Aflatoxin in Corn

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Aflatoxin, a poisonous byproduct of the fungus Aspergillus flavus, may develop in corn standing in the field and in holding wagons or storage. A. flavus is abundant in nature, grows primarily on organic matter or in soil and produces an abundance of spores which are readily spread by air currents. The fungus requires certain favorable conditions for growth and harmful toxin production. There are several strains of A. flavus but all strains do not produce aflatoxins. Further, those strains producing toxins do not produce aflatoxins under all conditions in which the fungus grows.

In most years, aflatoxin is not a problem in Kentucky. It can become more serious, however, when crops are grown under stress or when grain is not properly handled.

Factors Affecting Aflatoxin Formation

Temperature and Moisture

Both temperature and moisture play a vital role in the potential for aflatoxin development on corn. Conditions favoring the aflatoxin-producing fungus, A. flavus, are as follows:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Optimum</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>80 - 100°F</td>
</tr>
<tr>
<td>Relative Humidity</td>
<td>85 - 100%</td>
</tr>
<tr>
<td>Kernel Moisture</td>
<td>18% and above</td>
</tr>
</tbody>
</table>

Development of the fungus usually stops when the temperature is below 55°F and grain moisture is 12 percent or less.

Drought

Aflatoxin problems on corn are more severe when drought stress prevails during the latter part of the growing season. Corn is especially vulnerable after it reaches the dough stage; prior to this time, the kernel moisture is too high for infection by the aflatoxin-producing fungus. If severe drought stress is present, however, kernel moisture drops to where infection can occur. In addition, high temperatures and high relative humidities around the kernels further favor infection. The fungus also has less competition from other organisms under drought stress conditions.

Drought is now considered one of the major factors which predisposes certain crops to infection of aflatoxin-producing fungi. In 1977, drought-stressed corn in the southeastern states was plagued with aflatoxin. An inverse correlation between yields of drought-stressed corn and aflatoxin levels has been reported, thus suggesting the increased susceptibility of plants grown under stress.

Insect Damage

Aspergillus flavus is frequently associated with insect damaged corn. Researchers have

Values are taken from several sources and in some cases represent compromises among findings.
found that insects may directly transfer spores to healthy ears or at least expose the kernels to contamination by fungus spores from other sources. The corn earworm can transmit the fungus both under field and laboratory conditions. Other insects such as sap beetles also carry spores of *A. flavus*.

**Sampling and Detection**

Corn ears should be collected in each representative area of the field to determine the level of aflatoxin present. To determine if aflatoxin exists, select ears damaged by insects or other causes. Shelled corn in the wagon or grain bin may be sampled with a five foot compartmentalized grain trier or by hand sampling if a trier is not available. If sampling by hand, reach down at different levels and grab a fistful of corn. Take as many samples as possible (totaling at least 10 pounds) and combine all samples into one composite sample. Then take a subsample (at least 5 pounds) for aflatoxin testing.

Aflatoxin may be present in a relatively few kernels or in “hot spots” in stored grain. Smaller samples increase the risk of not detecting 20 parts per billion (ppb) aflatoxin in corn. Processes which mix the corn, such as shelling or loading, facilitate good sampling.

The blacklight provides an effective first step in the process of examining corn under an ultraviolet light (366nm) in a darkened chamber or room. Corn infected with *Aspergillus flavus* will exhibit a characteristic bright greenish-yellow fluorescence (BGYF) or “firefly glow” under a blacklight. A color standard is available from the Northern Regional Research Center, 1815 North University St., Peoria, IL 61604 to aid in identification of the fluorescences. Ideally, the entire sample should be cracked or coarse ground for examination.

What designates a positive blacklight finding is not well-defined but a good guideline is one glowing kernel, or one or two glowing particles per 2-pound sample. Remember that a positive blacklight finding only indicates the presence of the mold and the potential for aflatoxin. Studies indicate that blacklight findings are not a good measure of aflatoxin, and that a large percentage of samples showing positive will prove negative for aflatoxin with chemical analysis. Tips of corn kernels, corn bees wings, soybean hulls and weed seeds can produce a similar glow under blacklight. The blacklight is a valuable first screen but positive findings require further testing to determine if aflatoxin is present.

Three different methods of chemical analysis are commonly used for determining the presence of aflatoxin. The minicolumn is most widely used when it is desirable to test large numbers of samples and to get results in less than 1 hour. This procedure can detect aflatoxin levels down to 4 ppb which is well below the legally permitted level of 20 ppb. Minicolumn analysis can be performed by grain elevators with minimum laboratory facilities and is an excellent screening procedure for determining approximate aflatoxin levels.

