

Management of toxicants and anti-nutritional factors in poultry feed

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Presence of mycotoxins and anti-nutritional factors (ANFs) in poultry feed is one of the major constraints in utilization and maintaining feed quality as the mycotoxins are widely present in feedstuffs and ANF are inherent component of feedstuffs. Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi such as Aspergillus, Penicillium and Fusarium, which invade crops in the field and may grow on food during storage under favourable conditions of temperature and humidity. The occurrence of mycotoxins in agricultural commodities depends on region, season and the conditions under which particular crop is grown, harvested or stored.

Key words: Anti-nutritive factor, Mycotoxins, Poultry feed

CROPS grown under warm and moist weather are especially more prone to aflatoxin contamination. The mycotoxins of most concern, based on their occurrence and toxicity are aflatoxins, ochratoxin-A, trichothecenes, zearalenone, and fumonisin. The ingestion of ANF by bird produces various effects such as retarded growth, poor feed conversion, hormonal changes and sometimes histo-pathological alterations in vital organs.

Mycotoxins in poultry feed

Prevalent and relevant fungal toxin in feed are discussed here.

Aflatoxins: There are four main types of naturally occurring aflatoxins i.e., B₁, B₂, G₁ and G₂. The major producers of aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B₁ is the most toxic compound among all. Aflatoxins are primarily hepatotoxic, nephrotoxic and immuno-suppressive. Aflatoxicosis in poultry is characterized by listlessness, anorexia, poor pigmentation, jaundice, dehydration of combs and shanks,

decreased egg production, egg weight and hatchability; suppressed immunity, disrupted bone development, and decreased nutrient absorption and metabolism. One effect that is used as a presumptive diagnosis of aflatoxicosis in poultry is an enlarged, fatty, yellow and friable liver that occurs in broilers when they consume aflatoxin contaminated feed.

Ochratoxin-A: The term ochratoxin has been derived from *Aspergillus ochraceus* from where the ochratoxins were first isolated. Ochratoxin-A has been found widespread as a natural contaminant of cereal grains. Ochratoxin-A accrues in hot climates, while *Penicillium verrucosum* produces it in temperate climates. The Ochratoxin-A is nephrotoxic, characterized by pale and enlarged kidney. The symptoms of ochratoxicosis in poultry include reduced growth, decreased feed conversion efficiency, feed refusal, reduced immunity, impaired nutrient absorption and metabolism, and increased mortality.

Citrinin: Citrinin mycotoxin is chiefly produced by *Penicillium citrinum*, *Penicillium expansum* and

Penicillium verrucosum. Citrinin toxicity symptoms include reduced growth, decreased feed consumption and feed conversion. It is also a nephrotoxin like ochratoxin. Necropsy of birds with citrinin toxicity revealed the presence of pale and swollen kidneys and also enlarged liver. Citrinin toxicity in poultry leads to increased water consumption followed by diarrhea.

T-2 Toxin: T-2 toxin derived its name from the *Fusarium tricinum* T-2 strain, natural contaminants of grains such as maize, barley, oats, wheat, rye, beans, soybeans and some cereal-based products. Signs of T-2 toxin in poultry comprised decreased feed intake, growth depression, oral lesions, abnormal feathering, decreased egg production, thinner egg shells and impaired hatchability. It inhibits synthesis of protein, DNA and RNA.

Fumonisin: Fumonisin are predominantly produced by *Fusarium moniliforme*. Fumonisin B₁ (FB₁) and Fumonisin B₂ (FB₂) are the most potent toxicants. These mycotoxins inhibit the synthesis of lipoproteins sphingolipids

(sphinganine and sphingosine), the structural component of nervous system. In poultry, the gross lesions toxicity include enlargement of liver, kidney, pancreas, proventriculus and gizzard, atrophy of lymphoid organs and rickets. It has adverse effect on gut health characterized by villous atrophy and goblet cell hyperplasia.

Zearalenone (ZEN or F-2 toxin): ZEN is mainly produced by *Fusarium roseum*, *F. tricinatum*, *F. Graminearum*, *F. oxysporum* and *F. moniliforme*. Poultry is found to be less susceptible to the estrogenic effects of ZEN. At large concentrations in feed, the ill effects of ZEN in poultry may include vent enlargement and secondary sex characteristics. Although poultry appears to be resistant to the toxic effects of ZEN, its detection in poultry feed is suggested to be used as a biomarker for unknown *Fusarium* toxins.

