

## The Problems of Mycotoxins in Dairy Cattle Rations<sup>1</sup>

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Certain kinds of molds in feedstuffs are a periodic problem to livestock producers, although many molds are quite beneficial to the well-being of man. Penicillin, soya sauce, and roquefort cheese are examples of products derived from beneficial molds. Therefore, all molds must not be considered undesirable, but rather should be judged on the basis of the individual mold and its habitat.

Mycotoxins are the poisonous substances that can be produced by certain molds under certain conditions. The term "mycotoxin" is derived from myco meaning fungi and toxin meaning toxicant of biological origin. Mycotoxins are secondary substances or metabolites produced by a wide range of fungi, principally molds. There are over 100 species of molds that produce mycotoxins. Most mycotoxins of importance in Florida agriculture are produced by species in the genera *Aspergillus*, and *Fusarium*. Mycotoxin-induced diseases in animals are called "mycotoxicoses," whereas diseases caused by fungi are termed "mycoses." Until about 1960, the problem of concern was centered mainly on poisoning of animals and associated economic loss. More recently, the issue is food safety due to the food-chain transfer of residues. Also, advancements in analytical procedures have provided a means for

identifying and quantitating mycotoxins and their metabolites in meat, dairy, and poultry products.

The mycotoxins of greatest concern to dairymen are aflatoxin B<sub>1</sub> and B<sub>2</sub>. Two others that are being studied and frequently appear to be of concern to ruminant animals are zearalenone (F-2 toxin) and deoxynivalenol (vomitoxin). The effects of zearalenone on ruminant animals is still somewhat controversial. Some reproductive problems in ruminant animals have been blamed on the presence of zearalenone even though such reports have not been experimentally documented. The two mycotoxins that will be discussed in this paper are aflatoxins and vomitoxin.

### Susceptibility of Feedstuffs

The susceptibility of different feedstuffs to mold infection is directly related to their adequacy as substrates. The seed or kernel, because of its richness in carbohydrates, is a frequently targeted plant part. However, other species of molds utilize cellulose and therefore prefer the more fibrous parts of the plant. Even so, aflatoxins grow primarily on grains such as corn. Many factors that enhance mycotoxin production do so by increasing the availability of

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substrate to the fungus. *Aspergillus flavus* and *Aspergillus parasiticus* produce four major toxins: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>. These were named according to their fluorescence properties under shortwave ultraviolet light on thin-layer chromatographic plates; B<sub>1</sub> and B<sub>2</sub> fluoresce blue, whereas G<sub>1</sub> and G<sub>2</sub> fluoresce green. Metabolites of toxicological significance include: aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) AFB<sub>2</sub> and AFM<sub>1</sub>. On soybeans, only negligible strains concentrations of AFB<sub>1</sub> are produced by both species. *Aspergillus* species is capable of colonizing most of the important grain crops including corn, small grains, peanuts, cottonseed, and most nut crops.

Damage to the seed coat (pericarp) of corn induced by insects, drought, hail, frost, or mechanical harvesting favors fungal invasion. Moreover, insects can also serve as carriers of fungal spores. The three crops with high potential for invasion by *Aspergillus* species during growth, harvest, transportation, or storage are corn, peanuts and cottonseed. Colonization of soybeans and small grains generally occurs in storage.

The occurrence of preharvest mycotoxins in grain and other feedstuffs from different areas is primarily a function of climate. Toxigenic fungi that produce aflatoxins thrive in the warm, humid environments that exist in the southern United States. Other mycotoxins, such as the *Fusarium* species that produce trichothecenes and zearalenone, require both warm and cool temperatures.

Aflatoxin-producing strains of *Aspergillus* are distributed worldwide in soil and air. When environmental conditions are favorable, and substrate (feed or seed) is accessible as a nutrient source, colonization and mold growth can easily occur. In general, mold growth and aflatoxin formation require a moisture content greater than 14 percent, a temperature of at least 77 °F, and some degree of aeration.

**Corn**. The elevated content of aflatoxins found in corn in any given year is usually the result of increased invasion by molds prior to harvest. Contributing factors include drought, insect damage, and kernels with damaged seed coats. Other contributing factors include corn harvested late, competition from weeds, and inadequate fertilization.

Corn placed in storage inadequately dried will probably be invaded by molds.

**Cottonseed**. Aflatoxins found in cottonseed are usually due to some sort of field problem such as heavy rainfalls during late July and August. Again, *Aspergillus flavus* penetrates the seed through damaged areas such as exit holes of the pink bollworm. Cotton harvested late due to rainfall and before the moisture has evaporated is subject to aflatoxin contamination.