Thin layer chromatography and high pressure liquid chromatography give more accurate quantification and identification of aflatoxin. These procedures are not practical for rapid screening and require highly trained laboratory personnel. However, they are desirable when more definitive testing is required. Contact your county Extension agent for information on laboratories offering services for quantitative aflatoxin analysis.

**Clinical Effects**

**Dairy**

The federal Food and Drug Administration has set 0.5 parts per billion (ppb) aflatoxin as the maximum allowable level in milk. To maintain aflatoxin levels in milk below this amount, lactating dairy cattle should not be fed feed containing more than 20 ppb aflatoxin. This “tolerance level” of 0.5 ppb may seem low; however, considerable data indicate that the young of all species are more susceptible to aflatoxicosis than adults. The potential hazard to infants and small children is apparent. Because milk and milk products constitute a large portion of the diet of children, rigorous measures must be taken to prevent unnecessary intakes of the toxin.

Dairy calves are more susceptible to aflatoxin than adult animals. However, because these animals are not secreting milk for human consumption, a level of 100 ppb is acceptable in the feed. Pregnant, non-lactating cows should not be fed feed containing more than 100 ppb aflatoxin. Higher levels may result in the birth of small, weak calves, lower milk production and/or possible abortions. This level assumes feeding...
Table 1.—Dilution Rate for Corn Containing Various Levels of Aflatoxin

<table>
<thead>
<tr>
<th>Aflatoxin in Corn (ppb)</th>
<th>Maximum Aflatoxin in Grain Mix</th>
<th>Aflatoxin (ppb) in Grain Mix</th>
<th>Total Ration2</th>
<th>Safe to Feed to Calves</th>
<th>Safe to Feed to Yearlings</th>
<th>Safe to Feed to Lactating Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>40</td>
<td>800</td>
<td>40</td>
<td>20</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>200</td>
<td>40</td>
<td>800</td>
<td>80</td>
<td>40</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>400</td>
<td>40</td>
<td>800</td>
<td>160</td>
<td>80</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>1000</td>
<td>40</td>
<td>800</td>
<td>640</td>
<td>320</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1000</td>
<td>20</td>
<td>400</td>
<td>320</td>
<td>160</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>2000</td>
<td>10</td>
<td>200</td>
<td>200</td>
<td>100</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1Guthrie, L. D. Aflatoxin in Dairy Rations, Animal Science Fact Sheet, Univ. of Georgia.
2Based on 50% of dry matter from grain and 50% of dry matter from forage.

less than 10 pounds of the contaminated feed per day and that the forage is uncontaminated. Open heifers over six months of age can tolerate large concentrations of aflatoxin in a given feed. High levels may reduce reproductive efficiency and lower feed utilization (Table 1).

Remember that once aflatoxin is in a feedstuff, it remains regardless of the method of preservation. Proper ensiling will certainly prevent the production of aflatoxins, but fermentation will not destroy any aflatoxin present in the feed when it is ensiled. If aflatoxin contamination is suspected, the silage should be analyzed.

**Beef Cattle and Sheep**

Cattle and sheep, especially mature animals, are less susceptible to the effects of aflatoxins than other farm animals such as hogs or poultry. Feeding aflatoxin-containing feeds to cattle within safe levels can be one way of utilizing the contaminated feed. Feed with relatively high levels of aflatoxin must be diluted to safe levels. Sheep are more tolerant of aflatoxin than beef cattle, though generally less is known about the effects of aflatoxin in sheep.

In beef cattle, contaminated feeds may reduce growth rates and increase the amount of feed per pound of gain. Calves are more susceptible than adults. In affected calves, there may be severe rectal straining and a prolapsed rectum in some cases. Lactating cows show a significant reduction in milk yields.

Studies have shown that feeding 700 ppb of aflatoxin B, to 450-pound beef cattle results in liver damage. Feeding 1,000 ppb to these animals reduces growth rate and lowers feed efficiency.

The level of aflatoxin in the ration, length of feeding period and age of animal will have a bearing on the effect of aflatoxin fed to cattle. The following are guidelines for feeding aflatoxin-contaminated feeds to beef cattle:

1) Creep feeds and rations for lactating beef cows should not receive more than 20 ppb in the ration dry matter.

2) Rations for non-lactating cows and bulls should not contain more than 200 ppb (dry matter basis).

