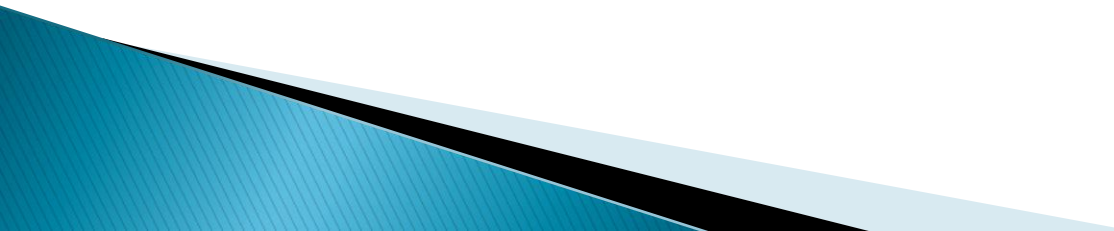


Mycotoxins

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.
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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

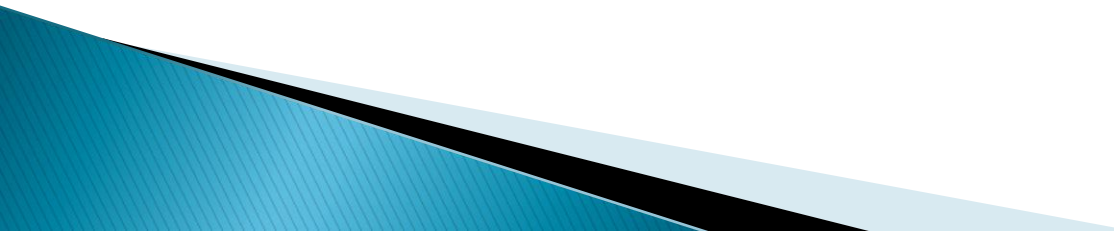
- Storage fungi which invade the grains after harvest during drying and in storage
 - e.g. *Aspergillus*, *Penicillium*



Species	Toxins
<i>A.flavus</i> and <i>A.parasiticus</i>	Aflatoxins
<i>A. ocheraceus</i>	Ochratoxin
<i>Fusarium roseum</i>	Trichothecane (t-2) toxin
<i>Penicillium citrinum</i>	Citrinin
<i>A.nidulans</i> and <i>A.versicolor</i>	Sterigmatocycin

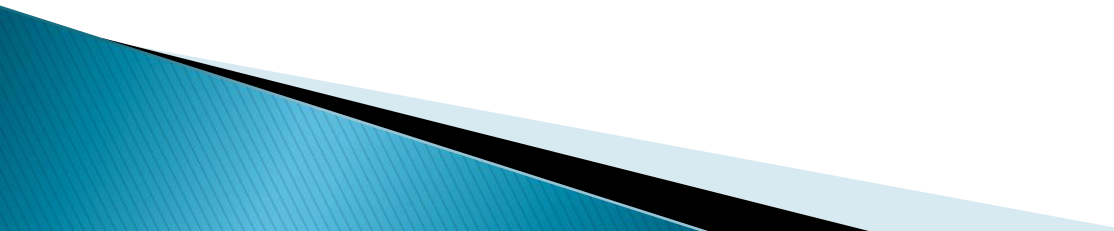
CHARACTERISTICS OF MYCOTOXINS

- ▶ 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.
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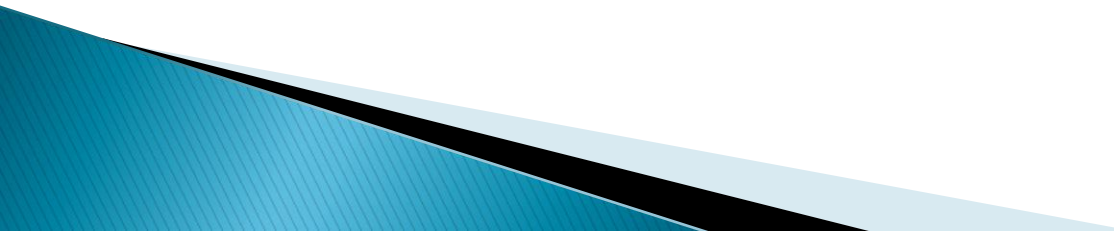
- ▶ They can activate at low concentrations.
 - ▶ Toxic effects- carcinogenic, mutagenic, teratogenic & immunosuppressive.
 - ▶ Acquired immunity does not occur following exposure.
 - ▶ Each toxin affects specific target organs or tissues.
 - ▶ Human exposure may result from excretion in milk or accumulation in food-animal tissues
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Target organs/ tissues	<u>Toxins</u>
Vascular system	Aflatoxins
Digestive system	Aflatoxins
Mucous membrane	Trichothecane (t-2) toxin
Urinary system	Ochratoxin
Reproductive system	Zearalenone (<i>Fusarium</i> toxin)
Cutaneous system	Sporidesmin

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
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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.
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- ▶ 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.

