[c]	2.07[c]
Gain/feed, lb	0.175	0.164	0.159	0.155[e]	0.152[e]

[a]One steer died on the 59th day.

[b,c]Comparable means with a different letter superscript are significantly different at the 1% level.

[d]Dry matter.

[e]Eight steers from each of these groups were confined in metabolism cages for a one-week period.

comparable in size to the offspring from control sows (Table III).

The boars were autopsied after 8 months on the aflatoxin ration, and the sows after 11 months. Histological examination of liver (and other tissues) revealed only minimal lesions in the livers of the sows and questionable hepatocyte hypertrophy in the livers from the boars. The pigs produced from the sows fed aflatoxin had no detectable liver lesions at six weeks of age.

Serum albumin, blood urea nitrogen and liver isocitric dehydrogenase were lowered and plasma isocitric dehydrogenase was increased in the blood from sows fed aflatoxin. Similar effects also prevailed in the boars, but to a lesser degree (3).

From these results it was concluded that rations containing up to 450 ppb aflatoxin would impose no adverse economic threats to swine breeding stock.

Beef Cattle

In the beef cattle feeding trial, 50 young cross-bred steers, divided into groups of 10, were fed rations containing from 0 to 1000 ppb aflatoxin B_1 (5).

Two steers fed the 1000 ppb level died, one on the 59th day and one on the 137th day of the feeding trial. Weight gains were significantly lower when the level of aflatoxin in the ration was 700 ppb or higher, and the feed efficiency values decreased as the level of aflatoxin fed increased (Table IV).

No adverse effects related to aflatoxin intake could be detected from the hematocrit, hemoglobin or red and white blood cell counts. Blood cholesterol levels were also unaffected by the ingestion of aflatoxin.

The organ weights per 100 lb body weight are tabulated in Table V. Significantly increased liver and kidney weights were observed for the two highest levels of aflatoxin intake. Livers from 3 of 8 steers fed 1000 ppb and 2 of 10 steers fed 700 ppb were grossly abnormal being greyish in color, enlarged and having a fibrous rubbery texture.

The serum enzymes, alkaline phosphatase and malic dehydrogenase, increased in activity in response to aflatoxin, while the level of lactic dehydrogenase decreased (6). Blood urea nitrogen, serum albumin and total serum protein all decreased.Glutamic-oxaloacetic transaminase and isocitric dehydrogenase values were not affected. Liver glutamic-oxaloacetic transaminase, malic and isocitric dehydrogenases all became depressed in response to aflatoxin, even at the 300 ppb level. Liver lipid and vitamin A values decreased at the 700 and 1000 ppb levels, whereas alkaline phosphatase, lactic dehydrogenase and glycogen values were unaffected at all levels of aflatoxin intake.

The microscopic findings in the cattle tissues were less definitive than those found in swine fed aflatoxin. In

TABLE V

Effect of Aflatoxin B_1 on Organ Weights of Beef Cattle

Ration[a] Aflatoxin B_1, ppb	Mean organ wt., g/100 lb body weight Liver ± S.E.	Kidneys ± S.E.	Spleen	Heart	Adrenals
Control	605 ± 12	90 ± 3	98	193	1.5
+ 100	665 ± 18[b]	99 ± 12	103	194	1.9
+ 300	610 ± 10	97 ± 3	83	189	1.8
+ 700	739 ± 54[b]	107 ± 3[c]	92	208	2.1
+ 1000	720 ± 51[b]	107 ± 3[c]	89	193	1.6

[a]Ten steers per group; two steers died on 1000 ppb group.

[b]$P < 0.05$.

[c]$P < 0.01$.

TABLE VI

Aflatoxin M in Milk from Cows Following Withdrawal of Aflatoxin B1

Cow	Aflatoxin M in dry milk, ppb					
	Week before withdrawal	Days after withdrawal				
		1	2	3	4	7
1	82	44	17	5	[a]	5
2	96	55	27	[a]	3	1
3	1500	718	434	285	160	a

[a]Not analyzed.

TABLE VIII

Effects of Aflatoxin on Laying Hens

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

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

general varying degrees of hepatic cell enlargement, enlarged nuclei and bile duct proliferation were present, the severity of which was not directly related to aflatoxin intake. The reduced intensity of hepatocyte alteration, greater irregularity in location of bile duct proliferation, and fibrosis distinguished these lesions from those found in swine.

There was no chemical evidence of aflatoxin in the meat of steers fed the highest level of aflatoxin. Blood samples, on the other hand, from the 1000 ppb level did show traces of B_1 and M_1. However, when five steers on the 1000 ppb level of aflatoxin were changed to a control ration for 72 hr prior to slaughter, there was no detectable evidence of aflatoxin in the blood, thus indicating rapid clearance when the intake of aflatoxin was discontinued.

From these results it was tentatively concluded that the aflatoxin no-effect level in this beef cattle study was about 300 ppb.

