

# Aflatoxin M<sub>1</sub> in Milk

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## Introduction

Aflatoxins are mycotoxins of major concern to the dairy industry. Most mycotoxins are found in grain, usually grown in a drought, although they may contaminate pasture grass and occasionally hay. Most frequently, aflatoxins are found in corn (see Figures 1-3) and cottonseed, and sometimes in their byproducts, but also on rare occasions in soybeans or distiller's grain. Peanut products also may be contaminated with aflatoxins and can result in aflatoxins in milk.

Because aflatoxins are carcinogenic to animals and perhaps humans, they are monitored closely in the food supply. Aflatoxins are the most carcinogenic natural compounds known. Milk that is sold commercially is checked for aflatoxin M<sub>1</sub>. When aflatoxin M<sub>1</sub> is found at concentrations of 0.5 parts per billion (ppb) or greater, the milk is discarded because it cannot be used for products that go into the human food supply. On occasion, milk processors may also use a level less than the 0.5 parts per billion or 500 parts per trillion as a guideline for allowing milk into the human food supply.



**Figure 1. *Aspergillus flavus* in standing corn**

Lactating cows that eat feed containing 20 ppb or greater aflatoxins may produce milk that exceeds the tolerance level for aflatoxins in milk. It usually takes the cow approximately two to three days on aflatoxin-free feed for milk concentrations to fall below tolerance levels. The length of time required for milk to become aflatoxin free is dependent on the concentration of aflatoxins in the feed (and milk) as well as the diet being fed to the cow.



**Figure 2. Close-up of *Aspergillus flavus* on corn**



**Figure 3. *Aspergillus flavus* in stored corn**

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## Source of Aflatoxins in Milk

Aflatoxin M<sub>1</sub> contamination of milk results primarily from the conversion of aflatoxin B<sub>1</sub> that is metabolized by enzymes found primarily in the liver. After aflatoxin M<sub>1</sub> is formed, it is excreted in the urine and milk of the cow. The action level for aflatoxin B<sub>1</sub> is 20 parts per billion for feed fed to lactating dairy cows. **As both aflatoxins B<sub>1</sub> and M<sub>1</sub> may cause cancer in humans, the action level of 0.5 parts per billion of aflatoxin M<sub>1</sub> in milk is strictly enforced by the United States Food and Drug Administration (FDA).**

Aflatoxin B<sub>1</sub> in feed is a mycotoxin produced by *Aspergillus* molds that grow on grain, especially corn (Figures 1-3), cottonseed and sometimes peanuts. It is rarely found in forages and is usually not present in high enough concentrations in corn silage to be of concern. Feed does not contain aflatoxin M<sub>1</sub> as it is found only in milk. Aflatoxins refer to a group of several chemicals produced by the *Aspergillus flavus* fungus and related species. The most common aflatoxins are B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub> and M<sub>2</sub>. Aflatoxin M<sub>1</sub> is the mycotoxin found in milk and is converted normally from aflatoxin B<sub>1</sub> in feed although other conversions may occur.

Once a level of contamination of 20 ppb in a feedstuff has been determined, the feed can no longer be used to feed milking dairy cattle. It also cannot be blended with other feeds to reduce the level of contamination, as the Food and Drug Administration does not allow this. However, young animals and breeding animals or dry dairy cows can be fed levels of feed with aflatoxin ranging up to 100 parts per billion. Feed that has aflatoxin levels of 100 to 200 ppb can be fed to finishing hogs and beef cattle. For levels of aflatoxin from 20 to 300 ppb, the use of the feed is limited to finishing beef cattle. When feed levels contain greater than 300 ppb, there are no legal feeding options for the grain. Grain with high levels of aflatoxin can be used for ethanol production. The resulting distiller's grain or maltage is likely to contain high concentrations of aflatoxin since aflatoxin is heat stable and, therefore, should not be fed to lactating dairy cows.

Blended grain products with high levels of aflatoxins can be used for direct feeding on the farm after they have been blended with other feeds. Federal law prohibits the sale of grain blended to reduce the aflatoxin levels. However, blending of the grain on the farm is the best option for using the grain with high levels of contamination of aflatoxins. The contaminated feed can be blended with clean grain or other products so that the level of contamination is diluted to an acceptable level. Blending is not recommended for corn or other products fed to lactating dairy cows because of the risk of pockets of contaminated feed. Instead, consider feeding the contaminated grain to other animals such as heifers or beef cattle. As a general rule, aflatoxin-infected corn

should be stored only for short periods of time as levels of contamination may increase in storage. When storing, it is critical to maintain low moisture levels (e.g., 13 to 15 percent if possible). Maintain the grain in storage at low temperatures, usually below 40 degrees F if weather permits.

