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Molds and Mycotoxins in Feeds

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Introduction

Some molds or fungi when growing in grains, seeds, and other plant parts can produce compounds that are toxic when consumed. These compounds are called mycotoxins, and the diseases caused are called mycotoxicoses.

Although it has been known for more than 100 years that some kinds of moldy grains, when eaten, could cause illness, intensive study of mycotoxins and mycotoxicoses only dates from the 1960s, when a toxic compound was extracted from cultures of the fungus *Aspergillus flavus* isolated from a batch of peanut meal that had been proven to be toxic. The compound was soon purified, chemically characterized, and named aflatoxin. Feeding tests with laboratory animals soon showed that aflatoxin in amounts of a few parts of toxin per million parts (ppm) of feed or a few parts per billion (ppb) could cause serious injury, including fatal liver cancer, to animals. This work on aflatoxin led to work on other fungus toxins, some of which, produced by *Fusarium*, now are of major importance worldwide in animal health.

We do not yet know all we need to know about mycotoxins and mycotoxicoses, but we do know enough to say that they constitute real and present problems for growers, marketers, processers, and users of agricultural products.

Aflatoxin

Aflatoxin is produced by the fungus *Aspergillus flavus*, and is found more commonly in some feed ingredients than others, and more often in some years and some regions than others. Then, too, some animals are more sensitive than others to injury by aflatoxin, and so it is undesirable in any animal feed.

Some of the aflatoxin consumed by animals may be passed along to humans in meat, or in changed but still toxic form, in milk or eggs. U.S. Food and Drug Adminstration (FDA) regulations and enforcement limit the amount of aflatoxin in foods and feeds or feed ingredients in interstate (between states) commerce; some state agencies have established similar limits of permissible aflatoxin contamination in grains or other products for intrastate (within the same state) commerce. Grains or other products with levels above the permissible amounts are subject to confiscation. Mixing high and low aflatoxin-content corn to achieve a blend which meets FDA standards constitutes adulteration, and is subject to severe FDA penalties. However, under some circumstances FDA and state departments of agriculture regulations permit the blending of aflatoxin-contaminated and sound grain to obtain mixes that can be fed to some nonlactating animals. Such feed can be used on the farm where it is produced, but cannot be sold.

Conditions Favorable or Necessary for Aflatoxin Production

The Fungus. *A. flavus*, like many other fungi, is found in all kinds of decaying plant materials and in soils worldwide. It is one component of the fungi that cause microbiological deterioration and decay of seeds when stored too moist (figure 1).

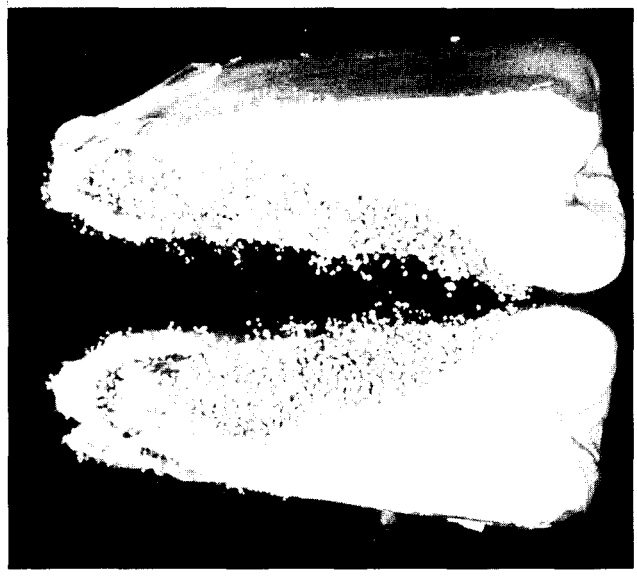

Figure 1. Apergillus flavus growing in the germ of corn.

A. flavus constitutes what the students of *Aspergillus* call a "group" species, of countless strains, biotypes, or varieties. These are all referred to as *A. flavus*. One species of *A. flavus*, has been used for close to 2000 years in the Orient to convert starch to sugar in rice so that it can be fermented into a Japanese liquor called sake. Another is grown in wheat bran in the United States and elsewhere to produce the enzyme, diastase, used in baking and brewing. Two members of the group, *A. flavus* and *A. parasiticus*, can produce aflatoxin. More accurately, some strains of these two species, when growing in certain materials under special conditions, can produce aflatoxin. *A. parasiticus* is found more commonly in the tropical and subtropical areas, and *A. flavus* more commonly in the cooler regions. *A. parasiticus*, for example, is seldom encountered in corn in the U.S. Corn Belt states, but is common in peanuts and corn in the southeast and south. Most of those working with aflatoxin problems simply call the fungus *A. flavus*, and do not haggle about which species it may be. The fungus will be referred to here simply as *A. flavus*.

Not all isolates or strains of *A. flavus* produce aflatoxin, but aflatoxin-producing strains are common in some regions. Of 283 isolates of *A. flavus* from rice in Texas, 268 produced some aflatoxin when grown in pure culture in the laboratory, and 88 produced a lot of aflatoxin. Yet, none of numerous isolates of *A. flavus*, isolated from a number of feed samples suspected of being involved in illness of cattle in Minnesota, produced any aflatoxin at all, even when grown in pure culture in the laboratory under optimum conditions for aflatoxin production. And none of the feed samples from which these strains of *A. flavus* were isolated contained detectable amounts of aflatoxin. One

conclusion from this is that the presence of *A. flavus* in a given sample of feed tells nothing about whether aflatoxin is or is not present in the feed. Many isolates of *A. flavus* are not capable of producing aflatoxin under **any** conditions that have been tested, and the presence of *A. flavus* in a given feed sample does not imply that the feed is unwholesome.

Moisture Content. The minimum moisture content for the growth of *A. flavus*, and production of aflatoxin, is that in equilibrium with a relative humidity of 80-85 percent. In the starchy cereal seeds such as maize or corn, sorghum, millet, wheat, oats, barley, and rice, this is a moisture content of about 18.0-18.5 percent, wet weight basis, which is the usual basis for expressing moisture content in these grains in commerce. In soybeans it is a moisture content of 17.0-17.5 percent, in peanuts 8.5-9.0 percent, and in copra (dried coconut meat) 12.0-12.5 percent. In the late 1970s some work purported to show that *A. flavus* could grow in and produce aflatoxin in rice and corn with a moisture content of 15.0-15.5 percent. This conclusion was based on tests on grains contaminated with spores of *A. flavus*, conditioned to moisture contents of 15.0-15.5 percent, held for a time, then surface disinfected and cultured on an agar medium and incubated until the fungus grew out and could be identified. If *A. flavus* grew out from these surface disinfected kernels or kernel fragments, it was assumed that the fungus had grown in them during the holding period. There was one flaw in this work, however: no seeds or seed fragments were cultured at zero time, immediately after surface disinfection; the workers **assumed** that the surface disinfecting procedure used would kill all the spores of *A. flavus* used to contaminate the seeds at the start of the test. This was a false assumption. If kernels of rice, corn, or other grains are contaminated with spores of *A. flavus*, or with spores of any of a great number of other fungi, and then are surface disinfected by shaking them for a minute in a 1-2 percent solution of sodium hypochlorite, followed by a rinse in sterile water (the procedure used by those workers) and are cultured immediately, the fungus will grow in from 50 to 100 percent of them; the surface disinfection is a surface disinfection in name only. Anyone who questions this can easily test if for themselves. The tests purporting to show that *A. flavus* could grow in rice and corn with 15.0-15.5 moisture proved what was not so.

There is no upper limit of moisture for growth of *A. flavus* for aflatoxin production; in the laboratory *A. flavus* often is grown in a liquid medium for production of aflatoxin.

Temperature. The lowest temperature for aflatoxin production is about 54°F (12°C), the optimum 81°F-86°F (27°C-30°C) and the maximum or highest temperature is 104°F-108°F (40°C-42°C). *A. flavus* will grow slowly below 54°F (12°C), and rapidly up to 131°F (55°C)—it is a major fungus together with *A. candidus* in heating grains and many other plant materials up to that temperature—but it will not produce aflatoxin at temperatures below 54°F (12°C) or above 104°F-108°F (40°C-42°C).

Time. Under optimum conditions for growth *A. flavus* can produce some aflatoxin within 24 hours and a biologically significant amount in a few days.