Dairy Cattle

Several laboratories have reported the detection of aflatoxin in the milk of dairy cows fed rations containing aflatoxin. British workers, for example, have found aflatoxin M_1 in milk when the aflatoxin B_1 intake exceeds 0.5 mg per cow per day (7). The aflatoxin level in the ration, in other words, should not exceed 50 ppb, to insure that only negligible amounts of aflatoxin will be excreted in the milk. When high levels of aflatoxin are fed to cows, approximately 1% to 3% of the mycotoxin ingested is transmitted into the milk as M_1 (8). Of practical importance also is the observed rapid drop of M_1 in the milk when the intake of aflatoxin is discontinued (Table VI). It is encouraging to note that negative results were obtained when a survey of commercial milk and dairy products for M_1 content was carried out by experts at the Eastern Regional Research Laboratory in Philadelphia.

Broilers

Day-old Arbor-Acre hybrid cockerels were fed a ration containing 1600 ppb aflatoxin for a period of eight weeks under simulated practical conditions (9). At weekly intervals during the trial, chicks from control and aflatoxin fed groups were autopsied to obtain blood and liver samples for hematology and biochemical analyses. The mean body weights of the aflatoxin group lagged behind the control group during the eight-week period although not significantly, and there was no enhancement in mortality (Table VII). These results correlate well with those reported by North Carolina workers (10) who found that significant growth inhibition was not observed until the level of aflatoxin approached 2500 ppb. These same workers also reported the results of an important practical experiment showing that growth inhibition was reversible when chicks fed diets containing as high as 5 to 10 ppm of aflatoxin for one week were returned to an aflatoxin-free diet.

Upon termination of the feeding trial at eight weeks, sections of various tissues were preserved in formaldehyde for microscopic examination. Samples of meat, blood and liver were also composited, lyophilized and preserved at -10 F pending chemical analysis for aflatoxin residues.

Histological results for the chicks fed 1600 ppb aflatoxin indicated that the liver was the prime target organ. Microscopic lesions were present in most livers, the optimal time for detection of bile duct and hepatocyte changes being between 17 and 31 days from the start of the feeding trial (9). Concurrently, positive biochemical effects were also detected; namely, the plasma albumin was decreased, and the levels of nucleic acid and succinic dehydrogenase in the liver decreased.

No evidence of aflatoxin residues were detected by chemical analysis of lyophilized meat, liver or blood from the chicks fed 1600 ppb aflatoxin for eight weeks. The method of analysis used was capable of detecting as little as 3 to 5 ppb aflatoxin (11).

Since positive effects were detected at the 1600 ppb level of aflatoxin intake, a second trial was run in which day-old chicks were started on a diet containing the equivalent of 800 ppb. The results clearly indicated that the same biochemical and microscopic effects were present as described above when twice the level of aflatoxin was fed, but the changes were much less severe. Also at eight weeks the mean body weight of the aflatoxin fed chicks exceeded that of the control group (9).

TABLE VII

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

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

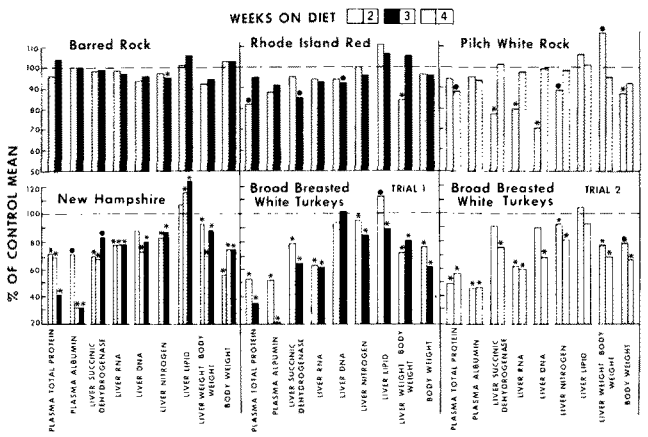

FIG. 1. Effect of aflatoxin (≃800 ppb B_1) in the diet on pure strain chickens and on turkeys. Significance of difference between test and control means: •, $P < .05$; *, $P < .01$. Two mean values for similar time periods indicate repeated feeding trials.

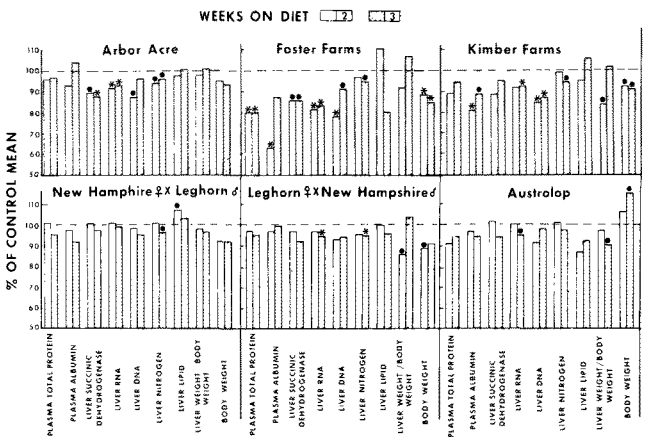

FIG. 2. Effect of aflatoxin (≃800 ppb B_1) in the diet on hybrid cross chickens. Significance of difference between test and control means: •, $P < .05$; *, $P < .01$.

