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MYCOTOXINS

What are Mycotoxins ?

Mycotoxins are toxic secondary metabolites produced by certain fungi infesting agricultural products. Their production is unavoidable and depends on different environmental factors in the field and/or during storage. Due to its unavoidable and unpredictable nature, mycotoxin contamination presents a unique challenge to feed safety. The most common mycotoxins are Aflatoxins , Ochratoxin A, Trichothecenes A, Zearalenone, and Fumonisins.

Fungal Infections in Grains

Plants may be contaminated by mycotoxins in two ways

- As fungi growing as pathogens on plants;
- Secondly, there are fungi growing on stored harvested plants.

In this context, it has to be considered that not all of these fungi are able to produce mycotoxins or produce them in different amounts depending on the substrate on which they are growing. However, high incidence rates of contamination of cereal grains and animal feed have been reported worldwide. Cereal grains constitute the major portion of animal feeds.

Fungal Infections in animals

Chickens and other animals are affected by fungal infections in four ways.

- Feed ingredients in the field before harvest.
- Feed ingredients during storage after harvest.
- Mixed feeds in bulk bins and on feeding equipment.
- The gastrointestinal or respiratory tract of chickens.

Table : 1: Factors influencing Mycotoxin contamination in Feed stuffs

High temperature	High Humidity	Mechanical Harvesting
Draught	Weather condition at harvest	Cropping practices
Frost	Soil Characteristics	Plant variety
Delayed Harvest	Improper storage	

The fungi which has developed by first three ways exert deleterious effects of at least three inter related types.

- Production of toxic metabolites.(mycotoxins)
- Modification of nutrient composition of feed ingredients and
- Modification of the nutrient utilization by the animals.

What are the effects of mycotoxicosis?

Genetic progress has produced breeds and hybrids in poultry capable of ever-higher meat and egg production. Due to their increased metabolic rate these breeds are less tolerant to mycotoxin contamination in feed. Mycotoxicosis is difficult to diagnose as symptoms are very common (Table 2) and affect more than one organ.

Although large doses of mycotoxins are needed to kill a broiler, contaminated feed can greatly reduce broiler performance resulting in serious economic losses for broiler producers. The synergy between different types of mycotoxins has deleterious effects on the birds. The effect of two or more mycotoxins fed to a bird simultaneously is often greater than the effect of either mycotoxin individually. Secondly it is also seen that it is not the case with all mycotoxins. For Example, Kubena, 1997(5) reported that there was synergy between Fumonisin and T-2 but not between Fumonisin and DON.

Table 2: Symptoms of Mycotoxicosis in Poultry

Severe depression in body weight gain and diarrhoea	Lesions on the margins of the beak and esophageal Mucosa
Fatty liver hemorrhagic syndrome	Enlarged liver, kidney and spleen
Increased requirement for Methionine, antioxidants and other nutrients	Anemia/ reduced hematocrit
Depressed egg production and weak shells	Lower Hatchability
Poor feathering and skin pigmentation	Impaired immunity
Increased incidence of tibial dysplasia	Regression of bursa of Fabricius
Focal necrotic lesions on the liver.	

Table 3: Major Mycotoxins , Fungal species, Feed Ingredients affected and the toxic effects.

Mycotoxins	Fungal Species	Feed ingredients affected	Possible toxic effects on animals
Aflatoxins (B_1, B_2, G_1 and G_2)	*A. flavus* and *A. parasiticus*	Cereals Grains, Groundnuts, soybeans	Hepatotoxin: carcinogenic; reduced growth rate; hemorrhagic enteritis; suppression of natural immunity to infection; decreased production of meat, milk and eggs
Ochratoxins	*A. ochraceous* *P. viridicatum*	Cereal Grains	Toxic to kidneys and liver, Poor feed conversion, reduced growth rate. General unthriftiness; reduced immunity to infection
Patulin	*P. urticae,* P. expansion P. claviforme and *A. clavatus*	Cereal Grains	Hemorrhages of lung and brain; edema; toxic to kidneys; possibly carcinogenic
Citrinin	*P. citrinum*	Cereals especially in Rice and Groundnut	Kidney damage. Haemorrhages, Lymphoid necrosis. Immunosuppression in poultry
Fusarium Toxins **a**. Zearalenone	*F. graminearum* *F. tricinctum*	Cereal grains	Infertility, stunting and even death
b. Vomitoxin, Dexoynivalenol or DON	*F. graminearum*	Cereal grains	Reduction in weight gain
c.Trichothecenes (T-2, HT-2, Diacetoxyscripenol or DAS)	*F. tinctum* , some strains of F. graminearum F. equuiseti F. lateritium F. poae and *F. sporotrichoides*	Cereal grains	Severe inflammation of gastrointestinal tract and possible hemorrhage; oedema; vomiting and diarrhoea; infertility; degeneration of bone marrow; death; reduced weight gain, slow growth, sterility.
Fumonisin B_1, B_2	*P. monoliforme*	Maize	Leuco/encephalomalacia

(Source : Auburn university , Alabama)

How To Manage Mycotoxins?

