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Mycotoxins; A serious threat for nutrition world

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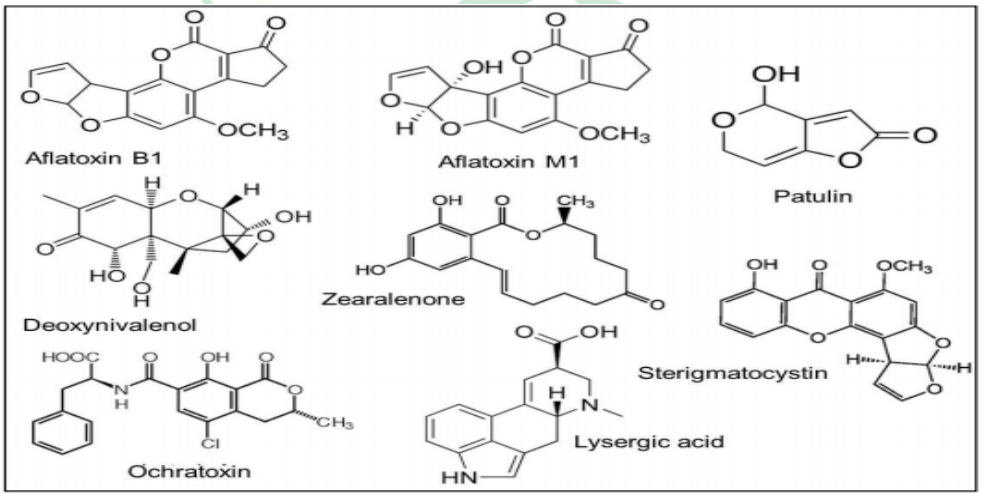
Background

The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100,000 turkey poults died. When this mysterious turkey X disease was linked to a peanut (groundnut) meal contaminated with secondary metabolites from *Aspergillus flavus* (aflatoxins). Mycotoxin, a greek word "Mykes"- mould & "Toxicum"-poison. Thus, mycotoxin means, toxic/poisonous secondary metabolites produced by moulds contaminating feed & feed ingredients which are carcinogenic, mutagenic and teratogenic in chronic state. Mycotoxin originates from fungi, those are eukaryotic organisms that do not contain chlorophyll, but have cell walls, filamentous structures. Fungi create a huge threat to safety and security of livestock and human being. Fungi cause serious economic losses damaging upto 25% of worlds poultry crop (FAO). Thus, Mycotoxin control is crucial for production economics, animal welfare, products quality and food safety.



Chemical structures of a few mycotoxins

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Characteristics of fungal mycotoxins

Approximately 300 various kinds of mycotoxins exist in biological system having following characteristics,

1. Funagl toxins have high physical and chemical stability.

2. It varies greatly in their severity.

3. Molecular weight ranges from 200 to 500 kD.

4. Grow by producing long filamentous structure called hyphae.

5. Hyphae form a network responsible for cementing kernels together.

6. This complex forms clumps of grain that cannot be separated.

7. Grain-mould fungi also produce spores capable of aerial dispersal in the field.

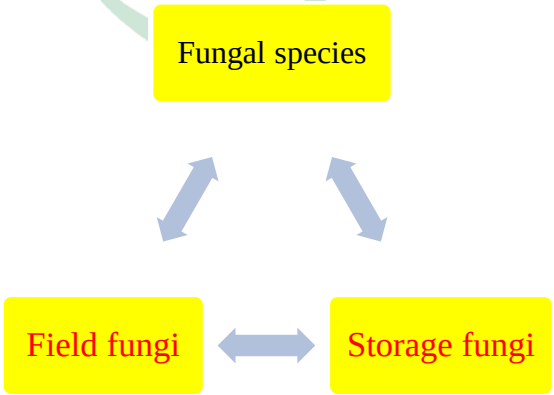
8. Masses of these spores give the mould a characteristic colour.

