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Aflatoxicosis; Diagnosis and Treatment in Livestock

Deep Narayan Singh[1], Mamta[2], Ajay Kumar[3], Rajneesh Sirohi[4]

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Abstract

Aflatoxicosis is a fungal borne toxicosis that may affect almost all species of livestock in India as well as abroad. Aflatoxins are major class of mycotoxins produced primarily by Aspergillus flavus i.e. most common grain mold fungi. The fungus grows on carbohydrate rich feeds such as peanuts, cottonseed, corn, sorghum and cereal grains when they are stored in hot conditions without adequate drying and aeration. Aflatoxin M1 found in milk of dairy cattle is the metabolite of Aflatoxin B1 that occurs in feed materials. Aflatoxicosis is a problem in livestock, most notably swine and cattle. Gross lesions include hepatic enlargement, congestion, yellow discoloration, and friability; petechiae or more generalized hemorrhage; and edema and ecchymotic or petechial hemorrhages of the gall bladder. Cattle are more resistant than pigs, but the typical lesions of aflatoxicosis, as described above, can be found following exposure. Fibrosis and bile duct proliferation may be extensive and found together with fibrotic veno-occlusion of the central veins. Sheep are resistant to aflatoxin.

Keywords: Aflatoxin; Corn; Fungus; FDA.

INTRODUCTION

Many different fungi may grow as molds on stored grains. Fusarium and Aspergillus fungi are among the most common grain molds. Not all fungi produce toxins, but Aspergillus flavus, which produces aflatoxin, is among the most common grain mold fungi.

The development of aflatoxins depends on the infestation and growth of the Aspergillus mold in grain. High temperatures and high humidity favor the infection of corn kernels through the silks by the Aspergillus fungi. High humidity prone areas are more susceptible for aflatoxin poisoning. Below normal soil moisture (drought stress) has also been found to increase the number of Aspergillus spores in the air. Therefore, when drought stress occurs during pollination, the increased inoculums load (spores in the air) greatly increases the chances of infection. Further more, drought stress, nitrogen stress, and other stresses that affect plant growth during pollination can increase the level of aflatoxins produced by Aspergillus fungi.

Aflatoxins are poisonous, carcinogenic by products produced during the growth of several species of the mold fungus Aspergillus flavus. These byproducts are produced as the fungi grow

Author Affiliation: [1]Associate Professor, Department of Livestock Farm Complex (LFC)/Livestock Production Management (LPM), Bihar Veterinary College, Patna, Bihar Animal Sciences University, Patna-800014, India., [2-4]Assistant Professor, Department of LPM, College of Veterinary Science & Animal Husbandry, U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura 281001, Uttar Pradesh, India.

Corresponding Author: Deep Narayan Singh, Associate Professor, Department of Livestock Farm Complex (LFC)/ Livestock Production Management (LPM), Bihar Veterinary College, Patna, Bihar Animal Sciences University, Patna-800014, India.

E-mail: drdeep25@gmail.com

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in feed grains, processed feed, and food products. Aflatoxins are primarily a problem in corn but can also occur in other grain crops. Aflatoxins are highly toxic to livestock, poultry, and people. Consumption of low concentrations by animals sensitive to aflatoxins can lead to death in 72 hours. In general, at nonfatal levels, the health and productivity of animals fed contaminated feed are seriously impaired. As a result, the Food and Drug Administration (FDA) has set an action level for aflatoxins in corn at 20 parts per billion (ppb). Corn containing aflatoxin levels of 20 ppb or more cannot be sold in interstate commerce, and, in general, should not be fed to young poultry, swine, and livestock, or to lactating animals, and must not be milled for human consumption.

