

MYCOTOXINS AND MYCOTOXICOSES

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Toxigenic Fungi

Mycotoxins are toxic fungal metabolites that cause intoxication when consumed by animals, including humans. Fungi that produce mycotoxins are called toxigenic fungi. The toxigenic fungi do not produce mycotoxins after they have been ingested by animals and humans.

Toxigenic fungi grow in corn, cereals, soybeans, sorghum, peanuts, silage and other food and feed crops or hay in the field, in grain during transportation. Mycotoxins can be produced in storage under conditions favorable for the growth of the toxin-producing fungus or fungi.

Mycotoxins can be found in any animal feedstuff or human foodstuff that has previously supported growth of toxigenic fungi. It is estimated that there may be 20,000 to 300,000 unique mycotoxins and only a relative few (<50) have been well characterized. These toxins can be found in processed foods and feeds produced from contaminated feedstocks. The most common mycotoxins are produced by fungi in the genera; *Aspergillus*, *Penicillium* and *Fusarium*. However, fungi in the genera; *Alternaria*, *Stachybotrys*, *Claviceps* and *Epichloe* produce common and important mycotoxins.

Mycotoxicoses

Mycotoxicoses is the disease caused by animals and humans consuming feedstuffs and foodstuffs contaminated with mycotoxins. Mycotoxins can cause death or mycotoxins can cause chronic ill health resulting from mycotoxins damaging the kidneys, and liver. Mycotoxins can also damage the immune, cardiovascular, endocrine, reproductive and nervous systems. Mycotoxins also cause hemorrhage, abortion, reproductive disorders, tremors, convulsions, immune system dysfunctions, skin disorders and gangrene of appendages. In addition,

mycotoxins cause economic losses from unthriftiness, reduced growth rate, poor feed conversion, feed refusal, increased restlessness, agalactia, and lameness (Table 1 – page 25).

Historic records exist of mycotoxins causing disease in humans and animals exist. Alimentary toxic aleukia (ATA) was linked with consumption of grains that had overwintered in the field. The wheat, barley and prosomillet were shown to be infected by *Fusarium* species that are potent producers of trichothecene mycotoxins. Alimentary toxic aleukia was observed in tens of thousands of people in Russia and central Asia from 1941-1947. In 1934 a malady called "moldy corn disease" occurred in the Midwest. More than 5,000 horses died because of mycotoxins contaminating feed. This group of mycotoxins was linked to "Moldy Corn Disease" are the fumonisins. In 1972, Gibberella ear rot caused extensive feed-refusal problems in swine in the Corn Belt. Aflatoxin has caused problems in several animal species in the southeastern USA for many years, and fescue toxicosis has been a common problem for many years with fescue pastures in the Mid-southern and Southern regions.

Human suffering from mycotoxicoses also includes "Holy Fire" – "St. Anthony's Fire" which is linked to consumption of ergot alkaloids in rye and wheat flour. A disease called "Yellow Rice Disease" was associated in Asian countries with humans consuming rice colonized with *Penicillium* molds. A disease called "Acute Cardiac Beriberi" also was associated with yellow rice. This disease is linked to neuro- cardiotoxic mycotoxin citreoviridin being produced by *Penicillium* species. Aflatoxins are linked to liver cancer in humans, esophageal cancer has been linked to consumption of grain infected with *Fusarium moniliforme*. Several mycotoxins have been linked to increased incidence of cancer in humans. Zearalenone has been associated with precoces breast development in girls. Ochratoxin A is suspected as a cause of the Balkan endemic nephropathy. Gliotoxin, a mycotoxin, is suspect in multiple sclerosis.

The adverse effects of feeding moldly grains and other feedstuffs has long been known by livestock and poultry producers. The specific implication of a specific mycotoxin did not occur until 1960 after the outbreak of "Turkey X Disease" in great Britain. The contaminated feed was traced to peanut meal imported from Brazil. The chemicals that became known as aflatoxins were identified, and the mycotoxicosis was reproduced by dosing poultry and livestock with aflatoxins. Thus, aflatoxins were discovered and identified as etiology of the death in >100,000 young turkeys, ~20,000 ducklings, pheasants, partridge poult and numerous

other livestock. Identification of aflatoxins stimulated research on toxigenic fungi and the mycotoxins they produce (Table 1).

Toxigenic Fungi

Three genera of fungi namely *Aspergillus*, *Penicillium*, and *Fusarium* (*Gibberella*) are most frequently involved with cases of mycotoxin contamination in corn, small grains, and soybeans (Table 1). *Aspergillus flavus* produces aflatoxins in starchy cereal grains (e.g., corn, wheat, sorghum, oats, barley, millet, rice) and mold growth and mycotoxin production essentially starts at a moisture content of about 18 percent (0.85 aw, equilibrium with 85 percent relative humidity), and at temperatures of 54° to 108 °F (13° to 40°C) with optimum growth at 81° to 86°F (25° to 35°C). The critical moisture content for growth of *A. flavus* in soybeans is 15 to 15.5% and for peanuts 8 to 9%. The upper limit of moisture for growth of *A. flavus* and aflatoxin production is about 30%. *A. flavus* will grow slowly below 54 °F (13 °C), and most rapidly grow at 98 °F (37°C) but will not produce aflatoxins at temperatures below 54 °F (13°C) or above 108 °F (40 °C). Under optimum conditions for growth, low levels of aflatoxins can be produced by *A. flavus* within 24 hours and a biologically significant amount can be produced within a few days.

Other toxigenic fungi grow on grain at moisture contents of 17 to 40% and a wide range of temperatures from below freezing (<0 °C) for some species of *Penicillium* to over 131°F (55°C) for some species of *Aspergillus*. The quality of the grain and its suitability for storage are adversely affected by (1) a high moisture content, (2) physical damage to the kernels, and (3) the extent to which storage fungi have invaded the seed before the grain goes into storage.

Toxigenic fungi may grow under a given set of conditions but do not necessarily produce mycotoxins. The substrate is important. For example, *A. flavus* flourishes on many crop plants, but does not produce equal amounts of aflatoxin. *A. flavus* grows equally well on peanuts and soybeans. *A. flavus* produces more aflatoxins when growing on peanuts than when growing on soybeans. The risk factors for preharvest production of aflatoxins are warm-to-hot, humid conditions, drought-stressed and insect-damaged plants; these conditions are the most common in the southeastern United States.

Aflatoxins and Aflatoxicosis

The fungi, *Aspergillus flavus* and *A. parasiticus*, are common in most soils and are usually involved in decay of plant materials. They commonly cause stored grains to heat and decay and commonly invade corn, peanuts and cottonseed in the field before harvest. The problem is serious in subtropical and tropical regions of the world where cereals, peanuts, corn, and copra are important in the human diet. Aflatoxin B₁ is one of the most potent, naturally occurring animal carcinogen and is formed in corn, corn silage, all cereal grains, sorghum, peanuts, and other oil-seeds. All species of animals appear to be susceptible to aflatoxins and susceptibility varies from species to species. Aflatoxins were identified as the cause of epidemic liver cancer (hepatoma) in rainbow trout and 4 ng/kg of diet fed for ~16 months causes liver cancer. Aflatoxins are classified as a confirmed potential human carcinogen. Young animals are more sensitive to aflatoxins. Cows are less sensitive to aflatoxins than calves. Monogastric animals including horses are more sensitive to aflatoxins than mature ruminants. Animals and humans on a protein-deficient diet are more sensitive to aflatoxins than animals on a protein adequate ration.

