Mycotoxicosis

Dr. Poonam Shakya
GENERAL FEATURES OF MYCOTOXIN FORMATION

More than 100 known species are capable of elaborating mycotoxins.

Same mycotoxin can be produced by different fungi & the same fungus can produce different mycotoxins.

Toxin production occurs only under specific conditions of moisture, temperature, suitability of substrate & appropriate oxygen tension.

The optimum conditions for toxin production are relatively specific for each fungus.

For e.g. *Fusarium* elaborates its toxin at freezing temperature, while *A. flavus* requires a temperature of 25°C.
The susceptibility of different crops to mould infection is governed by the presence of suitable substrates.

Damage to the seed coat by insects, mechanical harvesting, severe frost or other factors may predispose crops to fungal attack.

Insects may also serve as carriers of fungal spores.

The fungi associated with cereal grains have been divided into two types.
Field fungi

- Field fungi which invade the grains before harvest and require greater water activity for growth
  - e.g. *Fusarium*, *Helminthosporium* and *Cladosporium*
Storage fungi

- Storage fungi which invade the grains after harvest during drying and in storage
  - e.g. Aspergillus, Penicillium
<table>
<thead>
<tr>
<th>Species</th>
<th>Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus</em> and <em>A. parasiticus</em></td>
<td>Aflatoxins</td>
</tr>
<tr>
<td><em>A. ocheraceus</em></td>
<td>Ochratoxin</td>
</tr>
<tr>
<td><em>Fusarium roseum</em></td>
<td>Trichothecane (t-2) toxin</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>Citrinin</td>
</tr>
<tr>
<td><em>A. nidulans</em> and <em>A. versicolor</em></td>
<td>Sterigmatocytosin</td>
</tr>
</tbody>
</table>
CHARACTERISTICS OF MYCOTOXINS

- Mycotoxin - Greek word – ‘mykes’ meaning ‘fungus’ & Latin word – ‘toxicum’ meaning ‘poison’.

- Group of compounds produced by some strains of certain fungi that cause illness or death when ingested by man or animals.

- Low molecular weight, non-antigenic, heat stable secondary fungal metabolites.
They can activate at low concentrations.

Toxic effects- carcinogenic, mutagenic, teratogenic & immunosuppressive.

Acquired immunity does not occur following exposure.

Each toxin affects specific target organs or tissues.

Human exposure may result from excretion in milk or accumulation in food-animal tissues.
<table>
<thead>
<tr>
<th>Target organs/ tissues</th>
<th>Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular system</td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>Digestive system</td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>Mucous membrane</td>
<td>Trichothecane (t-2) toxin</td>
</tr>
<tr>
<td>Urinary system</td>
<td>Ochratoxin</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Zearalenone (Fusarium toxin)</td>
</tr>
<tr>
<td>Cutaneous system</td>
<td>Sporidesmin</td>
</tr>
</tbody>
</table>
MYCOTOXICOSIS

- Mycotoxicosis is disease syndrome that result from the ingestion of mycotoxins

- Neither infectious nor contagious, but they cause heavy economic losses to the poultry and cattle farmers by affecting growth and production performance
Factors affecting severity of mycotoxicosis in animals

- Species of toxigenic fungus
- Concentration of mycotoxin in the food
- Age, sex and health status of the exposed animal
- Target organs or tissue affected
- Duration of exposure to contaminated feed.
Route of entry—ingestion, inhalation or direct skin contact.

Mycotoxicosis occurs in 2 forms.

- **Acute**
  - Produced when high to moderate amounts of mycotoxins are consumed.
  - Causes marked signs of disease or death.

- **Chronic**
  - Moderate to low levels of mycotoxin intake.
  - Cause low productivity, slow growth rate, reduced reproductivity and inferior market quality.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Fungus</th>
<th>Crop or substrate</th>
<th>Mycotoxin</th>
<th>Animals affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aflatoxicosis</strong></td>
<td><em>Aspergillus flavus</em> <em>Aspergillus parasiticus</em></td>
<td>Ground nut, maize and nut crops</td>
<td>Afaltoxins B1, B2, G1,G2</td>
<td>Cattle, pig, poultry and dogs</td>
</tr>
<tr>
<td>Ergotism</td>
<td><em>Claviceps purpura</em></td>
<td>Seed heads of many grasses and grains</td>
<td>Ergotamine and ergometrine</td>
<td>Cattle, Sheep, Pig, Horse and Poultry</td>
</tr>
<tr>
<td>Facial Eczema</td>
<td><em>Pithomyces charatarum</em></td>
<td>Pasture, litter</td>
<td>Sporidesmin</td>
<td>Sheep and Cattle</td>
</tr>
<tr>
<td>Oestrogenism</td>
<td><em>Fusarium graminareum</em></td>
<td>Maize, Barley and cereals</td>
<td>Zearalenone</td>
<td>Pigs</td>
</tr>
<tr>
<td>Leukoencephalomalacia</td>
<td><em>Fusarium moniliforme</em></td>
<td>Maize</td>
<td>Fumonisins B1 (A1, A2, B2)</td>
<td>Horses and Donkey</td>
</tr>
<tr>
<td>Trichotheccane toxicosis</td>
<td>Many <em>Fusarium</em> species</td>
<td>Cereals</td>
<td>T-2 toxin, diacetoxy-seripenol</td>
<td>Many species</td>
</tr>
<tr>
<td>Ocharatoxicosis</td>
<td><em>A. ochraceus</em> <em>P. viridicatum</em></td>
<td>Barley, wheat and Maize</td>
<td>Ochratoxin -A</td>
<td>Pigs and Poultry</td>
</tr>
</tbody>
</table>
CLINICAL FEATURES OF MYCOTOXICOSES