Several other factors play a role in the development of Aspergillus mold and aflatoxin production. Because drought stress plays such an important role, practices that reduce drought stress in plants should reduce the levels of infection and aflatoxin production. Irrigation has been shown to be very effective in reducing Aspergillus infection and aflatoxin development, even if done only during pollination. Tillage practices have not been as effective and have only been demonstrated to reduce aflatoxin by sub soiling in areas with hardpans. Occasionally during droughty periods, hybrids of differing maturities or those planted early will pollinate during periods when drought stress is less often observed in South Dakota. Escaping drought with planting dates and hybrid maturity may differ from one year to the next. Time of harvest has also been shown to be important in influencing the occurrence and levels of aflatoxin because Aspergillus does not compete well with other molds when corn is above the 20 percent moisture content. Harvesting corn when moisture content is above 20 percent followed by rapid drying to at least a moisture content of 14 percent within 24 to 48 hours of harvest keeps further Aspergillus growth and toxin production at a minimum.

Aflatoxicosis and Livestock:

Aflatoxicosis is a disease caused by the consumption of aflatoxins. Aflatoxins are secondary mold metabolites produced by some strains of Aspergillus flavus and other related species of Aspergillus fungi. The four most common aflatoxins are B1, B2, G1, G2 and M. Contaminated grains and grain by products are the most common sources of aflatoxin. Corn silage may also be a source of aflatoxins, because the ensiling process does not destroy toxins already present in silage. Aflatoxins are metabolized in ruminants by the liver and are excreted in the bile. Aflatoxin B1 is the most potent mycotoxin (toxic substance produced by a mold) to affect cattle. B1 increases the apparent protein requirement of cattle and is a potent carcinogen (cancer causing agent). When significant quantities of B1 are consumed, the metabolite M1 appears in milk within 12 hours. Research suggests M1 is not as carcinogenic or mutagenic as B1, but it does appear to be as toxic as its parent compound.

Aspergillus thrives at high grain moisture levels (22% to 26%) and temperatures of 82 °F to 90°F. Drought stressed corn is especially susceptible to aflatoxin contamination.

The swine are also affected by Aflatoxicosis. The effects of feeding aflatoxin contaminated corn depends on the age of the pig and the concentration of the toxin in the feed. Low levels (20 to 200 ppb) can affect pig performance through reduced feed in take and suppression of the immune system. High levels (1,000 to 5,000 ppm) can result in death. A metabolite of aflatoxin (aflatoxin M) can be found in milk from sows fed diets with aflatoxin. Lactation diets with 500 to 750 ppm aflatoxin can result in mortality and reduced growth of nursing pigs. There are no maximum a safe levels of aflatoxin in swine diets, as effects can be found with low concentrations (20 ppb). There are published maximum a tolerable@ levels in complete diets for some classes of swine: pigs less than 50 pounds - 20ppb; finishing pigs - 200ppb; breeding swine - 100ppb. If the fungus is present and viable, aflatoxin levels can increase during grain and feed storage. One severe case of aflatoxicosis (850ppb aflatoxin in the finished feed) resulted from feeding corn with less than 20 ppb aflatoxin, but the feed was stored in large, outside feeders for seven to 14 days. There is a risk of aflatoxin toxicity in feed from low levels of aflatoxin in corn. The first step in addressing aflatoxin in swine on the farm is prevention. The potential for aflatoxin is reduced by drying (less than 15% moisture), removal of foreign material and cracked kernels, and routine aeration of stored corn. Suspect corn should be analyzed for aflatoxin B1 and aflatoxin B2, through a commercial lab or the veterinary diagnostic lab. If aflatoxin is present, the concentration will determine the next step. The simplest method of dealing with aflatoxin contaminated corn for on-farm use is blending.

Contaminated corn can be mixed with a clean corn to reduce aflatoxin to an acceptable level. For example, mixing 500 pounds of corn containing 400ppb aflatoxin with 1500 pounds of aflatoxin free corn reduces the aflatoxin level to 100ppb. If the contaminated corn contains viable fungus, the