Disease	Fungus	Crop or substrate	Mycotoxin	Animals affected
<u>Aflatoxicosis</u>	<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i>	Ground nut, maize and nut crops	Afaltoxins B1, B2, G1,G2	Cattle, pig, poultry and dogs
Ergotism	<i>Claviceps purpurea</i>	Seed heads of many grasses and grains	Ergotamine and ergometrine	Cattle, Sheep, Pig, Horse and Poultry
Facial Eczema	<i>Pithomyces charatarum</i>	Pasture, litter	Sporidesmin	Sheep and Cattle
Oestrogenism	<i>Fusarium graminearum</i>	Maize, Barley and cereals	Zearalenone	Pigs
Leukoencephalomalacia	<i>Fusarium moniliforme</i>	Maize	Fumonisin B1 (A1, A2, B2)	Horses and Donkey
Trichothecene toxicosis	Many <i>Fusarium</i> species	Cereals	T-2 toxin, diacetoxyscirpenol	Many species
Ochratoxicosis	<i>A. ochraceus</i> <i>P. viridicatum</i>	Barley, wheat and Maize	Ochratoxin -A	Pigs and Poultry

CLINICAL FEATURES OF MYCOTOXICOSIS

- ▶ 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.
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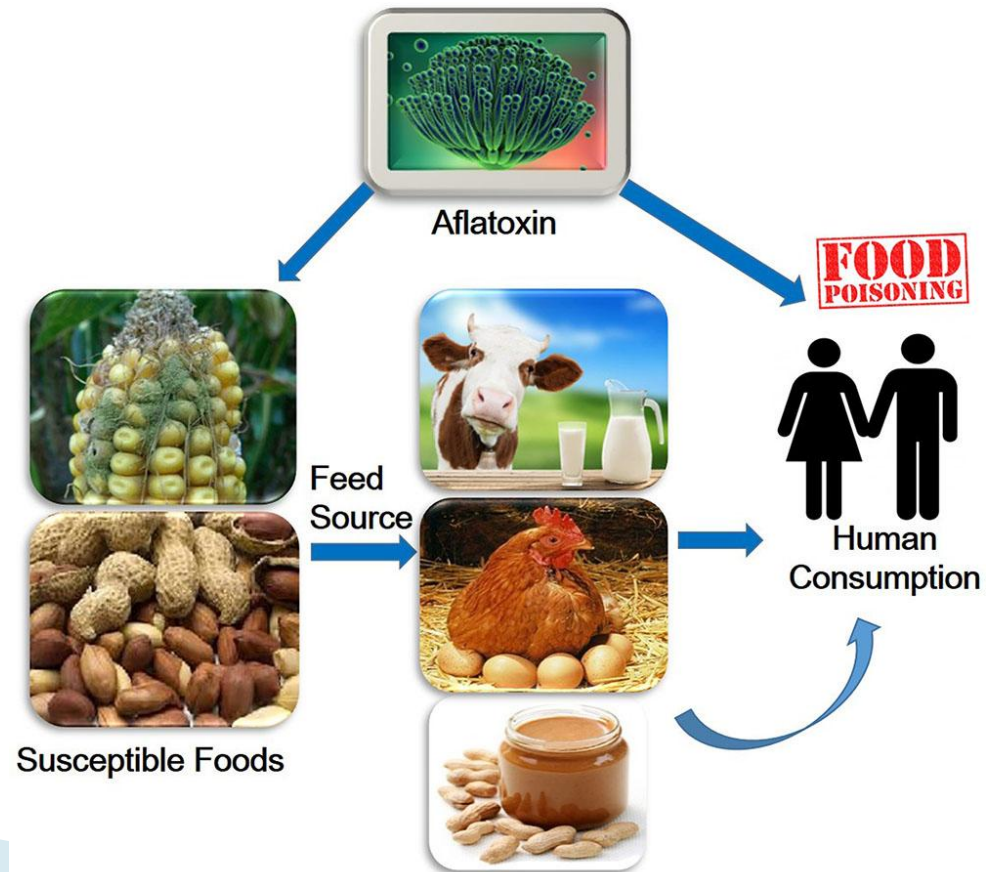
- ▶ Recovery generally depends on the type and amount of mycotoxin ingested and the duration of the exposure to contaminated feed.
 - ▶ The only acceptable evidence for the presence of mycotoxicoses in animals is the laboratory demonstration of mycotoxins in suspected food, or in the tissues, secretions or excretions of affected animals.
 - ▶ Characteristic lesions in target organs of affected animals are important supporting diagnostic evidence.
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AFLATOXICOSIS

- ▶ 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.

