AFLATOXIN IN CORN
New Perspectives

North Central Regional Research Publication 329
Research Bulletin 599
Iowa Agriculture and Home Economics Experiment Station
Iowa State University, Ames, Iowa
June 1991

Prepared by Members of the North Central Regional Committees NC-151
and NC-129, O.L. Shotwell and C. R. Hurburgh, Jr., eds.

Agricultural Experiment Stations of
Illinois, Indiana, Iowa, Kansas, Michigan,
Minnesota, Missouri, Nebraska, North
Dakota, Ohio, South Dakota, and
Wisconsin with the U.S. Department of
Agriculture cooperating.
Requests

This bulletin (North Central Regional Research Publication 329) is published by the Iowa Agriculture and Home Economics Experiment Station. Requests for copies may be sent to Agriculture Information Services, 304 Curtiss Hall, Iowa State University, Ames, Iowa 50011.

Distributed in cooperation with the North Central Region Educational Materials Project, Cooperative Extension Service.

The participating agricultural experiment stations and government agencies provide equal opportunities in programs and employment.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture and the participating experiment stations over other firms or similar products not mentioned.

This publication was prepared by the North Central Regional Committees NC-151, Marketing and Delivery of Quality Cereals and Oilseeds, and NC-129, Occurrence of Mycotoxins in Feeds and Food and Their Effects on Animal and Human Health. Members of the regional committees are:

**NC-151 Technical Committee**

Illinois Agricultural Experiment Station: L. D. Hill  
Indiana (Purdue) Agricultural Experiment Station: R. L. Stroshine  
Iowa Agriculture and Home Economics Experiment Station: C. R. Hurburgh, Jr.  
Kansas Agricultural Experiment Station: K. C. Behnke  
Louisiana Agricultural Experiment Station: H. D. Traylor  
Michigan Agricultural Experiment Station: F. W. Bakker-Arkema  
Minnesota Agricultural Experiment Station: R. V. Morey  
Missouri Agricultural Experiment Station: A. L. Karr  
Montana Agricultural Experiment Station: F. V. Dunkel  
Nebraska Agricultural Experiment Station: L. B. Bullerman  
North Dakota Agricultural Experiment Station: L. F. Backer  
Ohio Agricultural Research and Development Center: R. C. Pratt  
Texas Agricultural Experiment Station: L. W. Rooney  
Washington Agricultural Experiment Station: Y. Pomeranz  
Wisconsin Agricultural Experiment Station and Stored Product Insects Research Unit, ARS, USDA: W. E. Burkholder  
National Economics Division, ERS, USDA: M. N. Leath  
National Center for Agricultural Utilization Research, ARS, USDA: J. A. Bietz  
Federal Grain Inspection Service, USDA: D. Koeltzow  
U. S. Grain Marketing Research Laboratory, ARS, USDA: J. L. Steele and C. R. Martin  
Cooperative State Research Service, USDA: L. F. Flora  
Industry Advisory Committee: R. Swanson

Project Coordinator: N. D. Schmidt  
Administrative Adviser: J. H. Brown
Processing of Aflatoxin-Contaminated Corn

Rodney J. Bothast
Agricultural Research Service,
U.S. Department of Agriculture

Abstract

Contamination of agricultural food and feed products by aflatoxin is a problem not only because the products themselves are lost, but also because disposal is costly. This review focuses on ammoniation and ammoniation coupled to fermentation as practical process strategies for salvaging aflatoxin-contaminated corn. Extensive laboratory, pilot-scale, and farm-scale experiments at the Northern Regional Research Center led to an ammoniation procedure to reduce aflatoxin in corn from amounts in excess of 1,000 µg/kg to < 10 µg/kg. Numerous feeding studies support the ammoniation process as having no adverse effects on animals. Subsequently, a process was developed for converting aflatoxin-contaminated corn to alcohol fuel by combining ammonia inactivation with liquefaction during the traditional ethanol fermentation process. Superior ethanol yields were achieved with ammonia addition, and aflatoxin concentrations were reduced 80-85% in the spent grains. Unfortunately, FDA has not sanctioned the use of ammonia to destroy aflatoxin because of "incomplete" information on the potential toxicity and carcinogenicity of reaction products. Thus, many questions still remain about commercial utility of these processes.