Aflatoxins have been classified by IARC as a confirmed potential human carcinogen. Outbreaks of human and animal aflatoxicosis have occurred in India and Africa. In the years 1977 and 1980, before harvest >60% of the corn grown in the southeastern United States contained ≥ 20 ppb aflatoxin B₁, and the majority of the pre-harvest corn exceeded the 20 ppb levels permitted by the U.S. Food and Drug Administration (FDA).

Regulated Levels of Aflatoxins

Aflatoxins, fumonisins and deoxynivalenol are regulated by the FDA (Table 2). The FDA regulates aflatoxins (sum of B₁, B₂, G₁ and G₂) at 20 ppb for human foods. Some state agencies and foreign countries have established more restrictive limits (no more than 5 ppb) of permissible aflatoxin contamination in grains or other products in interstate/ international commerce. Grains or other products with levels above 20 ppb but less than 100 ppb may be shipped under specific conditions within the USA for cattle feed. Grains over 100 ppb may be subject to confiscation. Mixing high and low aflatoxin-contaminated corn to achieve a blend, which meets FDA standards constitutes adulteration, and is subject to severe FDA penalties.

Table 2. Regulatory limits for mycotoxins in the USA.

Mycotoxin	Regulatory limit
Aflatoxin B ₁ , B ₂ , G ₁ , G ₂	20 ppb for all products for human food, immature animals and dairy cattle, animal feeds other than corn or cottonseed and grain for export 100 ppb for grain intended for breeding beef cattle, breeding swine or mature poultry 200 ppb for grain intended for finishing swine of 100 lbs. or greater 300 ppb for grain intended for finishing beef cattle or cottonseed meal intended for beef cattle, swine or poultry
Aflatoxin M ₁	0.5 ppb for milk
DON	1 ppm for all finished wheat products, e.g. flour, bran and germ that may be consumed by humans 10 ppm for all grains or by-products destined for beef cattle older than 4 months and for chickens; these ingredients should not exceed 50% of the diet 5 ppm all grain or by-products destined for swine; these ingredients should not exceed 20% of the diet 5 ppm all grains and by-products for all other animals; these ingredients should not exceed 40% of the diet
Fumonisin total of B ₁ , B ₂ and B ₃ (proposed regulations-FDA, 2000)	2-4 ppm Human foods 5 ppm (<20% of diet) Horses and other equids and rabbits 20 ppm (<50% of diet) swine and catfish 30 ppm (<50% of diet) breeding ruminants, poultry, mink, dairy cattle, laying hens 60 ppm (<50% of diet) ruminants >3 months before slaughter and mink for pelt products 100 ppm (<50% of diet) poultry raised for slaughter 10 ppm (<50% of diet) all other species or classes of livestock and pet animals
Ochratoxin A	No regulation in the USA but the European Union has a 5 ppb limit for raw cereal grains, 3 ppb for all grains and cereal products destined for human consumption and 10 ppb for dried vine fruits
Zearalenone	No regulation in the USA but 1 ppm is advised.
Other mycotoxins	No regulation in the USA

However, under some circumstances FDA and state department of agriculture regulations have permitted the blending of aflatoxin-contaminated and clean grain to obtain mixes that can be fed to some nonlactating animals. Such feeds can be used on the farm where it is produced, but cannot be sold. The aflatoxin M₁ metabolite is regulated at 0.5 ppb (0.5 ng/kg dry weight). Corn with 100 ppb of aflatoxin can be fed to nonlactating animals and 300 ppb can be fed to finishing cattle and 150 ppb can be fed to finishing pigs without residues of aflatoxins and their

metabolites in edible portions of the animals.

Field Contamination

The potential seriousness of the aflatoxins contaminating crops before harvest is illustrated by the years 1983 and 1988. In these two years, a general drought extended across the Corn Belt from Nebraska and Iowa to Illinois, Indiana and Ohio. Thousands of corn samples were taken from fields in these states and 5% to 10% of the samples contained >20 ppb of aflatoxin. In 1983, corn sampling at 118 elevators in Indiana showed none of the corn contained more than 100 ppb of aflatoxin, and only five samples contained more than 20 ppb.

It is essential that the growers and livestock producers be aware of the aflatoxin hazard locally and, if necessary, they should have the corn assayed for aflatoxins. If a producer is purchasing feed out of the local area, he should be aware of the aflatoxin situation in the area from which the grain is purchased and require aflatoxin analysis if there is reason for concern. This also applies to the *Fusarium* sp and other mycotoxins discussed in later sections.

Risk Factors for Production of Aflatoxins

Aflatoxins B₁, B₂, G₁, and G₂ are produced by *A. flavus* and *A. parasiticus* in grains before harvest and during storage. Infection is most common after the kernels have been damaged by insects, birds, mites, hail, early frost, heat and drought stress, windstorms, and other unfavorable weather. The presence of *A. flavus* or *A. parasiticus* in a given feed sample does not show that aflatoxins are present. The presence of the toxigenic fungi does increase the risk for aflatoxin production. Aflatoxins persist under the majority of environmental conditions, and aflatoxins are not destroyed during feed manufacturing processes. Pelletizing feeds may eliminate fungi present in the stock, but will not reduce or eliminate aflatoxin present in any of the ingredients. Food processing and baking does not destroy aflatoxins. Aflatoxins are not destroyed during alcohol production, and on a dry matter bases, aflatoxins are concentrated in stillage and distillers solubles.

Decontamination and Binding of Aflatoxins

Roasting, ammoniation at ambient temperatures and some microbial treatments may sharply reduce but not eliminate the aflatoxin content. Ammoniation has been shown to be most

effective in reducing aflatoxin levels. Currently, these treatments have limited application with roasting being the least effective. The addition of binding agents such as hydrated sodium calcium aluminosilicate (HSCAS) and bentonite clays to corn has been shown to decrease the toxic effects of aflatoxin when fed to swine. These compounds probably work by nonspecific binding (adsorbing) to the mycotoxin and thereby reducing absorption of aflatoxins. Although not specifically approved for this purpose, various products which have this ability are approved as binding or anti-caking agents.

Toxicology of Aflatoxins

All animal species are susceptible to aflatoxicosis, and the sensitivity varies between species. For example, monogastric animals such as birds, fish, dogs, and swine appear to be more susceptible than mature ruminants. In poultry, liver and kidney disorders, leg and bone problems and increased occurrences of bruising can develop as well as outbreaks of diseases such as coccidiosis. Aflatoxins decrease native resistance to disease and this phenomenon may cause vaccines to fail. Immunosuppression is manifested by increase susceptibility to disease and increased occurrences of disease especially diseases that normally would not have fatal outcomes. Liver disease causes a decreased in blood clotting factors and an increase in trimming and condemnation of the birds occurs because of massive bleeding and bruises. Less carcass pigmentation is exhibited and egg yolks are paler. The hatchability of eggs can decrease, and reduced indicis of production in the birds may be noted. Growth is reduced and mortality rate increases, especially during the growing period.

