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MYCOTOXIN RESIDUES IN POULTRY PRODUCT: THEIR EFFECT ON HUMAN HEALTH AND CONTROL

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Mycotoxins occur sporadically both seasonally and geographically. The formation of mycotoxins in nature is considered a global problem. They occur naturally in a wide variety of feedstuffs used in livestock feeds only under aerobic condition. Fungi are extremely adaptable organisms being able to metabolize a large variety of substrates over a wide range of environmental conditions. Mycotoxins are a heterogeneous group of harmful secondary fungal metabolites. Most of these mytocoxins belong to the three genera of fungi: Aspergillus, Penicillium and Fusarium. Although over 300 mytocoxins are known, those of most concern based on their toxicity and occurrence, are aflatoxin, vomitoxin, ochratoxin, zearaleone, fumonisin and T-2 toxin. They are produced in cereal grains before, during and after harvesting in various environmental conditions (Shareef, 2010).

Table 1. Favourable conditions for the growth of fungi

i) Moisture content in feed	12-14% or higher
ii) Relative humidity in the go-down	Above 70-75%
iii) Physical condition of grains	Damaged seed coat due to insects and improper harvesting
iv) Ambient temperature	Moderate to high (25-30°C) for *Aspergillus*. Low (15-20°C) for *Fusarium*
v) Storage	Leaky roof in the godown and moist

Mycotoxins are metabolized in the liver and kidneys and also in the digestive tract. The possible presence of toxic residues in edible animal product such as meat and eggs may have some detrimental effects on human health (Chen et al., 1984). Fungal contamination affects both the organoleptic characteristics and the alimentary value of feeds and entails a risk of toxicosis. The biological effects of mycotoxin depend on the ingested amounts, number of occurring toxins, duration of exposure to mycotoxin and animal sensitivity (Saleemi et al., 2010). Mycotoxins have attracted worldwide attention over the last few decades. This is due to three main reasons, firstly the effect of mycotoxins on human health, secondly due to the huge economical losses associated with contaminated feeds and the loss of livestock productivity and thirdly due to the impact of mycotoxin contamination on international trade in commodities. So, controlling mould growth and mycotoxin production is very important to the feed manufacturer and livestock producer.

Table 2. Common mycotoxins in feed stuffs, commodity affected and health effects

Mycotoxins	Commodities	Fungal source(s)	Species susceptibility	Effects of ingestion
Aflatoxins B1, B2, G1 and G2	Corn, peanuts	*Aspergillus flavus* *Aspergillus parasiticus*	Human, All domestic animals & poultry	Aflatoxin B1 - potent human carcinogen (IARC). Risk of human toxicosis. Hepatoxic, carcinogenic, immunosuppressive
Cyclopiazonic acid		*Aspergillus flavus*		
Sterigmatocystin		*Aspergillus versicolor*		
Ochratoxin A	Barley, wheat	*Aspergillus ochraceus* *Penicillium verrucosum*	Human, Mainly pigs & poultry	Carcinogenic, Nephrotoxic, gout
Citrinin		*Penicillium citrinum*		
T-2 toxin, Diacetoxyscirpenol (DAS)	Peanuts	*Fusarium tricinctum*	Mainly pigs & poultry	Mouth lesions, loss of appetite, Reduce egg production in laying hen
Deoxynivalenol (DON), Nivalenol (Vomitoxin)	Wheat, corn, and barley	*Fusarium graminearum* *Fusarium crookwellense* *Fusarium culmorum*	Human, Mainly pigs & dairy animals	Human toxicoses, Dermatotoxic, feed refusal
Zearalenone	Corn, wheat	*Fusarium graminearum* *Fusarium culmorum* *Fusarium crookwellense*	Mainly pigs and dairy animals	Possible carcinogen. Estrogenic and reproductive disorder
Fumonisin B1, B2	Corn	*Fusarium moniliforme* *Fusarium proliferatum*	Human, Mainly pig, poultry & horse	Carcinogenic, Neurological disorders, liver damage, Cause of equine euco-encephalomalacia in horses.