Management of mycotoxins

Mycotoxin problem in poultry is difficult to manage, but the impact of mycotoxins can be reduced. The best way to prevent contamination of mycotoxins in feed is to prevent the fungus growth in feed ingredients. Fungal growth may occur in the field itself, at the time of harvest and during post-harvest drying and storage. Good agricultural and good storage practices make the primary line of defence against mycotoxin contamination of feeds. The tolerance level of mycotoxins varies greatly depending upon genetic variability, age, sex, nutritional status and exposure to stress of birds. The tolerance levels of aflatoxins in diets are 50 and 100 ppb in white broiler chick, 150 ppb in WL chick and layer, and 300 ppb in quail, 50 ppb in turkey, and 20 ppb in duck. The toxic levels for other mycotoxins are 100 ppb for ochratoxin A, 5 ppm for fumonisin, 2-10 ppm for Deoxynivalenol, 100 ppb for T-2 toxin and 100 ppm for Zearalenone.

General strategies: The management issues to alleviate the effect of mycotoxicosis in poultry flock include immediate change of feed contaminated with mycotoxins with fresh un-contaminated feed; thorough washing and cleaning of

feeders, feed handling and storage appliances and waterers; avoiding storage of feed for a longer period, and improving sanitation and hygiene at the farm.

Good agricultural practices: Repeated monoculture of maize in the same field enriches the soil with fungal spores, which increase the risk of contamination. Removal, burning or burial of crop residues is likely to reduce *Fusarium* inoculums. Seed varieties that are resistant to seed infecting fungi; and recommended for a particular region should be grown. Minimize insect damage and fungal infection in the vicinity of crop by proper use of registered insecticides, fungicides and other appropriate practices. *Fusarium* can produce more mycotoxins once a crop has ripened if harvest is delayed due to wet weather. Grain should be harvested as soon as possible once ripe. The equipment to be used for collecting and transporting the harvested grain should be clean, dry and free of visible fungal growth. During harvesting operation, mechanical damage to the grain and contact with soil should be avoided. Immediately after harvest, the grains should be dried to below 12% moisture for safe storage.

Good storage practices: Mould can grow and produce mycotoxins within few days during storage, if grains are not dried and cooled thoroughly. Grain spoilage during storage can be prevented by controlling grain moisture and temperature. The amount of foreign materials and damaged kernels in crop to be stored should be minimized. The storage area should be dried and protected from rain and groundwater. Protection from rodents and birds should be provided. Lower concentrations of oxygen and higher carbon dioxide inhibit aflatoxin production in stored grains. Moisture below 9% will inhibit the growth and development of rice and corn weevils. During the larval stage, insect produces water and heat which is sufficient to start mould growth. In the feeds having moisture level of 12-15 % and a combination of the mould and insect can lead to heating. Certain organic acids such as propionic,

acetic, butyric, fumaric, formic, sorbic, benzoic, tartaric and citric acids or their salts like calcium dipropionate, sodium propionate and potassium sorbate can effectively be used to prevent mould in stored grains. The level of organic acids depends on moisture level in grains and type of acid. Recommendations for propionic acid application in poultry feed for one-month storage varies from 0.25% for feed with 13% moisture to 0.5% for feed having 15% moisture. Recommendations for benzoic acid application in poultry feed for one-month storage varies from 0.30% for feed with 13% moisture to 0.5% for feed having 15% moisture content. Recommendations for fumaric, citric and tartaric acids are 0.20, 0.45 and 0.40%, respectively in poultry feed with 13% moisture. Most of the mould inhibitors help in preventing mould contamination but are unable to protect the birds from mycotoxins that have already formed in the feed. Moreover, mould inhibitors are not effective indefinitely; most of the mould inhibitors lose their effectiveness after a short period of time.