Regular or occasional consumption by livestock of feedstuffs contaminated with aflatoxins range of <100 ppb to a few hundred parts per million (ppm) may result in decreased feed consumption, decreased feed conversion, stunting, and decreased flesh growth, wasting of body condition and death. Decreased productivity is accompanied by damage to the liver and additional pathology such as hemorrhaging into the gastrointestinal tract, muscles and body cavities may be observed. As with poultry, suppression of natural immunity occurs and decreased effectiveness of vaccines and increases in diseases associated with animal production occurs. Signs of neurologic dysfunction may also be observed. Once the damage has been done, the animals may not fully recover even if returned to a toxin-free ration.

Aflatoxins in Milk

Aflatoxins M₁ and M₂ are metabolites of aflatoxins B₁ and B₂, respectively, that are excreted in milk from dairy cows fed aflatoxin contaminated feeds. Lactating cows consuming feed containing 20 ppb or less of aflatoxin will have less than 0.1 ppb of aflatoxin M₁ in milk. The dietary threshold for cows to excrete aflatoxins in milk is ~15 ppm in the diet. Generally, the levels of the M₁ metabolite are 1% of the aflatoxin content of feed and range from 0.17% to 6.3% of the dietary aflatoxin. The percentage of dietary aflatoxins excreted in milk increases with milk yields, and cows in early lactation excrete higher levels of aflatoxins in milk. For comparison, humans excreted 0.09% to 0.43% of the dietary aflatoxins in milk.

Aflatoxins in Grain Dust

Aflatoxins are present in the spores of *A. flavus*, and these spores can be produced in great abundance on the ears of fungus-infected corn. When corn is unloaded and mixed at elevators or other transfer points, considerable grain dust (fungal spores and mycelia plus broken grain) is formed and grain dust can contain aflatoxin. In 1980, dust collected in Georgia near a combine harvester contained from 2,030 to 41,200 ppb of aflatoxin. The aflatoxin content of the dust at the elevator receiving this corn ranged from 621 to 1,480 ppb. Aflatoxin produced in other stored cereal grains will also be present in “grain dust”. Dust masks must always be worn when handling obviously moldy grain. Inhaling aflatoxin-contaminated dust is a health hazard. Workplace exposures to aflatoxins have been associated with increased occurrences of cancer. Grain handlers have been shown to have more respiratory problems than the general population. Farmer's lung, a disease which afflicts grain handlers, is a pulmonary disease which occurs in farmers when they inhale large amounts of grain dust containing fungal hyphae and spores. Farmers' lung is a tissue-damaging allergic reaction (extrinsic allergic alveolitis) to fungal spores and other material in grain dust. Mycotoxins in grain dust are frequently associated with skin irritation, fever, wheezing, breathlessness, cough and ulcers. This latter disease is due to the direct effect of the fungal toxins, not an allergic reaction. Grain invaded by *Aspergillus* species is highly friable, therefore great care should be taken when feeding grain screenings. Broken grains often have very high levels of aflatoxin concentration.

Zearalenone, Zearalenol - Estrogenic Syndrome

Zearalenone (F2 toxin) and zearalenol are produced almost exclusively by *Fusarium* species. These species of *Fusarium* contribute to ear and stalk rot and scab on the heads of cereal grains (scab) standing in the field. *Fusarium* can also be a storage disease of corn. The zearalenone mycotoxins can be found up to 5 ppm in corn silage and delayed harvest soybeans. When consumed by swine at dietary concentrations at 0.1 to 5 parts per million (ppm) (mg toxin/kg feed), these compounds cause the estrogenic syndrome, which is characterized in females by a swollen and edematous vulva with enlarged mammary glands, and in young males by a shrinking of the testes and swollen anal area. Young gilts may show nymphomania, vagina prolapse and gilts, bores and barrows may have prolapse of the rectum. Anoestrus and false pregnancy may be observed in gilts and sows. Abortions generally do not occur, but reduced litter size may be observed. If lactating sows consume zearalenone-contaminated feed, piglets may develop enlarged vulva and anal regions. Splay-legged piglets are linked to sows consuming zearalenone-contaminated feed during late pregnancy. These impacts on reproductive performance cause financial loss to hog industry. Zearalenone poisoning (estrogenism) in swine and dairy cows is usually more prevalent in the winter and early spring because once the fungus is established in the grain or silage, it generally requires a period of relatively low temperatures to produce biologically significant amounts of zearalenone. Some strains of *Fusarium graminearum* when growing in corn produce zearalenone and a mixture of mycotoxins. In zearalenone poisoning, the animal is generally exposed to a mixture of mycotoxins. In addition to estrogenism, severe stunting and other deleterious effects can be observed in swine. Nymphomania, decreased fertility, prolonged estrus and swelling of the vulva and decreased milk production are signs in dairy cows fed rations containing zearalenone. The offending grains usually are corn, barley, corn silage and occasionally hay. In incidents where zearalenone was linked to estrogenism in dairy cattle, the zearalenone level detected in the concentrate was 1.5 ppm with 1.0 ppm DON. The effects of zearalenol are similar to zearalenone but zearalenol is generally considered to have 5- to 10X greater estrogenic effects.

Broiler chicks and laying hens, unlike swine and dairy cows, are affected very little by dietary zearalenone, even when fed massive doses. Pure zearalenone fed to broiler chicks and finishing broilers at rates from 10 to 800 ppm resulted in no effect on weight gain, feed consumption, and feed gain ratio. The weights of the liver, heart, spleen, testicles, oviduct,

comb, and bursa were similar to those in the controls that received no 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) they developed greatly enlarged vents within four days, but there were no other gross effects.

Deoxynivalenol (DON, Vomitoxin) - Feed Refusal in Swine

Deoxynivalenol is produced by a number of fungal species. Important producers of deoxynivalenol are *Fusarium graminearum* (sexual state *Gibberella zeae*) which causes red ear rot of corn, and *F. culmorum* and *F. graminearum* which cause Fusarium head blight (scab) of wheat and barley (Table 3).

Table 3. Fusarium species associated with Fusarium head blight (scab) and the mycotoxins they produce in wheat and cereal grains.

<i>Fusarium</i> species	Mycotoxins Produced					
	DON ¹	DAS ²	nivalenol	T-2	HT-2	zearalenone
<i>acuminatum</i>		x		x	x	
<i>avenaceum</i>						
<i>culmorum</i>	x		x			x
<i>equiseti</i>		x	x			x
<i>graminearum</i>	x		x			x
<i>Poae</i>		x	x	x		
<i>sporotrichioides</i>		x		x		
<i>tricinctum</i>						

1. deoxynivalenol and its acetylated derivatives
2. diacetoxyscirpenol

These fungi generally produce other mycotoxins including zearalenone. The DON contaminated feed is usually unpalatable to swine. Field-infected corn with visibly damaged kernels of more than about 5 percent is commonly refused by pigs. Feed refusal may be accompanied by swollen vulvas and reproductive problems from zearalenone and DON being present in the same ration. Swine producers often encounter serious problems when they improve consumption by applying molasses or other similar materials. A mixture of mycotoxins can produce bazaar effects.