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(Source: Accensi et al., 2004; Lee-Jiuan and Li-Mien, 2006; Saleemullah et al., 2006, Hanif et al., 2006; Fraga et al., 2008)

The mycotoxin standards are required by:

- Poultry and livestock industry
- Feed manufacturers
- State and Central Govt. research institutes
- Analytical and Quality control laboratories
- Food processing industry / Food exporters
- Diagonistic laboratories

PROBLEMS WITH MOULDS

i) Reduction of nutrients in feed

Moulds utilize the nutrients present in the grain for their metabolism and propagation and thereby reduce the nutritional quality of grain. Energy, crude protein and crude fat contents of moldy maize may go down up to 5, 7 and 63%, respectively (Akande et al., 2006). Mould growth reduces all amino acids in the diet, particularly lysine and arginine. Loss of vitamins A, D, E, K, B12, B1, B2, niacin, pantothenic acid, pyridoxin etc. is also another problem (Raju and Rama Rao, 2004).

ii) Reduced organoleptic quality of feed

Mould growth in feeds causes feeds to cake or clump, change color, consistency and smell and become less palatable leading to their refusal by birds.

iii) Co-contamination

Fungi producing different mycotoxins require diverse environmental conditions for their growth. However, co-occurrence of several mycotoxins in a single feed stuff is not unlikely (Chandrasekaran, 1996) as the feed stuffs are exposed to varied climatic conditions in the field and during transit and storage, favouring their production by the respective fungal species. Besides, grains and oilseed by-products, which normally constitute the poultry rations, are derived from crops, grown in different climatic conditions. Thus, the mixed feeds, made of these feed stuffs, contaminated with individual mycotoxins may have all the mycotoxins present in the individual ingredients.

Co-contamination of feeds by mycotoxins in poultry

- Aflatoxins & Ochratoxin (Johri et al., 1996)
- Aflatoxins & T-2 toxin
- Aflatoxins & Diacetoxyscirpenol (DAS)
- Ochratoxin & T-2 toxin
- Ochratoxin & Citrinin
- Vomitoxin & Fusaric acid

iv) Production losses

The economic losses to poultry industry by way of reduced production performance due to mycotoxins are calculated to be over Rs 500 million every year. Feeds contaminated with mycotoxins, if fed to birds, reduce feed intake, weight gain (Raju and Devegowda, 2000) and egg production (Rogers et al., 1991). The other adverse effects include immunosuppression (Raju and Devegowda, 2002) and increased susceptibility to diseases (Ramadevi et al., 1996), damage to organs, lowered reproductive performance (Sharlin et al., 1980), low egg shell quality (Glahn et al., 1991) and poor egg yolk and skin pigmentation

(Schaeffer and Hamilton, 1990). Toxins produced by fungi greatly differ in their toxicity and the clinical signs shown by the affected flock.

Aflatoxin causes significant impact on health of chicken which include reduced feed intake and growth rate, immune suppression and vaccination failures, enlarged and fatty liver, kidney congestion, reduced skin and yolk pigmentation, embryo malformations and reduced hatchability in breeders. T-2 toxin causes yellow caseous plaques at the angle of mouth, on tongue and palate and edges of beak, ochratoxin and citrinin primarily damage kidney, yellow staining of egg shells and dark meat spots in egg white are noticed with ochratoxin, oosporein causes gout and necrosis and gangrene of extremities like comb, beak and toes are seen with ergot alkaloids. Simultaneous feeding of poultry with more than one mycotoxin, in most of the cases leads to aggravated/cumulative toxicity (Raju and Devegowda, 2000; Tessari et al., 2006).

MYCOTOXIN TRANSFER TO POULTRY EGGS AND MEAT

There are more than 300 known mycotoxins, however, due to their presence and concentration in food, and toxic potential , only a few are relevant to consumer protection. These include the aflotoxins, oc hratoxins and tric hoth ecenes like deoxynivalenol , zearalenone and fumonisins (Blank , 2002). It is impor tant to consider to what extent mycotoxins might be carried over into the edible tissues like meat and eggs when contaminated feed is fed to poultry. A review by Blank (2002) showed that the carryover of mycotoxins into edible tissues is relatively low and is dependent on the specific mycotoxins and animal species.

Mycotoxin residues in poultry products

The problems with mycotoxins do not end with production losses as many of them are readily transferred to the poultry products viz. meat and eggs (Bintvihok et al., 2002). Aflatoxin is metabolized in the body into active metabolites. In chicken B1 is metabolized into M1 and B2a in the liver. NADP linked enzyme system reduces B1 and B2 to cyclopentanol and aflatoxinol. However, in layer both aflatoxin B1 and aflatoxinol accumulates in eggs and aflatoxinol is the major metabolites in muscle and blood. In turkey, aflatoxinol is a minor metabolite and B1 and M1 are the major metabolites (Gargees and Shareef, 2009). For every 1200 parts of aflatoxin intake, 1 part is deposited in meat and for every 2200 parts, 1 part is transferred in to eggs (Coker et al., 1984). Similarly, ochratoxin, T-2 toxin etc. are also transferred in to poultry products and therefore are of great significance from human health point of view. Furthermore, after a short withdrawal period, combined with the feeding of non-contaminated diets, most of the mycotoxins except for ochratoxin, are no longer detectable in the edible tissues of poultry. On the other hand the mycotoxins such as DON, ochratoxin, alatoxins and aurofusarin may have detrimental effects on embryo development and hatchability (Ellis et al., 1991).