Competing Microflora. Under some circumstances *A. flavus*, when growing with a mixture of other fungi that normally accompany it in grain undergoing spoilage by molds, will produce little or no aflatoxin, but aflatoxin has been found in small amounts in freshly harvested high-moisture corn awaiting drying. Again, the presence of the fungus is not proof of the presence of the toxin; proof of the presence of the toxin depends on detection of the toxin itself.

Persistence of Aflatoxin

Once aflatoxin is formed it is extremely durable under most conditions of storage, handling, and processing of seeds or other plant parts, or in foods or feeds made from them. It is relatively heat stable at temperatures up to and above boiling (212°F or 100°C). Pelletizing feeds may eliminate fungi present in the stock and may reduce, but not eliminate, aflatoxin present in any of the ingredients. Chemical detoxification of aflatoxin-contaminated materials will be discussed later.

Aflatoxin in Field Crops

High Aflatoxin Risk Crops. Peanuts, cottonseed, copra, Brazil, and pistachio nuts and in some years and areas, corn or maize, are at relatively high aflatoxin risk.

Low Aflatoxin Risk Crops. The major food and feed grains other than corn—wheat, oats, barley, rye, rice, sorghum, and millets are at very low aflatoxin risk, and soybeans, even when they may at times be heavily invaded by *A. flavus* when in storage, also are at very low aflatoxin risk.

One reason some crops are at high aflatoxin risk is that the seeds may be invaded by *A. flavus* while the seeds are developing on the plants in the field. If the conditions then or later become favorable for the continued growth of *A. flavus*, it develops as a practically pure culture. *A. flavus* may invade through the flowers or, in corn, through the silks; other fungi may invade the seed via the same route. In the high-aflatoxin-risk crops *A. flavus*, under some conditions, is able to develop almost alone or to predominate over the associated microflora. In the low-aflatoxin-risk crops *A. flavus* almost never invades the seeds before harvest; in tests of many tens of thousands of samples of these low-aflatoxin-risk seeds from many sources and over several decades, *A. flavus* has rarely been found in the seeds before harvest. In years of moist preharvest or pre-combining weather, seeds of these grains may be heavily invaded by a variety of fungi such as *Alternaria*, *Helminthosporium*, *Cladosporium*, and *Fusarium* (more than 100 species of fungi have been isolated from barley seeds) and some of these may seriously damage seed quality for various uses, (*Fusarium* produces toxins), but *A. flavus* is almost never found among them. *A. flavus* may, however, develop during storage at moisture contents high enough to permit or promote microbiological deterioration and spoilage, but when *A. flavus* is growing with a mixture of many other filamentous fungi and yeasts, it produces little or no aflatoxin. Soybeans are just not a favorable material for aflatoxin production, and even when stored soybeans are invaded, heated, and decayed by a variety of fungi in which *A. flavus* may predominate, little or no aflatoxin is produced. USDA investigators tested 866 samples of soybeans from inspection offices and found 7 to 10 ppb of aflatoxin in two samples, none in the others.

High Aflatoxin Risk Crops

Peanuts or Groundnuts. Peanuts or groundnuts blossom above ground, but soon after pollination send a peg down into the ground where the fruits are later formed. Spores of *A. flavus* may germinate on the pistils of the flowers just after pollination, and mycelium of the fungus grows down through the pollen tubes into the embryo of the seed and produces aflatoxin there within the developing seed. At harvest the peanut plants are lifted from the ground and piled in windrows for several days where the nuts undergo a necessary aging process. Then if the weather is moist, *A. flavus* present in the seeds may continue to grow, and also may invade sound nuts through shells

broken during harvesting. After this aging process, the seed pods are removed and stored. If the seeds have a high enough moisture content, any *A. flavus* already present will continue to grow and sound peanuts may be invaded through broken shells and become contaminated with aflatoxin.

A few examples will illustrate the seriousness of the aflatoxin problem in peanuts when it first came to be recognized in the 1960s: of 173 samples of groundnuts tested in the Sudan in Africa, 71, or 41 percent, contained aflatoxin; 30 of the samples contained more than one million ppb, or more than one part per thousand. Individual seeds within some of these lots had considerably higher concentrations of the toxin. Of 52 samples of peanuts and peanut products (whole nuts, meal, cake) Denmark imported for feed, 45 contained aflatoxin, and one sample contained 3,465 ppb. A single peanut kernel containing one part per thousand of aflatoxin is sufficient to contaminate 100 pounds of peanut butter with 25 ppb of the toxin.

In many countries where peanuts constitute an important source of protein in the diet, strict surveillance to avoid aflatoxin in the food is not possible. The peanuts deemed unfit for human consumption are likely to be fed to animals, and both humans and their domestic animals could be consuming enough aflatoxin to injure them.

Soon after the aflatoxin hazard in peanuts was first recognized, in the early 1960s, U.S. growers and processors of peanuts and peanut products began an intensive program to eliminate aflatoxin from marketed products. This meant developing harvesting, combining, storage, and sorting practices, accompanied by constant in-line sampling and testing for aflatoxin, to assure that peanuts and peanut products marketed in the U.S., even in worst-case years, would contain less than 5 ppb of aflatoxin.

If aflatoxin is present in the stock, crude peanut oil obtained either by pressing or by chemical extraction will contain some of the toxin, but the refined oil contains none. In India, where crude peanut oil is used for cooking, and aflatoxin commonly is present in the peanuts in some seasons and some areas, the people consume aflatoxin with the oil. In the U.S., peanut meal and cake, like other feed ingredients, are limited to no more than 20 ppb of aflatoxin, and all countries that import peanut meal and cake have strictly enforced regulations limiting the amount of aflatoxin permitted in these products. They also have the sampling and inspection procedures to enforce these regulations.

Where peanuts are grown as a garden crop, as in many semitropical and tropical regions, and consumed by the growers themselves and fed to their livestock, aflatoxin must at times be consumed.

Cottonseed. Aflatoxin occurs in cottonseed wherever cotton is grown, and in some years it is common, especially in irrigated cotton in Texas, Arizona, and California. Infection may occur through the flowers, as it does in the other high-aflatoxin-risk crops, but injury to the bolls by insects or other agents may be necessary for the formation of large amounts of aflatoxin in the seeds. Boll rot caused by *A. flavus* is common, and some seeds from individual bolls were found to contain from 200,000 to 300,000 ppb of aflatoxin. In a 1969 survey, 15 percent of 2,780 samples of cottonseed collected throughout the U.S. contained aflatoxin. In cottonseed stored at 21.8 percent moisture and 85°F (30°C), alfatoxin increased from a relatively small amount to more than 18,000 ppb in less than 30 days. Cottonseed meal from which the oil has been removed is a common ingredient of many animal feeds; it obviously is important that any batch of cottonseed or cottonseed meal or cake intended for feed be checked for aflatoxin. In the early research on aflatoxin toxicity, an outbreak of fatal hepatomas (liver cancers) in hatchery trout was traced to cottonseed meal in the ration.

Coconuts and Copra. Interior tissues of young coconuts are commonly invaded by filamentous fungi and yeasts, presumably via the flowers. Once the husks of mature coconuts have been removed, fungi easily enter the interior of fruits through the germ pore of the shell. If *A. flavus* is among these entering fungi, once the coconut shell is cracked or broken, or the meat removed and the oil expressed, *A. flavus* may develop rapidly and produce large amounts of aflatoxin. Copra is an excellent material for aflatoxin production. Copra to be used for feed should be checked for aflatoxin before being incorporated in the ration.

Brazil, Pistachio, and Other Nuts. When incoming shipments were first tested for aflatoxin in the late 1960s and early 1970s, 20 percent of the Brazil nuts, and 80 percent of the pistachios were refused because of high aflatoxin contamination. After exporters of these nuts established their own sampling and testing procedures, the number of cargoes refused because of high aflatoxin contamination was reduced to less than 3 percent. This does not mean, however, that the cargoes in which no aflatoxin was found were free of aflatoxin. Any sampling procedure used to detect the presence of irregularly distributed material in a large bulk is of limited value. The samples taken amount to only an infinitesimal portion of the total bulk so high levels of aflatoxin in other portions could be missed.

