MYCOTOXINS: HOW TO DEAL WITH THE THREAT OF MYCOTOXICOSIS

INTRODUCTION

Many species of fungi produce secondary metabolites known as mycotoxins. Several of which, when ingested by humans and animals above a certain concentration, will cause a toxic response called mycotoxicosis. Mycotoxin-producing molds also damage crops, which can cause significant economic losses at all levels of food and feed production.

Toxin production requires a) the presence of a mold; b) a suitable substrate; and c) a suitable environment. If a mold is present, toxin production is influenced by moisture, temperature, oxygen, and nature of substrate. Most plant feedstuffs will provide a suitable substrate. Molds not only produce mycotoxins, but also reduce the nutrient value of feed (30).

Parameter	Good Corn	Moldy Corn	Reduction (%)
Total Fat (%)	3.8	2.4	36.8
Fatty Acid Content Palmitic (16:0)	11.3	9.1	19.5
Metabolizable Energy (kcal/kg)	3350	2510	25.1
Carotene (mg/kg)	3.1	2.3	25.8

Table 1. Mold growth decreases the nutritional value of corn.

MAJOR MYCOTOXINS IN POULTRY

The most significant mycotoxin-related risks in poultry are associated with fungi of the genera *Aspergillus*, *Fusarium*, and *Penicillium*. These fungi and their mycotoxins are produced either pre-harvest, during harvest, in storage, or during feed processing whenever conditions are favorable. *Fusarium* species are field fungi that invade grains during the growth of the plant, and *Aspergillus* and *Penicillium* species are storage fungi which generally develop after harvesting.

Some fungal strains can produce more than one mycotoxin and a single mycotoxin can be produced by more than one fungus, meaning birds are generally exposed to not just one mycotoxin but to several toxins at the same time. The most important mycotoxins for poultry and the fungi that produce them are shown in Table 2.

Molds	Mycotoxins	Ld_{50}[1] (µg/kg)
Aspergillus flavus and *Aspergillus parasiticus*	Aflatoxins B1, B2, G1 and G2	6.5
Aspergillus flavus	Cyclopiazonic acid	100
Aspergillus ochraceus	Ochratoxins	3.6
Aspergillus versicolor	Sterigmatocystin *Penicillium* toxins	- -
Penicillium viridicatum	Ochratoxins	3.6
Penicillium citrinum	Citrinin	95
Fusarium tricinctum, *Fusarium graminearum*, *Fusarium solani*	T-2, HT-2, DAS DON MAS	4.9 – 5.2 3.8 – 5.9 140
Fusarium moniliforme	Fumonisins B1	300*
Fusarium moniliforme	Moniliformin	5.4
Fusarium graminearum	Zearalenone	-
Fusarium roseum		-
Ergot	Claviceps	-

[1] LD_{50} (µg/kg) = Dose at which 50% of the test animals die.
* Not LD_{50}, birds fed this concentration had severe growth depression.

Table 2: Molds and mycotoxins important in intensive poultry production and their respective LD_{50} (13).

AFLATOXINS

Aflatoxins are the most widespread and most studied group of all mycotoxins. The toxin occurs in warm and humid climatic conditions and is not considered a problem in colder climates; however, the global availability of feed stuffs means contaminated materials can be transported anywhere in the world.

Aflatoxin B1 is the most common and biologically active of all the aflatoxins and causes decreased egg production and mortality. Clinically, the signs are anorexia, visceral hemorrhages, embryo toxicity, and increased susceptibility to stressors. Histopathology reveals fatty liver, liver necrosis, and bile duct hyperplasia. Aflatoxin B1 also suppresses the immune system and reduces vaccine response.

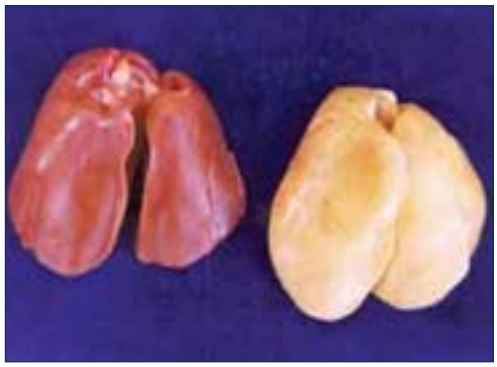

Figure 1. Fatty liver (right) associated with Aflatoxin B1.