Risk factors are wet, rainy, warm and humid weather occurring from anthesis stage of flowering on to maturity promotes *Fusarium* infections of corn, wheat, barley and other cereals. These infections result in ear rot in corn, and scab or head blight in wheat, barley, oats, and rye. Low temperatures following infection may increase the production of DON. The mycotoxins already present in corn at harvest may increase in ear corn stored in cribs due to continued mold growth and mycotoxin production. Improperly stored high moisture corn and silage can have high levels of mycotoxins. Grains stored at < 20% moisture and free of the toxin at harvest have not been observed to develop either DON or zearalenone mycotoxins in storage. The *Fusarium* fungi cannot grow at moistures less than 20%.

Feeds that contain 1 ppm of DON may result in significant reductions in swine feed consumption and weight gain. Vomiting is rather uncommon in field cases because pigs will refuse to eat the contaminated feed. Clinical signs and lesions in affected swine included feed refusal, increased restlessness and fighting, banging of the feeders, increased occurrences of sows stepping and laying on piglets, instances of vomiting within a shortly after eating, episodes of diarrhea and signs of abdominal pain, decreased weight gain, poor feed efficiency, and a failure of mature sows to return to estrus. The pathology of DON in pigs are erosions on the oral mucosa, and irritation and congestion of the gastrointestinal tract. In experimental studies, pathology of the pancreas has been reported. The pigs may become anemic and have low serum protein, calcium and phosphorus. In all field cases investigated in detail, the problems were reduced or disappeared when the pigs were given sound feed. Some pigs may not have compensatory gain. Dairy and beef cattle are relatively insensitive to dietary concentrations of DON likely to be found in feeds. Young birds are more sensitive to DON than older birds. Levels of 5 ppm in feed to chicks for age 1 day to age 21 days caused changes in the intestine, but did not alter performance parameters.

Trichothecene Mycotoxins, T-2, HT-2, Diacetoxyscirpenol (DAS)

Fusarium species that produce DAS, T-2, nivalenol, and other trichothecenes are listed in Table 1. These fungi commonly attack grains and can grow at temperatures from slightly above freezing to about 86 °F (30°C). T-2 and HT-2 toxins are produced over a temperature range of 46° to 77 °F (8° to 25°C), with the maximum production at temperatures below 59 °F (15°C). This group of mycotoxins produced by *Fusarium poae* and *F. sporotrichoides* were

associated with Alimentary Toxic Aleukia (ATA) a disease that killed thousands in the USSR in 1913, and in the Ukraine during the 1940 to 1947 interval.

All domestic animals are susceptible to poisoning by dietary intake of T-2, HT-2, and DAS in the range of a few ppm. In poultry T-2 toxin in feed contaminated with 1 to 3.5 ppm of T-2, and 0.7 ppm of HT-2 (a closely related toxicant) may produce lesions at the edges of the beaks, abnormal feathering in chicks, a drastic and sudden drop in egg production, eggs with thin shells, reduced weight gains, hemorrhages in various tissues, increased susceptibility to infections and increased mortality. The same feed fed to turkeys results in reduced growth, oral erosions and less immunity to infection.

T-2 and DAS in cattle feed results in unthriftiness, decreased feed consumption, slow growth, lowered milk production, diarrhea, abdominal pain, anemia, decreased white blood counts, abortions, bleeding and bruising, decreased immune function and sterility. An outbreak of the hemorrhagic bowel syndrome and death of some animals can occur in herds of cattle and swine.

Pigs are susceptible to the trichothecene mycotoxins. T-2 toxin and/or DAS in amounts sufficient to cause toxicoses in pigs have been found in un-harvested corn, in silage, in soybeans, and in finished feeds using corn and soybeans as ingredients. Feed refusal is generally the first sign that the feedstuffs contain trichothecene mycotoxins. The second sign is decreased weight gains and this can be accompanied with bouts of diarrhea and lethargy can be observed. Abdominal pain and teeth grinding can occur. Hemorrhages can occur including bleeding from the intestinal tract. The trichothecenes target all cells with rapid division. These are the cells that line the gut, precursor cells that form the red and white blood cells, and precursor cells that produce spermatozoa in the testicle. Abortions have been associated with trichothecenes poisoning of sows. Infertility with uterine and ovarian lesions, result from consumption of feed contaminated with 1 to 2 ppm of T-2 toxin. Pigs, placed on feeds that do not contain mycotoxins, recover but residual effects may be observed.

Occupational health hazards exist in handling trichothecene contaminated grains and feed. The trichothecene mycotoxins can cause severe skin and eye irritation. Trichothecene contaminated grains and feed is diverted to ethanol production. Personnel must wear protective clothing and respirator when handling contaminated grain.

***Fusarium equiseti* - Tibial Dyschondroplasia in Poultry**

Tibial dyschondroplasia (TDP) is a disease in poultry that have rapid growth rates, and is an economically important disease. In tibial dyschondroplasia the growth-plate cartilage of the tibia does not mature and ossification does not occur. This results in deformity, growth plate fractures and infections. The most likely cause of this deformation is a toxin called fusarochromanone produced by *Fusarium equiseti*. When added to the diet of broiler chicks at 75 ppm, 100% of the chicks developed TDP. This toxin may be largely responsible for the TDP syndrome in poultry.

Stachybotrys and Stachybotryotoxicosis

Stachybotrys chartarum (syn. *atra*, *alternans*) and perhaps other *Stachybotrys* species produce the trichothecene mycotoxins; verrucarins B and J, roridin E, satratoxins F, G, H and G plus an unrelated toxin stachylysin. In addition some isolates also produce cyclosporins, trichoverrols trichoverrins, spirolactams, spirolactones, spirocyclic drimanes and phenylspirocyclic drimanes. Because of the numerous mycotoxins produced by this fungus many analytical laboratories limit the analyses to the verrucarins. These mycotoxins are potent inhibitors of protein and DNA syntheses. Intoxication has been seen in cattle, horses and humans associated with ingestion or inhalation of spores and mycelia. Signs of intoxication are dermatitis, leucopenia, fever, various chest and upper airway symptoms, inflammatory disorders of the mouth, rhinitis, conjunctivitis, and neurological disorders. Generally symptoms will start within 2 to 3 days of exposure, and without new exposure occurring, signs may last for 3 weeks. The *S. chartarum* fungus grows at moistures in equilibrium with humidities of 93% or greater and requires high cellulose content substrates with low available sugar and nitrogen. Clinical signs of stachybotryotoxicosis have been observed in humans living in moldy buildings and after handling wall board contaminated with black mold.

***Fusarium verticillioides* (formely *F. moniliformae*) - Fumonisin**

Fumonisin are structurally similar to sphingolipids. This group of mycotoxins are primarily produced by *F. verticillioides* and *F. proliferatum* and eleven other *Fusarium* species. Fumonisin are generally considered to be the cause of “moldy corn poisoning” in horses, mules, and donkeys. This disease in horses is known as equine leukoencephalomalacia. Fumonisin

also cause liver disease in horses and have cardiac effects, cause pulmonary edema of swine, and are strongly associated with esophageal cancer and neural tube defects of humans.