**Peanuts**. Peanuts can be colonized by *Aspergillus* species in the ground before digging, during curing and drying in windrows or stacks, and in storage. Before digging, invasion has been attributed to drought-induced stress, damaged pods, or over maturity. After digging, a moisture content of 14 percent to 30 percent is conducive to mold invasion and subsequent aflatoxin formation, but is prevented when the moisture is very high. When peanuts are being cured, retardation of drying by rainfall or humid weather conditions usually results in some degree of aflatoxin production.

## Metabolism of Aflatoxins

Aflatoxins are easily absorbed from the gastrointestinal tract, are extensively metabolized, and the majority of the dose is eliminated from the body in a relatively short period. The metabolic fate and distribution of aflatoxins in livestock is of concern for two reasons: (1) metabolism accounts for much of the extreme toxicity and carcinogenicity of aflatoxins, and (2) distribution of the metabolites to various body locations of food animals can impart hazardous residues to products used as food for man.

Aflatoxin B<sub>1</sub> in the feed of an animal is converted in part to a hydroxylated compound, aflatoxin M<sub>1</sub>, which appears in the milk of lactating cows. Studies reveal that relatively small amounts of AFM<sub>1</sub> appear in milk within a few hours after aflatoxin AFB<sub>1</sub> containing feed is consumed. The level of AFM<sub>1</sub> in milk disappears within a few milkings after the source of AFB<sub>1</sub> is removed from the diet.

The concentration of AFM<sub>1</sub> in milk increases proportionally with the amount of AFB<sub>1</sub> in the diet of the lactating dairy cow. When ingestion is continuous, milk concentrations will increase until an equilibrium with intake is established. The conversion rate of AFB<sub>1</sub> to AFM<sub>1</sub> varies some with source of contamination. Recent studies indicate that a greater percentage of AFB<sub>1</sub> is secreted in milk as AFM<sub>1</sub> than was earlier reported. The data shows that the reported ratio of 300:1 probably underestimates the actual transfer of AFB<sub>1</sub> to AFM<sub>1</sub>. The mean ratio as reported in more current studies varies from about 58:1 to 75:1, depending on the commodity studied and the level of milk production. The average transfer in table 1 for cottonseed meal is 1.71 percent (58:1) and 1.33 percent for corn (75:1). The transfer varies some depending on level of milk production.

The present actionable FDA guidelines for AFM<sub>1</sub> in milk is 0.5 ppb and for AFB<sub>1</sub> in feed is 20 ppb. According to the average transfer value of 66:1 obtained from Table 1  $[(58 + 75) \div 2]$ , a concentration of 20 ppb AFB<sub>1</sub> in feed would result, on average, of 0.3 ppb AFM<sub>1</sub> in milk  $(20 \div 66)$  which is below the legal maximum of 0.5 ppb but still can result in damage to the animals (see following section Effects of Toxicity from Aflatoxin and Vomitoxin). A concentration of 33 ppb AFB<sub>1</sub> in feed would result, on average, in an AFM<sub>1</sub> concentration in milk near 0.5 ppb  $(33 \div 66)$  thus making the milk illegal. These values are simply good guidelines, not precise numbers. The amount of aflatoxins transferred from the feed to the milk will be influenced by the type of feed source containing the aflatoxin (corn, cottonseeds, or peanuts) and by the amount of milk produced by the cows fed the contaminated feedstuffs.

### Stability of Aflatoxins

Aflatoxins and their metabolites are highly stable during normal milk processing procedures. In one study, the AFM<sub>1</sub> concentration in milk was not reduced by either vat pasteurization or high temperature short time pasteurization. After 17 days of storage at 39 °F the concentration of AFM<sub>1</sub> in pasteurized milk was the same as the aflatoxin concentration in raw milk.

### Identification and Guidelines

The blacklight, 365 nanometers, can be utilized to screen corn prior to purchase, sale, or use for feed. Generally, one "greenish-yellow glowing" kernel per pound represents about 20 ppb; 10 positive kernels per pound, about 200 ppb.

False positives or negatives occur with this screening method in about 30 percent of the samples examined. Therefore, assay or testing by official methods should be used to accurately identify the level of aflatoxin indicated by the use of the blacklight. This requires submitting a good representative sample (about 10 lbs) to laboratories such as the State Department of Agriculture and Consumer Services laboratory located in Tallahassee. The laboratory should be qualified for analyzing for mycotoxins (see **selected laboratories** section 8).