Dietary manipulation: The protein level of the new or changed diet should be increased to 25-30%. Alternatively, 1-2 kg methionine and 1.5 kg lysine hydrochloride may be supplemented per tonne of feed with normal protein content. Addition of DL-methionine (DLM) 0.1% above the NRC recommendation in starting (0.6% methionine) and finishing diet (0.48% methionine) in the aflatoxin (250 ppb) contaminated diet diminished the adverse effects of aflatoxicosis in broiler chickens. Supplementation of DLM at 500 ppm or methionine hydroxy analogue (MHA) at 769 ppm level in 500 ppb aflatoxin contaminated diet ameliorated the adverse effects of aflatoxicosis in Japanese quails. Both supplementation of DLM at 500 ppm and MHA at 769 ppm level in aflatoxin contaminated diet were equally efficacious in ameliorating the adverse effects of aflatoxicosis in growing Japanese quails. Fat and oil are beneficial in reducing aflatoxicosis and improving energy value of feed. Supplementation of selenium

Table 1. Anti-nutritional factors present in feeds of plant origin

Feedstuff	Toxic factor
Raw soybean and its meal (solvent or expeller processed)	Trypsin inhibitor, phytohaemagglutinin, antigens, lipoxygenase, goiterogen, saponin, estrogen, phytic-acid, oligosaccharides, rhamnogalactans
Groundnut and its meal	Trypsin inhibitor, goiterogen, tannins, oligosaccharides and lectins (also susceptible for <i>Aspergillus</i> growth)
Mustard or rape seed and its meal	Goiterogens (glucosinolates), tannic acid, erucic acid, sinapine (cholinester), pectins and oligosaccharides
Safflower seed and its meal	Estrogenic factor, two phenolic glucosides (Bitter flavour) and Fibre
Sunflower seed and its meal	Chlorogenic acid, quinic acid and fibre
Sesame seed and its cake	Phytate (5 g/100g) and oxalates (35 mg/100g)
Linseed and its cake	Linamarin (cyanogenic glucoside), antipyridoxine (Linatin) factor and mucilage (HCN Level @ 10-300 mg)
Kapok seed meal (seeds of silk cotton tree)	Tannins, tyrosine and fatty acids with cyclopropene rings
Copra meal (coconut meal)	Fibre (mannans) and estrogenic factor
Palm kernel meal	Fibre (half of the fibre-NDF high levels of galactomannans -(1,4)-D mannans) and sharp shells
Cotton seed and its meals	Gossypol (phenol like compound), cyclopropenoid fatty acids, tannins
Cotton seed and its meals	Gossypol (phenol like compounds) cyclopropenoid fatty acids, tannins
Guar meal (<i>Cyamopsis tetragonoloba</i>)	Guar gum (18-20%), antitrypsin factor and antivitamin E factor
Castor seed and its meal	Ricin (toalbumin)-phytohaemagglutinin, ricinine (toxic-alkaloid), ricinus allergen (protein polysaccharide)
Neem seed and its meal (<i>Azadirachta indica</i>)	Bitterness @ limonoids @ triterpenoids bitter principles: Protomeliacins, limonoids, azadirone, gedunin, vilasinin and secomeliacins nonisoprenoid polyphenolics-flavanoids, tannins and coumarin viz. nimbin, salannin and azadirachtin dried seeds – limonoids – 0.001 to 0.1%, azadirone, 0.45% and epoxy azadirone, 0.72%, azadiradione, 0.7% and salanin, 0.95%
Mahua cake (<i>Madhuca latifolia</i>)	Mowrin (saponin) and tannins
Karanja cake (<i>Pongamia glabra</i>)	Fat bound toxic factors – karajinin and pongamol (flavanoids)
Lupin meal	Quinolizidine alkaloids, pectins, oligosaccharides, high manganese, saponin.
Peas	Protease inhibitors, tannins, lipoxygenase and lectins
Rubber seed meal	Hydrocyanic-acid (20-40mg/kg)
Maize	Selenoamino acids, estrogen (mouldy), trypsin inhibitor
Wheat	Tyramine, trypsin-inhibitor, NSPs (11.4%), arabinoxylans
Rice	Estrogen and haemagglutinins
Rye	Amylase and protease inhibitor and NSPs (13.2%)
Oats	Amylase inhibitor and estrogens
Triticale	Trypsin and chymotrypsin inhibitor (NSPs)
Some varieties of barley	b - glucan (NSPs – 16.7%)
Sorghum	Tannins (0.35-1.2%)
Rice-polish and rice bran	Trypsin inhibitor and antithiamine factor
Chunies	Antitryptic factor
Sal seed and its meal	Tannic acid (tannins)
Tapioca meal (<i>Cassava</i>)	Cyanogenic glucoside (HCN – 1000 – 3000 mg/kg DM)
Fish meal and meat meal	Gizzerosine and histamine (Biogenic amines)

