AFLATOXINS IN IOWA Updated October 1, 2005 Dr. Gary Osweiler Iowa State University

OCCURRENCE OF AFLATOXIN IN IOWA AND NEARBY STATES

Conditions in eastern and southeastern Iowa as well as extensive areas of adjacent states to the south and east during the 2005 growing season have favored invasion of corn with *Aspergillus flavus* mold. This fungus has the potential under adverse conditions of drought stress and insect damage to produce toxic metabolites known as aflatoxins. Aflatoxins are potent toxins and experimentally are known also to cause cancer in some animals. Other feed grains, especially sorghum, milo and cottonseed can also be infected and support production of aflatoxins.

Aflatoxin can be produced in standing grain before harvest. If conditions of moisture and temperature support continued mold activity after harvest, aflatoxins can continue to be produced during storage, especially at moisture content above 12% and temperatures greater than 70 °F. Aflatoxin, once produced, is quite stable to heat, milling, pelleting and many chemicals. While four specific aflatoxins are generally produced (designated B1, B2, G1, G2), the most frequent and toxic is aflatoxin B1.

Early testing at elevators or other collection points is often done in two ways. A "black light" (ultraviolet light) illuminating aflatoxin-contaminated grain causes a firefly-like greenish-yellow fluorescence which is caused by a non-aflatoxin product that results from growth of *A. flavus* mold. This is not aflatoxin, but serves as a marker that aflatoxins may be present. Overall, this test is subject to inaccuracy of around 13% and in some locations can result in as much as 25% incorrect answers. This technique should not be used to measure the amount of aflatoxins in the grain. A rational decision about use of grain cannot be made on the basis of the black light alone. The second method involves quick tests based on chemical or antibody detection of aflatoxins itself. These tests require some basic chemistry to conduct, but are often conducted on-site or in laboratories nearby where grain is collected. They are generally useful and can give approximations of the amount of aflatoxins in a specific sample. Some commercial laboratories and state laboratories also offer full service chemical and instrumental testing to confirm and quantify aflatoxins. The Iowa State University Veterinary Diagnostic Laboratory (515-294-1950; http://www.vetmed.iastate.edu/departments/vdpam/vdl/) routinely tests for aflatoxins in grain.

SAMPLING

Laboratory testing of aflatoxins is recommended whenever there is a positive black light result and the grain is intended for livestock use. Testing is only accurate if a representative sample is collected. Sampling is an estimate and inattention to sampling technique can cause large errors in results. Because aflatoxins can vary widely in a field or storage bin, some specific sampling techniques are strongly recommended.

Field sampling is difficult and subject to inaccuracy. A suggested method for collecting a field sample is to make one or more trips across a field with the combine. Then, as the hopper is emptied into a wagon, pass a cup through the stream of grain every 30 seconds and collect these to make a total of 10 pounds or more. Mix the collected sample thoroughly and submit as least 5 pounds for testing. If the field is large, sample several times at different locations across the field. Sampling ear corn is less accurate, and if this is done at least 30 to 50 sites with several ears per site should be collected.

Moisture content of samples to be tested should be less than 14% for short term shipping to a laboratory. If grain moisture is higher, seal the bag, freeze the grain and keep it cold until it reaches the laboratory. An alternative is to dry the grain for 6 - 12 hours at 140 °F, then ship to the laboratory in a paper bag.

Sampling from stored grain should be done by the moving stream method as described above for sampling from a combine. Probe sampling is acceptable where grain has been recently blended. Multiple samples (10-30) from several levels of the bin should be sampled. Never trust a "grab" sample as representative of the entire grain supply.

EFFECTS OF AFLATOXIN ON LIVESTOCK

When crop conditions or other factors require use of aflatoxins contaminated grains in livestock, special care must be taken to avoid adverse effects in the animals and to prevent residues of aflatoxins in foods from animals.

Aflatoxin is a potent toxin. However, for production and disease effects in animals it does follow the rules of dosage and response. This means that small amounts cause mild or negligible effects and larger amounts cause increasingly serious effects. Aflatoxins bind to nucleic acids and also impair protein formation in the body. They may thus cause organ damage and/or cancer from prolonged exposure.

Low levels of aflatoxins in feeds - sometimes less than 1 part per million (PPM) – can cause poor growth, interfere with the immune system and result in liver damage and bleeding. High dosages cause acute loss of appetite, depression, hemorrhage, diarrhea and death. Signs of aflatoxin poisoning can include slow growth, reduced milk production, hemorrhage and jaundice (yellow color of skin and eyes). With continued exposure, there will be liver damage and suppression of the immune response and ability to resist infections or to respond adequately to vaccinations.