Present FDA regulations for the feeding of aflatoxin-contaminated rations to dairy cattle suggest a maximum allowable level of AFB<sub>1</sub> in the total ration of 20 ppb, which could possibly result in a level of 0.27 ppb AFM<sub>1</sub> in milk (75:1). The present actionable guidelines for AFM<sub>1</sub> in milk is 0.5 ppb.

### Effects of Toxicity From Aflatoxin and Vomitoxin

**Aflatoxins** . Early indications of aflatoxin toxicity include reduction in feed intake followed by weight loss or decreased rate of gain. Also, there is usually a decrease in feed efficiency, increased susceptibility to stress, and decreased reproductive performance. Calves are more susceptible than older animals. Chronic aflatoxicosis is characterized by unthriftiness; anorexia; a drying and peeling of skin on the muzzle; prolapse of the rectum; liver damage; elevated levels of blood constituents such as cholesterol, bilirubin, and serum indicator enzymes; and edema in the abdominal cavity. Milk production may be dramatically decreased in dairy cows fed aflatoxin-contaminated feed. Almost any level of aflatoxin-contaminated feedstuff in the ration may lead to some liver damage, especially in young animals.

**Deoxynivalenol (Vomitoxin)** . Deoxynivalenol is more commonly called vomitoxin or "DON" in the

field. As the name implies, vomiting and feed refusal are associated with the consumption of contaminated feeds, especially in swine and ducklings. Vomitoxin is a mold toxin produced by species of fusarial molds. It has been associated with reduced feed intake, unthriftiness, reduced weight gains, and decreased performance. Other symptoms include diarrhea, abortion, hemorrhage, hematological changes and nervous disturbances.

The U.S. Department of Agriculture has not established official DON maximums and there are no federal regulations to control it. A potential hazard has been suggested at levels of 2 ppm in the feed. Higher levels have been reported. The presence of DON in a feedstuff is considered a possible marker for the potential presence of other fusarium toxins.

### Management Considerations

Good management practices will help reduce the problem of mycotoxins in dairy rations. The first preventive measure is to purchase ingredients that are relatively free of mycotoxins. Beware of feedstuffs that appear suspicious. Store grains at moisture levels less than 14 percent. Commence a clean-up program around the feed mill or feed troughs to eliminate damp areas suitable to mold growth. Be very cautious in the purchasing of corn and/or peanut meal during seasons when crops are being harvested following a drought.

Aflatoxin-contaminated products must be excluded from the food chain. Sources of contaminated feedstuffs must and should be avoided in feeding programs. There are some alternative methods to salvage a damaged product such as corn. Of the available methods, ammonium treatment appears to offer the best alternative. The combination of ammonia (0.6 percent to 4 percent), heat, and moisture (10 percent to 20 percent) is particularly effective. Decontamination is usually accomplished by placing the product in a sealed polyethylene bag. Since ammonia is dangerous, contact an experienced operator for conducting the process.

### Selected Laboratories for Mycotoxins

Romer Labs, Inc.

P.O. Box 2095125

Washington, MO 63090

314/239-3009

Dairy One

Forage Testing Laboratory

730 Waren Road

Ithaca, NY 14850

607/257-1272

Waters Laboratory

P.O. Box 38

Camilla, GA 31738-0382

912/336-7216

Woodson-Tenent Labs, Inc.

2 Box 1097

Gaines, GA 30501

404/536-5909

**Table 1.** Effect of Source of AFB<sub>1</sub> contamination on levels in milk.

| Treatment       | ppb(ug/kg) | Milk prod. | AFB <sub>1</sub> Intake (ug) | MY(lb) | AFB <sub>1</sub> in milk (ug/lb) | AFB <sub>1</sub> Transferred Percent |
|-----------------|------------|------------|------------------------------|--------|----------------------------------|--------------------------------------|
| Cottonseed meal | 44         | High       | 966                          | 66.9   | 0.28                             | 1.95 (51:1)                          |
|                 |            | Low        | 742                          | 40.7   | 0.27                             | 1.47 (68:1)                          |
|                 |            | Ave.       | 854                          | 53.8   | .275                             | 1.71 (58:1)                          |
| Corn            | 49         | High       | 1116                         | 68.6   | 0.23                             | 1.42 (70:1)                          |
|                 |            | Low        | 885                          | 44.0   | 0.25                             | 1.24 (80:1)                          |
|                 |            | Ave.       | 1000.5                       | 56.3   | 0.24                             | 1.33 (75:1)                          |

ug/kg = Micrograms/kilograms | Frobish et al. J. of Food Protection, Vol. 49, Oct 86.