@ 2-3 g, vegetable oil @ 10-20 kg, with 300 g Choline chloride and 1 g of vitamin E per tonne of feed is beneficial. Water-soluble vitamins and vitamin A should be added twice the amount of required level. Supplementation of ascorbic acid @ 300 mg/kg diet is beneficial to counteract some of the adverse effects (viz. depressed egg production and

electrolyte imbalances of ochratoxin in laying hens under normal as well as elevated ambient temperature, at which ochratoxin is more toxic. Supplementation of additional zinc at 40 ppm diet (total 80 mg/kg diet) to the aflatoxin contaminated diet diminished the ill effects of 250 ppb aflatoxin in broiler chickens. Synthetic antioxidant like butylated

hydroxy toluene (BHT) inclusion in the diet of broiler chickens at 1,000 ppm level provided moderate protection against the adverse effects of aflatoxicosis in broiler chickens.

Mycotoxin adsorbents: Adsorbents or toxin binders can be used when the feed is contaminated with mycotoxins. Various toxin binders are available with varying adsorption efficacy for various mycotoxins. Diatomaceous earth, sodium bentonite and zeolite at 0.5 or 1% level, alone or in combination, were partially efficacious in ameliorating the adverse effects of 300 ppb aflatoxin B₁ in broiler feed. Use of mannan oligosaccharides (MOS) and *Saccharomyces cerevisiae* (at the rate of 0.05%, 0.1%, and 0.2%) and their combination ameliorated the effect of aflatoxin partially or completely in dose dependent manner. The 0.2% level of MOS and SC is more effective than 0.05% and 0.1% level in counteracting the 300 ppb of aflatoxin in the feed. Other toxin binders with varying efficacy are also available in market. Herbal mixture containing *Acacia catechu* 25%, *Phyllanthus niruri* 40%, *Andrographis paniculata* 25% and base material 10% @ 0.5 to 0.75 kg per ton reduces the effect of aflatoxicosis.

Processing of contaminated feeds for toxin inactivation

The physical and chemical treatments found useful for inactivation of aflatoxins in contaminated feeds or ingredients include:

- raising the moisture levels up to 20% and autoclaving at 5 PSI for one hour followed by drying in an oven at 80°C with or without addition of sodium hydroxide (15 g/kg).
- agitation of feed with Ca(OH)₂ @ 2% followed by addition of formaldehyde at 15% moisture followed by autoclaving at 15 PSI for half an hour and drying.
- ensiling after addition of liquor ammonia (6%, v/w) at 20% moisture for 20 days followed by drying at 35°C in an oven.

However, the feasibility of such processing methods has not reached the farmers' door. Moreover, such

processing methods may not be suitable for poultry feeds. Therefore, the best way of using contaminated feeds are accurate estimation of contents for different mycotoxins for the determination of the safe incorporation of such feeds in compounded feeds.

Anti-nutritional factors (ANFs)

There are certain anti-nutritional factors, which reduce feed intake and nutrient utilization. The anti-nutritional factors are generated in naturally occurring feedstuffs by the normal metabolism of the species from which the material is generated. They act by different mechanism, e.g. decomposition or inactivation of some nutrients, diminution of the digestive or metabolic utilization of food, alteration of hormone level, etc. Some examples of anti-nutritive factors are soluble non-starch polysaccharides, protease inhibitor, tannins, haemagglutinins, gossypol, oxalates, saponins, mimosine, cyanogens, etc. as given in Table 1.