Leukoencephalomalacia typically occurs in horses, mules, or donkeys foraging corn left standing in the field after harvest, or fed grain screenings heavily infected with *F. verticilloides*. The toxins fumonisin B1 and B2 are produced only by certain strains of *F. verticillioides*. This toxicant is also carcinogenic in laboratory animals. Dietary levels of >60 ppm of feed caused leukoencephalomalacia within 25 days. Dietary levels of >100 ppm fumonisins cause pulmonary edema in swine to occur within 4 days. *F. verticillioides* is common even in food-grade corn and is often abundant in ground feeds and in silage particularly when corn is produced under drought conditions and where insect (e.g. European Corn Borer, Corn Earworm) damage to ears is common. Corn infected with *F. verticillioides* is very friable and thus easily broken, therefore horse owners should avoid feeding screenings to horses. Research on the fumonisin toxins began only recently and current thought is that concentrations of >5 to 10 ppm are necessary for mycotoxicosis in horses and >10 to 20 ppm for swine. The U.S. Food and Drug Administration has proposed but not yet adopted regulatory levels. As with other toxigenic fungi, various strains of *F. verticillioides* vary greatly in their mycotoxin producing ability.

Ochratoxin, Citrinin, and Penicillic Acid (PA) - Nephrotoxins

Ochratoxins (A, B, C) are primarily produced by *Aspergillus alutaceus* var. *alutaceus* (syn. *A. ochraceus*), *Penicillium verrucosum* (Dierckx) and *P. viridicatum* (Westling). Several other *Aspergillus* and *Penicillium* species have been reported to produce one or more of the ochratoxins. Ochratoxin A is the most common and most studied, and has been found in wheat grain, in all milled fractions, and has been identified in bread and pasta products. The *Penicillium* species are the most important in temperate climates and *A. alutaceus* var. *alutaceus* in tropical climates. All of these fungi grow under storage conditions when in equilibrium with 80 to 85% moisture (~16 to 18% for starchy cereal grains) and when temperatures are as low as 50 °C. Ochratoxin A contamination by *Penicillium* sp. is common where grain is lodged and wet weather delays harvest in temperate climates. In the field, intoxication from ochratoxin poisoning has primarily been reported for poultry and swine.

Ochratoxin A and citrinin mycotoxicoses primarily involves kidney, liver and immune system damage. Clinical signs vary with the species affected. Poultry have signs of listlessness

weakness, decreased feed consumption, increased water consumption, wet litter, increased bone fractures and decreased productivity. High levels of ochratoxin and citrinin cause visceral gout in chickens. Decreased feathering may occur. Clinical signs of ochratoxin and citrinin poisoning (porcine nephropathy) in pigs are increased water consumption and increased urination of dilute urine containing protein. Pigs are lethargic may have elevated body temperature. Ochratoxins can cause tonsillitis in pigs, and pigs can have immunosuppression. Ochratoxin mycotoxicoses in adult cattle, other adult ruminants and horses are not well characterized. Non-ruminated young ruminants are more susceptible to ochratoxins and citrinin.

Humans are sensitive to ochratoxins. The Balkan endemic nephropathy is associated with the consumption of ochratoxin contaminated foods. Human exposure to ochratoxins and citrinin can be from ingestion of contaminated grain or by inhalation of contaminated grain dust. Pork and chicken meat can contain residues of Ochratoxin A. Processed meats, such as sausages and cured hams, will have equivalent levels of those found in the fresh meat.

Maximum limits for Ochratoxin A contamination have been established in a number of countries with member countries of the European Unions setting limits of 5 (ppb) ng/g for raw grain and 3 ng/g for cereal grains intended for direct human consumption. Several countries have set 100-300 ng/g as the allowable level for animal feeds.

Toxicosis due to citrinin and ochratoxin A occurs most often in Denmark and other Scandinavian countries and is associated with *P. viridicatum* in barley. 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.

Sterigmatocystin

Sterigmatocystin is produced by several *Aspergillus* species including; *A. versicolor* (Tiraboschi), *A. fumigatus* (Fresen), *A. nidulellus* (Samson and Gams). (Syn. *nidulans* (Eidam) G. Wint., *A. terreus* (Thom)., *A. sydowii* (Bainier and Sartory), members of the *A. glaucus* (Link:Fr. group with *Eurotium* perfect stages) and *Bipolaris sorokiniana* (Sacc.) This mycotoxin is considered to be an important mycotoxin found in stored wheat and other cereals in Canada. The molds involved are relatively common in stored grains in both temperate and tropical regions. It is likely that these common saprophytes will be found in wheat stored at moistures in excess of an equilibrium with 70-75% Rh or ~14-15% moisture. This mycotoxin is

considered to be carcinogenic and causes liver damage. Clinical signs of bloody diarrhea, low milk production and deaths have been reported in a field poisoning incident. Sterigmatocystin is a precursor in the synthetic pathway for aflatoxins. The toxicology is similar to aflatoxin and it is considered to be less toxic. Only a few countries have regulations regarding sterigmatocystin contamination for food and feed.

Slobber Syndrome and Facial Eczema - Slaframine

The fungus *Rhizoctonia legumicola* growing in red clover and other legumes (black patch disease) produces the mycotoxin slaframine that when consumed by horses and cattle results in profuse salivation (slobber syndrome). This mycotoxicosis is relatively common throughout the Midwest. The syndrome in horses is characterized by excessive salivation and can be accompanied by anorexia, diarrhea, polyuria and stiffness. Abortions have been reported. In cattle slobbering and episodic increases in lacrimation occur. This can be accompanied by watery diarrhea and bloat. Difficult breathing has been reported in sheep and pigs. The compound itself is not toxic before being consumed, but is transformed by the animal into a toxic compound.

Alternaria toxins

The mycotoxins; alternariol, alternariol methyl ester, altenuene, alterntoxin and tenuazonic acid have been found in wheat where wet weather delayed harvest. These toxins have been found in whole wheat breads made from contaminated wheat grain. *Alternaria alternata* (Fr.:Fr, Keissl.), *A. triticina* (Pras. and Prab.) and perhaps some other *Alternaria* sp. have been shown to produce these toxins. These fungi grow only when wheat grain is in equilibrium with 95 to 100 % Rh. or greater than 22% moisture. These toxins have mutagenic effects and have been linked to the occurrence of esophageal cancers in China.

Claviceps sp - Ergot and Ergotism

Ergot toxicity, caused by the fungus *Claviceps purpurea*, differs from other mycotoxicoses, in that the mycotoxins are present in the developing and mature sclerotia. The mycotoxicosis occurs when the ergot sclerotia (fungal tissue) are consumed. Ergot poisoning in grazing animals typically occurs where headed grasses are grazed, sometimes selectively,

following favorable condition for infection by the *Claviceps* sp. The mature, dry ergot sclerotia are brittle and break during grain handling. The broken sclerotia are found in screenings. A large number of mycotoxins (ergot alkaloids) are found in the ergot sclerotia (ergot bodies). These include a variety of ergopeptine and clavine alkaloids that when consumed regularly in small amounts result in a complex of signs collectively called ergotism. Ergotism is characterized by are skin necrosis, necrosis of the ears, poor hair condition, gangrene or loss of extremities, lameness, agalactia and poor performance. Clinical manifestations will vary depending on the mixture of ergot alkaloids found in the sclerotia. In the USA, wheat of any class having more than 0.05% ergot by weight is declared ergoty and cannot be sold for human consumption. Levels >0.1% ergot in complete feeds may have adverse effects on livestock performance.

Risk factors increase during damp cool weather. The ergot fungus infects the flowers of cereals and many grasses when flowering occurs during predominantly cool, moist weather. Infected florets show characteristic black, spur-like sclerotia that replace the seed. Cattle can be attracted to seeds infected with *Claviceps* sp in the honey-dew stage of the infection.