Table 3. Relevence of naturally occurring mycotoxins as residues in poultry products

Mycotoxins	Poultry production	Carry over in meat and egg
Aflatoxins B1	+	Liver
Ochratoxin A	+	Hatching eggs
Cyclopiazonic acid	+	Meat and eggs
Deoxynivalenol (DON)	+	Hatching eggs
Zearalenone	+	Eggs
T-2 toxin	-	-
Diacetoxyscirpenol (DAS)	-	-
Fusarochromanone	+	Hatching eggs
Aurofusarin	+	Eggs

SAFE LEVELS OF MYCOTOXINS

Since, the response of birds to dietary mycotoxins depends to a great extent on many other associated factors like the nutrition, bio-security, prevalence of disease causing organisms in the environment, genetic back ground of the bird etc. it is extremely difficult to suggest the maximum levels of mycotoxins that show no adverse effect on birds. It may be considered that there is truly no safe level and increasing levels of mycotoxins carry increasing risk (Hamilton, 1984).

Table 4. Tolerance levels (ppb) of dietary aflatoxin in different poultry birds.

Different poultry birds	Tolerance levels (ppb)
Cross-bred broilers	400
Pure bred broiler chicks	200
White Leghorn chicks	150
Quail chicks	300
Quail layers	300
Guinea fowl keets	1500
Layers	600

PREVENTION & CONTROL OF MOULDS AND MYCOTOIXINS IN FEEDS

Preventing mould growth and mycotoxin production is very important to the feed manufacturer and livestock producer. Control of mould growth in feeds can be accomplished by keeping moisture low, feeding fresh, cleaning equipments and using mould binders and inhibitors. Moisture content in grains/feed ingredients higher than 11-14% makes them prone for mould infestation. The storage environments having high relative humidity levels influence the moisture content of feed ingredients considerably. Good ventilation in the godown facilitates removal of moisture from the material and the go-down. Aeration of grain bins is important to reduce moisture migration and keep the feedstuff dry (Jones, 1987). Grains with broken kernels are highly prone for mold growth than those with intact kernels. Storing the feed for long time facilitates mold development. More frequent delivery of feed with shorter residence time reduces the buildup of mycotoxins. Mixed feeds stored for one week or more have high aflatoxin incidence than fresh ones (Gowda et al., 2002).

Mycotoxin detoxification/counteraction

I. Physical treatments

i. Exposure to sunlight in thin layers for about 12-14 hours degrades aflatoxin up to 70-90% (Shantha et al., 1986). Some mycotoxins like aflatoxin, trichothecenes, zearalenone, ochratoxin, patulin, penicillic acid etc. are highly stable and others like ergot alkaloids and citrinin are relatively easy to be destroyed by heating. This inexpensive method can be used to reduce the aflatoxin content of rice and other food grains.

ii. Drying at 120 °C for 2-3 hrs also results reduction of aflatoxin content by 60-90%.

iii. Autoclaving at 1kg/sq.cm at 120 °C also are useful (Coomes et al., 1966).

iv. Electrochemically produced ozone provides protection against aflatoxin B1.

v. UV & ionising radiation of feeds may of some use.

vi. Pelleting is another method to inhibit mould growth.

II. Chemical agents

A number of chemicals have been proved to effectively destroy or modify mycotoxin so as to reduce or eliminate the toxic effects which include:

i. Alkali: Calcium hydroxide, sodium hydroxide, ammonia. Exposure of ground nut meal to ammonia at 30 psi for 15 min. results in total degradation of aflatoxin (Park, 1993).

ii. Acid: Solid or liquid forms of sorbic acid, benzoic acid, gentian violet and copper sulphate (0.04-0.05% in feed) work well, provided the chemical is evenly distributed in the feed. Generally, the acid form of

a mould inhibitor is more active than its corresponding salt (Jones et al., 1994).

iii. Oxidizing agent: Hydrogen peroxide , ozone(Clavero et al., 1993)

iv. Reducing agent: Sugars, bisulpphite

v. Chlorinating agent: Sodium hypochlorite (Coker et al., 1984)

vi. Solvents: Aqueous acetone, iso propanol and ethanol (Park and Liang, 1993).

vii. Miscellaneous: formaldehyde, urea (Shantha et al., 1986), methylamine etc.