Aflatoxins decrease the activities of several digestive enzymes, resulting in reduced feed conversion efficiency. Aflatoxins are known to interfere with vitamin D metabolism, contributing to reduced bone strength and leg weakness (19). Pale bird syndrome is as a result of poor pigmentation of skin and egg yolk caused by reduced absorption of fat and carotenoid pigments in affected birds.

Aflatoxin (µg/kg)	Fertile Eggs (%)	Hatchability (%)	Phagocytic Macrophages (%)
0	98.6	82.8	35.8
10	92.4	35.3	9.7

Table 3. Effect of aflatoxin on breeder performance.

Suppression of hepatic protein synthesis is the main factor resulting in growth suppression and reduced egg production. Aflatoxins are also associated with poor fertility and hatchability. High levels of aflatoxins fed to hens resulted in a dramatic reduction in reproductive performance (34), as shown in Table 3.

Aflatoxin (µg/kg)	IBD	ND
0	6180±195[a]	5800±199[a]
100	3800±212[b]	3025±208[b]
200	3046±220[c]	2650±214[c]
400	2200±225[d]	1850±217[c]

Table 4. Effect of aflatoxin B1 on antibody titers against infectious bursal disease (IBD) and Newcastle Disease (ND) in broiler chickens at weeks of age (40).

Perhaps the most important effect of aflatoxins is the immune-suppressing effect (9, 10) and consequent vaccine and therapeutic drug failures. Aflatoxin-induced immunosuppression results in reduced antibody levels, cell mediated immunity, and abnormal development of the thymus and bursa (see Table 4).

Aflatoxicosis has also been shown to increase the susceptibility to *Salmonella* infection (13).

The effects of aflatoxins on bird performance are dose dependent (see Table 5).

Aflatoxin (mg/kg feed)	Effect
2.5	Egg production reduced
10	50% reduction
20	100% reduction

Table 5. The effect of aflatoxin level on layer bird performance.

Consideration must also be given to the potential human health risk, residues of aflatoxins can occur in poultry meat and eggs, as shown in Table 6.

Aflatoxin in Feed (µg/kg)	Aflatoxin in Eggs (µg/kg)
100	0.23
200	0.78
400	1.40

Table 6. The relationship between the aflatoxin content of layer feed and the aflatoxin concentration in eggs (23).

TRICHOTHECENES

Type A trichothecenes, which include T-2, HT-2 toxin and diacetoxyscripenol (DAS), are a major concern and cause economic losses in productivity. They can be found in cereals, cereal by-products, and feeds. Jewers, 1990 reported an 11% to 24% reduction in body weight in growing chicks fed with T-2 and diacetoxyscirpenol, which was caused by severe oral lesion dermatitis (Figure 2) and intestinal irritation (45). T-2 toxins are often referred to as 'the feed refusal' toxins. These mycotoxins result in reduced feed intake, reduced body weight, induced abnormal feathering, decreased egg production, thinning of eggshells, and regression of ovaries in laying birds (9, 44). The effect of T-2 toxin on laying hen performance has been demonstrated at different dosage levels (see Table 7).

T-2 toxin (ppm)	Egg Production (%)	Egg Weight (g)	Body Weight (g)
0.0	96.29	52.45	1332
0.5	93.81	51.77	1313
1.0	91.75	51.35	1286
2.0	86.65	51.33	1285

Table 7. Effect of T-2 toxin on laying hen performance (36).

T-2 toxins have also been known to cause gizzard erosions and necrosis of the proventricular mucosa. They are the second most immune suppressive mycotoxins after aflatoxins, occurrence of both toxins is the most immunosuppressive combination of toxins (36).

OCHRATOXINS

Ochratoxin type A (OTA) is a common contaminant in a variety of feedstuffs, produced mainly by *Aspergillus* and *Penicillium* species. OTA is a nephrotoxin and significantly depresses feed intake, growth, feathering, egg production, and feed conversion efficiency (19). Eggshell quality can be affected along with yellow staining of eggshells and blood spots (12, 38). OTA is three times more toxic to young birds than aflatoxins. Severely affected birds show urate deposits in joints and in the abdominal cavity (see Figure 3) at higher doses. Diarrhea, tremors, and other neural malfunctions can also be observed (12). Acute OTA toxicity results in acute renal failure, leading to death.