Introduction

Contamination of agricultural food and feed products by aflatoxin is a problem not only because the products themselves are lost, but also because disposal is costly. The value of the crop does not just drop to zero, but actually becomes a liability that must be buried or otherwise eliminated. Consequently, the development of processes by which aflatoxin-contaminated agricultural products can be reclaimed or salvaged for animal feed or alcohol fuels has been a major research priority. Crops such as corn, peanuts, and cottonseed are subject to contamination, but each of these agricultural commodities presents specific problems in reducing or eliminating aflatoxin (19).

Various approaches for reducing aflatoxin in corn are addressed in this discussion. A direct route is to blend the aflatoxin-contaminated corn with "clean" corn. Blending usually is not recommended because of possible synergistic effects from undetected or unidentified toxins. But exceptions have been made by the U.S. Food and Drug Administration (e.g., 1977, 1980, 1983, 1988), which permitted the blending of aflatoxin-contaminated corn with "clean" corn to achieve acceptable feeds for poultry, swine, and beef cattle (18, 39). When the aflatoxin level of 1,000 bu of contaminated corn is at 1,000 µg/kg, a very large amount of good corn (49,000 bu) is needed to reduce the average contamination of the blend to less than 20 µg/kg.

Other methods for removal or inactivation of aflatoxin include physical, chemical, or biological processes (1, 13, 19, 29, 30, 37). Physical separation methods are highly desirable, being relatively inexpensive, but seem limited to rather special situations in which the contamination is confined to a small proportion of the seed, with sufficient difference in seed properties to make the separation possible (7). For corn, neither the wet-milling nor dry-milling process removed or inactivated aflatoxin (5, 8, 43). Although aflatoxins are relatively stable to heat, roasting of corn reduced aflatoxin (14). Most recently, inorganic adsorptive compounds (subject of the paper by T. D. Phillips et al., see page 359) have been used to bind and neutralize aflatoxins in the gastro-intestinal tract of animals (15). Results are promising, but many questions remain concerning efficiency for ruminants and the binding of nutrients.

In weighing approaches or processes for detoxification of aflatoxin in corn, several criteria should be met: (a) economic feasibility, (b) reduction of toxin concentration to safe levels, (c) freedom from toxic residues, and (d) little or no loss in nutritional value or in acceptability of the treated grain. These restrictions have narrowed the types of agents likely to succeed in a commercial-scale detoxification process to chemicals such as oxidizing agents, acids, and bases. Of these agents, ammonia seems most promising.

Ammonia Detoxification Process

Ammonia is one of the more effective reagents for treatment of cottonseed and peanut meals...
Figure 1. Large-scale ammoniation procedure to destroy aflatoxin in corn, from Brekke et al. (11).

(38) and offers a number of potential advantages for corn (3, 38). First, it is inexpensive and is available in the large quantities required. Second, ammonia as a farm chemical is well known to the farmer and its use for an "on-farm" process is quite feasible. Finally, although there are hazards in the use of ammonia, as with any chemical, these hazards are well known and can be contained.

In developing a large-scale process at the Northern Regional Research Center (NRRC) for on-farm or elevator use to detoxify corn containing aflatoxin (3), researchers took several steps:

Step 1. Preliminary studies in the laboratory and in a pilot plant were conducted to investigate parameters, such as corn moisture, ammonia concentration, and temperature, that could affect the inactivation process.

Step 2. Preliminary bioassay of the pilot-scale decontaminated corn was performed, using rainbow trout, ducklings, and broiler chicks, to ensure that the decrease in the chemically assayed amount of aflatoxin was actually matched by a decrease in biological activity.

Step 3. Animal acceptance and utilization trials were carried out to ensure that the process developed actually produced corn that would be accepted by the animals in the final full-scale feeding trials.

Step 4. Treated and control corn were prepared on a 1,000-bushel scale for use in feeding trials for poultry, swine, and cattle.