9. Spores can lay dormant for months or years

10. No efficient decontamination procedures are available for mycotoxin. Thus, mycotoxins that are present at the time of harvest will reach the final animal diet during feed consumption.

11. Exert additive, synergistic and antagonistic effects on poultry health.

Fungal growth



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Field fungi

They invade the seeds while the crop is in the field before harvest and require high moisture conditions (20-21%) such as, Fusarium, Alternaria, Clodosporium, Diplodia, Gibberella, Helminthosporium.

Predisposing factors

1. Environmental stress.

2. Hybrid vigour (crop variety).

3. Presence of fungal spores in field.

4. Agronomic practices (Soil cultivation, crop rotation).

5. Poor handling.

6. Annual variation in rainfall and climate.

Storage fungi

They invade grains or seeds during storage and require less moisture than field fungi (13-18%) such as, Aspergillus, Penicillium.

Predisposing factors

Moisture- *Aspergillus flavus* can start to produce aflatoxin at 83% RH. However, *Aspergillus ochraceus* needs at least 97% to produce ochratoxins.

Temperature- Mycotoxin well proliferates in temperature range of 10 - 30°C. *Aspergillus flavus* produces aflatoxins at 10°C - 25°C. *Fusarium tricinctum* produce T_2 toxin at 1°C - 4°C (may be up to 15°C). *Aspergillus ochraceus* produces ochratoxin from 12°C -20°C (may be up to 30°C) and *Penicillium viridicatum* produces ochratoxin between 4°C -31°C.

Substrate- Starch, Zinc in feed proliferates fungal growth

Apart from such predisposing factors, carbon dioxide (CO_2) concentration, fungal abundance, insect presence infestation (vectors of fungal spores, mechanical damage to the

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grain favoring the entry of moulds), pH -4-8, harvesting method of crop, Non-availability of acceptable quality grains for poultry feed etc are also significantly influences the mycotoxicosis.

Classification of mycotoxins

Clinical: Mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth.

Cell biological: Put them into generic groups such as teratogens, mutagens, carcinogens, and allergens.

Chemical: Have attempted to classify them by their chemical structures (e.g., lactones, coumarins).

Biochemical: According to their biosynthetic origins (polyketides, amino acid-derived,etc.).

Physical: By the illnesses they cause (e.g., St. Anthony's fire, stachybotryotoxicosis).

Mycological: By the fungi that produce them (e.g., *Aspergillus* toxins, *Penicillium* toxins).

Various mycotoxins

Several hundred mycotoxins are known. However, some most concern feed hazardous toxins are, Aflatoxin, Trichothecenes (DON, T_2 toxin, DAS, etc), Ochratoxin A, Zearalenone (ZEN), Fumonisins (FUM), Moniliformin, Cyclopiazonic acid (CPA), Navalenol (NIV)

Origin of fungal mycotoxins

Mycotoxin	**Responsible fungi**	**Affected feeds and their processed products.**
Aflatoxin(highly hepatocarcinogenic)	*Aspergillus flavus* *Aspergillus parasiticus* *Aspergillus nomius*	Corn, cotton seed, peanuts, soy