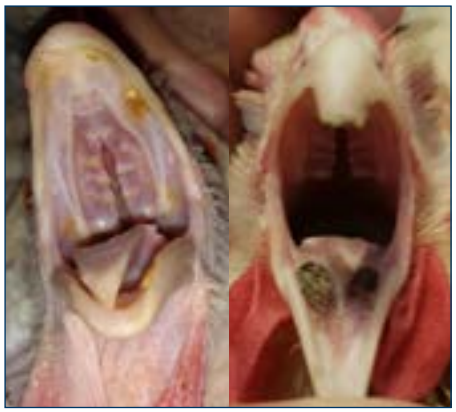

Figure 2. T-2 toxin oral lesions and necrosis.

ZEARALENONE (ZEA) AND DEOXYNIVALENOL (DON)

Zearalenone (ZEA) is responsible for reproductive disorders due to its estrogenic effect at high concentrations. Poultry are quite resistant to ZEA; however, at high concentrations, vent enlargement and enhanced secondary sex characteristics are seen. Layers are considered resistant to zearalenone even when fed at up to 800mg/kg (1); however, ZEA will contaminate eggs, which is a concern from a human health point of view, but also in terms of reproductive performance. Chicks derived from hens fed ZEA-contaminated feed contained ZEAs (5).

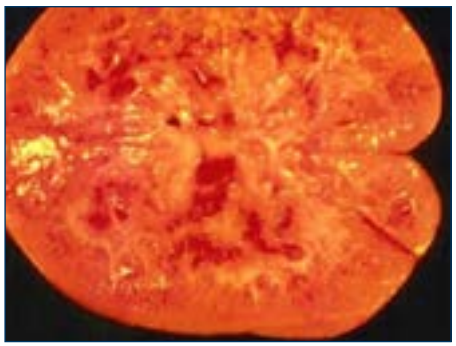

Figure 3. Kidney affected by ochratoxins.

Poultry are also quite resistant to deoxynivalenol (DON); however, there is an association with reduced feed intake in layers and breeders. The toxin is sometimes considered an indicator that other more potent *Fusarium* are present.

FUMONISINS

Fumonisins are found in tropical and temperate climates. Fumonisin B1 (FB1) is produced mainly by *Fusarium verticillioides* and naturally occurs in corn. Relatively high levels of Fumonisin B1 are required in order to show negative effects in poultry; however, when in combination with other mycotoxins such as aflatoxins, DON, and ZON, poultry are at greater risk (22). Performance-related effects include reduced weight gain and poor FCR. Clinical signs are spiking mortality, paralysis, extended legs and neck, poor gait, gasping, increased liver weight, and liver necrosis.

CO-CONTAMINATION OF FEEDS BY MYCOTOXINS

Co-contamination of mycotoxins appears to exert greater negative impact on health and productivity than do single toxins. For example, both aflatoxin and ochratoxin are extremely toxic to poultry and they act synergistically; the toxicity resulting from dual exposure to aflatoxin and ochratoxin is much greater than the sum of their individual toxicities. The effects of T-2 and DAS were additive in laying hens for feed intake, oral lesions, mild changes in plasma enzyme activities, and reduced egg production (16).

Fungi do not occur in feedstuffs as pure cultures, so the number of possible combinations of toxins is very significant. The scientifically established co-contaminants are listed in Table 8.

	Aflatoxin	DAS	DON	Fumonisin B	Fusaric Acid	Ochra-toxin	T-2 Toxin
Aflatoxin		++	+	-	-	++	++
DAS	++		-	+	-	-	++
DON	+	-		-	++	-	-
Fumonisin B	-	+	-		-	-	+
Fusaric Acid	-	-	++	-		-	-
Ochratoxin	++	-	-	-	-		++
T-2 Toxin	++	++	-	+	-	++	

+ signifies an additive effect of toxins, ++ signifies a synergistic effect, - no known additive or synergistic effect

Table 8. Co-contaminating mycotoxins in poultry (13).

The key point is that a feedstuff testing positive for a particular toxin signifies that growing conditions were favorable not just for that fungi, but also for others; therefore, testing the feedstuff for other co-contaminants is important.

MYCOTOXIN TESTING

A testing schedule should be put in place to continuously assess the mycotoxin threat to the feedstuff, and also to assist in identifying contaminated lots.