Fescue Toxicosis

Many tall fescue pastures in the U.S. are infected with a systemic fungus, *Neotyphodium (Acremonium)* and *Epichloe species*. This fungus is harmless to the host plant, but is responsible for a variety of symptoms known as fescue toxicosis, summer syndrome, and summer slump when infected plants are consumed by cattle. The fungus is endophytic, meaning it grows within the tillers, culms and inflorescence of the grass without invading the host as do most of the other saprophytic or pathogenic fungi producing mycotoxins. In fact, there is evidence that the endophyte has evolved a mutualistic relationship with the grass, conferring a survival advantage in some situations. In cattle, symptoms of fescue toxicosis include reduced average daily gains, decreased milk production to agalactia (reduced prolactin levels), reduced reproductive potential, elevated body temperature, rough hair coat and sloughing of the tail. "Fescue foot" is like ergotism in that feet or other extremities may become gangrenous and drop off. "Summer slump" in cattle is characterized by rough hair coat, low milk production, low weight gains, decreased conception rate, and decreased growth and maturity. Horses typically show agalactia, abortions, thickened placenta, retained placenta,

prolonged gestation resulting in large foals and dystocia (difficult birth). The ergopeptine alkaloids similar to those produced by ergot (*Claviceps* sp) have been identified in endophyte-infected fescue, but other alkaloids may be involved as well. Rye grass staggers is caused by ingestion of the mycotoxin lolitrem B and other associated indole diterpenes found in infected perennial ryegrass. Clinical manifestations are similar to fescue poisoning.

Mixtures of Mycotoxins

Feed grains can be infected with multiple toxigenic fungi with production of multiple mycotoxins. When one mycotoxin group is present, there is a distinct possibility that mycotoxins from a different group may be present. The toxic effects observed for multiple mycotoxins can be unique in that they do not mimic the toxicology reported for any particular mycotoxin. When horses and pigs consume DON with other trichothecene mycotoxins that are not generally included in mycotoxin analytical screens, the toxic effects of DON also increases. Fusaric acid is considered to be synergistic with DON. A source of fusaric acid can be forage. The combined effects of fumonisins and aflatoxins are considered to be additive. It is important to consider the presence of multiple mycotoxins when estimating the safe levels of mycotoxins that can be fed. The predominant interaction between mycotoxins is an additive effect. There is also evidence that synergism and potentiation can also occur.

Laboratory Testing for Mycotoxins

Evidence that mycotoxins are responsible for illness in humans or animals is generally based on:

- The occurrence(s) of the disease observed is linked with feeding a particular feed or consuming a particular food.
- Examination of the suspect feed shows evidence of fungal activity.
- The disease is not transmissible from animal to animal.
- Laboratory testing of the affected animal or person does not clearly identify an infectious agent.
- Young, older and pregnant animals are generally the most susceptible and are the first to show the malady.

When one or more of these criteria are met, laboratory testing of the suspect food or feed should be done. If a mixture of mycotoxins are present, a bioassay should be considered.

For chemical analyses, the mycotoxins in the feed need to be extracted, compounds that interfere with the assay removed, the mycotoxin identified and quantified. Procedures have been developed for the extraction, purification, and quantification of the common mycotoxins such as aflatoxins, zearalenone, T-2, DAS, DON, ochratoxin A, citrinin, fumonisins, ergot alkaloids, sterigmatocystin, patulin and some of the other trichothecene toxins. Table 4 (page 26) provides information on methods for identification and quantification of mycotoxins. To achieve reliable results, these procedures require considerable expertise in the performance and interpretation of the analytical results plus sophisticated and relatively expensive laboratory equipment. Preliminary screening for toxins can be done with commercially available kits. Kits are available for aflatoxins (B₁, B₂, G₁, G₂, and M₁), zearalenone, DON, T-2, fumonisin (B₁, B₂, B₃), ochratoxin A, sterigmatocystin, citrinin, and patulin. Sources of test kits can be found in Table 5. The majority of these kits use some kind of an immunoassay. False negative and false positive results generally exceed those observed in analytical chemistry.

Table 5. Commercial kits available for mycotoxin analysis

Manufacturer and web address	Tests available
Neogen Corporation www.neogen.com	Aflatoxins, DON, zearalenone, fumonisins, T-2, Ochratoxin A
Pickering Labs www.pickeringlabs.com	Aflatoxins, ochratoxin A, zearalenone, DON
Romer Labs www.romerlabs.com	Aflatoxins, DON, zearalenone
Tepnel Biosystems	Aflatoxins, ochratoxin A, fumonisin
Vicam www.vicam.com	Aflatoxins, ochratoxin A, fumonisin, T-2, zearalenone

Routine handling of contaminated grain--particularly heavily contaminated grain or hay may present a significant health hazard to technical personnel. Therefore, samples should be handled only by trained individuals working in appropriate facilities and within the guidelines of an acceptable safety protocol in compliance with state and federal occupation health and safety laws and guidelines. Mycotoxin analysis is available on a fee basis from Romer Labs (www.romerlabs.com) and veterinary toxicology centers at North Dakota State University, Iowa State University and several other state land grant universities.

Sampling for Mycotoxins and Sample Preparation

An adequate and representative sample of suspect feed grain or other feed should be obtained. This can be difficult in livestock feeding operations because the majority of the suspect feed has been consumed. It may be necessary to remove feed from the corners of the feeders or retrieve feed from the corners of the storage unit.

Proper sampling is essential because one kernel in 1,000 kernels of grain may be a source of significant mycotoxin contamination and contamination may occur only in pockets (hot spots) in the feed mass. Occasionally a biased sample may be more revealing than a truly representative one. For example, in studying stored grain or feed that shows evidence of moisture damage, heating, or "caking," a sample of damaged grain may be more appropriate than a composite one from an entire lot. Typically a 10-pound (5-kilogram) sample is usually collected by using a probe from random sites in the feed mass or continuously taken from a stream or flow of grain. This sample can be subdivided such that a representative but smaller sample is submitted for chemical analysis. The sample must then be finely ground so that it will pass through a screen of 15 to 20 mesh and be thoroughly blended to obtain an aliquot appropriate for chemical analysis. The objective of any sampling procedure (protocol) is to acquire a representative sample. A representative sample may require random sampling of feed from all areas of a feed mass whereas in freshly mixed feed (after harvest or following handling), a representative sample may be easily acquired by taking a few aliquots.

Samples stored for analysis should be placed in a paper bag or cardboard box and kept under cool, dry conditions that will not permit fungal growth and a possible continued production of mycotoxins. Care must be taken to keep samples in the same condition as at the time of sampling. For example, moist grain samples stored in plastic bags under warm, humid conditions may have significant aflatoxin contamination occur during sample storage. When probing a ship hold, grain bin, vehicle or hopper car, numerous random probes may be required and site selective probing should be done if signs of moisture leakage, insect activity or hot spots are identified.

It is often most practical to determine which fungi are present and then test for the mycotoxins produced by the fungi present because testing for mycotoxins can be expensive. Samples submitted for fungal identification should be chosen and stored as suggested above. Services for fungal identification are available from extension plant pathologists in most states.