The mycotoxins problem in poultry has been well–defined. Researchers are using their knowledge of the chemistry and biology of mycotoxins to construct prevention and neutralization mechanisms.

Mycotoxin Management

The threat of mycotoxins is minimized by operating at two levels i.e

- Prevention of mycotoxins
- Treatment of mycotoxins

Prevention of mycotoxins start at the crop or plant levels, where the crop is harvested and grains are stored for the further usage in feed production. Following methods may be adopted to minimize mycotoxins after harvest:

Harvest at the maturity and as soon as the moisture content allows minimum grain damage: This can be done when the moisture for Maize is 23-25 % ;Sorghum (Jowar)-12-17%; Soybeans 11-15 % etc.

Adjust the harvesting equipments for minimum seed or kernel damage and maximum cleaning.

Dry all grain to at least 15 % moisture as rapidly as possible, not to exceed a 24-48 hour period after harvest. Safe, long term storage can be achieved at uniform moisture level of 13 % or somewhat below. Slow drying (accomplished by low heat or natural air drying) is being used increasingly, but the grain can contain no more than 20-21% moisture in full bin drying. Another possibility is high temperature (60-65° C.) drying until the grain reaches 20-21 % moisture followed by low –heat drying to 13 % moisture.

Cool the grain after drying and maintain dry storage conditions. When possible continue cooling until the grain temperature reaches 36° to 41° F.

Thoroughly clean the grain and all bins before storage to remove dirt, dust, and other foreign matter, crop debris, chaff, and cracked or broken seeds and kernels. Caution has to be taken as mold infected kernels are friable and easily broken. Broken or damaged kernels are more likely to be mycotoxin contaminated. The use of seed – or – grain –cleaning equipment can significantly reduce the mycotoxin content (particularly aflatoxin) of a grain lot.

Storage has to be done in sealed airtight structures.

Continue periodic aeration and probing for "hot spots" at intervals of 1 to 4 weeks throughout the storage period. The analytical tests now in use can detect levels of aflatoxin lower than those of toxicological significances in raw materials and finished food products and feeds.

Use of propionic acid or a mixture

with ammonium isobutyrate, on high moisture grain during storage. Although this acid will not remove any aflatoxins already present in the grain, it will prevent the growth of fungi if properly applied. ***Grains treated with propionic acid can be used only for livestock and poultry feeds.***

Where feasible, choose varieties of grain that are resistant to insects, diseases, and mechanical damage. Any damage to the grain provides a route of entry for *Aspergillus flavus* and other toxin forming fungi. Once the fungus or fungi has invaded the plant, then the appropriate environmental conditions will lead to toxin formation.

Management Strategies to Curb Mycotoxins in Feeds

The poultry farmer usually relies on grain producers and traders to get quality raw ingredients for poultry feed. All the above parameters are not in his hand to ensure quality of his raw material. The different measures, which can be used, are discussed below.

Dietary Manipulations

Various Nutritional Strategies have been employed to alleviate the adverse effects of Mycotoxins.

- <u>Crude Protein</u> : Increasing dietary Crude Protein helped to alleviate but did not eliminate the adverse effects of Ochratoxin A on body weight and feed conversion, mortality rates did not appear to be affected. However, increasing protein levels is a costly approach to mycotoxins control.
- <u>Methionine Supplementation</u>. Veltmann (9) observed that increasing the dietary total sulphur amino acids to level in excess of NRC, protected chicks from the growth depressing effects of aflatoxin, possibly through an increased rate of detoxification by Glutathione, a sulfur amino acid metabolite.
- <u>Dietary Lipids</u>: Lipids exert their effects in part by interfering with absorption of the aflatoxin (8). Diets containing higher levels of linolenic acid supported better-feed conversion and lower mortality in chicks fed diets with aflatoxin.
- <u>Vitamin Supplementation:</u> Supplemental vitamins did not differ in their response to dietary aflatoxin as compared to birds fed diet with four times the NRC Recommended levels.
- <u>Antioxidants</u>: Antioxidants differ in their ability to alleviate aflatoxicosis. BHT was able to relieve the adverse effect of aflatoxicosis, when added at levels of 8-30 times the normal usage level but in no case were the adverse effects of aflatoxicosis were completely overcome.

Pelleting

Pelleted poultry feed with or without a propionic acid based mold inhibitor indicated that pelleting and propionic acid interacted to reduce the mold count in a model system. It appears that the effectiveness of propionic acid as a mould inhibitor can be greatly increased by the pelleting process.

Use of Mold Inhibitors

A number of products like low molecular weight organic acids and their salts (For Eg. Propionic acids) are widely used as mold inhibitors. It is important to note that these are fungistats and not fungicides; that is, they only inhibit growth of molds and do not inactivate any toxins already present. Mold inhibitors will not keep mold growth in check indefinitely.

