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Aflatoxin in Corn: Ammonia Inactivation and Bioassay with Rainbow Trout

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Four samples of corn were compared with respect to their hepatocarcinogenicity in rainbow trout. One corn sample was found by chemical analysis to contain no detectable aflatoxin. A second sample was contaminated with aflatoxins at a level of 180 μg/kg. Each of the above-mentioned samples was divided, and one-half of each was ammoniated. These four samples were added to a semipurified basal diet and fed to a sensitive strain of rainbow trout. It was found that ammoniation inactivated the aflatoxins and reduced the carcinogenicity of the contaminated corn to a level that was not significantly different from that with the basal control diet. It was also found that the ammoniation process did not reduce the nutritive value of the corn.

Aflatoxin B_1, a metabolite of the mold *Aspergillus flavus*, is a potent toxin and hepatocarcinogen occasionally occurring as a contaminant in feeds and food products (6). Initially it was found in peanut meal, and later it was found in cottonseed meal, corn, wheat, and other agricultural products.

Several domestic and experimental animals are sensitive to aflatoxin. At relatively high levels, it is toxic to most organisms that have been tested. At lower levels, it produces a carcinogenic response in trout and rats; teratogenic effects have been demonstrated in hamsters, and it may act as a mutagen in certain plants and animals. Although there is good evidence that it is capable of hepatocarcinogenicity in monkeys, there is only indirect evidence that the high incidence of human hepatoma in certain countries may be due to the presence of aflatoxin-contaminated foods. Excellent reviews by Goldblatt (9) and Detroy et al. (7) discuss the aflatoxin problem in all its aspects.

Certain strains of rainbow trout (*Salmo gairdneri*) are especially sensitive to aflatoxin hepatocarcinogenicity. The Mt. Shasta strain, used in the present studies, appears to be the most sensitive; dietary levels as low as 0.4 μg/kg are capable of inducing a 14% incidence of hepatoma in 15 months (11).

Government and university workers, and food industry and feed manufacturers concerned about a possible mycotoxin hazard in their products, have collaborated in the development of means for detecting and for reducing or eliminating the aflatoxins that occasionally are encountered (7). Studies by Masri et al. (M. S. Masri, H. L. E. Vix, and L. A. Goldblatt, U.S. Patent 3,429,709, February, 1969) and Gardner et al. (8) have demonstrated that aflatoxins, present as natural contaminants in peanut and cottonseed meal, may be inactivated by treatment of the meal with ammonia under elevated or atmospheric pressure in combination with heat and moisture. This report pertains to the inactivation of aflatoxins present in white corn by a modified treatment using aqua ammonia at atmospheric pressure and to the biological assay of treated corn by using sensitive rainbow trout.

MATERIALS AND METHODS

Corn. A sample of white corn, U.S. grade no. 3, known to contain aflatoxins (160 μg of B_1 per kg, 10 μg of B_2 per kg, 9 μg of G_1 per kg and a trace of G_2, for a total of 180 μg/kg), was divided in two portions. One portion remained untreated; the other half was ammoniated by the method described below. Another sample of white corn, U.S. grade no. 1, found by chemical analysis to be free from aflatoxin, zearalenone, and ochratoxin, was divided; one half was untreated and the other was ammoniated as described.

Ammoniation of corn. Ammonium hydroxide (ammonia concentration, 21.8 wt%) was added to 57 kg of contaminated corn in sufficient quantity to yield 1.5% ammonia based on dry-matter content of corn. Tap water was added to adjust the moisture from 12 to 17.5% (wet basis exclusive of ammonia). The mixture was blended in a 208-liter (55-gallon) stainless-steel drum, with mixing flights and gas-

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keted cover, for 30 min, and the closed container was held overnight at 25°C. The drum and contents were held for 12 days in a heated chamber (forced-convection dryer operated at 49 ± 1°C) to inactivate the aflatoxin.

After ammoniation, the corn was ground (95% passing through a 40-mesh U.S. standard sieve, i.e., 0.42-mm opening) by two passages through a hammer mill, aerated, and heated to 42 to 48°C in a 10-cubic foot (ca. 0.28 m^3) jacketed, steam-heated blender for 8 h to remove the odor of ammonia.

The lot of aflatoxin-free corn (11.4% moisture) was ammoniated, ground, and deodorized in a similar manner, except that the ammoniation holding time at 49°C was reduced from 12 to 6 days. The shorter time was used because data from the contaminated corn indicated that the ammoniation could be terminated in 6 days.