There is significant variability in the process of testing for mycotoxins brought about by the variability in sampling, sample preparation, and analytical variation. Table 9 shows the variability associated in measuring aflatoxin in a lot of contaminated corn; variation through sampling contributes to more than 75% of the overall error of testing (43).

	Variance	Ratio (%)
Sample = 910 g	268	75.5
Subsample, 50 g	56	15.9
Immunoassay, 1 aliquot	30	8.6
Total	355	100

[1] Sampling, sample preparation, and analyzes errors account for about 75.5, 15.89 and 8.6% of the total errors, respectively.

Table 9: The variability measured by the variance associated with a 910 g sample, 50 g subsample, measuring aflatoxin in 1 aliquot by immunoassay in a lot of shelled corn at 20 ppb aflatoxin.

Sampling error is large because of the extreme distribution among contaminated particles within a lot; it is estimated that only 6 kernels in 10,000 are contaminated in a lot containing a concentration of 20 parts per billion (ppb) aflatoxin (25).

A single spot sample or probing point is satisfactory if the contaminated particles are evenly distributed through the lot; however, mycotoxins generally occur in isolated pockets through the lot (39). Increasing the number of samples taken from a lot can increase the chances of identifying contaminated lots. Procedures used to take a sample from a bulk lot are extremely important; every individual item in the lot should have an equal chance of being chosen.

The sample should be an accumulation of many small portions taken from many different locations throughout the lot (4). The general recommendation is to take incremental portions every 200 kg (441 lb) of product (17). The accumulation of several incremental portions is called a bulk sample or composite sample (see Figure 4). If the bulk sample is larger than desired, the bulk sample should be blended and subdivided until the desired sample size is achieved. The smallest sample size that is subdivided from the bulk sample and ground in the sample preparation step is called the test sample (42).

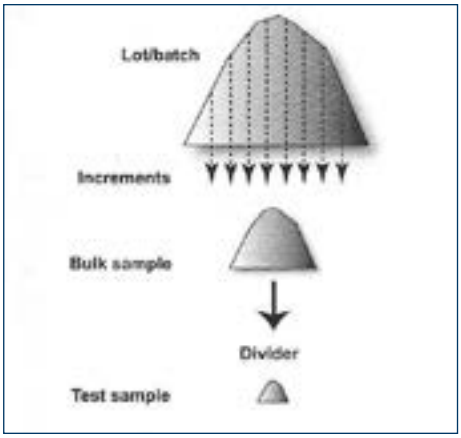

Figure 4: Test sample (42)*

*A test sample is removed from a bulk sample. A bulk sample is the accumulation of many small incremental portions taken from many different locations in the lot.

When drawing a sample from a bulk container, a probing pattern should be developed so that product can be collected from different locations in the lot. An example of a probing pattern referred to by the United States Department of Agriculture (USDA) is shown in Figure 5.

The sampling probe should be long enough to reach the bottom of the container when possible. When sampling from bags, the sample should be taken from many bags dispersed throughout the lot. Lanes between sacks allows access to sacks at interior locations. The recommended number of sacks sampled varies, from one in four for small lots, to the square root of the total number of sacks for large lots (17). When sampling from a moving stream, e.g. a moving belt, small increments should be taken along the entire length of the moving stream. Samples can be taken by an automatic crosscut sampling device or by hand; whatever collection method is used, it is important that the samples are taken frequently, at uniform intervals, and of the entire stream. Composite all the increments to obtain a bulk sample. If the bulk sample is larger than required, blend and subdivide the bulk sample to obtain the desired size test sample.

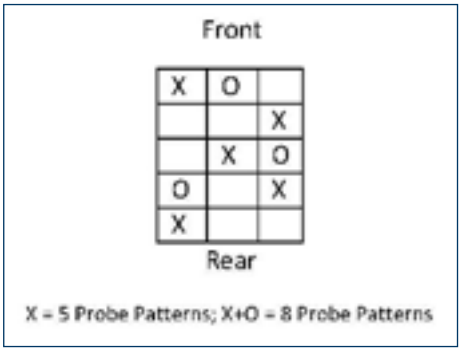

Figure 5. An example of a 5- and 8-probe sampling pattern (43).

Sample preparation involves reducing the size of the test sample to a quantity which can be analyzed. Granular products, such as maize grains, are ground prior to taking a sub sample, to reduce the particle size to as small as possible. This increases the homogeneity of the test sample, which will give a more accurate assessment of mycotoxin concentration (8).