3) Feeder calves should receive no more than 200 ppb and finishing cattle less than 100 ppb in the ration dry matter. These levels refer to the ration dry matter at feeding time. Animals destined for slaughter should be fed aflatoxin-free rations for at least 2 weeks before slaughter. It is recommended that aflatoxin-contaminated feeds be fed at the very lowest level and the shortest period of time practical considering feeds available.

**Swine**

Swine are adversely affected by consumption of aflatoxin contaminated feed. Symptoms exhibited by growing pigs include reduced growth rate and feed efficiency, weight and appetite loss and unthriftiness. In addition, liver damage and gross hemorrhages result, especially in the ham areas. The hemorrhages result in muscular incoordination which causes pigs to sit in a doglike position. The immune system is impaired.
which allows pigs to be more susceptible to secondary diseases. Death can ultimately occur in severe cases. In sows, abortions are likely. Growth of newborn pigs can be affected when consuming milk from sows fed aflatoxin-contaminated feed.

The same level of aflatoxin can affect swine in various ways. Factors which affect the influence of aflatoxins on swine performance are:

1) age (young pigs are more susceptible than older ones),
2) dietary protein and vitamin K levels,
3) amount of previous liver damage caused by chemicals and parasites, and
4) amount and duration of dietary contamination.

Various research reports indicate that the following traits are affected by aflatoxins at the level shown. These levels should be considered as guidelines only.

<table>
<thead>
<tr>
<th>Toxic Response</th>
<th>Parts Per Billion (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased Growth Rate</td>
<td>150-400</td>
</tr>
<tr>
<td>Reduced Feed Efficiency</td>
<td>200-400</td>
</tr>
<tr>
<td>Organ Change or Damage</td>
<td>300-500</td>
</tr>
<tr>
<td>Death (gradual)</td>
<td>810</td>
</tr>
<tr>
<td>Death (1-3 days)</td>
<td>2,000</td>
</tr>
<tr>
<td>Abortions, stillbirths</td>
<td>450-1,500</td>
</tr>
</tbody>
</table>

**Horses**

There has not been sufficient research to determine what level of aflatoxin is toxic to horses. It is known that horses are extremely sensitive to mold. Many classic cases of leucoencephalomalacia and liver disease in horses have been traced to their diets containing moldy feed or feed which was exposed to excess moisture.

Horses have been fed round bales left in the field with no known problems. Horses are more finicky eaters and generally will not eat musty or moldy hay if good hay is available in the remainder of the bale.

Some institutions have recommended not feeding horses feed that has more than 20 ppb of aflatoxin. Others suggest diluting moldy feed 1 to 10 with fresh feed for safety. Until more is known, the feeding of moldy or musty feed to horses is not recommended.

**Poultry**

Contamination of feed by aflatoxin can result in a variety of symptoms in poultry. Some of these include hemorrhaging, bruising, pseudo-rickets, fatty liver, encephalomalacia, exudates, poor growth and feed conversion, decreased hatchability, decreased egg production, increased blood clotting time, decreased immune response, decreased digestibility of dry matter and amino acids, and decreased energy utilization. Ducks and turkeys appear to be much more sensitive to mycotoxins than chickens. Detrimental effects due to levels of aflatoxin as low as 30 ppb in ducks and 125 ppb in turkeys have been observed. Much higher levels are required for negative effects in chickens.

Many of the symptoms of aflatoxicosis are similar to signs of various vitamin deficiencies, suggesting problems with absorption or utilization of these nutrients. The symptom that develops depends on several factors, including the particular species involved, the toxin involved and the nutrient which is most limiting in the diet.

There are several steps which should be taken if a suspected problem with aflatoxin occurs in a poultry flock. First, remove the contaminated feed from the birds and save representative samples for analysis. Clean out feed bins and feeding equipment. Besides supplying fresh feed, it may also be helpful to supply vitamins, minerals and/or electrolytes in the water for several days. Because turkeys, ducks and all breeding birds are quite sensitive to aflatoxin, diluting contaminated feed with fresh feed is not recommended.