In 1975, a shipment of Brazil nuts arriving at San Francisco was cleared by FDA inspectors as aflatoxin free. But the importers tested their own samples, found aflatoxin, and refused the shipment. They submitted some of these samples to us, and we found some of the nuts to be heavily invaded by a variety of fungi, including *A. flavus*. This stimulated us to buy packages of Brazil nuts in retail stores in St. Paul. All of these packages were labeled U. S. Grade No. 1. The nut meats in approximately 3 percent of these 15 samples were badly decayed by fungi, including *A. flavus*; a few of the nuts had masses of yellow spores over much of the surface. We do not know what inspection procedures or what nut characteristics are used to determine U. S. Grade No. 1 in Brazil nuts, but they obviously do not exclude all moldy nuts.

The USDA tested 1,768 samples of U.S.-grown almonds, filberts, pecans, and walnuts and found an average of 50 ppb of aflatoxin in 5 percent of them. Supposedly *A. flavus* invades and produces aflatoxin in nuts while the nuts are still on the tree.

Corn or Maize. Corn kernels on developing ears in the field can be invaded by *A. flavus* growing in through the silks, but the fungus also can grow or be carried in through holes in the husks made by insects such as borers, ear worms, or weevils. Intact kernels are somewhat resistant to invasion by *A. flavus* that grows in through the silks and the fungus may not develop much or at all unless the ears are injured by insect feeding. In one test in Kentucky, where beetles free of *A. flavus*, and other beetles contaminated with *A. flavus* were allowed to attack developing ears of corn, 100 times as much aflatoxin was formed in the ears with beetles-plus-fungus as in the ears invaded by beetles without the fungus. There, invasion through the silks was of minor significance in aflatoxin contamination of the ears.

Stress induced by drought and high temperatures also favors invasion of corn by *A. flavus* and the formation of aflatoxin in those ears. What actually is involved in this stress is not known, but there is no question that aflatoxin in corn is a far greater problem during a dry and hot growing season than one

of normal rainfall. In 1977 and in 1980 drought prevailed during much of the growing season in the southeastern U.S., and the aflatoxin problem in both corn and peanuts reached near calamity proportions. Much corn could not be fed or sold because of high aflatoxin content, and flocks and herds suffered severe losses from eating aflatoxin-contaminated feed. Costs associated with the aflatoxin outbreak there in 1980 were estimated at $100 million, and lawsuits amounting to $8 million were filed to recover damages to farm animals by aflatoxin-contaminated feed. In 1977, of 994 samples of corn from fields in Florida, 23 percent had more than 2,000 ppb of aflatoxin. In late 1977, the FDA established an "action level" of 0.5 ppb of aflatoxin in milk (had the zero amount level then in force been continued, it was estimated that 80 percent of the Georgia-produced milk in 1977-78 would have had to be dumped). Many livestock growers and milk producers in the region most affected questioned whether the hazards posed by aflatoxin might not be too great to make it worthwhile for them to continue their operations. Even wild game birds and deer in the region had detectable amounts of aflatoxin in their tissues. This illustrates what damage *A. flavus* and aflatoxin can do when the conditions are favorable for invasion and toxin production in corn.

In 1983 a general drought extending across the Corn Belt states from Ohio to Iowa and Nebraska seemed likely to trigger an outbreak of aflatoxin similar to that in the southeastern states in 1977 and 1980, but it turned out not serious. Of nearly 800 samples from fields in several of the states where the drought was most severe, about one in 10 contained more than 20 ppb of aflatoxin. The corn merchandizing and processing firms were alerted to the possibility that corn with more than the allowable amount of aflatoxin might be coming to market. Corn sampling at 118 elevators in Indiana showed none with more than 100 ppb of aflatoxin, and only five with more than 20 ppb. Corn with 100 ppb of aflatoxin can be fed to nonlactating animals without damage to the animals themselves or to passing harmful amounts of aflatoxin or aflatoxin derivatives along to humans in the edible portions of the animals. The corn milling industries set up their own surveillance procedures and diverted to feed any corn with more than 20 ppb of aflatoxin. Corn marketing proceeded normally, without scare stories or any emergency measures.

Drought stress probably will occur in some corn-growing areas every year, but the sampling and testing procedures now in place are designed to keep corn that contains more than the 20 ppb permitted by the FDA regulations out of marketing channels. Individual farmers and cooperatives feeding their own corn to their own animals probably should test corn for aflatoxin content in years when surveys indicate aflatoxin might pose a problem.

Aflatoxin is apt to be much more of a problem in the southeastern states from Virginia south to Florida and west to Mississippi and Louisiana, than in the Corn Belt. Aflatoxin is rarely found in corn in Michigan, Wisconsin, Minnesota, Iowa, Nebraska, or the Dakotas. It does not appear to be a problem in export corn—both the exporters and importers are well aware of the risks of aflatoxin contamination and do sufficient sampling and testing to make sure that export cargoes do not contain more than the permissible amounts of aflatoxin.

Aflatoxin in High Moisture Corn Silage. Usually silage is fermented by a mixture of fungi, yeasts, and bacteria, with bacteria predominating. We, however, encountered several instances of *A. flavus* producing harmful amounts of aflatoxin in high moisture corn silage. In every instance the silage was unloaded from the top with a circulating surface auger. *A. flavus* became established in a surface layer an inch or two deep. In this surface layer the fungus grew vigorously enough to raise the temperature of the corn so that few or no other fungi could grow: *A. flavus* established itself as a nearly pure culture. Most of this layer was removed each day by the circulating auger, allowing *A. flavus* to continue to grow as a nearly pure culture in the newly exposed layer, perpetuating itself from day to day. The solution to this problem was simple—remove enough silage to get rid of the *A. flavus* dominated area. This was done and the problem disappeared.

Presumptive Test For Aflatoxin in Corn. A presumptive test for the presence (not the amount) of aflatoxin in corn has been used in some country and terminal elevators for several years. It involves examining a 10-pound sample of corn in a layer one kernel deep, under ultraviolet or so-called "black" light. A characteristic bright yellow-green fluorescence (BYGF) indicates possible presence of aflatoxin. The fluorescing material actually is kojic acid, not aflatoxin. The test gives both false positives (BYGF but no aflatoxin) and false negatives (no BYGF, but aflatoxin present). Kits to make this test are available from several commercial sources.

According to fairly extensive tests with hundreds of samples of both white and yellow corn, even a single BYGF kernel or particle per kilogram of corn (about 2 pounds) or five fluorescing particles or kernels in a 10-pound sample, indicates that the corn should have laboratory analysis for accurate determination of the amount of aflatoxin present. A rapid mini-column test estimates rather precisely the amount of aflatoxin in a test sample, but requires apparatus, chemicals, and some know-how. Testing laboratories charge about $25 to $50 for aflatoxin analysis. The Minnesota State Department of Agriculture laboratory also tests for aflatoxin and other mycotoxins at reasonable prices. A fast and inexpensive quantitative enzyme-linked immuno assay (ELISA) for aflatoxin shows promise as a recent analytical tool. Moderately extensive testing indicates a good correlation between the results obtained by the ELISA test and those obtained by high pressure liquid chromatography (HPLC). In corn, formation of aflatoxin often is accompanied by heavy sporulation of *A. flavus* on the surface of the kernels and sometimes on the husks, detectable to bare eye examination.

Aflatoxin in Edible Animal Products

General. A portion of the aflatoxin in animal feed ends up in altered but still toxic form in the human food chain: milk of dairy cows, edible organs and flesh of beef and pigs, and eggs and flesh of poultry. The proportion of the aflatoxin in the feed to the aflatoxin in the milk or edible portions of the animals is several hundred or several thousand to one. That is, for 1 ppb of aflatoxin to appear in the milk or meat of these animals, the feed must contain several hundred to several thousand ppb of aflatoxin.

Milk. In eight studies, the ratio of aflatoxin in feed to aflatoxin in milk ranged from 31:1 to 1600:1, with an average of 244:1. That is, if the cow consumed feed with 24.4 ppb of aflatoxin, just over the level permitted in feed, the milk would have 0.1 ppb of aflatoxin, the smallest amount detectable in milk, and one-fifth the amount that causes liver tumors when fed for 20 months to rainbow trout (the animals most sensitive to injury by aflatoxin).

Beef and Pork. The ratio of aflatoxin in feed to aflatoxin in edible portions of beef and pork ranges from about 200:1 in the liver to several thousand:1 in muscle tissue.

Poultry and Eggs. As with beef and pork, the ratio of aflatoxin in the feed to aflatoxin in the flesh or eggs of poultry ranges from several hundred to several thousand:1.