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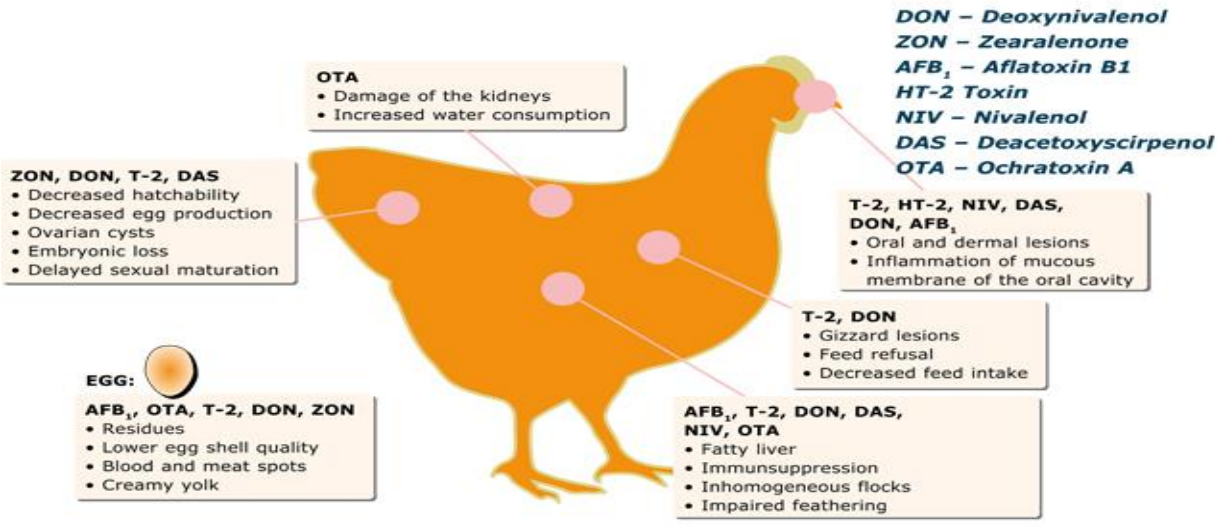
Ochratoxin A	*Aspergillus ochraceus* *Aspergillus nigri* *Penicillium verrucosum*	Wheat, barley, oats, corn
Trichothecenes (DON, T-2, DAS, etc)	*Fusarium graminearum* *Fusarium culmorum* *Fusarium poae* *Fusarium sporotrichoides* *Fusarium acuminatum* *Fusarium sambucinum* *Fusarium equiseti*	Corn, wheat, barley
Zearalenone	*Fusarium graminearum*	Corn, Wheat, Barley, Grass
Fumonisin	*Fusarium verticillioides* *Fusarium proliferatum*	Corn
Moniliformin	*Fusarium moniliforme*	Corn
PR toxin, patulin	*Penicillium roqueforti*	Silage, Grass

Effects of various mycotoxins on poultry health



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Mechanism of mycotoxin damage