**Management**

**Harvesting**

Aflatoxin can be produced on standing grain in the field. Fortunately, corn has effective protection layers composed of the shucks and the kernel coat which provide adequate protection against the invasion and growth of *A. flavus*. It has been suggested that the fungus must be physically introduced through the shuck and coat of the kernel for it to grow most effectively and produce aflatoxin. The fungus can enter by insect or mechanical damage to the protective layers. Aflatoxin is more likely to occur in the field in years when climatic conditions favor abundant growth of insects or when normal harvest is delayed and insects have longer to develop and invade the grain crop. If the grain is not infested in the field, normal harvesting and handling operations with modern combines will damage a number of kernels which could become infested with spores of the fungus. Managing grain harvest and storage to decrease kernel damage is important, particularly during warm weather.
Typically, corn may be harvested when it reaches a moisture content of 30 percent or less. The optimum starting moisture content for harvesting depends primarily on the relative prices of corn and drying fuels and is usually in the range of 25 to 27 percent. Early harvest may reduce the severity of insect invasion. However, early harvested grain also will be high in moisture which will necessitate more rapid drying to a moisture content below that for which aflatoxin can be produced. Generally, all shelled corn will require some artificial drying. If aflatoxin is present in the grain as it comes from the field, normal drying operations will not destroy it; it will merely restrict further development.

The time required for the fungus to grow and produce measurable quantities of aflatoxin after inoculation on corn is estimated to be from 24 to 48 hours. This time is affected by many factors including moisture level of the kernel, ambient temperature and extent of inoculation.

**Drying**

Four commonly used drying systems include natural air or low temperature, layer, batch-in-bin, and high temperature. Drying air is forced up through the grain mass in each of these systems. The top layer is always the last to dry. Therefore, the top layer is most likely to create conditions for aflatoxin production. The top layer should be dried to below 18% moisture as soon as possible.

**Natural Air and Low Temperature Drying**
The possibility exists for aflatoxin production under Kentucky conditions with this drying method. Stirring conditions which adequately mix all of the grain from top to bottom in the drying bin several times during the first 24 to 48 hours of drying might be helpful in reducing the potential for aflatoxin production.

**Layer Drying**—Typically, grain is placed in the drying bin in several separate layers. Subsequent layers are placed in the bin only after the preceding layer has been dried. This process is continued until the bin is filled to the desired level. The filling rate must be scheduled to dry the grain before aflatoxin can develop.

**Batch-In-Bin Drying**—Grain is placed in the drying bin in a layer 2 to 5 feet deep. These systems are designed and operated so grain harvested each day is dried and removed from the bin before the next day’s harvest begins. The probability of aflatoxin production is low. Variations in the typical operating procedures could result in conditions where problems might develop.

Batch-in-bin systems are usually designed for 10 cubic feet of air per minute per bushel of grain and drying air temperatures of 140°F. These drying conditions allow for drying times of less than 14 to 18 hours so that a farmer can dry a batch of grain each day. These drying conditions would be safe, assuming 48 hours as the time required for aflatoxin production. A problem may develop if grain is held overnight or for longer periods of time before drying.

**High Temperature Drying**—Drying times for these systems are 0.5 to 2 hours. The major concern for these systems would be extended holding times before drying begins. Grain should be dried within 24 hours after harvest and certainly held no longer than 48 hours even if aeration is provided during the holding period.

Wet holding bins are used primarily with high temperature drying systems to provide a continuous supply of wet grain for the dryer. Wet grain is added to the wet holding bin during the day as is necessary to unload wagons or trucks. Grain is removed from the bin upon demand from the dryer.

Two modes of unloading generally occur in grain bins—plug flow or enveloping flow. In plug flow, the entire mass of grain moves down the bin to the outlet orifice. The last grain into the bin would be the last to come out. In enveloping flow, the grain forms a funnel in the top of the grain mass. Grain flows down this funnel into a flow column which forms in the center of the grain mass. The last grain to be placed in the bin is the first to flow from the bin. If wet grain is added frequently to bins which unload by enveloping flow, it would be possible for much of the wet grain contained in the lower outside parts of the holding bin to remain in the bin for long periods of time. This would be a prime location for the production of aflatoxin and spoilage. Often this grain is difficult to remove from the bin after it becomes “molded” or allowed to pack. To minimize the risk of aflatoxin, the entire wet holding bin should be emptied every day so as to ensure that no high moisture grain is subject to contamination.

**Controlling Aflatoxin Levels**

**Organic Acid Treatments**

A method for preventing growth of aflatoxin-producing fungi on grain in storage is the use of
organic acid. These include acetic acid, propionic acid, isobutyric acid, mixtures of these, and ammonium isobutyrate. Organic acids will prevent A. flavus growth if applied properly to the grain as it is augered into the bins. However, these acids will not remove any aflatoxins which formed within the grain before the fungus was killed. Since aflatoxins can accumulate in corn before harvest, these treatments have little value when the problem is in the field. Grain treated with acids can be used only for livestock and poultry feed. Some of these acids are very corrosive and should not be used in metal storage bins unless the metal is amply protected. Carefully follow manufacturer's label instructions.

