

Aflatoxicosis in Food Animals: A Clinical Review

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Introduction

The term mycotoxin is used to describe secondary metabolites produced by various fungi which are toxic in varying degrees to humans and animals. Mycoses involve invasion of a pathogenic fungi within animal tissues. Mycotoxicoses imply toxic conditions in livestock and possibly humans due to ingestion of mycotoxin-contaminated food. Mycotoxins were not studied and identified with clarity as an entity until the early 1960's, although mycotoxicoses have been suggested in the past in humans, especially with ergotism in rye flour.² Many attempts to diagnose mycotoxicoses have been difficult as the disease is often subclinical, chronic, and endemic.³ In 1960, a tremendous outbreak of disease swept through turkey flocks in Great Britain with high mortality rates. The etiology was unknown at the time, so the disease was coined "Turkey X Disease." In 1961 the disease was traced to a toxin in the feed produced by *Aspergillus flavus*, and was subsequently called aflatoxicosis.² Interest in aflatoxins then led to more research into food mycotoxins and the discovery of many other mycotoxins.

Conditions for Feed Contamination

Aflatoxins are elaborated when certain strains of *Aspergillus flavus* or *A. parasiticus* grow on a substrate under conditions favorable for toxin formation.⁴ The presence of *A. flavus* or *A. parasiticus* growth or hyphal elements in the grain do not necessarily indicate toxin formation, as there are many non-toxin producing strains of these molds.¹

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The substrates favored by *Aspergillus* spp. are high carbohydrate containing grains and feedstuffs, such as peanut meal, corn, sorghum, and cottonseed.^{1,4} Laboratory work has indicated four major factors which influence the formation of aflatoxins: moisture, temperature, aeration, and substrate.⁵

Inoculation of corn probably occurs in the field. *A. flavus* conidia rapidly colonize silks in the yellow-brown stage with high temperatures. Growth of the fungus can then proceed down the silk to the kernel, although this mechanism of action is not understood.⁶ Internal infection of the kernel occurs during the denting or dry-down process. Insects play an important role as they may damage the seed coat and allow *A. flavus* to spread from kernel to kernel.⁶ Under storage conditions, *A. flavus* and *A. parasiticus* can grow at moisture levels of 15% or greater, at temperatures from 6°–46°C and in an aerobic environment.⁵

The aflatoxins were initially divided into two main groups, that being B and G, which fluoresced blue and green, respectively, under ultraviolet light. Later, in 1963, it was shown that a dihydro-derivative of both aflatoxin B and G existed, and these were labelled aflatoxin B2 and G2.⁴ In 1963 also, it was reported that milk fed to ducklings from aflatoxin-fed cattle produced liver lesions identical to those produced by aflatoxin B1.⁷ It was indicated by the study that aflatoxin B1 was biotransformed in the mammary gland to another aflatoxin metabolite by a lactating animal. This toxin was labelled aflatoxin M, and later subdivided into aflatoxin M1 and M2.⁴

Isolates of *A. flavus* vary greatly in the quantity and quality of toxin produced.⁸ Since the organism is also very ubiquitous in the environment, the chance of toxin produc-

TABLE 1 Surveys of cereals and soybean for aflatoxin²

Commodity	Number examined	Number positive	Percent positive	Concentration (p.p.b.)	Moisture range (%)	Year	Authors
Wheat	531	2	0.38	B-1 = 7 G-1 = 2	12	1964	Shotwell et al.
Oats	304	3	0.99	6	12	1964	Shotwell et al.
Grain sorghum	533	6	1.13	3-19	11-14	1964	Shotwell et al.
Maize	1311	35	2.7	3-19	13-24	1964 & 1965	Shotwell et al.
Soya bean	866	2	0.23	7-10	13-16	1964 & 1965	Shotwell et al.
Maize	283	6	2.12	12-25	14-18	1967	Shotwell et al.
Maize	293	8	2.73	6-25	13.8-15.4	1968	Shotwell et al.