In the U.S. and in many other countries the regulations and the monitoring of foods for aflatoxin are strict enough to protect the consumer from injury by aflatoxin in products sold in markets and stores: that is, in commercially packaged or processed foods. The levels of aflatoxin permitted in animal feeds (15-20 ppb, and for limited times and places up to 100 ppb in feed for mature, nonlactating animals) are below those known to injure animals consuming it. If the grower produces home-grown feed, it is essential to be aware of the aflatoxin hazard locally and, if necessary, have this feed checked for aflatoxin. This also applies to the *Fusarium* toxins discussed in the following section. Mycotoxins have been publicized so frequently in newspapers and farm journals in the last 20 years that by now they should be recognized as one of the hazards of agriculture.

Effects of Aflatoxin on Animals

General. Different kinds of animals, and even different varieties within a given species, may vary greatly in susceptibility to injury by aflatoxin consumed in the ration. Chickens, ducks, and turkeys are highly susceptible to aflatoxin injury and for quite a few years the "duckling test" was used to check the presence of aflatoxin in suspect feed or product. If day-old ducklings died shortly after being put on the feed, it was presumptive evidence of the presence of aflatoxin, and if characteristic hyperplasia (cell enlargement) had occurred in the bile duct tissues of the affected birds (detectable by pathologists examining sections of the tissues) the presence of aflatoxin was firmly established. Chemical tests to identify aflatoxin have long since replaced this biological test.

Swine, sheep, and cattle are less sensitive than chicks, ducklings, or turkey poults to aflatoxin injury. Young animals are more sensitive to injury by aflatoxin than old ones and animals on a protein-deficient diet are more sensitive to aflatoxin injury than are those on a well-balanced ration. Rainbow trout hatchlings are extremely sensitive to injury by dietary aflatoxin.

Organs Affected. In animals studied, the organ most seriously affected is the liver. Continuous and prolonged consumption of even very small amounts of aflatoxin may result in cancerous liver tumors; aflatoxin is said to be the most potent naturally occurring carcinogenic (cancer-causing) agent known. In one strain of rainbow trout, 0.5 ppb of aflatoxin in the ration will eventually result in cancerous liver tumors. This is 1 gram of aflatoxin in 2 billion grams of rations, or 1 gram in 2,000 metric tons, or 1 ounce of aflatoxin in 62,500 tons of rations.

Some Specific Cases. Broiler chicks fed a ration containing 200 ppb of aflatoxin for 10 weeks gained less weight than those on a ration free of aflatoxin, and at the end of the test had liver lesions characteristic of aflatoxin poisoning. Broiler chicks kept 3 weeks on a ration containing aflatoxin, then put on a ration free of aflatoxin, gained weight at less than normal, and had an increased susceptibility to bruising, which showed up as discolored areas in the flesh from hemorrhaging in small blood vessels. Hemorrhaging into the muscles or body cavities also is characteristic of aflatoxicosis. Broiler chicks on a well-balanced commercial ration with a 75 ppb of aflatoxin had decreased breast size, lower than normal dressed weights, poor color, and fatty livers.

Pigs given a ration containing 200 ppb of aflatoxin were anemic and stunted. The same amount of aflatoxin consumed regularly by calves and steers caused stunting and liver damage. Beef cattle showed no signs of injury after 10 weeks on a ration containing 440 ppb of aflatoxin.

Unthriftiness. Aflatoxin consumed regularly or intermittently in amounts too small to result in any obvious signs or lesions can cause unthriftiness, poor appetite, and below normal feed conversion. They lack vigor and spirit, and if they could talk they probably would say they are feeling lousy. Unthriftiness can result from many other causes besides aflatoxin poisoning.

Suppression of Immunity. One insidious effect of aflatoxin and also of some other mycotoxins is suppression of the natural immunity to infection. The animals become susceptible to infection by bacteria such as *Salmonella* and to various viruses and other infectious agents always lurking around the farmyard, feedlot, or poultry house, and that normal, healthy animals ward off.

This makes it possible to blame aflatoxin, real or imagined, for almost any disease in a herd or flock. An outbreak of disease occurs in the animals, samples of feed are sent to a testing laboratory, *A. flavus* and other fungi are found in them, and on this basis the feed producer is sued for damages suffered. To conclude, from such evidence, that the feed might have been toxic is wrong. All feed grains carry various kinds of molds, as do just about all food grains. Molds are an integral, essential, and natural part of the world: humans eat moldy foods and breathe moldy air. To suggest that a given batch of feed is toxic because a potential toxin-producing fungus is present is unreal.

To sum up the effects of aflatoxin in farm animals: regular or occasional consumption of feed containing aflatoxin in the range of less than 100 ppb to a few hundred ppm will result in decreased feed consumption, poor feed conversion, stunting, and decreased production of whatever it is that the animals are grown for—flesh or eggs in poultry, milk in dairy cows, and meat in pigs and beef cattle. The reduced growth and productivity may be accompanied by damage to the liver, hemorrhaging into the muscles or body cavities, and suppression of natural immunity to parasites and pathogens always present in the environment. Once the damage has been done, the animals will not fully recover even if returned to a toxin-free ration.

Aflatoxin in Dust Inhaled by Workers

Aflatoxin is present in the spores of *A. flavus*, which sometimes are produced in great abundance on the ears of fungus-infected corn. When corn is combined, and unloaded at elevators and at other transfer points, it generates much dust and some of this dust may contain aflatoxin. Dust collected near a combine in Georgia in 1980 contained from 2030 to 52,200 ppb of aflatoxin, and aflatoxin content of the dust at the elevator receiving this corn ranged from 621 to 1480 ppb. Dust masks are recommended for these workers, but in hot and humid weather they are uncomfortable to wear. In the severe aflatoxin outbreaks in the southeastern U.S. in 1977 and again in 1980, some workers on farms, in elevators, and feed mills must regularly have inhaled large amounts of aflatoxin-containing spores and dust. That inhaling aflatoxin-contaminated dust could be a health hazard is suggested by the following:

- A chemical engineer working on methods of sterilizing aflatoxin-contaminated peanut meals developed bronchial cancer. He died in a few months. An autopsy found his lungs contained aflatoxin.
- A professor and graduate student died of colon cancer several years after working on aflatoxin identification by thin layer chromatography (TLC), scraping off the aflatoxin spots without using a fume hood.
- In the Netherlands, 11 of 60 to 70 workers exposed to peanut meal containing aflatoxin, developed cancer, while

only four of a similar group not exposed to peanut meal dust, developed cancer. Airborne dust collected in the processing plant had 250-500 ppb of aflatoxin.

These instances strongly suggest that materials known to contain aflatoxin be handled with care. Wearing dust masks, though uncomfortable, seems the better choice.

Prevention

General. Prevention comprises a five-pronged approach:

- Varieties resistant to insects or other agents that give entry to or carry *A. flavus*, and to aflatoxin formation once the fungus has invaded them;
- Field practices that lessen the likelihood of damage to the parts susceptible to invasion by *A. flavus* (applicable to peanuts, but not to corn);
- Maintenance of postharvest and storage conditions that will not allow *A. flavus* to grow if it already is present, or that will keep it from invading the crop if it is not already present;
- Sampling and testing to detect contaminated lots in the marketing chain, and excluding them;
- Chemical inactivation of aflatoxin present in a given lot of contaminated product.

Resistant Varieties. Some varieties of peanuts and corn are much more resistant than others to invasion by *A. flavus* or to aflatoxin formation, or both. Geneticists and breeders are engaged in research on the development of varieties resistant to aflatoxin production; success is likely to come slowly.

Field Practices. In peanuts, careful cultivation and lifting at harvest will reduce to a minimum the mechanical damage to the pods that leads to infection by *A. flavus*. Corn should be combined with cylinder settings that do as little seed damage as possible. Also some varieties of corn are much more resistant than others to mechanical damage and this characteristic presumably could be bred into commercial varieties to make them less susceptible to postharvest invasion by *A. flavus*. Since most of the aflatoxin found in corn is formed in the field before harvest, the value of this approach for reducing aflatoxin contamination in corn is open to question. However, storage at moisture contents too low to permit damaging invasion by any and all fungi is an essential part of the maintenance of quality in all kinds of seeds, including those of the high aflatoxin risk crops: peanuts, cotton, and corn. If good storage principles and practices are applied, damage from fungi during storage can be eliminated or held to a nonthreatening minimum.