**Detoxification with Anhydrous Ammonia**

Treating high levels of toxic concentrated corn with ammonia before feeding is an effective method of detoxification to levels below 20 ppb. If the grain is to be fed to hogs, all ammonia should be removed before use. If it is to be fed to cattle, the grain may be mixed with silage without aeration. Ammonia is not registered by the Federal Food and Drug Administration for use on corn or other grains to be shipped out of state but it may be used on corn remaining in the state. It can best be used as a salvage treatment. Research on this process and the feeding of ammonia-treated corn is continuing.

Anhydrous ammonia is very corrosive and should not be used in unprotected metal bins or similar containers. It is harmful to humans and animals exposed directly to it, and it is explosive. Care and caution must be taken in designing, installing and operating ammonia injection and air injection distributor systems.

**Blending**

Another method of reducing average levels of aflatoxin contamination is to blend grain. Accurate sampling is essential if blending is to be successful. If contamination levels in one lot are in fact much higher than measured, the entire blended lot may become unacceptable. To ensure uniform mixing, lots to be blended should be fed into a common auger at the uniform respective rates desired to obtain the proper blend.

Blending is not an approved practice by the Federal Food and Drug Administration for commercial distribution. Blending is a practice more intended to reduce aflatoxin to acceptable levels for on-farm usage.

Blending can be accomplished any time that one of the lots of grain is at a lower concentration level than the desired final mixture. The following equation may be used to determine the appropriate ratio:

\[
\frac{\text{Ratio of least contaminated grain to most contaminated grain}}{= \frac{\text{Aflatoxin concentration in least contaminated grain, ppb}}{\text{Aflatoxin concentration in most contaminated grain, ppb}}} = \frac{\text{Aflatoxin concentration in blended grain, ppb}}{\text{Aflatoxin concentration in blended grain, ppb}}
\]

For example, suppose grain with 100 ppb was to be blended with grain that had no contamination to obtain a final product having 10 ppb. Using the above equation:

\[
\frac{100 - 10}{10 - 0} = \frac{90}{10} = 9
\]

Therefore 9 units of the least contaminated grain (0 ppb) are required for each unit of the most contaminated grain (100 ppb) to obtain a final blend of 10 ppb. If the least contaminated grain contains an aflatoxin concentration of 5 ppb:

\[
\frac{100 - 10}{10 - 5} = \frac{90}{5} = 18
\]

You would need 18 units of the least contaminated grain (5 ppb) for each unit of the most contaminated grain (100 ppb) to obtain a final concentration of 10 ppb in the blended grain.

Obviously, one of the grains to be blended must be lower in concentration than the desired final concentration. Also, the units of grain (bushel or pounds) to be blended must be at the same moisture content. The above examples illustrate the importance of obtaining an accurate measure of concentration. Slight differences can radically change the blend ratios.

**Screening (Cleaning)**

Grain should be cleaned either before or after it is dried to remove trash and broken kernels. Cleaning should be considered if the probability of aflatoxin contamination is large. Cleaning may reduce the economic return from the sale of grain. Broken and damaged kernels are the most likely locations for growth of microorganisms. Their
removal can 1) decrease the quantity of toxin present in grain, and 2) remove the prime location for potential growth of microorganisms and/or production of aflatoxin. Cleaning will also help reduce storage and handling problems by preventing accumulation of fines and trash in the center of the bin. It is difficult to force cooling or drying air through collections of fines and trash, and these areas may not dry or cool properly.

**Aflatoxin Corn in Ethanol Production**

Damaged grains which are contaminated with aflatoxin and/or other molds may be used as feedstocks in the brewing and distilled spirits industry. Chemical removal of aflatoxin from contaminated grain is not necessary before it is used in alcohol production. A recent study involving contaminated corn in the distilling industry revealed the following results:

1) Aflatoxin does not appear in distilled alcohol even when corn has relatively high levels of toxin.

2) Little toxin degradation occurs during distillation and is thus concentrated in the distillers grains.

3) Several decontamination procedures offer some hope in reducing or eliminating the toxin in stillage. The addition of chemicals and heat applied directly to stillage immediately after removal of the alcohol offers the most efficient method for detoxification.

Further research is needed to develop an efficient and reliable procedure for detoxifying distillers grain before it can be used as animal feed. However, alcohol may be distilled from contaminated corn for use as fuel alcohol and the wet solids discarded through land application (spray irrigation) or by burying. Distillers grains made from aflatoxin corn cannot be sold and should not be fed to animals since the toxin is not destroyed by heat in the fermentation process.