tion is significant if the growth requirements are met. A study on the incidence of aflatoxins in cereals and soybeans that covered the period from 1964-1968 showed that 2.6% of the corn samples were positive. The concentration of aflatoxin averaged 3.25 ppb. The incidence in soybeans, wheat, oats and sorghum was much lower (table 1).²

Toxicity and Pathogenesis

Toxicity levels that produce clinical signs vary with the species. Poultry seem especially susceptible to aflatoxins.^{1,2,3} In swine, young pigs are most sensitive, followed by pregnant sows and feeder pigs.¹

Cattle and sheep appear to be most resistant.¹

The liver is an organ of enzymatic degradation of toxins via the mixed function oxidase system (MFO). The MFO system is believed to also help in the detoxification mechanism by converting the toxins into a more polar structure. In aflatoxicosis, however, the MFO system in the liver seems to oxidize the aflatoxin to another metabolite that reacts with the chromatin of the nucleolus protoblast, thus impairing the template activity of the chromatin to produce M-RNA. Most research indicates the main cytotoxic effect is due to interference with messenger RNA, although some effects have been also shown to occur with DNA synthesis also.¹

The interference with cellular protein synthesis causes many lesions throughout the animal, with the most evident changes being in the liver. Mild, subacute aflatoxicosis causes variation in the size of the hepatocytes and their nuclei. Increasing doses of the toxin cause lipid vacuolization within hepatocytes due to impairment of fat mobilization secondary to disruption of enzyme synthesis.¹⁰

As the dosage and exposure increase, fatty retention by hepatocytes leads to multifocal lesions of lipid-laden hepatocytes, with cell

hypertrophy of medium sized biliary ducts and biliary ductules. Bile casts and dilated lymphatic vessels have also been reported.¹⁰ With the loss of centrilobular hepatocytes, a new vascular plexus develops around the central vein, leading to scattered necrosis of individual hepatocytes or coalescing into multifocal infarcts.¹¹

With severe chronic aflatoxicosis, hepatic lesions consist of fibrosis, necrosis, focal lipid accumulation with disseminated fatty degeneration, and atypical hepatocytes.¹⁰

Other pathologic changes occur with aflatoxicosis, many of which need to be researched further.³ Among these other lesions are nephrotoxic lesions, immunosuppression, and carcinogenicity.

Chronic, subacute aflatoxicosis may cause pyknosis and dilation of the distal convoluted tubular epithelial cells in the kidney. With an increase in dose to acute toxicity, the renal tubular epithelial cells will exhibit necrosis and may be filled with bile pigments, hyaline, and lipid.^{10,3}

Immunosuppression is observed in animals fed aflatoxin, but the pathogenesis is not completely understood. Aflatoxin appears to decrease the lymphocyte response to mitogens, inhibit macrophage migration, and decrease the effectiveness of humoral mediators such as complement.^{1,10} In one study, feeder pigs were fed aflatoxin B1 at a rate of 70 ppb.¹² No clinical signs or lesions were observed. When the animals were challenged with *Treponema hyodysenteriae*, the treated pigs showed more pronounced clinical signs in a shorter incubation period and a higher mortality rate than the control pigs.

Aflatoxin has also been shown to interfere with Erysipelas vaccination and acquired immunity when fed in subclinical doses to feeder pigs.¹³ Carcinogenicity of aflatoxin has not been thoroughly studied, although trout and rat hepatomas and occasional swine undif-

ferentiated neoplasms have been linked to aflatoxicosis.^{4,8,10}

Aflatoxin appears to have no direct effect on the fetus in a gestating animal, but fetal changes may be secondarily observed due to maternal toxic biochemical changes and hypovitaminosis A.^{10,14} When aflatoxin labeled with C14 is administered to gestating mice it appeared to localize in the fetal pigment layers of the eye and nasal mucosa, but toxicological significance of this finding was unknown.¹⁵

Clinical Signs

Clinical signs of aflatoxicosis are extremely varied and most go undetected due to the usual chronic exposure and due to cellular changes in various systems, rather than a specific organ. Acute toxicoses with massive doses would lead to death due to hepatic failure with no overt clinical signs. Acute signs, when observed, might include anorexia, depression, ataxia, and epistaxis or melena due to the substituted coumarin nucleus of the aflatoxin molecule.¹ Signs due to chronic exposure of aflatoxin include reduced feed efficiency, reduced milk production, icterus, and decreased appetite.