ANALYZES

Rapid strip tests: analyzes of feedstuffs for presence of mycotoxins can be conducted efficiently through the use of enzyme-linked immunosorbent assays (ELISA) testing kits, which have become a standard tool for rapid monitoring of mycotoxins (31). This method is satisfactory in order to establish if a specific feedstuff is either under or over a legal compliance level.

HPLC and GC-MS analyzes provides more accurate determination of the level and type of mycotoxins present in the feedstuff.

Some toxins can escape detection, as they may be masked by glycosides or proteins attached to the toxin, giving a false negative result; more refined analyzes methods are required to measure such toxins.

LS-MC/M is the latest technique, using liquid chromatography coupled to tandem mass spectrometry, which is capable of detecting hundreds of mycotoxins that include masked and emerging mycotoxins and metabolites simultaneously in a sample (28). Bioassays are used to establish presence of specific mycotoxins. An example is using crustacea, such as *Artemia saline* (see Figure 6) and assessing survival rate from a sample of material (21, 30).

Figure 6. Artemia Saline.

PREVENTATIVE APPROACHES

Assessing the mold levels of grain can indicate the likelihood of mycotoxins occurring.

Testing the material for the level and type of mold can sometimes indicate the likelihood of mycotoxin contamination; however, it is possible that molds may no longer be present in the material, but mycotoxins are. The best practice is to analyze for both molds and mycotoxins.

A general guide in terms of mold levels and possible actions are as follows:

Level Detected (per g)	Action
Up to 5,000/g	0.5 kg of Mold Killer[1]/Inhibitor[2]
Up to 50,000/g	1.0 kg/t Mold Killer/Inhibitor
Up to 500,000/g	1.0 kg/t Mold Killer/Inhibitor/binder
Up to 1,000,000/g	1.5 kg/t Mold Killer/Inhibitor/Binder
1–2 million/g	Caution, increase nutrient density of the diet.
> 2 million/g	Dilute with clean material divert less sensitive species or age of bird.
> 5 million/g	Discontinue use

[1] Mold killer: Acids – Propionic, formic, acetic, sorbic, butyric, benzoic, valeric and lactic acids. [2] Mold inhibitors: Salts – ammonium, calcium, sodium and potassium salts.

Table 10. Mold level (spore count per gram of feedstuff).

Mold killers (acids) give an instant mold kill, but can evaporate over time and therefore tend to offer short- to medium-term protection only. Salts give longer-term mold protection, as they release the acid in the presence of free water; they can be viewed as a reservoir of acid that is released whenever free water becomes available.

Molds such as *Aspergillus flavus* are extremely common in nature and assumed to be present in most maize crops. Development of molds in the field are dependent on high temperature, high humidity, and high rainfall. Damage or stress to the plant by diseases, insect or bird damage, weeds, frost, or drought permits easy entrance of molds and fungi and promotes rapid development of molds. Insect-damaged grain is more vulnerable to mold growth, so reducing insect infestations is critical in preventing mold growth in grains. Some toxins, such as aflatoxins, tend to occur in broken and damaged kernels and in foreign material.

Avoid harvesting grain at an excessively high moisture content. A moisture meter can be helpful in making these decisions. Keep grains in a holding bin using forced air to keep cool. Store the grain in weatherproof, well-ventilated facilities and monitor the temperature of stored grain. Drying the grain slowly and at low temperatures for long periods promotes aflatoxin development. All handling equipment and storage facilities must be kept well ventilated, clean, and dry prior to and during use. Storage facilities must be free of moisture leaks and all residue removed to reduce contamination.