In Montana, samples for fungal identification should be sent to the Schutter Diagnostic Lab, 121 Plant BioScience Building, Montana State University, Bozeman, MT 59717-3150 and marked attention Barry Jacobsen.

Detecting Mycotoxins

Some methods of mycotoxin analysis are presented in Table 4 (page 26). These methods differ in their sensitivities and are appropriate for only certain commodities. Typically aflatoxin, ochratoxin and fumonisin tests should be sensitive in the parts per billion (ppb) range, while tests for DON, T-2 and zearalenone are sensitive in the parts per million (ppm) range. It should be remembered that just detection of a mycotoxin is just part of the story. It is the dose that makes the poison and it is critical to understand what portion of the diet is from the mycotoxin contaminated feed as well as sensitivity of the animal species and animal age. It is also important to recognize that there can be significant variability in test results. There are published results of aflatoxin tests from 10 subsamples of a single lot of peanuts that show results varying from 0 to 230 ppb. Ingestion of multiple mycotoxins can change the dose response and it is important to remember that mycotoxins other than those identified can be present in the feed.

Regulatory Issues

Many countries including the USA have established regulatory limits that restrict the amount of mycotoxin permitted in foods and feeds. Table 2 provides information on regulatory limits for mycotoxins in food and feeds in the USA. Only levels of aflatoxins B₁, B₂, G₁, G₂ and M₁; DON are currently regulated by the FDA and there are proposed limits for fumonisins. It is important to understand that knowingly mixing lots of grains or feeds that exceed the regulatory limit with uncontaminated grain to achieve lower levels of mycotoxin concentration is considered adulteration and is subject to civil and criminal penalties.

Diagnosis

In animals, few mycotoxins produce clinical signs so characteristic that they permit unequivocal diagnosis. For example, the estrogenic syndrome in cattle can be caused by phytoestrogens in forage as well as by zearalenone and zearalenol in grain. Refusal of feed containing corn or cereal grains usually indicates mycotoxins produced by *Fusarium* sp. Other

substances in feed can also cause feed refusal. Some mycotoxins, including the trichothecenes and aflatoxins, may bring about reduced productivity or depressed growth, but certain environmental factors and nutrient deficiencies may cause similar effects. Lowered resistance to infections by microorganisms or opportunistic parasites and reduced protection from immunization may be the result of ingesting mycotoxins or it could be due to a deficiency in selenium. Therefore, diagnosis should be made based on symptoms observed, tissue pathology, and the presence of the mycotoxin and the estimated dose of the mycotoxin. Information of the microbial colonization of the suspect grain or feed is very helpful to determine the risk for multiple mycotoxins being present. Factors such as animal age and condition, duration of exposure and presence of more than one mycotoxin should be considered. The affects of exposure to multiple mycotoxins is largely unknown. Bioassays can be used to assess the toxicity of the suspect feed. Animals used in a bioassay should have detailed pathologic examination.

Minimizing Mycotoxin Problems

1. Harvest grains at maturity whenever possible and adjust harvesting equipment to minimize physical damage to the grain. Once harvested, grains or seeds should be stored at moistures that preclude the growth of mycotoxin producing fungi. If drying is not possible, the producer should consider the use of propionic or other acid storage aids. Such “acid” treated grain can only be used for livestock feed. Acid treated grains must be stored under the conditions and for the duration recommended by the manufacturer. Such storage aids can also be used for hay. Hay and grain stored beyond the recommended interval or under conditions different than those recommended by the manufacturer can have rapid growth of toxigenic fungi. When harvesting grains where harvest has been delayed by wet conditions extra care should be taken to assure the grain is dry enough for safe storage. Where scab is a problem in cereal grains extra air should be used during harvest so that much of the light weight “scabby grain” is left in the field. Screening out broken grain may reduce aflatoxin, fumonisin and ergot contamination. Use proper techniques to ensure proper ensiling of grain silages and haylage. Once conditions are anerobic and sufficient lactic acid is produced, the mycotoxin producing fungi cannot grow in silage or haylage.

2. Store cereal grains and oil seeds in weather proof structures that have been cleaned and treated for storage insects before storage. Cleaning grain to remove light weight “scabby grain”, broken grain and ergot sclerotia is helpful. Remember that mold infected kernels are very friable and easily broken. Commonly, cereals with high DON or aflatoxin levels can be reconditioned such that the heavily contaminated grain is cleaned out and levels of DON or aflatoxin are below regulatory levels. Controlling storage insects and rodents is critical.

3. Control mycotoxin producing molds in the field. Corn varieties with the Bt gene will typically have lower levels of fumonisin and aflatoxin because ear damaging insects are controlled. If scab is of concern in wheat, chose varieties that are more tolerant to Fusarium head blight and use an approved foliar fungicide at heading to anthesis. Fescue endophyte free seed is available and should be used for new seedings.

4. If aflatoxin is of concern, anhydrous ammonia treatment of contaminated grain will reduce aflatoxin levels by 30-50%. Once treated, the grain can only be used for animal feed.

5. Avoid feeding grain screenings (unless tested for mycotoxins), moldy silage, or moldy hay.

6. If grain is purchased for feeding from an area with know mycotoxin problems, have the grain tested before shipping or have this as a contract specification.

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Table 1. Major Mycotoxins and Toxin-Producing Fungi from Corn, Cereal, Soybeans, Peanuts, and Other Products and Some of their Effects on Animals.

Toxin or Syndrome	Fungal source	Feeds or foods affected	Possible effects on animals
<i>Aspergillus</i> Toxins- (primarily) Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ (B _{2a} , G _{2a} , M ₁ , and M ₂ are metabolites and seldom present in grain; M ₁ and M ₂ are important contaminants in milk)	<i>Aspergillus flavus</i> and <i>A. parasiticus</i>	Cereal Grains, peanuts, soybeans, and other foods	Hepatotoxin; carcinogenic; reduced growth rate; hemorrhagic enteritis; suppression of natural immunity to infection; decreased production of meat, milk and eggs, pulmonary mycotoxicosis
Ochraoxins (nephrotoxins)	<i>Aspergillus alutaceus</i> var. <i>alutaceus</i> (<i>ochraceus</i>) and <i>Penicillium viridicatum</i>	Cereal grains	Toxic to kidneys and liver; abortion; poor feed conversion, reduced growth rate, general unthriftiness; reduced immunity to infection
Sterigmatocystin	<i>Aspergillus nidulellus</i> , <i>A. glaucus</i> , <i>A. sydowii</i> <i>A. versicolor</i> and <i>Bipolaris sorokiniana</i>	Cereal grains	Toxemia; carcinogenic, hepatotoxic
Termorgenic toxin	<i>Aspergillus flavus</i> , <i>Aspergillus terreus</i> , <i>Penicillium cyclopium</i> , and <i>P. palitans</i>	Cereal grains, soybeans, peanuts, and other food feeds, etc.	Tremors and convulsions, death
Penicillium Toxins (primarily) Luteoshyrin	<i>Penicillium islandicum</i>	Rice	Hepatotoxic, tremors and convulsions
Patulin	<i>Penicillium urticae</i> , <i>P. expansum</i> , <i>P. clavirome</i> , and <i>Aspergillus clavatus</i>	Cereal grains, apple products	Hemorrhages of lung and brain; edema toxic to kidneys; possibly carcinogenic
Rubratoxin	<i>Penicillium rubrum</i>		Liver damage, nephrotoxic and hemorrhage
Citrinin	<i>Penicillium citrinum</i>		Kidney damage
Penicillic Acid	<i>Penicillium viridicatum</i> and several other <i>Penicillium</i> sp.	Cereal grains	Similar to ochratoxin
Ergot Toxins Ergopeptines	<i>Claviceps purpurea</i>	Cereal Grains	Vasoconstriction, loss of extremities (ears, tail, feet, etc.), skin necrosis, agalactia
Ergovaline	<i>Neotyphodium (Acremonium)</i> and <i>Epichloe</i> sp.	Fescue	Reduced weight gain, abortion, poor survivability of offspring, fescue foot