Sampling and Testing For Aflatoxin. This is an integral part of eliminating, from the food marketing chain, products containing more than permissible amounts of aflatoxin. The analytical tests now in use can detect levels of aflatoxin lower than those of toxicological significance in raw materials and finished food products and feeds. Aflatoxin and aflatoxicoses are problems mainly where aflatoxin-contaminated crops are fed on the farms where they were grown or where aflatoxin-contaminated ingredients are processed into feeds without the appropriate sampling and testing.

Chemical Inactivation of Aflatoxin. An ammoniation process has been developed to destroy or convert aflatoxin into metabolically harmless compounds, making aflatoxin-contaminated grain or feed usable. This process has been used on a small scale with some success, but there may be some complications that limit its value. In some samples of aflatoxin-contaminated feed treated with ammonia, the aflatoxin was in part converted to another but still toxic compound. Also, in Arizona in 1978 a large amount of stored cottonseed was unmarketable because it was contaminated with aflatoxin. In 1985, still in storage, some of it was treated with the ammoniation process to eliminate aflatoxin and shipped to California for processing as dairy cattle feed. However, tests by the receivers showed that it still contained aflatoxin and so it was refused. As a result, California prohibited any further imports of ammoniated cottonseed—in this instance ammoniation was decidedly unsuccessful.

Guidelines, Action Levels, and Tolerances for Aflatoxin Contamination

The FDA has established a guideline or action level of no more than 15 ppb of aflatoxin in peanut products used for animal feed, and no more than 20 ppb in all other feeds. Feed ingredients, or finished feeds with an aflatoxin content above these limits, are subject to confiscation. At the discretion of the FDA, the limits of aflatoxin contamination in this or that ingredient can be raised somewhat, locally and temporarily, as in 1978 when 20-100 ppb of the toxin were permitted in feed for mature, nonlactating animals in the southeastern U.S.

Recently consumer groups have instituted court action to require the FDA to establish tolerances instead of action levels or guidelines for aflatoxin in foods and feeds. In setting guidelines or action levels the FDA is not required to solicit public comment or to supply experimental evidence that the levels they establish are indeed safe. The FDA never has claimed absolute safety for its guidelines, but has tried to set levels that can be met with the technology available and well below those known to cause injury. To establish tolerances would require public hearings, and presumably would require experimental evidence to support whatever levels were established under the tolerances selected.

The analytical methods are continually being improved to detect smaller and smaller aflatoxin amounts. What if, in the near future, it is possible to detect one part of aflatoxin per trillion parts food or feed, and consumer groups get the tolerance set at that level—would this eliminate the dairy and beef industries of the southeast and perhaps elsewhere? The matter is before the courts now, and may eventually have to be decided by the Supreme Court.

Fusarium Toxins

Zearalenone and the Estrogenic Syndrome in Swine

The estrogenic syndrome in swine has been reported from the U.S., Canada, Mexico, Ireland, England, the USSR, Italy, South Africa, Japan, China, and Australia. In the U.S. it is common throughout the Corn Belt, where scattered cases occur every year, and widespread epidemics some years. Outbreaks of it have been reported as far south as Georgia, but it is much more common in the north.

Fungi That Produce Zearalenone

By far the major zearalenone-producing fungus is *Fusarium roseum* (*F. graminearum* and *Gibberella zeae* are synonyms of *F. roseum*). A common cause of ear and stalk rots, *F. roseum* is often accompanied by other species of *Fusarium* that contribute to the ear and stalk rots and that may produce some zearalenone as well as other toxins which can complicate the diagnosis of the estrogenic syndrome in swine.

Crops in which Zearalenone is Found

Corn and Feeds in Which Corn is a Major Ingredient. Corn unquestionably is the major source of zearalenone, although the compound has been found occasionally and in smaller

amounts in wheat, barley, oats, sorghum, sesame seed, hay, and silage. In the early 1970s, 65 samples of corn and commercially prepared swine feeds in which corn was a major ingredient, were tested for zearalenone. It was found in 45 percent of the 65 samples, in concentrations of 0.1 to 2909 ppm, based on dry weight of the feed. Levels of 0.1 to 5.0 ppm in corn fed to swine will cause tumefaction (swelling) of the vulvas. Zearalenone in amounts of 0.1 to 5.0 ppm was found in 17 percent of 223 samples of corn from commercial channels in the U.S. in 1972. Most of these samples were from the northern Corn Belt where ear rot caused by F. *graminearum* was common in 1972. In two other surveys of corn in commercial channels, in years when *Fusarium* ear rot was not common, much less zearalenone was found. In some years F. *graminearum* ear rot occurs erratically here and there, which accounts for the sometimes erratic distribution of the disease in swine.

Wheat and Other Small Grains. From 364 to 11,054 ppb of zearalenone were found in 19 of 102 samples of soft red winter wheat in the 1975 crop in the state of Virginia, when head blight caused by *Gibberella zeae* was epidemic there. Head blight or scab caused by F. *graminearum* was prevalent on one widely grown variety of wheat in Minnesota in 1978, but zearalenone was found only in trace amounts in several samples tested. Zearalenone has not been found regularly enough and in large enough amounts in barley or sorghum to be responsible for more than relatively minor and local toxicosis in swine.

Conditions That Promote Zearalenone Production

The combination of conditions that leads to the production of relatively large amounts of zearalenone in corn are:

- At least a moderate prevalence of F. *graminearum* ear rot in corn in the field before harvest.
- Storage of this infected ear corn in cribs, at moisture contents above 22-25 percent, so that the F. *graminearum* continues to grow slowly.
- A period of several weeks of low or fluctuating moderately low and somewhat higher temperatures, with the higher temperatures stimulating growth of the fungus and the lower temperatures stimulating production of zearalenone.

This combination of conditions prevails throughout much of the Corn Belt in late fall and early winter, following corn harvest. Ears of corn left unharvested in the field will be exposed to the same conditions and zearalenone can be formed in them, too.

Evidently little zearalenone is produced in corn in the field during the growing season. In Indiana, ears of corn in the field were inoculated with strains of F. *graminearum* known to produce zearalenone. Typical ear rot developed, but at harvest none of these ears contained more than 5 ppm of zearalenone and most had less. In 1972, when ear rot caused by F. *graminearum* was epidemic in Indiana, representative ears were collected at harvest and tested for zearalenone: eight ears contained 0.1 to 0.5 ppm, five contained 0.6 to 1.0 ppm, and three contained 5.6 to 10 ppm. It was estimated that the bulk shelled corn as it came from these fields would not have had more than one-fifth to one-twentieth the amounts found in the infected ears. From this it seems that even a moderately severe epidemic of F. *graminearum* ear rot would not result in enough zearalenone in the corn at harvest to produce the estrogenic syndrome in the Indiana swine that consumed it. In normal harvest years it seems the significant production of zearalenone is in ears of corn that come from the field with F. *graminearum* ear rot and are subsequently exposed to low temperature during storage in cribs.

Some strains of F. *graminearum* will produce moderate amounts of zearalenone at room temperatures in the laboratory, but these strains evidently are not common in the field, or are not aggressive producers of ear rot.

There is no evidence to suggest that zearalenone present in corn at harvest will continue to develop in stored shelled corn. F. *graminearum* requires a minimum of 22-25 percent moisture to grow, and if shelled corn is stored at that moisture content it is likely to be invaded by a mixture of other fungi, yeasts, and bacteria with which F. *graminearum* cannot compete. There might be situations, as in low temperature drying, in which F. *graminearum* could continue to grow for a short time, but there are no reported instances of this happening. Also there is no record of zearalenone being formed anew in high moisture shelled corn stored in silos. There is one Minnesota case on record where zearalenone was formed in high moisture corn treated with propionic acid. It was presumed that incomplete coverage by the acid accounted for its growth. There are statements in the literature to the effect that zearalenone in corn sometimes is a storage problem, but this occurs only in ear corn stored in cribs. Nor will F. *graminearum* continue to grow and produce zearalenone in mixed feeds. If a lot of mixed feed contains zearalenone, the zearalenone came from the corn component of the feed and existed before the feed was mixed.

Effects of Zearalenone on Animals

Swine. Zearalenone consumed by swine affects chiefly the genital system. In the prepuberal gilt, the vulva becomes swollen and this may progress to vaginal or rectal prolapse (figure 2). These outward changes are accompanied by characteristic changes in the interior tissues—the uterus of the affected animal is enlarged, swollen, and twisted, and the ovaries are shrunken. Young males undergo a feminizing effect, with atrophy of the testes and enlargement of the mammary glands. Litter size may be reduced, sometimes drastically. These effects can result from feeds containing several hundred ppb to 10-20 ppm of zearalenone. In concentrations of 66 to 5600 ppb, zearalenone has been found in samples of feed suspected of causing outbreaks of the estrogenic syndrome in U.S. and Canada herds.