Often chemical treatments are used in combination with physical treatments to increase the efficacy of decontamination.

III. Dietary manipulations

i. As the mycotoxin affect protein and amino acid metabolism, increasing the dietary level of protein can minimize the ill effect (Smith et al., 1971).

ii. Increased dietary concentration of sulpher amino acid like methionine (Devegowda et al., 1998), methionine hydroxy analogue (Sapcota et al., 2005a), N acetyl cysteine, pyridoxine, folic acid, riboflavin and choline (Johri et al. 1990) can protect the chick from growth depressing effect of aflatoxin. N-acetyl cysteine acts like an antidote forming a complex with aflatoxin B1 and aids in clearing the toxin.

iii. Antioxidants like α-tocopherol, ascorbic acid (Hoehler and Marquardt, 1996), selenium (Burguera et al., 1983), BHT and β-napthoflavone (Ehrich et al., 1986) offer considerable protection against aflatoxicosis. Some of these are known to block the activation pathway of toxins specially aflatoxin.

iv. Addition of certain organic acids like valeric acid, propionic acid or butyric acid at dietary levels of 0.05 to 0.25% is effective for prevention of mold growth, with the response being the highest with Fusarium sps (Higgins and Brinkhaus, 1999).

v. Certain herbs, spices and essential oils contain naturally occurring antifungal substances that may exert a protective effect at normal usage levels. Dietary supplementation of natural/herbal preparations like herbal vitamin C (0.025%), Spirulina (0.05%) etc. having liver protectant and immunostimulant properties is also found to have some beneficial effects during aflatoxicosis (Raju et al., 2004). Ocimum sanctum in feed (0.2%) ameliorates aflatoxin toxicity in broiler chicken (Sapcota et al., 2005b). Mustard, green garlic, cinnamon bark and hops inhibit mould growth, whereas peppers, cloves, thyme and green tea inhibit toxin production only. Cinnamon, cinnamon oil, clove and clove oil have strong antimycotic properties. All four substances inhibit growth and aflatoxin production. The essential oils of lemon and orange have been shown to be inhibitory to Aspergillus niger and Aspergillus flavus and to suppress aflatoxin formation. Honey has an antifungal effect against A. flavus and A. parasiticus and an even stronger antiaflatoxigenic effect (Gowda et al., 2004).

vi. Microbial enzymes capable of degrading mycotoxin molecule like dehydrogenase, reductase, lactonase, esterase and epoxidase have of late been contemplated as an effective tool in countering mycotoxins in chicken (Raju and Rao, 2004). Degradation of zearalenone by esterase and T-2 toxin by epoxidase has been experimentally proved (Pasteiner, 1997).

vii. Dietary inclusion of fats (rich in unsaturated fatty acids) like sunflower and soyabean oils is highly effective in countering aflatoxin in broiler chicken (Raju et al., 2005)

IV. Microbial agents

i. Several bacterial and fungal cultures have shown considerable degrading ability over aflatoxin, which include Lactobacilli, Saccharomyces cerevisiae etc. Saccharomyces cerevisiae significantly improves weight gain and other performance parameters and increases immune response in aflatoxin fed broiler chicks (Devegowda et al., 1994).

ii. Tetrahymena pyreformis (protozoa), Flavobacterium aurantiacum (bacteria), Lactobacillus acidophilus and others like Rhizopus oryzae, Corynebacterium rubrum, steroid hydroxylating fungi etc. are known to have considerable aflatoxin degradation capability.

iii. Other bacteria like Clostridium sporogenes, Lactobacillus vitulinus and yeast (Trichosporan) have also considerable mycotoxin degrading ability (Heidler and Schatzmayr, 2003).

V. Adsorbents

Binding agents such as zeolites, bentonite, aluminosilicates, activated charcoal, yeast cell wall preparations- MOS, humic acid (oxyhumate) (van Rensburg et al., 2006), spent canola oil, bleaching clays and alfalfa fiber are used in feeds containing mycotoxins to prevent intestinal absorption of the toxins thus lowering the bioavailability of mycotoxin (Garcia et al., 2003).

i. Mineral clay products such as zeolites, bentonites (Al-Jobory et al., 2001), aluminosilicates have highly reactive groups on their molecular surface, which can readily participate in ion exchange reactions. After ingestion, they are activated in the gut in the presence of moisture and adsorb mycotoxin molecule in an irreversible way through ion exchange. The resulting toxin-binder complex is not absorbed and goes excreted and thereby prevents it from entering into circulation. Addition of natural zeolite improved weight gain of broilers exposed to experimental aflatoxicosis by 29-41% (Harvey et al., 1993).