Chemical analyses. Approximately 23 kg of the contaminated corn and 45 kg of the ammoniated corn were ground, blended, and then sampled for the aflatoxin assay. Quantitative aflatoxin determinations were made on 50-g portions by the method recommended for corn by the Association of Official Analytical Chemists (2, 3). Ammoniated samples were neutralized to pH 5.7 before analysis. Reported values are the average of two determinations. For a single determination on unammoniated corn, the relative standard deviation has been reported as 37% (12), which decreases to 25% for two determinations and to 20% for three determinations. Aflatoxin values above 50 μg/kg have been rounded to the nearest multiple of 10, and those below 20 have been rounded to the nearest multiple of one. Values less than 1 μg/kg are reported as nondetectable.

Approved methods (1) of analysis were used for proximate analysis of the corn samples, except for total nitrogen, which was analyzed by the method of Uhl et al. (13). The method for analyzing corn for its content of water-extracted ammonia was as described previously by Lancaster et al. (10), who referred to it as "free" ammonia. Briefly, corn is soaked overnight in an excess of water, and the mixture is titrated with standardized acid to obtain the water-extracted ammonia content.

Preparation of diets. The basal control diet was prepared by the method of Castell et al. (5), which consists of mixing 35 g of the complete dry mix (Table 1) with 65 g of water and forming cubes of a size suitable for consumption by the trout.

In addition to the basal control diet, four modifications of the diet were fed to similar lots of fish by incorporating one of the four types of corn listed in Table 2. In these four experimental diets, the corn at a 25% level replaced the dextrin and a part of the casein and cellulose. All diets were isocaloric and isonitrogenous.

Biological testing for aflatoxin. Mt. Shasta strain rainbow trout with an average weight of 6.3 g each, reared on the basal control diet since hatching, were distributed randomly into 100-gallon (ca. 378.5-liter) fiber-glass tanks (80 fish per tank, duplicate tanks for each diet) (Table 3). Each tank was supplied with disease-free well water at a constant 13°C. The fish were fed ad lib three times daily.

TABLE 1. *Composition of semipurified basal control trout diet*

Ingredient	%
Casein	49.5
Gelatin	8.7
Dextrin	15.6
Salmon oil	5.0
Soybean oil	5.0
Mineral mix[a]	4.0
Carboxymethylcellulose	1.3
Cellulose (Alphacel)[b]	7.7
Vitamin mix[c]	2.0
Choline chloride (70%)	1.0
Vitamin E concentrate (α-tocopherol, 330 IU/g)	0.2

[a] Modified Bernhart-Tomarelli (4) salt mix (0.002% NaF and 0.02% $CoCl_2$ added).

[b] Nutritional Biochemicals Corp., Cleveland, Ohio.

[c] Vitamins supplied at the following levels (milligrams per kilogram): thiamine (HCl), 64; riboflavine, 144; niacinamide, 512; biotin, 1.6; calcium D-pantothenate, 288; pyridoxine (HCl), 48; folic acid, 19.2; menadione, 16; cobalamine (B_{12}), 0.159; isoinositol (*meso*), 2,500; ascorbic acid, 1,200; p-aminobenzoic acid, 400; vitamin A concentrate (250,000 IU/g), 200; vitamin D_3 (50,000 IU/g), 8.

Samples of 10 fish per tank were taken routinely at 4 and 8 months after the start of the experiments, with all remaining fish being used for the 12-month sample. All samples were weighed, the livers were removed for microscopic examination, and the incidence of liver cancer was determined. Nodules of doubtful classification were sectioned for routine microscopy, together with five livers taken randomly from each sample at each sampling date. All tissue sections were scanned microscopically for evidence of toxic alterations.

RESULTS AND DISCUSSION

Ammoniation of the contaminated corn reduced its total aflatoxin content from 180 μg/kg to a nondetectable level in 12 days (Table 2). The treatment could have been concluded 6 days earlier based upon analysis of an interim sample. Ammoniation increased the nonprotein nitrogen content of the contaminated corn by 0.35% and that of the noncontaminated corn by 0.30%. The effect on the crude fat, ash, and starch contents was minimal.