Finally, when an aflatoxin level of 400 ppb was fed, there were no detectable effects and it was tentatively concluded that this constituted a no-effect level for Arbor-Acre broilers.

The greater sensitivity of ducklings to aflatoxin compared with chicks, and the numerous reports of poultry breed and strain differences in response to aflatoxin (12,13) prompted a study of the degree of susceptibility of 17 breeds and strains of poultry and game birds to aflatoxin (14). A single dietary level of aflatoxin was fed in each trial (800 ppb), beginning with day-old animals for a period of two to six weeks when the aflatoxin effects would be expected to be the most pronounced. All of the various biochemical parameters tested for the aflatoxin-fed animals were plotted as a percentage of the respective control values.

As shown in Figure 1, the high degree of susceptibility of New Hampshire chicks and turkey poults is noteworthy. High mortality rates were also encountered in these two groups. In contrast, the relative inertness of Barred Rock chicks to aflatoxin can readily be seen. Rhode Island Red and Pilch chicks occupied an intermediate position in terms of aflatoxin susceptibility. In contrast to the high degree of susceptibility of New Hampshire chicks, the resistance of New Hampshire-White Leghorn hybrids to aflatoxin is impressive, suggesting that susceptibility may be under genetic control (Fig. 2). Of the three hybrid crosses shown, Foster Farms chicks appear to be somewhat more sensitive to aflatoxin than either the Arbor-Acre or Kimber Farms chicks. The Australop breed, on the other hand, showed considerable resistance, comparable to the Barred Rock.

Guinea fowl are also highly resistant to aflatoxin (Fig. 3). In contrast chuckars and Tennessee Red quail were highly sensitive, and Bob White quail and Japanese quail were intermediate.

Laying Hens

White Leghorn hens were used to study the effect of aflatoxin on egg production. A two-week period of equilibration preceded the 34-day period during which eggs were collected. The experimental group of 17 hens received a ration containing 2700 ppb aflatoxin (9). As indicated in Table VIII, there was no weight loss or mortality in the aflatoxin-fed hens, and an apparent increase in egg production over the control group. The yolks and whites of more than 100 eggs from each group were composited, lyophilized and analyzed for aflatoxin residues. Confirming other reports (12,15,16), no evidence of aflatoxin transmission into eggs could be detected by chemical analysis. Finally, the hens were bled, scalded and defeathered, and composite samples of blood, meat and liver were lyophilized and analyzed for aflatoxin. There was no evidence of aflatoxin residues in the tissues of the hens fed 2700 ppb aflatoxin in the ration for a period of 48 days. Histological examination of the liver sections revealed minimal to mild lesions associated with the ingestion of aflatoxin.

The salient biochemical effects observed in the hens fed aflatoxin were decreased liver nitrogen, protein and glycogen concentrations, greatly increased liver lipid, decreased liver nucleic acid concentrations, decreased serum proteins, and an increased rate of incorporation of radioactive leucine and uridine into protein and ribonucleic acid by liver slices (M.R. Gumbmann, personal communication).

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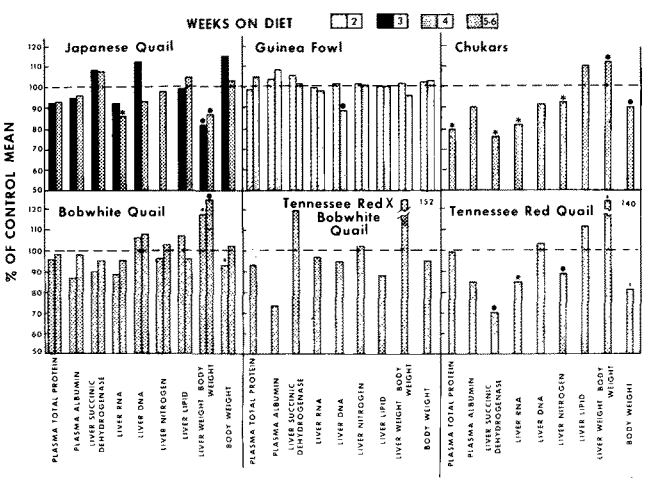

FIG. 3. Effect of aflatoxin (≃800 ppb B_1) in the diet on quail and guinea fowl. Significance of difference between test and control means: •, $P < .05$; *, $P < .01$. Individual body weights not obtained, ‡. Two mean values for similar time periods indicate repeated feeding trials.

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