aflatoxin levels will increase with high moisture and warm temperatures. Fungal inhibitors such as propionic acid can reduce or prevent fungal growth. These inhibitors can be expensive to use and difficult to apply properly. They will have no effect on the aflatoxin already present in the corn. The commercial feed industry often uses compounds called aluminosilicates to improve the pelleting and flow properties of feed. In the early 1980s, research showed some of these compounds reduced the negative effects of feeding aflatoxin contaminated corn. Mixing 10 pounds per ton sodium bentonite in feed using corn with 750ppb aflatoxin produced growth comparable to aflatoxin free feed. Similar benefits were found using a hydrated sodium calcium aluminosilicate. These compounds partially bind aflatoxin in the digestive tract and reduce their absorption. Aflatoxin is a problem that can often be controlled through proper grain handling. During drought conditions, however, it is a constant possibility. Suspect corn should be tested, and appropriate action taken based on the level of aflatoxin and the age and weight of the pigs to be fed. For more information, contact your local county Extension office or veterinarian, or the Alabama Department of Agriculture and Industries feed control office.

Development of Aflatoxin

Mature corn that remains in the field or corn that is stored without adequate drying can be subject to Aspergillus growth and aflatoxin production. Temperatures between 80°F and 100°F and relative humidity of 85 percent (corresponding to 18 percent grain moisture) are optimum for growth of Aspergillus. Growth of the fungus is poor below 55°F, but if the grain is moist enough, toxins can still be produced. However, simply reducing the moisture content to as low as 12 percent does not kill the fungus and does not reduce the levels of toxins that have already been produced. If moisture levels rise again above 12 percent anytime during storage, and temperatures are high enough, then mold growth and toxin production will resume. It is important to note that conditions favoring the growth of Aspergillus also favor the growth of other fungi that can have harmful effects on humans if they are inhaled or ingested while working in grain handling facilities. Always wear a dust respirator when working in grain or feed storage and handling areas.

Preventing Aflatoxin Contamination

Resistance to aflatoxin accumulation in corn kernels has been recently identified. Hybrids resistant to aflatoxin and other fungal toxins should become available in the near future.

Detecting Aflatoxin Contamination:

Once aflatoxin is produced, it is stable. Heat, cold and light do not affect it. It is also colorless, odorless and tasteless, and because of the low concentrations involved and the uneven distribution in grain bins, aflatoxins are difficult to detect. In the past, elevator operators and buyers used the black light test, but this test simply detects compounds that fluoresce (aflatoxins and others) and should only be used to select samples that require further testing. Similarly, mini-column tests are no longer recommended, as they were

Prone to give false positive results if used improperly.

However, aflatoxin contaminated feed can be tolerated by some livestock, particularly older animals. Obviously, the higher the level of contamination, the greater the risk in feeding contaminated corn to animals. Further more, continued proper storage is essential so that aflatoxin levels do not continue to increase in the corn or feed before use. Detoxification of feed continues to be an elusive goal. However, certain feed additives have been successfully used to inhibit mold growth and to reduce the incidence of aflatoxicosis in animals. Organic acids such as propionic, sorbic, and benzoic acids as well as their salts such as calcium propionate and potassium sorbate, and copper sulfate can be used to inhibit mold growth in feed. Mineral clays such as zeolite and bentonite as well as hydrated sodium calcium alumino-silicate (HSCAS) can bind to aflatoxin, protecting animals from absorbing the toxin that may be in the feed. These products, according to FDA rules, cannot as yet be labeled as mycotoxin binders, and are sold as anti-caking and free flow feed additives. There are no clear cut safe levels for different animal species regarding their resistance or tolerance to aflatoxins. The following section on aflatoxicosis and ruminants and the general guidelines for dealing with aflatoxin contaminated feed may assist you in deciding.

Sampling for Aflatoxins

Regardless of the test procedure used, the single most important factor for reliable and accurate testing of grain for aflatoxins is obtaining a representative sample. The ideal sample size should be at least 10 pounds of corn. The sample should consist of several smaller subsamples (10 or more 1 pound samples) that have been taken from different spots and then mixed together. Handle each bin or truck separately, and take a 10 pound

sample from each source. Place samples in a cloth or paper container that allows air exchange. Air tight containers or plastic bags allow condensation, which raises the moisture content, resulting in the possibility of continued growth and toxin production of the fungus. Send or take samples to a testing lab as quickly as possible.