Ammoniation of the corn under the conditions used turned the kernel surface from white to a deep brown or mahogany color. The deepest discoloration was limited to the bran coat and germ. The vitreous endosperm was slightly darkened; a tinge of tan was apparent in the floury endosperm. Deodorized meal from the contaminated corn had a deep tan color, and that from the noncontaminated corn was light

TABLE 2. *Analysis of corn before and after ammoniation, grinding, and deodorizing*

Sample identity	Aflatoxin (μg/kg)					Water-extracted ammonia (% db[a])	Total nitrogen (% db)	Crude fat (% db)	Ash (% db)	Starch (% db)
	B_1	B_2	G_1	G_2	Total					
Aflatoxin-contaminated corn										
Before ammoniation	160	10	9	TR[b]	180		1.72	4.75	1.51	71.3
After ammoniation	ND[c]	ND	ND	ND	ND	0.15	2.07	4.54	1.50	72.0
Aflatoxin-free corn										
Before ammoniation	ND				ND		1.65	4.51	1.26	75.3
After ammoniation						0.15	1.95	4.51	1.30	78.1

[a] db, Dry basis.
[b] Trace.
[c] None detected.

TABLE 3. *Hepatoma incidence, body weight, and food efficiency in trout fed corn-supplemented diets for 12 months*

Diet composition	Avg total body wt[a] (g)	Food/gain ratio	Total mortality	Hepatoma incidence
Basal control (BC)	483	1.04	4	1/56
	479	1.11	0	0/60
Modified BC[b] + 25% uncontaminated, untreated corn	515	1.15	0	1/58
	504	1.08	1	1/57
Modified BC + 25% uncontaminated, treated corn	523	1.11	0	0/60
	544	1.05	4	0/51
Modified BC + 25% contaminated, untreated corn	445	1.04	4	56/57[c]
	484	1.06	1	53/55[c]
Modified BC + 25% contaminated, treated corn	514	1.05	2	3/58
	505	1.09	1	0/58

[a] Average weight of fish at the beginning of the experiment (11 July 1973) was 6.3 g.
[b] Corn replaced the dextrin, some casein, and cellulose in the basal diet. All diets were formulated to be isonitrogenous and isocaloric.
[c] A 10% incidence of hepatoma (2/20) and a 95% (19/20) incidence of hepatoma were noted in the 4- and 8-month samples, respectively.

tan. This difference in color may have been due to the difference in time the two lots were held at 49°C.

The water-extracted-ammonia content of the noncontaminated corn (whole kernels) was 0.91% (dry basis) after ammoniation, and this quantity was reduced to 0.32% by grinding and to 0.15% by heating and aeration. Grinding and deodorizing the corn lowered the ammonia odor from a strong (i.e., intolerable) level initially to a nondetectable level and also removed a moderate, manure-like odor that was present after the ammoniated corn had been heated for 12 days.

Ammoniation of the corn increased the total nitrogen in both the contaminated and uncontaminated samples (Table 2). In other respects, the nutritive value presumably was not affected by ammoniation. In the following section, it will be noted that the diets which had been ammoniated and which had an increased nitrogen content also produced slightly greater growth in the trout; however, the food/gain ratios were very similar.

Trout feeding experiments. Ammoniation of the corn samples greatly reduced or eliminated aflatoxin carcinogenicity (Table 3). The incidence of hepatoma (in this case, small tumors) after 12 months on the experimental diets was 3/116 in the ammoniated portion of the contaminated sample. This is not significantly greater than the incidence in the untreated portion of the uncontaminated sample, 2/115, and is similar to the basal control diet in which the incidence was 1/116. In marked contrast was the incidence of hepatoma (i.e., many large tumors) in the fish fed the untreated, contaminated corn: 109/112. Examination of 20 fish fed this diet (40 μg of aflatoxin B_1 per kg) for 8 months showed a high tumor incidence, i.e., 19/20. Histological examination of the tissues from trout fed the uncontaminated or ammoniated, uncontaminated corn showed little or no abnormalities that could be attributed to aflatoxin. However, based on the appearance of the nuclei and arrangement of cytoplastic contents, a low level of toxicity is indicated. Although the ammoniation process was quite effective in inactivating aflatoxin B_1, perhaps other substances

may have been present in this corn which caused the slightly unusual histology mentioned above. Additional research is needed to clarify this point.

The nutritive value of the ammoniated corn to trout appeared to be equal or perhaps slightly superior to that of the untreated corn samples. The body weight of the fish fed the ammoniated corn was slightly greater than that of comparable fish fed untreated corn. The amount of food required to produce a unit gain in weight was similar in all groups.

We want to caution the reader that, before an ammoniation process for detoxifying corn can be recommended by the U.S. Department of Agriculture, approval by the Food and Drug Administration is needed of the corn and of the ammoniation process. Feeding tests to obtain such approval were started late in 1975 with swine and laying hens. The processing and feeding results will be described in subsequent papers.

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