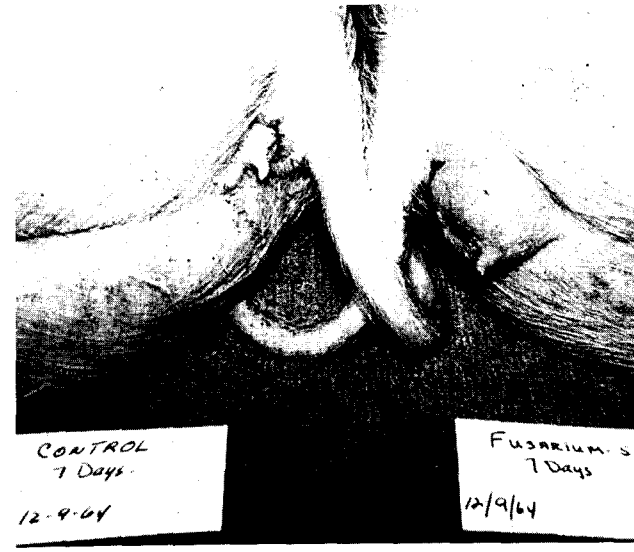

Figure 2. Swollen vulva in gilts caused by zearalenone in the diet.

Dairy Cows. Decreased fertility, prolonged estrus and swelling of the vulva are some of the signs associated with rations containing zearalenone as well as other natural products produced through natural infection of feed ingredients (corn, barley, hay). Animals vary as to their response but some will show standing estrus during mid cycle. In a controlled experiment involving 36 heifers, zearalenone lowered the conception rate significantly.

Poultry. Broiler chicks and laying hens are very little affected by dietary zearalenone, even when the compound dosage is relatively massive. Pure zearalenone was fed to broiler chicks and finishing broilers at rates of 10, 25, 50, 100, 200, and 800 ppm, with the controls receiving the same ration minus the zearalenone. Similar tests were made with laying hens. In the broilers, weight gain, feed consumption, and feed gain ratio were not affected, and the weights of the liver, heart, spleen, testicles, oviduct, comb, and bursa of *Fabricius* were similar to those in the controls which received no zearalenone. No prominent signs were detected in post-mortem examination, other than enlargement of the oviduct in some birds fed 800 ppm of zearalenone.

In laying hens, zearalenone had no effect on egg production, egg size, feed consumption, body weight, fertility, hatchability of fertile eggs, or reproductive performance.

When turkeys ate feed containing 300 ppm of zearalenone (a massive dose), within four days they developed greatly enlarged vents, but there were no other gross effects (figure 3).

Figure 3. Effects of zearalenone on the vents of 10-12 day-old turkey poults. Left, control; Right, zearalenone administered in feed rations at the rate of 300 ppm for 5 days.

Deoxynivalenol (DON, Vomitoxin) and Feed Refusal in Swine

Occurrence

Feed refusal resulting from consumption (or non-consumption) of feed contaminated with deoxynivalenol or DON has been found worldwide, especially in the temperate zones and sometimes over large areas. Swine may refuse feed for reasons other than DON in the ration, but DON is a common cause of refusal. Feed refusal may be accompanied by swollen vulvas and reproductive problems from zearalenone in the same ration, and sometimes a complex of effects as described below.

Fungi That Produce DON

DON appears produced only by *F. graminearum*, (= *F. roseum*, = *Gibberella zeae*), the same species that produces zearalenone, but evidently different strains of the fungus are responsible for producing the different toxins. Sometimes DON and zearalenone are both found in the samples of mixed feed or corn, but it is not known whether the toxins occur together in the same kernels.

Conditions That Favor Production of DON

Wet or rainy, or warm and humid weather from flowering time on promotes infection of corn and the small-grain cereals by *Fusarium*, resulting in ear rot in corn and in scab or head blight in barley, wheat, oats, and rye. Low temperature following infection may increase production of DON. In one case of DON toxicosis, corn harvested early contained 1.6 ppm of DON and that harvested later on the same farm contained 10.6 ppm. DON already present in corn at harvest may increase in ear corn stored in cribs, as does zearalenone. DON is not known to increase in shelled corn or in small grains that come contaminated from the field, nor would it be expected to since *Fusarium* growth requires a minimum moisture of 22-25 percent in corn. Grains that are free of DON at harvest will not develop it in storage. DON and zearalenone are sometimes spoken of as storage problems, but there is no evidence that DON increases in storage, other than in ear corn stored in cribs at moisture contents high enough for the fungus to continue to develop from infections that occurred in the field.

Effects on Animals

Swine. Feeds that contain more than 1 ppm of DON may result in significant reductions in animal feed intake and weight gain. Even smaller amounts may result in lowered feed consumption and lower than normal weight gain. Although the name "vomitoxin" suggests that vomiting occurs, vomiting is rather uncommon in field cases because pigs will ordinarily not eat enough of the DON-contaminated feed to cause vomiting. Besides feed refusal, other effects may accompany intoxication by DON. Investigations of a DON outbreak in Illinois in 1981 and 1982 are worth reviewing in some detail: cool, wet weather prevailed in Illinois and surrounding areas before and during harvest in 1981, favoring infection of corn and small grains by *Fusarium*. Reports of feed refusal and clinical signs of ill health appeared in farrowing operations, feeder pigs, and breeding sows in late 1981, and increased in 1982. Investigators gathered nearly 400 samples of feed, mainly corn and corn-based ground feeds, but also wheat and oats, from farms and swine operations where problems had appeared. They tested them for DON, zearalenone, T-2 toxin, diacetoxyscirpenol (another *Fusarium* toxin), and aflatoxin. In one investigation, feed samples were also analyzed for additional *Fusarium* toxins—HT-2, fusarenone, monoacetoxyscirpenol, and nivalenol.

DON was found in 80 percent of the nearly 400 samples, in concentrations of 0.1 to 41.6 ppm. Zearalenone was found in 12 percent of the samples, at concentrations of 0.1-8 ppm. Some of the feeds contained both DON and zearalenone, but none of the other suspect mycotoxins was found.

Clinical signs and lesions in the affected swine included feed refusal, a few instances of vomiting, lack of weight gain, poor feed efficiency, failure of mature sows to return to estrus, reduced fertility, high mortality of nursing pigs, intestinal tract inflamation, and acute diarrhea in young pigs. Examinations of

dead young pigs revealed hemorrhaging into the abdominal cavities and pale, friable livers.

In all four cases investigated in detail, the problems were reduced or disappeared when the pigs were provided with sound feed.

Another outbreak: In Australia in 1983, wheat, barley, and triticale grown on a farm in southeastern Queensland were infected with *F. graminearum* during prolonged wet weather before harvest. Some of this grain, when fed to swine, was refused by the animals. Gilts that consumed some of the feed developed enlarged and reddened vulvas. The grains were found to contain 0.1 to 34 ppm of DON and 0.1 to 6.2 ppm of zearalenone. The researchers who described this outbreak speculated that most of the DON and zearalenone probably developed in the grains during storage, but this seems unlikely; they mentioned only that grains had been stored at above 12 percent moisture, but even if the grains had been stored at 16-18 percent moisture (resulting in rapid microbiological spoilage) *Fusarium* still would not have been able to grow.

Both DON and zearalenone were found in a number of samples of feeds, including pellets, that were involved with feed refusal in swine herds in several midwestern states.

Cattle. Dairy cattle are relatively insensitive to dietary concentrations of DON likely to be found in feeds. No ill effects were noted in cattle that consumed rations containing up to 6.4 ppm of DON.

Poultry. Chickens suffered no detectable ill effects from rations containing up to 18 ppm of DON, when chickens ate a ration containing 9.18 ppm of DON, none was detected in the flesh or eggs. No ill effects were detected in turkey poults given a ration containing 5 ppm of DON.

In all these tests with cattle and poultry, the DON in the ration came from grain naturally infected with *F. graminearum* in the field. If DON might sometimes be accompanied by *Fusarium* toxins other than zearalenone, there was no evidence of this in these tests.