Three major factors determined the effectiveness of ammonia in reducing aflatoxin in corn (9): the ammonia level, the moisture level, and temperature. At 0.5% NH₃ and 15% moisture in the corn, an initial aflatoxin concentration of 600 µg/kg was reduced to < 20 µg/kg in about 3 weeks at 25°C, but in 3 days at 38°C. The inactivation reaction was temperature-sensitive from -18 to 60°C and became more effective as the corn moisture increased from 12.5 to 20%.

Feeding trials on ducklings, broiler chicks, and trout all confirmed the effectiveness of the ammoniation process in reducing the biological activity of aflatoxin in line with reduction in the chemical assay (9, 10). Acceptance studies showed few problems at less than 1% ammonia, and for laying hens, even ammonia concentrations up to 2.6% caused no taste problems with the eggs. Swine readily accepted and efficiently utilized mixed diets containing ammonia (1.5%)-treated aflatoxin-contaminated corn (21).

Figure 1 depicts scale-up of the ammoniation detoxification process developed at NRRC (11). The process was conducted in a sealed, metal (protected by epoxy paint) storage-drying bin with an
an elevated slotted floor for recirculation of the air-ammonia mixture through the contaminated bed of grain. Essentially, the treatment process consists of three steps: (a) adjusting corn moisture to 15-22%, (b) treating the corn with a gaseous ammonia (0.5-1.5% dry weight basis) and air mixture for 2 to 28 hours, then continuing to recycle gas flow for 20 additional hours and holding the mixture at ambient temperatures for 13 days to obtain detoxification, and (c) drying the treated corn for storage. The procedure has been used successfully to reduce the aflatoxin content of corn from 1,000 μg/kg to less than 20 μg/kg.

Estimated cost for ammonia detoxification ranges from 17 to 66¢/bu (G. E. Hamerstrand, personal communication), with an estimated cost of 27¢/bu for a plant processing 900,000 bu/yr. But the process has not been optimized, and experience in the field (subject of paper by W. C. Hammond, see page 377) with plastic containment, less ammonia, and shorter holding times will affect the cost of ammonia detoxification.

### Reaction Products

Studies on the chemical reaction between aflatoxin B₁ and ammonia have identified the formation of several decomposition compounds (4, 16, 26, 38, 41, 42), and the safety of these compounds is of major concern to the FDA. Hydrolysis of the aflatoxin B₁ lactone, the first step in the reaction, is reversible if the ammoniation process is carried out under mild conditions and allows for electrostatic and/or hydrogen bonding with protein constituents of the corn. When the reaction is allowed to proceed further, aflatoxin D₁, a 206 MW compound, and other compounds are formed that do not revert back to aflatoxin B₁. Type and amount of reaction products are dependent on temperature and pressure conditions used, whether liquid ammonia or ammonia gas is the ammonia source, and the corn constituents.

Selected reaction products have demonstrated some degree of toxicity in such tests as chick embryo, Salmonella/microsome mutagenicity, or covalent binding index (CBI) (20, 25, 27, 40). But the response of these reaction products is many orders of magnitude less than that from unchanged aflatoxin B₁ (Table 1). Also, the formation of these products in the feed matrix is usually <1% of the original aflatoxin contamination level. A large portion of the reaction products is bound to feed components such as protein and is potentially biologically unavailable to animals (38).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutagenic potential μg/plate</th>
<th>Chick embryo μg/egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>0.005</td>
<td>0.125</td>
</tr>
<tr>
<td>Aflatoxin M₁</td>
<td>0.16</td>
<td>—</td>
</tr>
<tr>
<td>Aflatoxin D₁</td>
<td>2.25</td>
<td>2.5</td>
</tr>
<tr>
<td>206 MW Compound</td>
<td>3.3</td>
<td>—</td>
</tr>
</tbody>
</table>

*From Park et al. (38).