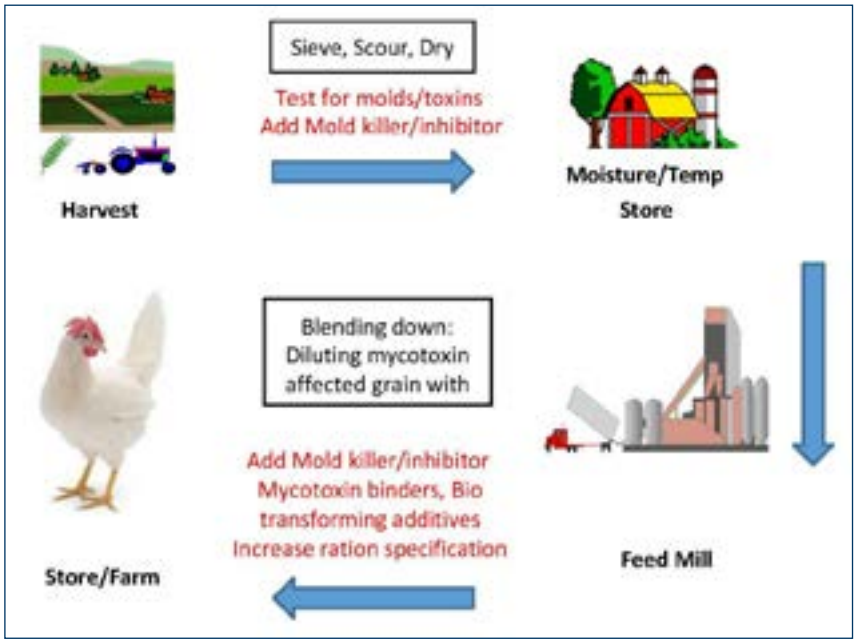

Figure 7. A schematic diagram showing the steps taken to reduce the risk of mycotoxin exposure, from harvest through to delivery of the feedstuff to the farm.

Apply liquid or dry mold inhibitors; use of organic acids such as propionic acid and ammonium isobutyrate will prevent mold growth if correctly applied as it is augured into the silo. Organic acids, however, will not destroy toxins already present in the grain (20).

Figure 8. Maize grains contaminated with mold.

VISUAL ASSESSMENT OF THE LOT

Look out for visual clues of contaminants. Grains can show signs of mold growth (see Figure 8) and/or insect damage and presence of "fines," which are associated with mold growth.

CLEANING

During the cleaning process of contaminated grain, dust, husks, hair, and shallow particles are blown away by aspiration or scouring. Grain cleaners have been shown to reduce the level of aflatoxin in maize grain by as much as 50%.

MECHANICAL SORTING AND SEPARATION

Managing mycotoxin exposure should start at harvest by removing heavily contaminated grains when possible. In this process, the clean product is separated from mycotoxin-contaminated grains. High feed losses are possible due to incomplete and uncertain separation; therefore, mechanical sorting and separation is not always considered cost-efficient. If mycotoxin-affected grains must be used, dilution of the affected batches of grain is a cost-effective measure for reducing the impact of mycotoxins to the animal; however, multiple sampling and mycotoxin analysis are needed to determine the concentration of mycotoxin in every batch of feed. "Blending down" material which has been analyzed higher than maximum permitted levels of toxins is not permitted in some regions (especially if the material is destined for breeding animals).

WASHING

Washing procedures using water or sodium carbonate solution result in some reduction of mycotoxins in grains.

PROCESSING OF FEEDS

Feed processing doesn't necessarily reduce mycotoxin risk. Short-term exposure to pelleting temperatures of 70-80°C (158–176°F) is not enough to eliminate fungi (18). Further, deficient cooling conditions during processing of pelleted feed can lead to unwanted condensation during storage, which can result in the growth of molds.

TREATMENT

Nutritional Approaches

- Increased levels of antioxidants, such as selenium and vitamins such as A, C, and E.
- Increased levels of methionine: detoxification of aflatoxins involves the glutathione system, which contains cysteine; metabolic levels of methionine are depleted, leading to poor growth and feed efficiency.
- Increased levels of choline: the presence of mycotoxins can have a negative impact on liver condition. Choline is synthesized in the liver and has a role in maintaining liver condition. Additional supplementation of choline maybe required to meet the bird's daily requirements, especially when mycotoxins are present.
- Form of vitamin D_3: vitamin D_3 undergoes conversion through two steps before it reaches the form that can be utilized by the bird. The first of these two changes occur in the liver. Feeding certain metabolites of Vit D_3 bypasses this first step, allowing more efficient and faster uptake of the required form of vitamin D_3 - $25OHD_3$. This approach is of particular importance if liver function has been compromised by mycotoxins.

Chemical Detoxification

Detoxification with ammonia or ammonia-related compounds is one of the most practical means of decontamination of aflatoxin in agricultural commodities (26). Dietary aflatoxin inactivation by ammonization for layer breeders had no detrimental effect on the immunological response elicited by Newcastle disease vaccination, as measured by hemagglutination inhibition (HI) titers (7). Hydrogen peroxide is an oxidizing agent acceptable in foods and has the potential to destroy up to 97% of aflatoxins. Similar effects have been found with treatment by organic acids and surfactants (6, 37).