Non – Nutritive adsorbents

One of the most promising aspects of control of mycotoxins is the use of

Table4: Effect of some of common mycotoxines on pullet and layer performance

Mycotoxin	Toxic Level in feed	Symptoms
Aflatoxin	50 ppb 1ppm 2ppm 2.7 ppm	Increased embryonic Mortality Decreased body weight and egg weight Decreased egg Production Decreased hatchability
T-2 Toxin	20 ppb 2ppm 3ppm 4 ppm 5 ppm 8 ppm	Oral Lesions Decreased hatchability and Vomiting Gizzard Lesions, dermatitis sub epidermal hemorrhages Decreased weight gain in pullets, poor feathering Feed Refusal in Pullets Feed Refusal in adult birds, Decreased production and thin shells. Decreased chick weight.
Ochratoxin	1 ppm 2 ppm 2.5 ppm 4 ppm	Delayed Maturity, decreased production, hatchability and progeny performance. Increased Mortality Decreased weight gain, poor feed efficiency. Feed refusal and decreased egg weight, cessation of production
Zearalenone	50 ppm 250 ppm	Decreased oviduct and Liver weight Decreased shell quality
Citrinin	220 ppm 400 ppm	Decreased weight gain Decreased feed efficiency
Deoxynivalenol (DON)	200 ppb 350 ppb 16 ppm	Feed refusal Fatty Liver Decreased Growth
Cyclopiazonic acid	50 ppm	Decreased Body weight. Enlarged Proventriculus and Mucosa

(Source : Dekalb Veterinary Services bulletin) various adsorbents to reduce their adverse effects. Sorbents act by reducing the bioavailability of mycotoxins by adsorption on their surface. Indeed, if a stable sorbent-mycotoxin complex is formed, the absorption of mycotoxins in the gastrointestinal tract can be reduced, decreasing both toxic effects for the animal and carry over in animal products for human consumption.

With this aim numerous sorbents from different sources have been tested, such as hydrated sodium calcium alumionosilicate (HSCAS), zeolites, bentonites, clays and activated carbons.

- HSCAS, a phyllosilicate derived from natural zeolite, is perhaps the most extensively investigated sorbent. It is ineffective in binding dangerous mycotoxins other than Aflatoxin B_1. Its protective properties are very low toward Ochratoxin and Zearalenone and nil toward Trichothecenes.
- Zeolites are hydrated alumionosilicate of alkali and alkaline earth cations characterized by infinite three-dimensional structure. The pore size distribution of synthetic Zeolites, as opposed to natural ones, varies very little, being generally concentrated within a narrow diameter range. If the size of the pores is compatible with those of the mycotoxins molecules, adsorption can occur. On the contrary, adsorption can be low or nil due to the absence of intermediate sized pores.
- Bentonites are sorbents with layered (lamellar) crystalline microstructure and variable composition. Their adsorption properties mainly depend on the interchangeable cations

Other clays such as kaolin, sepiolite and montmorillonite have a variable ability to reduce toxic effects of Aflatoxin B_1 as reviewed by Ramos et al. (7). However, their efficacy is limited to Aflatoxin B_1 and is lower than that of HSCAS and bentonite.

- Activated Carbons (AC) are another important group of sorbents. They are a family of carbonaceous substances manufactured by activation processes aimed at developing a highly porous structure. Overall evidence of the high ability of AC in binding mycotoxins in vitro has been seen. (*2,3-4*). The highest abilities have been observed in the adsorption of AFB_1 and OTA (Ochratoxin A), whereas the lowest in the adsorption of DON (Deoxynivalenol) . AC has been demonstrated to adsorb efficiently FB_1 simultaneously with AFB_1. When compared to HSCAS, AC showed much higher adsorption abilities toward all the tested mycotoxins. Thus, AC are capable of binding in vitro several mycotoxins and it is reasonable to consider their potential use as multi-mycotoxins sequestering agent, differently from other extensively studied sorbents, such as HSCAS and bentonite, which are not capable to adsorb efficiently mycotoxins other than AFB_1.

Conclusion

Minimizing the adverse effects of mycotoxins in poultry diets is not a simple task that can be accomplished with one intervention strategy. It begins with the purchase of quality grains (characterized by low moisture content and minimal broken kernels), Followed by proper handling of grains during mixing and delivery.

Use of mycotoxin binding agents seems to be a very promising approach to the detoxification of mycotoxins. The positive outcome from the initial investigations of AFB_1 binding to HSCAS and bentonite fostered additional interest in this approach and further studies were conducted on other sorbents and mycotoxin. Activated carbon is promising sorbent which has shown higher adsorption abilities towards all tested mycotoxins in *in-vitro* test. The adsorption properties of activated carbon are strictly dependent on the source material and physico- chemical parameters such as surface area and pore size distribution. Chemical treatments can strongly modify activated carbon surface characteristics.

Because of the ubiquitous nature of mycotoxins in nature, the problem of minimizing mycotoxicosis will not be easy to solve and will require constant attention to detail throughout the entire process of grain harvest, shipping, storage, feed manufacturing and animal production. No one segment of the animal production chain can totally protect or prevent the problems associated with Mycotoxicosis. A holistic approach is needed to minimize the adverse effects and enhance overall animal production.

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