### Feeding Trials

Extensive feeding studies (22, 23, 33-36, 38) of aflatoxin-contaminated corn and ammonia-detoxified corn generally support the ammoniation process as having no adverse effects on cattle, swine, or chickens. Switching from aflatoxin-contaminated feed to decontaminated rations significantly reduced or completely eliminated observable signs of aflatoxicosis in animals originally receiving the untreated product. Production parameters (e.g., milk and egg quality) were either significantly better or not adversely affected by the treatment. Weight gain and feed efficiency varied according to species, with ruminant animals showing positive results and nonruminants showing either no effect or decreased values. Protein Efficiency Ratio (PER) values were generally less, probably because of decreased nitrogen solubility and available lysine levels. Numerous feeding studies showed no toxic effects or lesions related to the ammoniation procedure (35). Metabolism studies suggest poor absorption of decontamination reaction products when compared with aflatoxin B₁ (34). The ammoniated product is readily accepted by the animal if adequate aeration is allowed to remove residual ammonia. Nevertheless, it must be emphasized that FDA has not approved ammonia detoxification of aflatoxin-contaminated corn (39).
Alcohol Production

A number of studies have been carried out on the fate of mycotoxins in contaminated grains used as substrates for the fermentative production of ethanol (12, 17, 24, 28, 29, 31, 32). Results common to these studies are: (a) little degradation of toxin during fermentation; (b) no toxin in the distilled alcohol; and (c) toxin accumulation in the spent grains. This last result presents a serious problem because the spent grains often are destined for animal feed. Consequently, practical detoxification procedures are essential. Lillehoj et al. (28) detoxified aflatoxin B₁ in wet postfermentation stillage with sodium hydroxide, ammonium hydroxide, sodium hypochlorite, and hydrogen peroxide. Substantial quantities of these chemical agents and high temperatures were required to achieve inactivation. Subsequently, Nofsinger and Bothast (32) reported that corn detoxified with gaseous ammonia (process previously described) can be used effectively for the production of ethanol and spent grains free of aflatoxin. Ethanol yields and conversion efficiencies were better on ammonia-detoxified corn than on contaminated corn. Aflatoxin accumulated in the spent grains of contaminated corn, and the concentration in the spent grains of ammonia-detoxified corn was less than 20 μg/kg. Because temperatures of approximately 90°C are commonly attained during the cooking step of the traditional ethanol process, and ammonia is useful both as a detoxifying agent for aflatoxin and as a nitrogen source for yeast (32), Bothast et al. (6) developed a procedure for combining detoxification and fermentation into a single, efficient integrated process. The integrated detoxification and fermentation process is diagrammed in Figure 2 and described by the sequential steps listed below for the fermentation of 1 bu of aflatoxin-contaminated corn.

Grinding

Aflatoxin-contaminated corn is ground into a fine meal to pass through a 10-mesh (2-mm) screen.

Slurrying

In the fermentation vessel, a mash (20% solids, wt/wt) is prepared by adding 23 gal of water to 56 lb of milled grain, then adding 1% ammonia as ammonium hydroxide, based on the "as is" weight of the grain (i.e., approximately 2 lb of reagent ACS ammonium hydroxide per bu). Then a bacterial alpha-amylase (0.11 lb per 56 lb of grain) is added for liquefaction. No pH adjustment is made.

Liquefaction

The alkaline mash (pH of approximately 9.5) is heated in a closed system with continuous agitation to a temperature of 90°C and held for 1 h.

Figure 2. Integrated detoxification and fermentation process, from Bothast et al. (6.)
Conversion
The mash is cooled to 60°C by addition of 6.2 gal of water, and the pH is adjusted to 4.2 with dilute hydrochloric acid. Fungal glucoamylase (0.4 lb per 56 lb of grain) is added, and the mash is held for 2 h at 60°C for conversion. The mash is then cooled to fermentation temperature (30°C), and the pH is adjusted to 4.5 with ammonium hydroxide.

Fermentation
Distillers yeast (1%, vol/vol) is added. Before inoculation, the yeast is grown in YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose) for 24 h at 30°C. The inoculated mash is held at 30°C for 3 days while fermentation proceeds.

Distillation
The fermented mash is distilled in a pot or continuous still, and the alcohol is recovered.

Feed Recovery
The spent grains are recovered by filtration screening or centrifugation, and the wet (65 to 80% moisture) material is assayed for residual aflatoxin. If the aflatoxin content of the stillage exceeds 100 μg/kg, then a second ammonia detoxification treatment may be necessary.