Mycotoxin Sequestering Agents

Supplementation with non-nutritive mycotoxin-sequestering agents is by far the most practical and most widely studied method for reducing the effects of mycotoxin exposure (15).

Activated Charcoal

Activated charcoal is an amorphous form of carbon heated in the absence of air and then treated with oxygen to increase porosity. There is some data to suggest activated charcoal is effective in absorbing some aflatoxins but not toxins derived from other species. Activated charcoal can also result in absorption of micronutrients in the feed.

Silicate Minerals (Clays)

Bentonine (Montmorillonite). Bentonites can be classified as calcium, magnesium, potassium or sodium bentonites. Several types of bentonites have been proven to bind aflatoxin B1 by as much as 66% by forming a complex with the toxin, both in-vitro and in-vivo. Formation of a complex with the toxin prevents absorption of the aflatoxin across the intestinal epithelium.

- Zeolites are a group of silicates consisting of interlocking tetrahedrons of SiO4 and ALO4, which attract positive cations within the structure. Liver concentrations of aflatoxin B1 were reduced with the use of zeolite at 2% inclusion levels in the diet when layers were fed 2.5 ppm aflatoxin (46)
- Hydrated sodium calcium aluminosilicate (HSCAS) is considered one of the most effective silicates for aflatoxin sequestration, due to its high affinity for and stable association with aflatoxin B1 (33).
- Use of sodium alumino-silicate, hydrated sodium calcium aluminosilicate, and sodium bentonite can absorb aflatoxins; however, clays are mostly effective against mycotoxins but don't appear to have significant effect on *Fusarium* and *Penicillium*-derived toxins. A potential negative effect in using clays is that they tend to reduce the utilization of manganese, zinc, magnesium chloride, copper,and sodium (13).
- Mineral-based absorbents and activated carbon are generally used at high concentrations in the feed, which is a disadvantage in high nutrient density monogastric diets. High levels of inclusion could provide excessive sequestration capacity that may decrease the bioavailability of important micronutrients (15).

Yeast Cell Wall-Based Adsorbents

Yeast cell wall derivatives, principally modified glucomannan, can adsorb higher levels of several mycotoxins at lower inclusion rates than inorganic binders (27). The specific mode of action of some yeast cell wall components suggests that their activity would not affect the availability of micro-nutrients. Modified glucomannan has been shown to bind *Fusarium*-derived toxin. Tests conducted at four concentrations of T-2 toxin (0, 0.5, 1.0 and 2.0 mg/kg) and two concentrations of a commercial preparation of modified glucomannan reversed the suppression of egg production by T-2 toxin; this effect was observed at the highest level of T-2 toxin (2mg/kg) (29). Layers given feed contaminated with several *Fusarium* toxins experienced reduced feed intake and egg production; supplementation with a modified glucomannan prevented these effects (11).

Biotransformation

Biological detoxification by enzymes and/or microorganisms degrades mycotoxins within the gastrointestinal tract, before resorption into the animal occurs. There are now enzyme and microorganism-based products effective in transforming specific toxins such as Fumonisins and Trichothecenes into nontoxic metabolites.

SUMMARY

- Prevent fungal growth on crops in the field, at harvest, and during storage of feedstuffs and processing of feed.
- Implement mechanical means of removing contaminated material from the feedstuff and consider addition of mold inhibitors/killers.
- Implement a mycotoxin testing and surveillance schedule. This is important not just from the point of risk assessment to livestock, but also from a regulatory and human health point of view.
- Apply a robust sampling plan. Increasing the number and size of samples taken from a lot can increase the effectiveness of testing and the chances of identifying contaminated lots.
- Detect and quantify the mold and mycotoxin concentration in the feedstuff, remembering many mycotoxins co-contaminate materials—detection of one toxin may indicate presence of another, more toxic mycotoxin.
- When the feedstuff has been identified as contaminated, act before the birds consume the feed, not after the birds are affected by the toxin.
- Remove and replace the feed or apply an appropriate mycotoxin binder or bio-transforming agent specific to the type of toxin recovered in the feed.
- Monitor the flock for any performance or clinical related signs of mycotoxicosis.

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