Clinical Pathology

The laboratory data of an aflatoxicosis case will often reflect the hepatocellular toxicity and secondary blood parameter changes. Both conjugated and unconjugated bilirubin will be elevated, depending on the chronicity and the dose of the toxin. Serum proteins will be decreased.^{1,10,11,12} SGPT levels are increased, and SAP levels might be increased.¹⁰ A differential leukocyte count may indicate a neutrophilia with a left shift and eosinopenia due to immunosuppression and secondary infection.^{10,11,12}

Diagnosis and Treatment

Once the clinical signs and laboratory data support the hypothesis of aflatoxicosis, the diagnosis should also include grain levels of aflatoxin. The usual method of detection of aflatoxins is thin-layer chromatography and also possibly cytotoxicity tests for those samples not demonstrated by conventional means.¹⁶

A quick screening test for aflatoxin level in shelled corn or ground feed is the Woods' light test. A black light is held over the sample and flourescing of a metabolite in the production of aflatoxin might be observed. This is only a screening test; subjective errors and false negatives are quite common.

Aflatoxin levels which are considered safe in animal feedstuffs are 20 ppb or lower. A concentration of aflatoxin in feed at 100-300 ppb caused chronic intoxication signs in swine, whereas acute lethal intoxication of swine was observed at feed levels of 1,000 ppb or greater.¹⁰ Cattle and sheep are relatively more refractory to the effects of alfatoxin, possibly due to rumenal microbial degeneration, whereas poultry are more sensitive to aflatoxin than swine.^{1,4}

Therapy is palliative to minimize the secondary pathological changes. The feed, if exhibiting high aflatoxin levels, should be immediately withdrawn and a low-fat, high quality protein ration should be implemented.¹ Environmental stress should also be minimized.

Necropsy findings and histopathology are often necessary for a definitive diagnosis of aflatoxicosis in conjunction with clinical signs and feed levels of the mycotoxin. Fatty, tan livers will usually be observed if there is any degree of chronicity. Multifocal areas of fatty necrosis as well as fatty degeneration will also

TABLE 2 Comparative Pathology in Animals Fed Aflatoxin-Contaminated Feed.¹

Liver Lesions	Calves	Cattle	Swine	Sheep	Duckling	Adult Duck	Turkey Poul	Chick
Acute necrosis and hemorrhage	—	—	+	—	+	—	+	—
Chronic fibrosis	+	+	+	0	—	+	—	—
Regeneration nodules	—	+	+	0	±	+	+	—
Bile duct hyperplasia	+	+	+	0	+	+	+	±
Veno-occlusive disease	+	+	—	0	—	—	—	—
Enlarged hepatic cells	+	+	+	0	+	+	+	—
Liver tumors	0	0	0	0	—	+	0	0

be observed in these cases (Table 2). Histopathology might also show renal tubular epithelial degeneration and detachment with cast formation.¹⁰ Petechial hemorrhages in the nasal cavity and gut have also been observed antemortem and postmortem.^{10,14}

Prevention

Feed contaminated with aflatoxin at a level greater than 20 ppb is not to be sold. Many attempts have been made to detoxify feed but none proven to be totally feasible.¹ Some methods include physical separation, milling, heat, biological inactivation with microorganisms, and chemical treatments such as with ammonia. Chemical detoxification with ammonia shows promise as a detoxification means, but prevention of the fungal growth during the storage of grain through moisture and insect control appears to be the most practical.