Humans. Until close to 1900 in eastern Europe and western Russia, where people made their own bread from their own grain (sometimes infected with *F. graminearum*), there were occasional human outbreaks of toxicosis, characterized by headaches, dizziness, shivering, vomiting, disturbances of vision and general malaise associated with *Fusarium* toxins in the bread. *Fusarium* toxicoses were widespread in the population of one USSR district in the early 1940s and still occur in portions of China where the people consume locally grown grains from fields invaded by toxin-producing strains of *Fusarium*.

In 1982 scabby wheat was common in the hard red winter wheat from Minnesota through Nebraska, Kansas, Missouri, and Oklahoma. Samples of this wheat contained up to 6.8 ppm of DON. Some of this wheat was experimentally milled and when the different milling fractions were tested for DON, it was found in every one, more in the bran than in the break flours. Whole wheat flour and bread made from samples of this wheat contained from 108 to 520 ppb of DON, so that the DON in the wheat was not removed (although reduced) by milling, nor destroyed by baking. DON was found in amounts of 39-45 ppb in rye breads marketed in New York: this had to have come from field-contaminated grain.

Control

As with other mycotoxins, the only control is to avoid grains contaminated with DON. The FDA has issued a suggested limit of 2 ppm of DON in grains destined for feeds, and Canada has suggested the same limit. State departments of agriculture, veterinary diagnostic laboratories, and private testing laboratories can analyze samples for DON content. Farmers or feed producers in areas where *Fusarium* ear rots of corn or scab or head blight of small grains is prevalent might do well to have samples tested before mixed into feeds to avoid including contaminated lots in mixed feeds for swine.

Fusarium Species, T-2, Diacetoxyscirpenol, and Toxicoses in Domestic Animals

Instances of toxicoses resulting from consumption of feeds contaminated by one or more of these potent toxins have been reported throughout the temperate zones of the world. The most publicized of these have involved a sudden and drastic drop in egg production in laying hens, an outbreak of the hemorrhagic bowel syndrome, with death of some of the animals, in herds of swine or cattle. After thorough examination and testing by veterinarians, mycotoxicologists were called in and detected T-2 toxin, or T-2 and diacetoxyscirpenol, or a combination of one or both and still other *Fusarium* toxins in the feed. When sound feed was provided, the troubles quickly disappeared. Many lesser instances of damage by these toxins must go undetected. Some investigators of *Fusarium* toxins and toxicoses believe these probably are much more common and of much greater importance in animal economy in many countries than now realized. Routine examination of feeds or feed ingredients for mycotoxins has not yet, but probably should, become a standard procedure in the diagnoses of unknown diseases in farm animals.

Fungi That Produce T-2 and Diacetoxyscirpenol

These toxins are produced mainly by a group species that Snyder and Hansen (*Fusarium* taxonomists) lumped together as *F. tricinctum*. Other taxonomists find this system "totally unacceptable" and insist that the toxin-producing members of the group are *F. poae* and *F. sporotrichioides*. Some strains or varieties of the *F. roseum* group also produce diacetoxyscirpenol, and probably other and yet unidentified toxins that may be associated with the toxicoses caused by T-2 and related trichothecene compounds.

Major Sources of T-2 and Conditions Favoring Production

T-2 and/or diacetoxyscirpenol have been found in barley, wheat, millets, safflower seed, field corn, sweet corn, and in many mixed feeds with any of these grains as a main ingredient. In 1973-1974, USDA workers collected 173 corn samples from marketing channels in the Midwest and found presumptive evidence of T-2 toxin in 54 percent. At that time methods of chemical extraction, purification, and quantitative analysis of T-2 were not available, but they extracted each sample, concentrated the extract, and applied it to the shaved skin of a rat. If a characteristic skin lesion developed, this was considered evidence of T-2 in the corn sample. Some of the T-2 positive samples were in the upper (better) grades of corn, including grade No. 1. Corn is a prime suspect as a carrier of many *Fusarium* toxins, in part because it constitutes a major feed grain throughout much of the world, and also because it is so commonly infected with *Fusarium* ear rots. T-2, with zearalenone, has been found in samples of mixed feed associated with bloody stools in swine in Nebraska; with DON and zearalenone in mixed feed

associated with vomiting in dogs in Iowa; in mixed feed associated with vomiting and diarrhea in swine in Minnesota; and with zearalenone and DON in the pith of corn stalks collected after harvest in Minnesota.

Conditions Favoring Production of T-2 and Diacetoxyscirpenol

If T-2 and/or diacetoxyscirpenol are present in corn at harvest, it or they can be expected to increase in ear corn in cribs at moisture contents that permit some continued growth of *Fusarium*, followed by low temperatures or by fluctuating moderate and low temperatures. These are the same conditions that promote increased production of zearalenone in ear corn stored in cribs. But small grains infected in the field before harvest, and affected with scab or head blight, may contain damaging amounts of T-2 or diacetoxyscirpenol, or both, at harvest. There is no evidence to suggest these toxins continue to increase in small grains in storage, nor will these toxins form anew in shelled corn or small grains in storage. T-2 in amounts of only 1-2 ppm in feeds has caused infertility in gilts and sows in Hungary, and drastic reduction of egg production by laying hens in Israel; it may be present in slightly greater amounts than this in scabby wheat or barley at harvest. T-2 in amounts of 1-2 ppm has been associated with unthriftiness and gastrointestinal hemorrhaging in swine and cattle in the U.S.

Effects of T-2 on Animals

Evidently all domestic animals are susceptible to injury by dietary T-2 and diacetoxyscirpenol in the range of a few ppm.

Cattle. Unthriftiness, decreased feed consumption, slow growth, lowered milk production, sterility, gastrointestinal hemorrhaging, and death can result from toxins eaten by cattle.

Swine. Infertility, with some lesions in the uteri and ovaries of swine, resulted from consumption of feed contaminated with 1-2 ppm of T-2 toxin.

Poultry. Drastic and sudden decrease in egg production resulted from consumption of feed contaminated with 3.5 ppm of T-2 and 0.7 ppm of HT-2. The problem disappeared when sound feed was provided. The feed containing the toxin produced more damaging effects than did the pure T-2 toxin administered in the same amount, indicating that other toxins might have been present in the feed.

In U of M tests in which T-2 was added to feed at 1-2 ppm and given to chickens, egg production was reduced, eggs had thin shells, the chickens had abnormal feathering and grew slowly. The same feed given to turkeys resulted in reduced growth, beak lesions, and less immunity to infection (figure 4).

Figure 4. Lesion on the edges of the beak of a turkey consuming 2-10 ppb of T-2 in its rations.

As small an amount as 2-10 ppb of T-2 in the ration has resulted in ill effects in poultry.

Control

As with the other mycotoxins, the only control is to avoid contaminated feeds. Private laboratories, those of state departments of agriculture and veterinary diagnostic laboratories, can test feed or grain samples for T-2 and diacetoxyscirpenol. If more than a small amount of *Fusarium* infection is present in feed grains at harvest, it might be well to test for these toxins before grains are compounded into feeds and fed to animals. Usually by harvest time in an area, any greater than normal amount of *Fusarium* ear rots in corn, or of *Fusarium* head blight or scab in wheat and barley, will be evident. There is always the possibility that in some variety or specific planted location of corn, wheat, or barley, there may be an unusually high infection by *Fusarium*, and it would pay farmers to be alert to these.

Fusarium equiseti and Tibial Dyschondroplasia in Poultry

Tibial dyschondroplasia (TDP) is a common bone deformation in growing broiler chickens and turkeys worldwide. The lesion shows up in a cone of cartilage extending distally from the proximal tibiotarsal physis (figure 5). This deformation is of economic importance in the poultry industry and until recently its cause was not known.

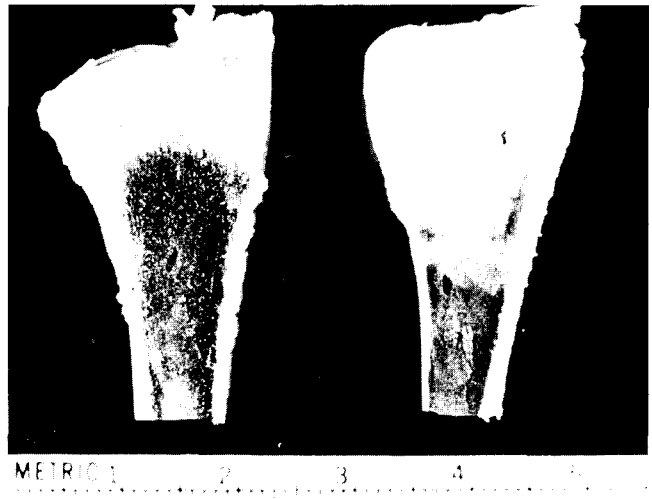

Figure 5. Bone lesion in poultry causing tibial dyschondroplasia as a result of eating grain infested with *Fusarium equiseti* and containing fusorochromanone. (Photo courtesy of Mary Walser, D.V.M.)