Effects of Aflatoxins on Animals

Effects of aflatoxin consumption are similar in all animals; the animal's susceptibility to aflatoxin, however, varies by species, age, and individual variation. In acute clinical aflatoxicosis, signs of acute hepatic injury are seen as coagulopathy, increased capillary fragility, hemorrhage, and prolonged clotting times. Blood pigments may appear in the urine and mucous membranes are icteric. The liver shows gross changes caused by centrilobular congestion and hemorrhage and fatty changes of surviving hepatocytes. Death of the animal may occur within hours or a few days. In chronic aflatoxin poisoning, most of the effects are still referable to hepatic injury, but on a milder scale. The most sensitive clinical sign of chronic aflatoxicosis is reduced rate of growth of young animals. Other signs include prolonged clotting time, in- creases in serum glutamic oxalacetic trans aminase, ornithine carbamyl transferase, and cholic acid levels. Hepatic pathology includes a yellow to brassy color, enlarged gall bladder, dilute bile, histologic signs of fatty changes in the hepato cytes, and bile duct proliferation. Frequently the signs of chronic aflatoxins are so protean that the condition goes undiagnosed for long periods. Chronic aflatoxin poisoning, however, is the man ner in which animals are most frequently affected and. The economic consequences are often considerable. Both acute and chronic aflatoxin poisoning can impair immune responses and native defense mechanisms. In addition to the liver, the thymus is also a target organ for aflatoxin; thymic involution results with loss of cortical thymocytes. It is primarily the cellmediated immune responses that are affected by aflatoxin; prominent among these are diminished responses in delayed cutaneous hypersensitivity, graft vs. Host reaction, leukocyte migration, and lymphoblastogenesis. Aflatoxin also reduces phagocytic activity in a dose related manner; this is important not only to the phagocytic clearance of invading organisms but also to presentation of antigens to other components of the immune system. Some humoral components are diminished by aflatoxin, including complement C4), interferon, IgG, and IgA, but not IgM. Usually only dramatically high levels of aflatoxin will affect antibody titers and gut associated lymph tissue or the bursa or Fabricius in Immunity acquired through vaccination procedures is impaired in fowl cholera and porcine erysipelas, but not New castle disease in chickens. Similarly, susceptibility to some infectious agents (e.g., Salmonella, Candida, Treponema, Eimeria, and infectious bursal disease virus) is increased, but not all infectious processes seem to be affected (Pier and McLoughlin, 1985). of considerable potential economic consequence is the fact that aflatoxin can suppress the immune system of young animals by in utero transfer across the placenta of the pregnant dam (Pier et al., 1985). In these cases the affected newborn animals lack resistance to infection and cannot respond well to vaccines. These are reactions of considerable consequence in colonized animals in which we rely on elective vaccination procedures in disease prevention. Several of the major mycotoxins exert their effects through different organ systems and different biological pathways. Aflatoxin, ochratoxin, and T-2 toxin all interfere with protein formation, but each does so in a different manner; aflatoxin binds to both RNA and DNA and blocks transcription. T-2 toxin blocks initiation of translation, and ochratoxin blocks phenylalaninet RNA synthetase, and thus blocks translation. Each toxin causes different effects on globulin formation; aflatoxin reduces IgG and IgA but not IgM and usually does not reduce antibody titers, ochratoxin reduces IgG and IgM and regularly reduces antibody responses, T-2 reduces IgM and IgA but usually not IgG and often reduces antibody response. Both aflatoxin and T-2 effect reduced complement activity, but in different ways; aflatoxin reduces C4 activity, whereas T-2 reduces C 3 activities. Thus, one might expect that when mixtures of mycotoxins are encountered in feed mixtures that some interaction of these toxins might be apparent. Experiments combining aflatoxin and T-2 toxin show a synergistic effect on lethality, but only additive suppression was seen on weight gain and selected immunologic traits (Pier et al., 1988). Another mycotoxin mixture (aflatoxin and cyclopiazonic acid) was studied in guinea pigs (Pier et al., 1989). This was an interesting mixture because these two toxins appear together in nature; some strains of A. flavus are capable of producing both toxins in corn and other substrates. Synergistic interaction was seen on lethality, weight gain, and histopathologic changes in the liver. However, an interesting observation was that cyclopiazonic acid over came the immunosuppressive effects of aflatoxin on cell poultry. mediated immune responses. From these and other studies it is

apparent that the immune system is a sensitive register for the effects of various mycotoxins and that mixtures of mycotoxins can profoundly affect the animal organism.