Corn in the field that has been subjected to drought conditions is more susceptible to aflatoxin contamination. In cases of severe contamination, yellowish-green mold colonies will form on the ears and will fluoresce when exposed to a black light. Most elevators screen incoming corn and cottonseed products with a black light, which can identify potential problems and aflatoxin contamination. However, less than half the samples of grain with contamination showed fluorescence under a black light in one study, although detectable levels of aflatoxin were present. Conversely, not all loads of grain that fluoresce will be positive for aflatoxins. Compounding the problem is that a load of grain rejected at one elevator as a result of a positive black light test may then be taken to another elevator where it will pass the test. If a load of grain fails the black light test initially, it should be sampled and sent to a laboratory for analysis. **Samples containing only 5 contaminated kernels of corn per million kernels may be above the legal limit to feed to lactating dairy cows.**

Reasons for failure to detect aflatoxin in grain with black light fluorescence may be related to the fact that *Aspergillus* metabolites may not show the same degree of fluorescence as the various aflatoxins. As a general rule, all loads of corn and cottonseed should be screened for aflatoxins and should not be fed to lactating dairy cows if fluorescence is seen. The grain can be fed to other animals as allowed by law.

An additional problem with aflatoxin is that it may be localized within specific parts of a load. This toxin is often located near the edges and corners of fields, where irrigation is poor. Sometimes, dairy producers unknowingly buy a small amount of contaminated grain. The grain is consumed by cows within one to two days and is detected in the milk two to three days later by the milk processor or marketing cooperative. Then, there is no remaining grain that shows high levels of aflatoxins.

## Symptoms of Aflatoxicosis

Aflatoxicosis is the disease caused by the consumption of aflatoxins. For most producers, no visual symptoms of aflatoxicosis will be observed in the animals. However, high concentrations of aflatoxins and/or prolonged duration may cause visual symptoms in cattle, especially young calves. Beef and dairy cattle are more susceptible to aflatoxicosis than sheep and horses, although other mycotoxicoses occur in these species, such as facial eczema in sheep and leukoencephalomalacia in horses. Young animals of

all species are more susceptible than mature animals to the effects of aflatoxin. Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals.

Feed refusal, reduced growth rate and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat and mild diarrhea may occur; anemia along with bruises and subcutaneous hemorrhages are also symptoms of aflatoxicosis. The disease may also impair reproductive efficiency, including abnormal estrous cycles (too short and too long) and abortions. Other symptoms include impaired immune system response, increased susceptibility to disease and rectal prolapse.

Clinical laboratory findings vary with the animal species, level of aflatoxin in the ration and the duration of feeding. There are no consistent diagnostic changes in hematocrit, hemoglobin and differential cell counts in animals fed aflatoxin.

Leukocytosis may occur in animals with secondary bacterial infections. Serum bilirubin levels may be elevated, and typically serum protein levels are decreased.

Lesions observed at necropsy related to either acute or chronic liver disease are dependent upon the level of aflatoxin and the duration of feeding. A majority of acute liver damage observed has been the result of experimentally high doses, while chronic liver damage is a more common field observation. The liver is usually pale tan, yellow or orange. Hepatic fibrosis and edema of the gallbladder may also be observed.

The diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions and the presence of infectious diseases due to the suppression of the immune system. On the farm, more than one mold or toxin may be present in contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult.

The later effects of aflatoxicosis depend upon the severity of liver damage. Once overt symptoms are noticed, the prognosis is poor. Treatment should be directed at the severely affected animals in the herd and further poisoning prevented. Most lactating cows that show positive for aflatoxins in milk will not show visual symptoms.

## What to Do if Milk Is Detected With Action Levels of Aflatoxin

If aflatoxin is detected in milk, it is critical that records be maintained of all feeds, feeding practices, milk quantities and contamination levels, plus animal health and performance. If the grain or related feed is fed to other animals, these records should be maintained also.

After milk has been detected with greater than 0.5 ppb of aflatoxin in one load, all grain products fed to animals should be removed from the ration immediately and new grain and/or related items replaced in the diet. As cottonseed and corn are the most likely sources of aflatoxin contamination, these grains should be tested to determine their level of aflatoxin. The exception to the preceding statement is when an additional milking has occurred on the dairy and the milk then tests below 0.5 ppb aflatoxin. In this case, the feed should be tested before it is removed from the feed supply.

**It is illegal to sell grain with levels greater than 20 ppb aflatoxin for lactating dairy cows, and the seller of the grain is responsible for damage resulting from the sale of grain.** However, in most cases, perhaps 60 percent of the time, the exact source of feed contamination is not determined. In cases of severe contamination and over a prolonged period of time, some data indicate that animals may experience adverse health effects which can affect both present and long-term performance. Calves also are more susceptible to aflatoxin and have died as a result of aflatoxin contamination in feed.