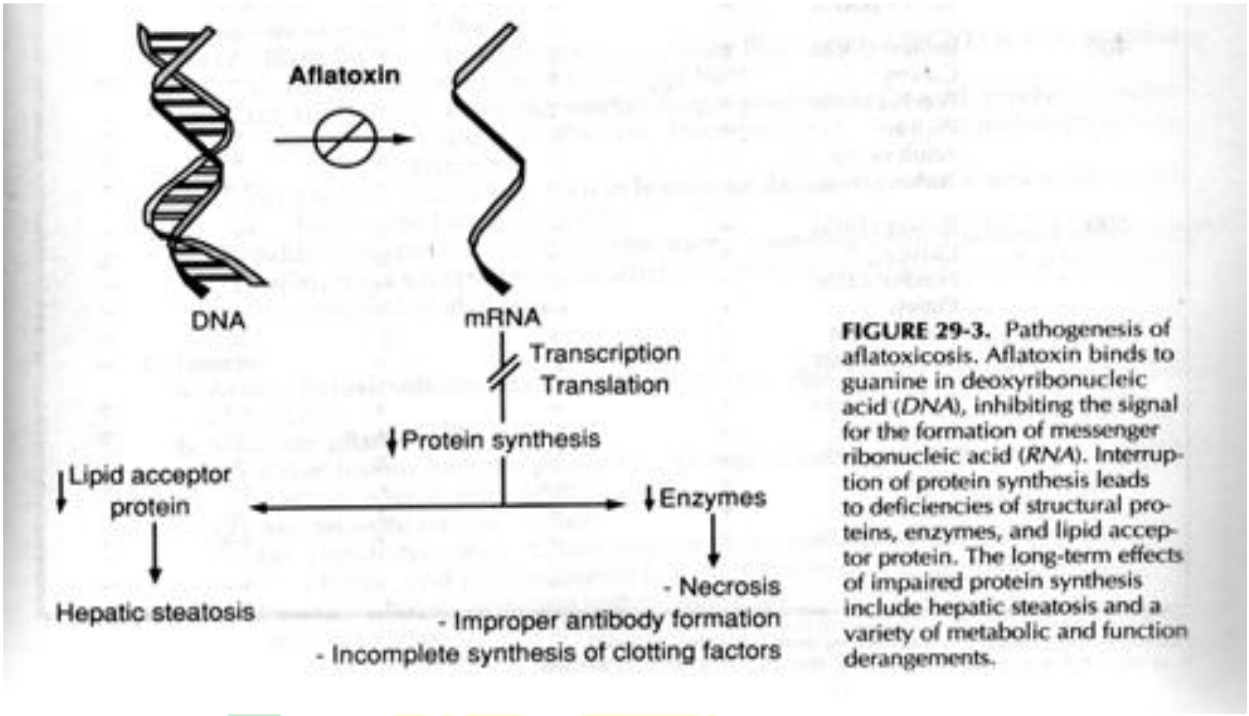

FIGURE 29-3. Pathogenesis of aflatoxicosis. Aflatoxin binds to guanine in deoxyribonucleic acid (*DNA*), inhibiting the signal for the formation of messenger ribonucleic acid (*RNA*). Interruption of protein synthesis leads to deficiencies of structural proteins, enzymes, and lipid acceptor protein. The long-term effects of impaired protein synthesis include hepatic steatosis and a variety of metabolic and function derangements.

Deleterious impacts of mycotoxins

Age, sex, physiological status and nutritional status of livestock predispose the fungal toxicosis by, reduction of feed intake or feed refusal, alteration in nutrient content of feed in terms of nutrient absorption and metabolism, effects on the endocrine, exocrine systems, immunosuppressant, poor growth, reduced egg production, reduced feed conversion, increased mortality, poor egg shell quality, reduced fertility, leg problems, carcass condemnation, increased susceptibility to disease.

Aflatoxins

Most well known, internationally common & most feared mycotoxin. Aflatoxin B_1, B_2, G_1 and G_2 refer to toxins which fluoresce blue (B) or green (G) under ultraviolet light and are separable by thin layer chromatography (TLC). Aflatoxin M_1 is the hydroxylated metabolite of Aflatoxin B_1 secreted in the milk of animals receiving Aflatoxin B_1. Ducks are the most susceptible for Aflatoxicosis. Aflatoxins utilize the nutrients present in the

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ingredients for their metabolism and propagation and thereby reduce the nutritional quality of ingredients.

Aflatoxin B_1 (AFB_1)

LD_{50} -0.5 mg/kg b.wt. in ducks &2-10 mg/kg b.wt. in other poultry. It is most prevalent toxin in cereals. Active hepatocarcinogenic. Inhibit nucleic acid synthesis by either direct interaction with enzymes involved or by a toxin-DNA template. Aflatoxin B_1 is metabolized into M_1 and B_{2a} in the liver and NADP linked enzyme system reduces B_1 and B_2 to cyclopentanol and aflatoxinol in broilers. In layers, AFB_1 and aflatoxinol accumulates in eggs and aflatoxinol is the major metabolite in muscles and blood. Corn is more liable to the most critical AFB_1 contaminations throughout the world.

Aflatoxicosis

Aflatoxin at high concentration (10-100 ppm or mg/kg) causes death of chicks. At moderate level (1-10ppm) in chicken causes reduced growth rate, decline in feed efficiency, pale and enlarged liver, bile duct proliferation and hemorrhages. Aflatoxins in layers feeding more than 2 ppm in feed results in reduced egg production.