- Diseases produced are not transmissible to incontact animals.
- Outbreaks are often seasonal and sporadic, and may be associated with certain batches of stored food or particular types of pasture.

- Initially, the signs of illness are decreased growth rate or immunosuppression.
- Treatments such as antibodies are usually ineffective.
Recovery generally depends on the type and amount of mycotoxin ingested and the duration of the exposure to contaminated feed.

The only acceptable evidence for the presence of mycotocoses in animals is the laboratory demonstration of mycotoxins in suspected food, or in the tissues, secretions or excretions of affected animals.

Characteristic lesions in target organs of affected animals are important supporting diagnostic evidence.
The name aflatoxin derives from *Aspergillus flavus* toxin.

Aflatoxins are a group of approx. 20 related toxic compounds produced by some strains of *A. flavus* and *A. parasiticus* during growth on a variety of cereal grains and food stuffs such as maize, cotton seed & groundnuts.
High humidity & high temperature during pre-harvesting, harvesting, transportation and storage, as well as damage to feed crops by insects, drought and mechanical injury during harvesting, favours the growth and toxin production of *Aspergillus flavus*. 

![Diagram showing the connection between aflatoxin, feed source, susceptible foods, and human consumption.](image)
Mould growth and toxin formation require a moisture content of the substrate greater than 15%, temp. 25°C and adequate aeration.

Toxic, carcinogenic, teratogenic and mutagenic activity.
The four major aflatoxins are B1, B2, G1 and G2. These mycotoxins are named according to their position and fluorescent colour on thin layer chromatography (TLC).

B1 and B2 produce blue colour and G1, G2 gives green fluorescence.

Aflatoxins M1, M2 are hydroxylated metabolites of B1 and B2 that are excreted in the milk of lactating animals such as dairy cows.
BIOLOGICAL EFFECTS OF AFLATOXIN

- Acute toxicity
  - Hepatic injury & nervous signs such as ataxia and convulsions.
  - Death may occur suddenly.

- Chronic toxicity
  - Reduction in efficiency of food conversion, depressed daily weight gain, decreased milk production in dairy cattle and enhanced susceptibility to intercurrent infections due to immunosuppression.
PATHOGENEITY

- **Symptoms**
  - Young animals are highly susceptible.
  - Aflatoxin B1 produce the most hepatogenic, carcinogenic, teratogenic and embryotoxic effects.
  - Calves- blindness, circling, grinding of teeth, diarrhoea, tenesmus & convulsions.
  - Cattle- aflatoxin M1 and M2 are excreted in the milk.
- Pigs - drowsiness, inappetance, jaundice, weight loss & yellow urine
- Ducklings - most susceptible avian species
- Signs include anorexia, poor growth rate, ataxia and opisthotonus, followed by death
- In birds over three weeks of age, subcutaneous haemorrhages of legs and feet
Lesions

- Principle target organ is liver.
- Depending on the severity of intoxication, hepatomegaly with necrosis & bile duct hyperplasia

- Acute hepatic failure & massive haemorrhage due to impaired blood clotting, increased capillary fragility leading to death

- Chronic toxicity, in addition to liver damage, degenerative changes in the kidney, thymus cortical aplasia leading to decreased cell mediated immune response
DIAGNOSIS

- Chemical identification of mycotoxins in food samples
- Biological assays for toxicity are important confirmatory steps

- Concentration of aflatoxin B1 in excess of 100μg /kg of feed are considered toxic for cattle

- Thinlayer chromatography and HPLC are more sensitive analytical methods for determining aflatoxins levels in the food.
- Radio immuno assay & ELISA
- **Biological assays**- Ducklings are mostly susceptible. Bile duct proliferation in one-day-old ducklings and chick embryo bioassay
CONTROL AND PREVENTION

- Prevention of contamination at all stages of food production, storage and use

- Decontamination procedures like physical removal and chemical treatment of aflatoxin contaminated feeds such as with acids, alkalies, aldehydes, oxidizing agents of selected gases (ammonia)

- High affinity inorganic compounds such as benzoic and propionic acid as preservatives for stored agricultural products.
Ergotism

- Caused by ingestion of grasses & cereals, particularly rye, infected with fungal species of the genus *Claviceps*, notably *Claviceps purpurea*.