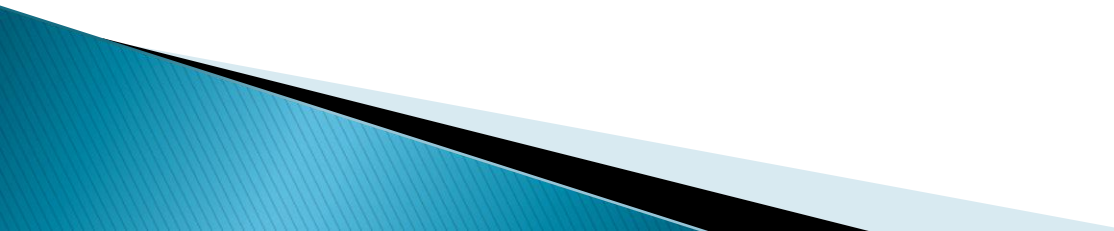


- ▶ High humidity & high temperature during pre-harvesting, harvesting, transportation and storage, as well as damage to feed crops by insects, drought and mechanical injury during harvesting, favours the growth and toxin production of *Aspergillus flavus*.

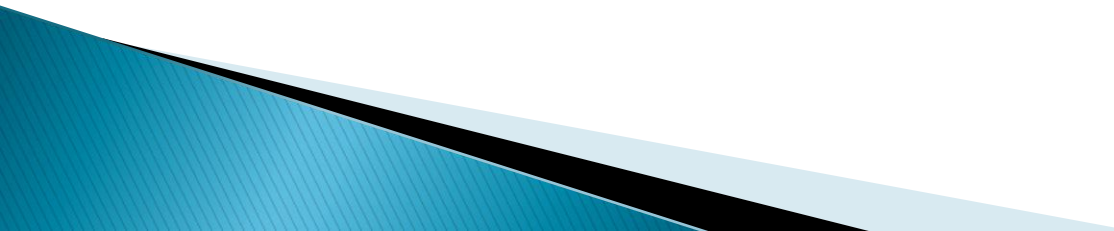


- ▶ Mould growth and toxin formation require a moisture content of the substrate greater than 15%, temp. 25°C and adequate aeration
- ▶ Toxic, carcinogenic, teratogenic and mutagenic activity



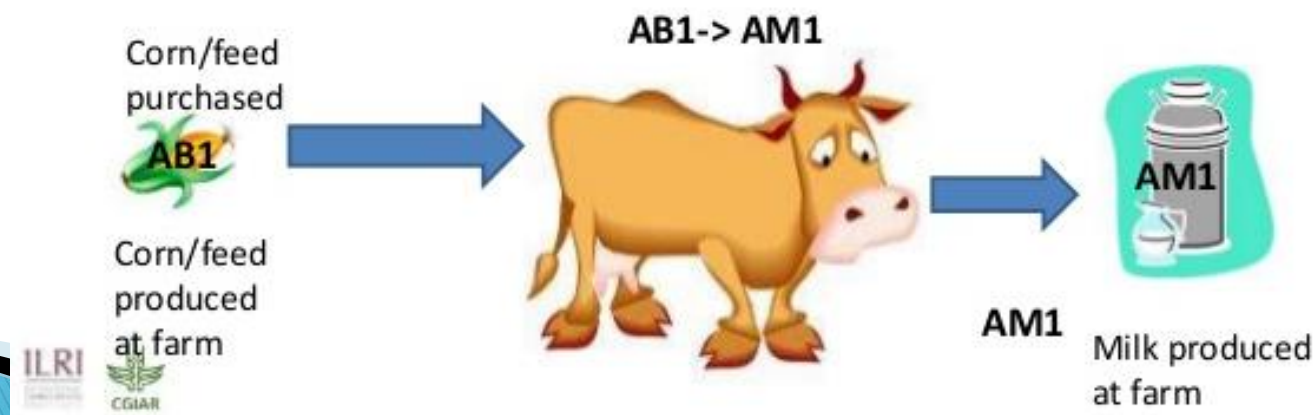
- ▶ The four major aflatoxins are B1, B2, G1 and G2. These mycotoxins are named according to their position and fluorescent colour on thin layer chromatography (TLC).
 - ▶ B1 and B2 produce blue colour and G1, G2 gives green fluorescence.
 - ▶ Aflatoxins M1, M2 are hydroxylated metabolites of B1 and B2 that are excreted in the milk of lactating animals such as dairy cows.
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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.
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PATHOGENECITY