Sign of Aflatoxicosis

In liver causes fatty change, hepathocyte degeneration, Bile duct hyperplasia, hepatic necrosis, suppression of hepatic protein synthesis, pale liver. In bones, impaired vitamin D metabolism, reduced bone strength, leg weakness. Digestion obstruction includes, reduced bile salt production, lipid digestion and reduce metabolism of minerals such as, Fe, P, Cu etc. It reduce prothrombin levels, increases the fragility of capillaries. It has carcinogenic effect. It causes pale bird syndrome (Bruising and downgrading). Reduced fertility and hatchability. Impaired pigmentation. Vaccination failures and poor antibody titers. Malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia and decreased concentrations of bile salts and pancreatic lipase, trypsin, amylase and RNase. Decreased hepatic gene expression of superoxide dismutase, glutathione S-transferase, epoxide hydrolase and increased gene expression of Interleukin 6 and cytochrome p450 $1A_1$ and $2H_1$. In chicks fed 2.0 mg/kg AFB_1, various hepatic genes associated with energy production and fatty acid

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metabolism (carnitine palmitoyl transferase), growth and development (insulin-like growth factor 1), antioxidant protection (glutathione S-transferase), detoxification (epoxide hydrolase), coagulation (coagulation factors IX and X) and immune protection (interleukins) were down regulated, whereas genes associated with cell proliferation (ornithine decarboxylase) were up regulated (Yarru *et al*., 2009b).

Ochratoxin (OTA)

At a cellular level, OTA interferes with DNA, RNA and protein synthesis by inhibiting the enzyme phenylalanine-tRNA synthetase. OTA affects renal carbohydrate metabolism through a reduction of the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in gluconeogenesis. The effects of OTA on DNA, RNA and protein synthesis are thought to be due to the phenylalanine moiety of the toxin competing with phenylalanine in the enzyme catalyzed reaction. Ochratoxin A is nephrotoxic (acute renal failure, pale and grossly enlarged kidney). In young chicks, ochratoxin A is approximately three times more toxic than aflatoxin. LD50 for day old chick 2.14+0.57 mg/kg b.w. LD_{50} for day old chick 4.63 mg/kg b.w. LD_{50} for 3 weeks old poultry 7.84 mg/kg b.w. Polyurea with large volumes of wet faeces, urate deposition in joints and abdominal cavity, depletion of lymphocytes and with strong suppression of cellular immunity, poor egg shell quality, reduced feathering, reduced embryo viability by decreased hatchability, egg with blood and meat spots, reduced size of bursa, Elevated serum uric acid level, yellow egg shell stains (due to uric acid), lower absorption of carotenoids, glycogen accumulation in breast muscles. Ochratoxin A causes hypocarotenidemia in broilers.

Trichothecenes

Trichothecenes includes T_2 toxin, HT_2 toxin, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), neosolaniol, 8-acetoxyneosolaniol, 4-deacetylneosolaniol, nivalenol, 4-acetoxynivalenol (Fusarenone-X), DON (vomitoxin) and 3-acetyldeoxynivalenol. These are typical field mycotoxins. A primary inhibition of protein synthesis followed by a secondary disruption of DNA and RNA synthesis. Scab or head fusariosis seen in cereals. Poultry are most sensitive to T_2 toxin and DAS. Proven tissue irritants, Oral lesions, dermatitis and intestinal irritation. Loss of appetite, reduced feed

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intake, reduced weight gain. Oral lesions includes, circumscribed proliferate yellow caseous plaques occurring at the margin of the beak, mucosa of the hard palate and the angle between the mouth and the tongue, Gizzard erosion includes necrosis of proventricular mucosa, regression of ovaries. Tibia dyschondroplasia. Increased liver weight and peroxidative changes in liver, abnormal blood coagulation, leucopoenia and proteinemia and immunosuppression.

Deoxynevalenon (DON)

LD_{50} for day old chick is 140 mg/kg b.wt. Acure eccymotic hemorrhages throughout the carcass. Widespread deposition of urate. Irritation to upper GI tract. Increase in gizzard size. Necrotic lesions in mouth.