Implications

The economic consequences of aflatoxicosis on young growing animals are substantial and varied. Aflatoxins cause clinical illness and death when consumed in high quantity; at lesser levels they reduce the growth rate and feed efficiency of young animals and they reduce the animals' ability to cope with infections. Undoubtedly we will recognize yet other effects of the aflatoxins on animal and human health as investigations continue. Because of their exceptional biological activities and their propensity to peak in essential field crops during problem years, aflatoxins promise to be a continuing problem in animal production.

Symptoms of Aflatoxicosis

Beef and dairy cattle are more susceptible to aflatoxicosis than sheep or horses, although other mycotoxicoses occur in these species, such as facial eczema in sheep and leukoence phalornalacia 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 hemorrhage 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 Pathology

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 haematocrit, 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.

Diagnosis

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 the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult. The prognosis of aflatoxicosis depends 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 furt her poisoning prevented.

Serological tests for diagnosis of Aflatoxins:

Serological tests are now considered to be more reliable and their accuracy has been validated by comparison to more costly and time consuming analytical procedures. Serological test kits using such methods as ELISA do not require specialized labs, equipment, or training and when conducted according to manufacturer's instructions can give accurate results for the presence (qualification) and amount (quantification) of Aflatoxins in grain samples.

Determination of Aflatoxins in laboratory

Modified Romer's method:

Method: The aflatoxin is extracted with acetone, treated with Cupric carbonate and ferric gel to eliminate fluorescent material other than aflatoxin, washed with acid and alkali and extracted with chloroform, dried, rediluted with chloroform and spotted in an activated TLC plate with standards and ascertained the concentration by visual comparison method in a UV viewing cabinet.

Preparation and Requirement of reagent

1. 0.2 M NaOH: Dissolve 8 gm of NaOH in 1000 ml solution.
2. 0.41 M FeCl3: Dissolve 17 gm of FeCl3 to make 1 lit of solution with distilled Water.
3. 0.03% H2SO4: 0.3 ml of conc. H2SO4 in 1 lit of distilled water
4. 4.0.02 M KOH and 1% KCl: Dissolve 1.222 gm of KOH and 10 gm of KCl in 1 lit standard flask with distilled water.

Preparation of Activated TLC plate:

To prepare two plates (10cmx10 cm or 10cmx5cm)

of 0.2mm thickness dissolve 16 gm of silica gel (G) in 35 ml of distilled water, apply on the plate using applicators allow it to natural dry. Then keep the plate at 105°C for 1hour and cool. Draw lines with 1 cm space such that standards are at the middle of the plate and four sample spots can be applied on either side of the standard.

Procedure:

1. Take 10 gm of sample. Add 40 ml of distill water. Beat it in the mixie for 2 minutes.
2. Add 60 ml of acetone and again it for the 2 minutes. Contents may slightly be heated up. High temperature should be avoided.
3. Filter the contents. Take 30 ml of filtrate and add approximately 0.6 gm of cupric carbonate in beaker (A).
4. In another beaker (B), take 34 ml of 0.2 M NaOH + 6 ml of FeCl3 (0.41 M) and swirl the contents.
5. Add the contents in the beaker (B) to beaker (A) and again mix it slowly by swirling movements.
6. Filter the contents through Whatman No. 1 Filter paper.
7. Take 40 ml of filtrate in a 25 ml separating funnel.
8. Add 40 ml of (0.03%) H2SO4 and 10 ml of chloroform. Mix it slowly.
9. Collect the chloroform layer in a 100 ml beaker, add 10 ml of Chloroform, mix thoroughly, allow to settle and collect the chloroform in the same 100 ml of beaker.
10. In a second separating funnel, take40ml of 0.02 M KOH and 1% KCl mixture.
11. To this, add the collected 20 ml of chloroform extract. mix it slowly and collect the layer through anhydrous Sodium sulphate bed drop by drop to remove ant traces of moisture.
12. Keep the chloroform extract in an oven at 50 C till it becomes dry.
13. The dry afflatoxins film is rediluted with0.2 ml Chloroform and spot on the TLC plate taking exactly 5 micro liter, 10 micro liter, 20 micro liter and 40 micro liter besides the standard spots of 5 micro liter and 10 micro liter.