Fusarium Toxins

Zearalenone (Estrogenic syndrome) Zearalenol	<i>Fusarium graminearum</i> , <i>F. colmorum</i> , <i>F. equiseti</i>	Cereal grains, soybeans	Hyperestrogenism, infertility, stunting, and even death
Emetic or feed refusal Factor, (Vomitoxin) Deoxynivalenol or DON	<i>Fusarium graminearum</i> (sexual state), <i>Gibberella zeae</i> , <i>F. culmorum</i>	Cereal Grains	Food refusal by swine, cats, dogs; reduction in weight gain
Other trichothecenes (T-2, HT-2, Monoacetoxyscripenol or MAS, Diacetoxyscripenol or DAS)	<i>Fusarium graminearum</i> , <i>F. equiseti</i> , <i>F. poae</i> , <i>F. acuminatum</i> , <i>F. sambucinum</i> and <i>F. sporotrichoides</i>	Cereal grains, soybeans, potato	Severe inflammation of gastrointestinal tract and possible hemorrhage; edema; vomiting And diarrhea; infertility; degeneration of bone marrow; death; reduced weight gain; slow growth; sterility, abortion
Fumonishin B ¹ , B ²	<i>F. verticillioides</i> , <i>F. proliferatum</i>	Corn	Leukoencephalomalacia “moldy corn disease” in horses, pulmonary edema in swine, neural tube defects and esophageal cancer in humans

Table 4. Methods of Detecting Mycotoxins

Name	Mycotoxin	Description	Use	Remarks
Black light	Aflatoxins	Cracked grain or screenings are viewed in the dark under long-wave ultraviolet light (approx. 365 nm). Samples are checked for “glowers” or starchy endosperms that fluoresce a bright greenish yellow (BGYF). The BGYF compound is <u>not</u> aflatoxin but a substance produced by <i>A. flavus</i> or <i>A. parasiticus</i> when growing on living seed. This compound is not produced in dead seed. Grain may be cracked for testing with a cereal grain grinder.	A rapid, PRESUMPTIVE test for the BGYF compound (kojic acid), a metabolite usually cosynthesized with aflatoxin). Positive samples should be analyzed by the minicolumn, TLC, GLC, or HPLC tests before any action is taken. A standard should be used with each test, and fluorescing grain should be checked to see that the fluorescent compound is water-soluble and in the starchy endosperm and peripheral parts of the germ (embryo).	Quick but ONLY indicative of <i>Aspergillus flavus</i> or <i>A. parasiticus</i> . The test is neither quantitative nor qualitative. It should be used only by trained personnel because many types of foreign material, e.g., glumes, cobs, some weed seeds, and soybean fragments, may fluoresce but are not usually water-soluble. The training is minimal.
Fluorometric iodine rapid screenings or “F1-IRS” (See Applied Biochem. Vol 1).	Aflatoxins	Finely ground grain is extracted with solvent and zinc acetate-salt solution is stirred in before the sample is filtered. Iodine is added to the clarified diluted filtrate before estimating the amount of fluorescence ^b in samples containing aflatoxin.	Rapid (7 to 8 min.) and cheap. The test determines whether aflatoxin is present or not. This technique is more accurate than the black light test (see above).	Samples are quickly designated aflatoxin positive or negative. Positive samples may then be further analyzed.
Minicolumn ^d (for details see <i>J. Agric. Chem</i> 23: 1134-36, 1975)	Aflatoxins	Finely ground grain is extracted with solvents, purified by a precipitation procedure, and the extract washed through a column containing two absorbents. Migration and long wave UV light are used for detection.	Rapid (9 to 15 min.), simple, and semi-qualitative; requires inexpensive equipment; can detect aflatoxins down to 4 ppb. Romer’s mini-column procedure for feeds requires about 30 min. (see <i>Journal of AOAC</i> 58:500-506, 1975).	Quick but only qualitative. Can be used as a “go” or “no go” measurement above 4 ppb. The short minicolumn test is not suited for mixed feeds. Laboratories charge about \$25 to \$50 for aflatoxin analysis.

Thin-layer chromatography or "TLC" (see "Official Methods of Analysis." Chapter 26. Association of <i>Analytical Chemists</i> , 12 th Edition, 1975)	Aflatoxins, Zearalenone, Trichothecenes	Grain is extracted and the extract partially purified before placing on a thin layer chromatographic plate. UV light and migration are compared visually or densitometrically with standards used for identification of fluorescent aflatoxins or zearalenone. Trichothecenes do NOT fluoresce.	Can identify and quantitatively determine aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ . The detection limit for aflatoxins is 1 to 3 ppb. The sensitivity limit for zearalenone is 50 ppb. If necessary, confirmation can be made by additional chemical tests on the TLC plate.	Slow, somewhat expensive, but precise and reasonably accurate. Detection limits for trichothecenes are relatively low. Many compounds, especially trichothecenes, cause dermal reactions.
Gas-liquid chromatography or "GLC"	Zearalenone, Trichothecenes (T-2, MAS, DAS)	Grain is extracted and trimethylsilyl either derivatives are measured.	This quantitative method can accurately identify zearalenone, T-2, MAS, and DAS.	The sensitivity is far better than "TLC" for trichothecenes.
High-performance liquid chromatography, or "HPLC"	Aflatoxins Ergopeptines Fumonisin	Grain is extracted and the extract fractionated on either normal or reverse phase columns. The aflatoxins are detected using either UV absorbance or fluorescence detectors.	Can accurately and quantitatively identify aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ and their metabolites B _a , G _a , M, and M. Same for ergopeptines	The initial capital investment and technical expertise are the highest for this technique, and it is potentially the most sensitive.
Enzyme Linked Immunosorbent Assay or "ELISA" Many commercial kits available	Aflatoxin Zearalenone Ochratoxin A DON T-2 Fumonisin	Grain is extracted in methanol and placed in plastic well. Addition of antibody-enzyme conjugate and chromagen results in color which is quantitative measure of alkaloid.	Test is specific for target alkaloid but may be cross-reactive within members of an alkaloid group. Sensitive to 5 ppb (aflatoxin) and requires 10 minutes to complete.	ELISA requires a plate reader for accurate quantitation, but no other specialized equipment is necessary. ELISA is a good compromise of sensitivity, speed and expense.
Immunoaffinity column Many commercial kits available J. Assoc. Off. Anal. Chem. Intl. 80:941-949.	Aflatoxins (all) Ochratoxin A DON, T-2 Zearalenone Fumonisin (all)	Similar to ELISA	Similar to ELISA	Similar to ELISA