Animal susceptibility varies with species and age. In general, young animals (pre-weaning to early adolescence) are more affected than are adult animals. Species that are highly sensitive are trout, ducks, turkey poults and pre-weaning pigs. Animals that are moderately sensitive include all swine, growing turkeys, broiler chicks, pre-ruminant calves, dogs and horses. Animals most resistant are beef feedlot cattle, open cows, and sheep. Aflatoxin generally does not interfere with fertility or cause abortions. However, newborn animals nursing dams that consume aflatoxins can be poisoned by the aflatoxin M1 metabolite that is excreted in milk.

The clinical effects of aflatoxins may also be produced by some other diseases or toxins. Veterinary examination for differential causes of disease should always accompany a suspected aflatoxin poisoning case. Characteristic changes occur in liver which can be confirmed by microscopic examination. Aflatoxin metabolites (Aflatoxin M1) can be detected in liver, kidney, urine and milk to confirm exposure and to determine if residues are a problem. Aflatoxin is excreted rapidly from the body, so detectable levels may be gone within a few days to one to two weeks. Complete laboratory submission for diagnosis should include suspect grain or feed, fresh liver and kidney, urine if available, serum for laboratory tests of liver function and rumen or stomach contents. A portion of liver, kidney and other organs should be put in formalin for microscopic examination. Table 1 summarizes some effects in animals from different feed concentrations of aflatoxins

Table 1. RESPONSE OF DOMESTIC ANIMALS TO AFLATOXINS			
(Note: Values are in PPB. 1,000 PPB [part per billion] = 1 PPM [part per million])			
Species	PPB Aflatoxin in Feed	Effect	
Broilers	5,000 – 10,000	Liver necrosis, hemorrhage, death	
Broilers	2,500	Hemorrhage, reduced growth & feed efficiency	
Broilers	1,000 - 2,500	Decreased fat digestion, fat in droppings, decreased	
		gain and feed efficiency.	
Broilers	600	Bruising, reduced disease resistance	
Broilers	> 250	Possible reduced immune system function	
Swine	10,000 - 20,000	Single exposure can be lethal. Hemorrhage and	
		acute liver damage are expected.	
Swine	2,000 – 4,000	Lethal from multiple exposures. Liver damage,	
		jaundice and slow growth precede death.	
Swine	800 - 2,000	Subacute and may be lethal. Hepatic necrosis,	
		jaundice, liver fibrosis and hemorrhages	
		accompanied by slow growth and poor appetite.	
Swine	200 – 500	Reduced growth and feed efficiency. Immune	
		system suppression, reduced response to vaccines.	
Cattle	10,000 - 20,000	Icterus, hemorrhage, liver necrosis. Death in one to	
		two weeks.	
Cattle (Dairy)	2,000 - 4,000	Reduced milk production, rumen dysfunction, off	
		feed. Aflatoxin milk residues.	
Steers	700	Multiple doses cause reduced feed intake and feed	
(Yearling)		efficiency, liver damage and death over time.	
Calves	200	Fed 2 – 4 weeks will cause reduced weight gain,	
(Weanling)		hemorrhages and possible immune suppression.	

FDA (Food and Drug Administration) guidelines have established "action levels" for acceptable concentrations of aflatoxins in specified foods and feeds (http://vmcfsan.fda.gov/~lrd/fdaact.html). The following guidance levels from FDA are presented for information purposes. Keep in mind these may change, and one should always check directly with FDA or appropriate state authorities to verify acceptable levels.

Table 2. FDA Guidance Levels for Aflatoxin			
Fed to Food Animals			
Commodity	Amount		
Corn for interstate movement	20 PPB		
Corn for lactating cows	20 PPB		
Milk	0.5 PPB for fluid milk		
Corn for breeding beef cattle/swine or	300 PPB		
mature poultry			
Corn for finishing swine > 100 lbs	200 PPB		
Corn for finishing beef cattle	300 PPB		

TREATMENT AND PREVENTION

Aluminosilicate products such as hydrated calcium aluminosilicate (HSCAS) and sodium bentonite have proven effective in binding aflatoxins and preventing their absorption. Usually they are added to feed at 5 to 10 pounds per ton. They have been shown to reduce effects of aflatoxins on the liver and to reduce aflatoxin residues in milk. Recently modified glucan based adsorbents have also been developed and

marketed (MycosorbTM, Alltech). They may be similarly effective as the aluminosilicates and are used at lower levels in feeds.

Affected animals should be given high quality protein supplements. In addition, although Vitamin E and Selenium do not appear to protect against aflatoxins, Vitamin E may be depleted in mold contaminated feeds and testing for its status in animals or supplementing the ration is recommended.

Mold inhibitors, such as the organic acids, can prevent continued mold growth where moisture above 12 – 14% is a problem. Mold inhibitors prevent *A. flavus* growth, but do not destroy or modify aflatoxins.

Treatment of grains with anhydrous ammonia for 12-14 days reduces aflatoxin content, but regulations vary from state to state about the clearance of ammoniation for contaminated corn. Ammoniation is not yet cleared for commodities that would move in interstate shipment.