- The word ergot- French term - a rooster’s spur, accurately describes the compacted mass of hyphae that projects as a dark, purplish-black, misshapen replica of the original seed.
Ergot alkaloids

- Ergots contain the toxic alkaloids - ergotamine and ergometrine.
- Two forms of ergotism - gangrenous & convulsive ergotism.
- The ergot alkaloids, particularly ergotamine, stimulate and then depress the central nervous system when taken in large amounts.
When consumed in small amounts over long periods, they produce arteriolar spasm, capillary & endothelial damage resulting in vascular stasis, thrombosis, ischaemia and gangrene of the affected part.

Convulsive ergotism, characterized by neurotoxicity
Towards the end of pregnancy, ergot alkaloids may exert an oxytocinlike effect on the pregnant uterus.

- Abortions have been described in cattle consuming ergotized grass.
- Premature births, low litter size & mummified foetuses.
Clinical findings

- Gangrenous ergotism - Gangrenous necrosis of the extremities – nose, ears, tail, teats & limbs

- Cattle grazing on ergotized pasture or fed contaminated grain or silage develop lameness and gangrene as a major clinical sign of ergot toxicity.
Ergotism in a cow: a swollen right hind leg showing a line of separation and terminal gangrene. The left hind limb is unaffected.
A cold environment predisposes the extremities to gangrene. The affected part, which gradually loses sensation, may eventually slough.

The tips of the ears or tail may become necrotic and the teats and udder may appear unusually pale.

Nervous form of ergotism, muscular incoordination, tremors, blindness & convulsions.
Diagnosis

- Demonstration of fungus on pasture, in grains or in silage
- Extraction of ergot alkaloids
- Detection by chromatography, or biological testing

Prevention of ergotism

- Ergot infestation of grain fields can be minimized by using clean seed, crop rotation and deep cultivation
Fusarium Toxicoses

- The genus *Fusarium* is the largest single group of fungi with known toxigenic capability.

- Because of their close association with plants and their relatively high water activity requirements for growth, fusaria are usually well established in a crop before harvesting and may cause many problems in cereals following a late harvest after a wet summer.
Toxins

- Oestrogenic metabolites such as zearalenone (also referred to as F-2 toxin)
- Trichothecene toxins

- Fusarium species tend to produce highly coloured colonies, both obverse and reverse with banana-shaped macroconidia
Fusarium species on Sabouraud dextrose agar, seven days.
Fusarium species on Sabouraud dextrose agar, seven days. Reverse.
Fusarium species showing typical banana shaped macroconidia. (LPCB, ×400)
Oestrogenism

- This oestrogenic syndrome was first described in the USA more than 80 years ago.
- The disease, then termed vulvovaginitis, was associated with the consumption of mouldy maize by gilts.

Fusarium graminearum and other Fusarium species growing on maize, barley and other grains produce zearalenone, a phenolic resocyclic acid lactone with oestrogenic activity.

- Target organ system- reproductive tract and pigs are most commonly
Zearalenone can be demonstrated in feeds by thin-layer or gas chromatography.

Zearalenone is secreted into milk, if dairy cattle are fed *F. graminearum*-infected cereals and may be of public health concern.
Ochratoxicosis and Citrinin Toxicosis

- Several *Aspergillus* and *Penicillium* species, particularly toxigenic strains of *Aspergillus ochraceus*, *A. alutaceus* and *Penicillium verrucosum* produce ochratoxins.

- Group of related isocoumarin derivatives.

- Ochratoxin A is the principal nephrotoxic mycotoxin in this group.

- Natural production of ochratoxin occurs primarily in spoiled, stored barley, wheat and maize.

- Ochratoxin A is a stable compound which is only partially destroyed by heat processing and autoclaving.
The mycotoxin citrinin, which can also be produced by *A. ochraceus* as well as by *Penicillium citrinum*, *P. viridicatum* and *P. expansum*, is nephrotoxic.

Citrinin, frequently found together with ochratoxin A in affected foodstuffs, can enhance the effects of ochratoxin A.
• Pigs- reduced food intake, loss of body weight, depression, polydipsia & polyuria.

• Poultry- depressed growth rate, coagulopathy and poor-quality eggshells.

• Ruminants, especially adult ruminants, appear to be less susceptible to ochratoxicosis than monogastric animals. The flora of the adult rumen has been shown to degrade ochratoxin A.
Ochratoxins- cross the placental barrier and exert a teratogenic effect, immunosuppressive.

Ochratoxin formation is primarily a grain storage problem and detection of these mycotoxins requires solvent extraction followed by thin-layer chromatography of separated fractions.

Ochratoxins fluoresce yellow-green under ultraviolet light.