This protocol is based on experimental data (Table 2) obtained during 1- and 8-liter fermentations. Fermentations of aflatoxin-contaminated corn coupled with an ammonia treatment consistently produced more ethanol than fermentations of the same corn with no ammonia treatment. As noted previously (32), ammonia supplies needed nitrogen for the fermentative microorganism and should be included in any protocol for the fermentation of aflatoxin-contaminated corn. Inactivation of aflatoxin can simply be an additional benefit.

The primary difference between the fermentation process just described and the traditional ethanol fermentation is that a bacterial alpha-amylase was used in place of barley malt during slurring and liquefaction. The bacterial alpha-amylase has an optimum activity at 90°C and retains at least 25% activity at a pH of 10.0. Consequently, ammonia inactivation was combined with liquefaction at 90°C for 1 h.

This technology has subsequently been extended to 1- and 20-bushel fermentations of contaminated grain (400-500 μg/kg) at the University of Illinois and by a private alcohol producer. Approximately 72% of the aflatoxin was destroyed during the process. Ducklings and weaning pigs showed no adverse effects from diets containing stillage produced by the integrated process (W. B. Buck, personal communication).

Table 2. Combined detoxification and fermentation of aflatoxin-contaminated corn.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Volume (L)</th>
<th>Ethanol conversion efficiency (%)</th>
<th>Toxins in spent grains (μg/kg)</th>
<th>Toxin destruction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated Corn</td>
<td>1</td>
<td>61</td>
<td>1,205</td>
<td>—</td>
</tr>
<tr>
<td>Contaminated Corn with 1% NH₃</td>
<td>1</td>
<td>83</td>
<td>183</td>
<td>85</td>
</tr>
<tr>
<td>Contaminated Corn</td>
<td>8</td>
<td>74</td>
<td>1,116</td>
<td>—</td>
</tr>
<tr>
<td>Contaminated Corn with 1% NH₃</td>
<td>8</td>
<td>87</td>
<td>227</td>
<td>80</td>
</tr>
</tbody>
</table>

*From Bothast et al. (6).

Ammonia (weight of NH₃/weight of corn) added during slurring of corn naturally contaminated with aflatoxin (617 μg/kg).
A second ammonia treatment was tested to inactivate residual aflatoxin in wet spent grains recovered from the integrated process (6). After exposure to 1% ammonia (weight of NH₃/wet weight of spent grains) for 24 h at 25°C, the aflatoxin content of the dried spent grains was reduced 80% (227 μg/kg to 46 μg/kg). This second ammonia treatment may be necessary because total aflatoxin destruction achieved by using the process diagrammed in Figure 2 is dependent on the original aflatoxin content of the corn, and one ammonia treatment may not be sufficient to produce spent grains with sufficient reductions of aflatoxin content to be used safely in animal feed.

Recent summer drought problems (1988) in the Midwest and the South have been implicated in the increased occurrence of aflatoxin in corn (39). As a result, FDA revised the action levels (15) to 100 μg/kg for corn intended for breeding beef cattle, breeding swine, and mature poultry; 200 μg/kg for corn intended for finishing swine (> 100 lbs); and 300 μg/kg for corn intended for feedlot beef cattle. But the action level for corn intended for human food and immature animals still remains at 20 μg/kg. Also, major industrial alcohol producers have shown considerable interest in using the integrated ammoniation and fermentation process to salvage aflatoxin-contaminated corn to produce low-cost alcohol. Early in the harvest of 1988, contaminated grain was discounted from $0.60 to $1.75 a bushel (Chicago Tribune, October 18, 1988).

Conclusions

Ammoniation and ammoniation coupled with fermentation provide practical strategies for salvaging aflatoxin-contaminated grain for animal feed and alcohol fuel. Unfortunately, the FDA has not yet sanctioned commercial use of ammonia to destroy aflatoxin because of “incomplete” information about the potential toxicity and carcinogenicity of reaction products (2, 15). Consequently, many questions still remain about the commercial utility of these processes.

References