Nivalenol

LD_{50} for duckling is 2 mg/kg b.w. For duckling, it is 10-30 times more toxic than DON and 30 times less toxic than T_2 toxin.

T_2 toxins

LD_{50} is 4.97-5.25 mg/kg b.wt. for day old chick. Excellent indicator of toxicosis seen by yellowish white caseous oral lesions. Necrotic lesions in gizzard. Abnormal feathering. Abnormal wing positioning. Loss of righting reflex. Reduction in lymphoid organs (bursa, spleen and thymus). Increased sensitivity to salmonella infection.

HT_2 toxins

LD_{50} for day old chick is 7.22 mg/kg b.wt. Inappetance, Asthenia, Diarrhoea, Panting. Symptoms arises within 4-10 hrs of exposure.

Zearalenole (ZEA)

Often occurs with DON in naturally contaminated cereals. Responsible for reproductive disorders due to its oestrogenic effect at high concentrations. At high concentrations (more than 300 ppm) causes, vent enlargement, enhanced secondary sex

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characteristics, increased growth of ovary and bursa, fluid filled cysts on ovary duct, decreased comb size in male, decreased egg production in layers.

Fumonisin (FUM)

First isolated from cultures of *Fusarium verticillioides* (moniliforme). Six different FUM have been identified (A_1, A_2, B_1, B_2, B_3 and B_4). Broilers and turkeys are resistant to acute fumonisins toxicity. Spiking mortality (paralysis, extended legs and neck, wobbly gait, gasping). FUM are specific inhibitors of ceramide synthase (sphinganine/sphingosine N-acyltransferase), a key enzyme required for the synthesis of ceramide and more complex sphingolipids m leads to an increase in tissue concentrations of the sphingolipids sphingosine (SO) and sphinganine (SA) and a change in the SA:SO ratio. Reduced growth rate, increased organ weights, hepatocellular hyperplasia, multifocal hepatic necrosis, poor vaccination response, increased extramedullary hematopoiesis.

Diagnostic methodology of mycotoxins

Fungal toxicity can be studied through thin layer chromatography (TLC), PCR, immune based diagnosis kits, ELISA, high performance liquid chromatography (HPLC), gas liquid chromatography (GLC) coupled to (tandem) mass spectrometry (LC-MS/MS).

Mycotoxins detection in poultry feed

Fumonisins in corn-based food can be detected through LC with fluorescence (FD) & MS detectors. Aflatoxins B_1, B_2, G_1, G_2, OTA, ZEA & fumonisins FB_1 & FB_2, DON in corn can be detected through HPLC – postcolumn photochemical derivatization. Aflatoxins (B_1, B_2, G_1 & G_2) in peanut butter sesame paste can be detect through LC. Aflatoxins (B_1, B_2, G_1 & G_2) in nuts, cereals, dried fruits & spices can be detected through LC–MS. Aflatoxins (B_1, B_2, G_1 & G_2), ochratoxin, ZEA DON, fumonisins, T-2, HT-2 in different cereal food can be detected through ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC/MS/MS). Zearalenole (ZEA) in cereal can be detected through direct competitive enzyme-linked immunosorbent assay (DC-ELISA). Aflatoxins (B_1, B_2, G_1 & G_2), OTA, DON, ZEA, T_2 toxin, HT_2 toxin & others in peanut, pistachio, wheat, maize, cornflakes, raisins, figs can be detected through LC-MS/MS. Macrocyclic lactone