An isolate of *F. equiseti* from overwintered oats in Alaska, grown in autoclaved moist corn and fed to chicks as 3 percent of the ration, produced a high percentage of leg lesions typical of TDP. The same feed caused near zero hatchability of fertile eggs.

A toxin called fusarochromanone isolated from cultures of this fungus, when added to broiler chicks diet at a concentration of 75 ppm, resulted in TDP in 100 percent of the chicks. This toxin may be largely responsible for the TDP syndrome in poultry; it also kills chick embryos in fertilized eggs.

Fusarium moniliforme

Up into the 1930s occasional outbreaks of blind staggers (technically known as *equine leucoencephalomalacia*) sometimes occurred in horses foraging corn left standing after harvest. Results of limited tests suggest that a toxin or toxins produced by some strains or species of the *F. moniliforme* group, specific to horses, are responsible for this disease. Recently, a newly discovered toxin called fumonisin was cited as the cause of the disease. It is produced by certain isolates (not all) of *F. moniliforme*. This toxin is also carcinogenic in laboratory tests. An effort is being made to find this toxin in U.S. grains. *F. moniliforme* also has at times been suggested as the possible or probable cause of other diseases in farm animals, mainly on a basis of the presence of the fungus in samples of the suspect feed. However, *F. moniliforme* is common in corn—even in food-grade corn—and it often is abundant in ground feeds and in silage. Yet, growing pigs have been fed a ration containing 78-82 percent corn heavily invaded by *F. moniliforme*, and these pigs grew as well as the control pigs given a ration of sound corn.

Ochratoxin

High-Ochratoxin-Risk Crops and Areas. Ochratoxin has been found in occasional samples of most food and feed grains, in peanuts, and in cocoa and coffee beans, but it is a serious problem only in feed grains in limited areas. Several outbreaks of ochratoxin poisoning from contaminated corn and corn gluten have been reported in commercial flocks of poultry in the southeastern U.S. If other instances have occurred in the U.S. they have gone unrecognized or unreported. Ochratoxicosis, the disease, is a recurrent problem in swine in Denmark and a lesser problem in Sweden. In Denmark in 1971 ochratoxicosis in swine ranged from 0.6 to 66 cases per 10,000 pigs; in some years a large percentage of the animals in some herds may be affected.

Fungi That Produce Ochratoxin. In the laboratory, ochratoxin can be produced by several species in the *Aspergillus ochraceus* group and by a number of species of *Penicillium*, especially *P. viridicatum* and its near relatives. The species and conditions in nature most responsible for production of ochratoxin in stored grains is not known, for some of the following reasons.

Conditions That Favor Production of Ochratoxin. Since the toxin has not been found in grains before harvest, it most likely is produced by the growth of fungi on high-moisture grain in storage. In extensive work with deterioration of stored grains and seeds by fungi, we have seldom recovered *A. ochraceus* from more than a small percentage of seeds of any samples of grains in any stages of spoilage, from the earliest light molding to the final total decay. Usually *A. ochraceus* does not appear until spoilage is well underway. *A. ochraceus* inoculated as a pure culture on almost-fungus-free seeds of wheat can invade them slowly at moisture contents of 15-16 percent, and rapidly at moisture contents about 17 percent. But when it is inoculated with other common storage fungi such as *A. glaucus* and *A. candidus* that almost invariably accompany it in seeds undergoing microbiological deterioration, it cannot compete with them. This probably explains why it is almost never found as more than a trace of the mixed fungus flora responsible for spoilage in stored grains.

Species of *Penicillium* that produce ochratoxin require moisture contents in the range of 20-22 percent to grow in the starchy cereal seeds such as wheat, oats, barley, rye, and corn, but they can grow well at temperatures of about 40°F-50°F (5°C-10°C) and under those conditions they may at times predominate. In the regions where ochratoxin has been a recurrent problem—Denmark and Sweden—the preharvest and harvest weather often is cool and moist, and the postharvest storage temperatures are relatively low, favoring the growth of *Penicillium*. If ochratoxin is to be prevented by maintaining storage conditions that would limit the growth of the toxin-producing fungi, it would be good to know what combinations of moisture content, temperature, and fungus species are necessary for its production.

Animals Affected, Signs, and Lesions. All kinds of laboratory animals tested have been sensitive to injury by ingested ochratoxin. In the field, however, injury from ochratoxin poisoning has been chiefly (or only) in poultry and swine. Regular consumption of a ration containing several hundred ppb of ochratoxin will result in poor feed conversion, reduced growth rate and general unthriftiness, accompanied by reduced immunity to infection by bacteria and viruses. At slaughter the kidneys may be found to be enlarged and pale, with an uneven cortical surface, and cortical fibrosis. Lesions may also be evident in the liver. Ochratoxin damage to the kidneys of swine is characteristic enough to have been given the designation of "porcine nephropathy," recognizable and recordable in commercial slaughtering.

Control. The only control, as with other mycotoxicoses, is to avoid contaminated feed grains. Where ochratoxin is a recurrent problem, knowledge of the conditions under which it is produced in grains in storage would greatly facilitate development of an effective control program. The principles and practices of good grain storage are known, and where they are applied, damage to stored grains from fungi, including those that produce toxins, can be avoided. Adequate sampling and testing of feed ingredients for mycotoxins before the feeds are compounded should enable the animals' producers to identify and reject lots contaminated with ochratoxin.

Slobber Syndrome and Facial Eczema

The fungus *Rhizoctonia leguminicola* growing in red clover produces a compound that when consumed by cattle results in profuse salivation, whence the inelegant but descriptive name, "slobber syndrome," which is relatively common throughout the Midwest. Actually the compound itself is not toxic as consumed, but is transformed by the metabolism of the animals into a toxic compound.

Another fungus, *Pithomyces chartarum*, when growing on the dead leaves of forages and pasture grasses, produces a compound called sporodesmin, which is hepatotoxic when consumed by sheep. The skin of affected animals becomes sensitive to sunlight, resulting in the development of facial eczema. This is prevalent in some areas of New Zealand and Australia: a similar disease has been described in U.S. cattle.

Ergot and Ergotism

Ergot toxicity differs somewhat from the other mycotoxicoses because it results from the consumption of a considerable amount of fungus tissue in which the toxin(s) are found. In the other mycotoxicoses the toxins are secreted into the plant tissues in which the fungus is growing, and very little fungus material itself is consumed. *Claviceps purpurea* infects the flowers of a number of grasses, including wheat, rye, barley, triticale (the wheat-rye hybrids), and crested wheat grass, and forms characteristic spur-like sclerotia. These contain toxic alkaloids that when consumed regularly in small amounts result in a complex of signs collectively referred to as ergotism.

Ergot in whole grains can be easily recognized, but in ground feed special techniques are required to detect it and at times the ergot removed from food grains ends up, along with other screenings, in ground feed. Another species of the fungus, *C. paspali*, infects *Paspalum dilatatum*, commonly known as water grass, Dallis grass, or paspalum grass, an important forage grass in the southern U.S. and various countries. Paspalum ergot evidently is more toxic than the ergot on cereal grains.

Some animals apparently are much more susceptible than others to ergot poisoning. In controlled feeding tests, as little as 0.06 percent of ergot (from triticale) caused injury in beef cattle, but turkey poults fed a ration containing 0.5 percent of ergot, nearly 10 times as much as caused injury in beef cattle, suffered no detectable harmful effects.

Corn Smut

Some smut (*Ustilage maydis*) almost always is present in field corn, and relatively heavy infections are common in some regions in some years. Smut infection was heavy in many corn fields in western and southern Minnesota in 1976, and we received many inquiries as to whether silage made with this corn might be harmful to animals. The fairly extensive evidence available indicates no harmful effects in animals eating smutted corn. Many years ago some feeding tests appeared to show that smutty corn was toxic, but the toxicity found probably was caused by other fungi, such as *Fusarium*, also present in the corn.

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