ii. Hydrated sodium calcium aluminosilicate (HSCAS) at 1.0% of the feed (10 kg per tone) can significantly diminish the adverse effects of aflatoxin in chickens, pigs and cows and 0.5% level in diet has been the most successful one among them. It has also some protective effect against low levels of T-2 toxin, vomitoxin and zearalenone while no beneficial effect was seen with ochratoxin (Huff et al., 1992).

iii. Activated/ super activated charcoal is carbon that has been treated with oxygen results in a highly porous charcoal with greter surface area and @ 0.5% in diet improves performance of broilers fed aflatoxin (Edrington et al., 1997).

iv. Mannanoligosaccharide (MOS)- esterified-glucomannan, extracted from the cell wall of Saccharomyces cerevisiae, bound aflatoxin up to 81.6% has significant binding over zearalenone, vomitoxin, T-2 toxin and moderate binding over ochratoxin (Raju and Devegowda, 2002). This has advantage over other binding agents that it does not bind vitamins or minerals.

The ideal features of good mycotoxin binder are

i) Ability to bind a wide range of mycotoxins
ii) Low effective inclusion rate in feed
iii) Rapid and uniform dispersion in the feed during mixing
iv) Heat stability during pelleting, extrusion and during storage
v) No affinity for vitamins, minerals or other nutrients
vi) High stability over a wide pH range
vii) Bio-degradability after excretion

VI. The antifungal antibiotic

Natamycin (pimaricin) has strong antimycotic properties and 0.0005% (5ppm) natamycin delays the growth of seven mycotoxigenic species for 5 to 21 days i.e. Aflatoxin, Zearalenone, Vomitoxin, Ochratoxin, T-2 toxin and Fumonisins.

Table 4. The physico-chemical treatments for inactivation of preformed aflatoxins in contaminated maize and groundnut cake.

i.	Raising the moisture level upto 20%. Autoclaving at 5 PSI for one hour followed by drying in an oven at 80°C.
ii.	Adding sodium hydroxide (15 g/kg) and mixing. Raising the moisture content upto 20%, autoclaving at 5 PSI for one hour and drying in an oven.
iii.	Agitation of one kilogram of feedstuff with 20 g $Ca(OH)_2$ followed by addition and mixing of formaldehyde to raise the moisture content upto 15%. Autoclaving at 15 PSI for an hour and drying.
iv.	Addition of liquor ammonia to yield 6% concentration. Raising of moisture content up to 20%. Storing airtight for 20 days. Heating at 35°C and drying in an oven.

Table 5. Dietary additives and their dietary inclusion levels for protection to broilers against dietary aflatoxins

Detoxifying agents	g/qtl. Feed
Activated charcoal	100 – 200
Hydrated sodium calcium aluminosilicate (HSCAS)	100 – 200
Esterified glucomannan (EGM)	50 – 100
Herbal mixture (*Acacia catechu.* 25%, *Phyllanthus niruri*, 400%, Andrographis paniculata, 25%, base 10%)	50 – 75
Butylated hydroxyanisole	50 – 100
Dl-methionine*	100 – 200
Selenium**	0.200 - 0.300
Butylated hydroxy toluene*	50 -150
L-lysine HCI	150
Water soluble vitamins*	Double of the requirements
Increase the dietary protein level	Upto 26 to 28%

CONCLUSION

All poultry is sensitive to mycotoxins. This partly depends on the type, age and production categories of poultry, their living conditions and nutritive status and partly on the type, quantity and duration of mycotoxin ingestion. The presence of mycotoxins results in significant health disorders and a decrease in production performances. This leads to considerable economic loss for the poultry industry — either direct losses, i.e. death of the poultry or the indirect ones, i.e. the decrease in body mass, number and quality of eggs, greater food conversion, and immunosuppression. This results in increased sensitivity to infective agents and a bad vaccinal response. Morevover, mycotoxin residues in poultry meat, eggs and products derived from them pose a threat to human health. In order to prevent and reduce the negative implications of mycotoxins in the poultry production, it is necessary to create both global and national strategies for combatting mycotoxins, advance diagnostic techniques and procedures, intensify the control of food quality, introduce new limits on the maximum amount of mycotoxins allowed in food and poultry feed used for certain species and categories of animals, and synchronise it with the standards.

It is clear that mycotoxins will be of increasing importance for all those involved in feed manufacturing, farming and food production. Quality of raw materials, prevention of the occurrence of mycotoxins, control and testing systems are all essential to reduce the exposure of humans and animals to mycotoxins.

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