Preparation of Aflatoxins Standard

Carefully prepared the given Aflatoxin in a suitable standard flask with Benzene: acetonitile (98+2) mixture to give a concentration of 10 micro gram per ml. Standardize the concentration of stock solution using spectrophotometer. From this stock solution, prepare Aflatoxin solution in benzene: acetonitrile containing 4 micro gram per ml in a suitable standard flask which is the working standard. After spotting the standards and sample, develop the spots in an unsaturated developing time containing chloroform: acetone: water in the ratio 88:12:1. after developing 3/4th of the plate, the plate is carefully remove from the tank, dried well and viewed in UV cabinet viewer using long wavelength (364 nm)

Calculation:

$$\text{Aflatoxin content in ppb} = \frac{S \times C \times d \times 100}{T \times 1.714}$$

Where,

S = Standard which compare with the sample in the fluorescent intensity.

C= Concentration of standard.

d= dilution factor.

T= sample which compares standard in fluorescent intensity.

1.714= effective weight.

Treatment

Aflatoxicosis is typically a herd rather than an individual cow problem. If aflatoxicosis is suspected, the ration should be analyzed immediately. If aflatoxins are present, the source should be eliminated immediately. Levels of protein in the ration and vitamins A, D, E, K and B should be increased as the toxin binds vitamins and affects protein synthesis. Good management practices to alleviate stress are essential to reduce the risk of secondary infections. Secondary infections must receive immediate attention and treatment.

Aflatoxicosis can only be prevented by feeding rations free of aflatoxin. Preventing aflatoxin contamination is outlined on the preceding page, but since preventing contamination is not always possible, here are a *few keys facts to remember when dealing with contaminated feeds in animal rations:*

- The recommended feeding level is 0 parts per billion (ppb).
- The level of aflatoxin an animal can tolerate will depend upon the age and sex of the animal, its health status, and overall management level of the farm.
- To avoid contamination of milk, lactating dairy cattle should not receive more than 20 ppb in the total ration.
- Calves should not receive milk from cows fed

in excess of 20 ppb, because they can ingest aflatoxin from the milk.

- Beef cattle can tolerate slightly higher levels of aflatoxin, but yearlings and mature cows should not receive more than 400 ppb in the total ration. Weanlings should not receive more than 100 ppb in their total daily ration.
- Poultry and swine are more sensitive to aflatoxin contamination.

Under no circumstances should these livestock species be fed more than 20 ppb aflatoxin in their daily rations. The above are only guidelines. This does not suggest that feeding at these levels or below will reduce or eliminate the potential for aflatoxicosis. There are no clear cut safe feeding levels. Safe levels vary with each individual animal. Remember that ingestion of aflatoxins at levels even lower than those listed in the guidelines may cause some undesirable side effects and depends on such factors as age, sex, and general health of the animals. To feed at a level other than 0 ppb is a risk assumed by the person making the decision to do so. In all cases, monitor animal health closely and discontinue the use of contaminated feed immediately if undesirable effects are noticed.

CONCLUSION

Aflatoxins are highly toxic to livestock, poultry, and people. Even when fed at nonfatal levels, aflatoxin can seriously impair animal health and productivity. For lactating dairy cattle, do not exceed 20 ppb aflatoxin in rations to avoid exceeding the Food and Drug Administration level of 0.5 ppb in milk. Aflatoxin is just one of many mycotoxins that can adversely affect animal health and productivity.

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