In addition, fatty acid (erucic acid and other fatty acids), salt or ester (oxalates and phytates) and certain anti-vitamins are also anti-nutritional factors. Cereals, cereal by-products and oilseed residues, usually constituting about 90% or more of poultry diet contain soluble as well as insoluble non-starch polysaccharides (NSP) and toxicants. Protease inhibitors are widely distributed in leguminous seeds like soybean, guar, etc. and cause growth inhibition, reduced protein utilization and hypertrophy of pancreas. They are destroyed on heating or toasting. Trypsin inhibitor activity of solvent-extracted soybean meal is destroyed by exposure to flowing steam for 60 min. or by autoclaving under 5 Lb/in² for 45 min, 10 Lb/in² for 30 min, 15 Lb/in² for 20 min. or 20 Lb/in² for 10 min. Atmospheric steaming (100°C) destroys 95% of trypsin inhibitor content of de-hulled and defatted raw soybean flakes within 15 min. Dry heat is not effective in elimination of Phytohaemagglutinin (lectins) component but soaking and cooking are helpful. Glucosinolates may be removed from meal by extraction with hot water, dilute

alkali or acetone or decomposed with iron salts or soda ash. The soluble toxic NSPs increase viscosity of the gut content thereby reducing absorption of nutrients (starch, protein and lipids), led to wet and sticky droppings, more excreta volume, wet litter condition, poor feed conversion efficiency, excessive foul odour in poultry house, increases number of pathogenic organisms which induced necrotic enteritis and high mortality. The feed enzymes have been designed to curb the viscous effect of non-starch polysaccharides present in various feedstuffs. Similarly, phytase has proved to be highly effective in reducing the anti-nutritional property of phytate, present in all plant feedstuffs, besides augmenting availability of costlier mineral phosphorus. The goitrogenic products may be removed by extraction with acetone or water or by steam stripping of volatile isothiocyanates.

A satisfactory method of HCN extraction involves closed steam distillation with hydrochloric acid at 100°C for at least three hours. In general, feed ingredients containing cyanogens can be processed by cooking, followed by discard of cooking water or fermented or boiled then dried. Saponins can be extracted with hot water followed by extraction with ethanol or methanol. Dry or moist heat treatment or solvent extraction can achieve inactivation of estrogens. The heat generated during the commercial production of cotton seed meal helps to bind 80-90% gossypol with protein rendering it non-toxic. Higher levels of gossypol can be tolerated if iron salts are added to diet @ 1-2 ppm iron for every 1 ppm of free gossypol. Solid substrate fermentation involving certain fungi is capable of reducing 90% of free gossypol of cottonseed and eliminating its toxicity in chicks. Removal of tannins from de-oiled salseed meal, include cold water processing, boiled water processing, treatment with acids 0.1 N, HCL (1:10 w/v); 2.5% HCL), alkali [0.01 N, NaOH, 0.1 N, NaHCO₃, 0.05-5% Ca(OH)₂], Salt (3% NaCl), extraction with either acetone (30%)

or ethanol (4%) or methanol and autoclaving with tannin removal of 36-58, 42-80, 13-65, 12-68, 43-57, 41-49, 31-100, 38-53, 63-72, 33-38 and 17-100%, respectively. Autoclaving soya bean meal can reverse the increased requirement of metals. In both vegetable and animal kingdom, oxalic acid is found as free and in salt form. The nutritive value of linseed meal for chicks can be considerably improved after extracting the meal with water and autoclaving and supplementing with pyridoxine hydrochloride.

CONCLUSION

Mycotoxins can produce a significant reduction in the performance of poultry even at low concentration. Many chemical preservatives are used to prevent mould in stored grains. Organic acids such as propionic, acetic, butyric, fumaric, formic, benzoic, tartaric and citric acids can be effectively used. Change of feed contaminated with mycotoxins, keeping the poultry farm and utensils free from dust and fungi, use of suitable mycotoxin adsorbents in combinations, dietary manipulations and use of antioxidants are some of the measures for combating deleterious effects of aflatoxicosis in poultry.

Similarly, anti-nutritional factors are an inherent natural component of certain feedstuffs. Several processing methods have been applied to detoxify the anti-nutritional factors, however, those methods, except for toasting of legumes and free gossypol in cottonseed, have not received industrial attention due to excessive processing cost, nutrient loss in processing, cumbersome processing methods, etc. Therefore, the best way of management of the anti-nutritional factors and mycotoxins is suitable dilution of feedstuffs based on their concentration in feedstuffs to restrict their levels much below the tolerance levels of birds to be fed.

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