Immediately after aflatoxin is detected in milk, the ration should be reformulated with ingredients that contain less than 20 ppb aflatoxin. If the level of milk contamination exceeds 0.5 ppb on a second test, a special dietary chemisorbent should be added to the diet at recommended levels. These compounds include clays (bentonites) at 1 percent of the diet, activated carbon at 1 percent of the diet and glucomannan (Mycosorb®) at 0.05 percent of the diet on a dry matter basis. Some empirical observations indicate that the animal may consume levels of bentonite up to one pound a day. Limited data is available on the numerous compounds that are available to absorb the aflatoxin in the digestive system. However, in one study, about 1/4 pound of hydrated sodium calcium aluminino silicate (HACA – a compound approved for feed as an anti-caking agent) was shown to reduce aflatoxin M<sub>1</sub> in milk about 50 percent when cattle consumed feed containing 200 ppb aflatoxin. Silky clay loam soil and bentonite have a similar effect but have not been well studied. Many commercially available products also theoretically will bind aflatoxin M<sub>1</sub> and should result in lower aflatoxin in milk. Generally, the cost of using the commercially available products is greater than the cost of using bentonite to bind aflatoxin.

## Tests to Detect Aflatoxin

Records should be maintained for all feeds, feeding practices, milk contamination and animal health and performance for all cases of aflatoxin contamination of milk. There are simple, fast, semi-quantitative tests which can be performed to test for aflatoxin. Kits using ELISA (enzyme-linked immunosorbent assay) technology are available to test on the farm as well as commercially. The cost per

test on the farm is usually about \$20 to \$25 when prorating the cost of the incubator to conduct the test. Most laboratories will charge \$30 to \$75 per test for aflatoxin. An incubator to run the ELISA test will cost \$200 to \$400. The incubator also may be used to run tests for selected antibiotics in milk as well as aflatoxin.

To sample feed, take at least 8 to 12 samples at each of 3 to 5 locations in the feed bin or trough. Mix the sub-samples, and take a two-pound composite sample. Divide the composite sample into 2 one-pound samples. Store one sample in a cool, dry place, and use the other one for testing, either on the farm or at the laboratory. The second one-pound composite sample may be used for possible confirmatory testing or testing for other toxins.

Laboratories offer the ability to conduct basic mold counts, screen for specific molds and analyze for toxins. Testing capabilities and pricing will vary among labs. Some labs may not be recognized as accredited testing facilities, such as the Arkansas State Plant Board. In instances where possible litigation will occur, results must come from an accredited lab. Always verify upon initial contact with a lab if it is an accredited testing facility. Below are known laboratories that offer these services in or near Arkansas; however, testing for aflatoxin is not limited to these facilities.

Arkansas State Plant Board  
1 Natural Resources Drive  
Little Rock, AR 72205  
501-225-1598

A & L Analytical Laboratories, Inc.  
2790 Whitten Road  
Memphis, TN 38133  
901-213-2400; 800-264-4522  
[www.allabs.com](http://www.allabs.com)

A & A Laboratories  
1000 Backus Avenue  
Springdale, AR 72764  
800-962-7120  
[www.whitbeckgroup.com](http://www.whitbeckgroup.com)

ATC Scientific  
312 North Hemlock Street  
North Little Rock, AR 72114  
501-771-4255

Portions of this fact sheet were adapted from FS 907, *Aflatoxins: Hazards in Grain/Aflatoxicosis and Livestock*, South Dakota State University, Brookings; Figures 1-3 are from *Aflatoxin in Corn* by J. Allen Wrather and Laura E. Sweets, Missouri Agricultural Experiment Station, Delta Research Center, Portage.

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Additionally, test kits for aflatoxin are available commercially which do not require a reader, but these tests are primarily for feedstuffs. Although data indicate that the concentration of aflatoxin M<sub>1</sub> in milk is equal to 1.51 percent of the concentration of aflatoxin B<sub>1</sub> in the diet, there are many factors that affect the level of aflatoxin in milk.

## Summary

Mycotoxin production is found most frequently in pre-harvest grains that are harvested under high temperatures, prolonged drought and high insect activity. Thus, aflatoxin contamination in grain is much greater in states such as Arkansas which have warm temperatures and high humidity. Corn and cottonseed should be screened for aflatoxin if there is a likelihood of contamination. Generally, all corn processed at a feed mill or mixed in a total mixed ration for milking dairy cattle should be scanned with a black light for aflatoxins.

**If aflatoxin M<sub>1</sub> is detected in milk, the grain that is being fed to milking cows should be replaced with aflatoxin-free grain that contains bentonite and/or a commercially available dietary chemisorbent. An exception would be if the cows have been milked following the pick-up on the contaminated milk; then the milk should be tested to determine the level of aflatoxin. If the milk is above 0.5 ppb aflatoxin, the grain should be replaced; if the milk is below 0.5 ppb aflatoxin or has declined markedly, one might not replace the feed if it is likely future milk will be acceptable. In all cases, the grains should be tested for levels of aflatoxin.**

Records should be maintained for all feeds, feeding practices, milk contamination and animal health and performance for all cases of aflatoxin contamination of milk.

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