Conclusion

The effect of mycotoxins on food producing animals has only recently been realized. Economic impact has only been speculated, but may be significant. The clinical signs are often non-specific, and chronicity is often the rule. The practicing food animal veterinarian should always associate non-specific hepatic clinical signs with a possibility of mycotoxicosis, and correlate agricultural practices and feed evaluation with the animal status.

References

1. Buck, WB, Osweiler, GD, and Van Gelder, FA: *Clinical and Diagnostic Veterinary Toxicology*, 2nd ed. Dubuque, IA, Kendall-Hunt Publishing Co., 1976.
2. Buckle, AE: The Occurance of Mycotoxins in Cereals

- and Animal Feedstuffs. *Veterinary Research Communications* 7: 171-186, 1983.
3. Austwick, PKC: Fungal Nephrotoxins. *Veterinary Research Communication* 7: 145-154, 1983.
4. Heathcote, JG, and Hibbert, JR: *Aflatoxins: Chemical and Biological Aspects*. Amsterdam, Elsevier Scientific Publishing Co., 1978.
5. Lillehoj, EB: Effect of Environmental and Cultural Factors on Aflatoxin Contamination of Developing Corn Kernels. *Aflatoxin and A. Flavus in Corn*. Auburn University Ag. Ex. Sta. Res. Bul. 279:27-34, 1983.
6. Payne, GA: Epidemiology of Aflatoxin Formation by *A. flavus*. *Aflatoxin and A. Flavus in Corn*. Auburn University Ag. Ex. Sta. Res. Bul. 279:16-19, 1983.
7. Allcroft, R, and Carnaghan, RBA: Groundnut Toxicity: An Examination for Toxin in Human Food Products from Animals Fed Toxic Groundnut Meal. *Veterinary Record* 75:259-263, 1963.
8. Goldblatt, LA: Aflatoxin. New York, Academic Press, 1969.
9. Swick, RA: Hepatic Metabolism and Bioactivation of Mycotoxins and Plant toxins. *Journal of Animal Science*, 58:1017-1026, 1984.
10. Hoerr, FJ, and D'Andrea, GH: Biological Effects of Aflatoxin in Swine. *Aflatoxin and A. Flavus in Corn*. Auburn University Ag. Ex. Sta. Res. Bul. 279: 51-55, 1983.
11. Gayne, WE, Dungworth, DL, and Moulton, J.E.: Pathologic Effects of Aflatoxin in Pigs. *Pathol. Vet* 5: 370-384, 1968.
12. Joens, LA, Pier, AD, and Cutlip, RC: Effects of Aflatoxin Consumption on the Clinical Course of Swine Dysentery. *Journal of American Veterinary Research* 42:1170-1172, 1981.
13. Cyzewski, SJ, Wood, RL, Pier, AC, and Baetz, AL: Effects of Aflatoxin on the Development Of Acquired Immunity to Swine Erysipelas. *American Journal of Veterinary Research* 39:445-448, 1978.
14. Bodine, AB, and Mertens, DR: Biological Effects of Aflatoxin. *Aflatoxin and A. Flavus in Corn*. Auburn University AG. Ex. Sta. Res. Bul. 279:46-50, 1983.
15. Apelgren, LE, and Aorara, RG: Distribution Studies of C14 Labelled Aflatoxin B1 and Ochratoxin A in Pregnant Mice. *Veterinary Research Communications* 7: 141-144, 1983.
16. Robb, J, and Norval, M: Comparison of Cytotoxicity and Thin Layer Chromatography Methods for Detection of Mycotoxins. *Applied and Environmental Microbiology* Oct: 948-950, 1983.
17. Anderson, RA: Detoxification of Aflatoxin-Contaminated Corn. *Aflatoxin and A. Flavus in Corn*. Auburn University AG. Ex. Sta. Res. Bul. 279:87-90, 1983.