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mycotoxins (zearalenone, ZON, alpha-zearalenol, alpha-ZOL & beta-zearalenol, beta-ZOL) in Maize flour can be detected through Supercritical fluid extraction (SFE) & clean-up on florisil adsorption cartridge before Chromatograph. T_2 & HT_2 toxins in cereals can be detected through LC-FD. Fumonisins in maize can be detected through LC-MS-MS. Aflatoxins (B_1, B_2, G_1 and G_2) in rice (hull, bran, polished broken grains & polished whole kernels) can be detected through ELISA. Aflatoxin & ochratoxin in barley & wheat flour can be detected through optical waveguide light mode spectroscopy (OWLS) techn. in competitive & in direct immunoassays. Aflatoxins, ochratoxins, fumonisins, deoxynivalene zearalenone in a blend of naturally contaminated grains can be detected through HPLC. Ochratoxin A & aflatoxin B_1 was performed by using a reversed phase symmetry C-18 column (15 cm _ 4.6 mm, 5`IM particles) preceded by a Rheodyne guard 0.5 ` IM filter. The fluorescence detector used for emission of ochratoxin A emission for aflatoxin B_1. Fusariotoxin analysis (DON, ZEA, FB_1 etc.) in maize meal can be detected through liq. chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). Aflatoxin B_1 (AFB_1), citrinin (CIT), ochratoxin A (OTA) in rice can be detected through HPLC with fluorimetry detection equipped with an injector 20 µL loop, a C18 spherisorb column (3 lm C18, 0.46 ∗ 25 cm) and a fluorescence detector (Spectra physic 2000) can be used for different excitation & emission fluorescence parameters. Zearalenone (A & B) zearalenols, fumonisin B_1 in maize can be detected through HPLC coupled with MS (HPLC/MS), LC analysis by varian system, 2 pumps, polar modified RP-18 column. Aflatoxins & ochratoxin A in poultry feeds can be detected through TLC & HPLC. Aflatoxin B_1, fumonisin B_1, zearalenone, ochratoxin A in Rice, maize & peanuts can be detected through validated HPLC method with fluorescence detector. Aflatoxins in polished rice can be detected through HPLC-fluorescence detection (FD), confirmed using HPLC-electrospray ionization (ESI) –mass spectrometry (MS). Aflatoxins (AFs) (B_1, B_2, G_1 and G_2), zearalenone (ZEA) and ochratoxin A (OTA) in cereal grains can be detected through HPLC with fluorescence detection. 4-deoxynivalenol (DON/vomitoxin), DON & nivalenol in maize, wheat & barley can be detected through ELISA.

Maximum permissible limit of mycotoxins

Aflatoxin B_1 - 20 microgram per kg of feed (EU) & 200 microgram for feed ingredients (EU).

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Control of mycotoxins

Genetically engineer plants resistant to fungal infection, use feed additives that sequester toxins & prevent absorption from GIT. Have farmers select strains resistant to mycotoxins.

A. Chemical detoxification methods

a. Atmospheric pressure ammoniation where feed is sealed in plastic bag or bis for 2-3 weeks.
b. High temprature and pressure ammoniation for <1 hr.
c. Adverse effect-reduced content of lysine and S-containg amino acids (compensate by extra provision in diet).
d. 2% ammonia at 10-20% water content at 20^{O}C temp, for 4-6 weeks destroys most of ochratoxin. Addition of sodium bisulfite to detoxify aflatoxin, DON
e. Alkali, chlorine, hydrogen peroxide & Ozone treatment can be tried but effectiveness is very less.

B. **Physical detoxification methods**

Mycotoxins can be physically destroyed through temperature higher than 100^{o}C, UV radiation, sunlight, cleaning, dehulling, milling-sieving. But effectiveness of such physical methods are questionable.

Bibliography

(Anon, 2001b; Josephs *et al.*, 2001; Krska and Josephs, 2001; Bony, 2000; Chu, 2000; Miraglia and Brera, 2000; Stroka *et al.*, 2000a,b).

For detailed information on all aspects of mycotoxin research, including structure–function, test kits and methods, toxicology, fungal metabolism, epidemiology and surveillance, HACCP and prevention, the reader is referred to the following websites and links (www.eman@leatherheadfood.com; ***www.mycotoxin-prevention.com***;).