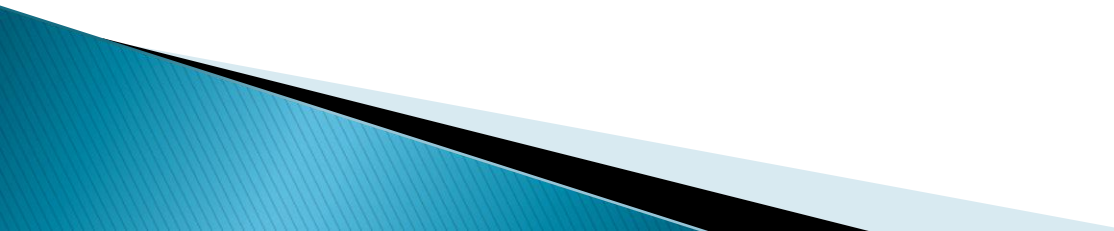
- ▶ **Symptoms**
- ▶ Young animals are highly susceptible.
- ▶ Aflatoxin B1 produce the most hepatogenic, carcinogenic, teratogenic and embryotoxic effects.
- ▶ Calves- blindness, circling, grinding of teeth, diarrhoea, tenesmus & convulsions.
- ▶ Cattle- aflatoxin M1 and M2 are excreted in the milk.



- ▶ 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

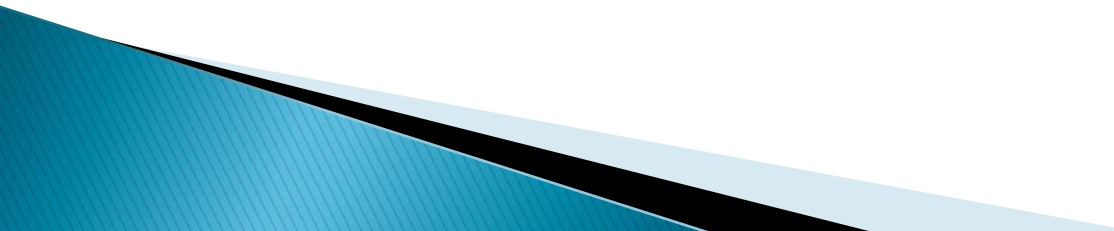
- ▶ Prevention of contamination at all stages of food production, storage and use
- ▶ Decontamination procedures like physical removal and chemical treatment of aflatoxin contaminated feeds such as with acids, alkalies, aldehydes, oxidizing agents of selected gases (ammonia)
- ▶ High affinity inorganic 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.
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- ▶ 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

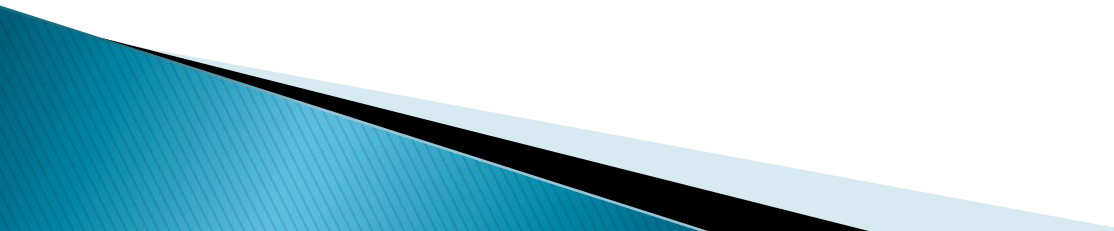
- ▶ Gangrenous ergotism - Gangrenous necrosis of the extremities – nose, ears, tail, teats & limbs
- ▶ Cattle grazing on ergotized pasture or fed contaminated grain or silage develop lameness and gangrene as a major clinical sign of ergot toxicity.



**Ergotism in a cow:
a swollen right hind
leg**

showing a line of
separation and terminal
gangrene.

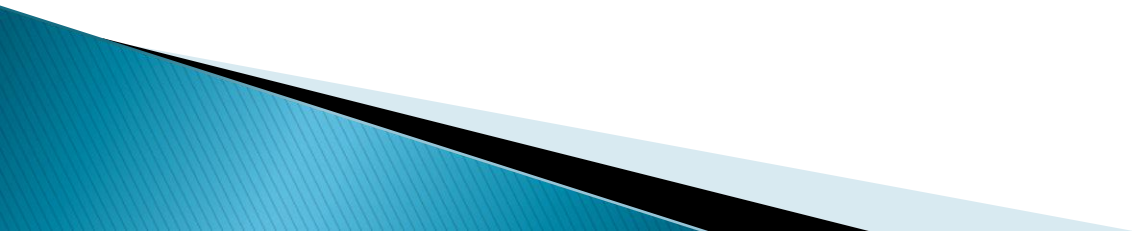
The left hind limb is
unaffected

- ▶ 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
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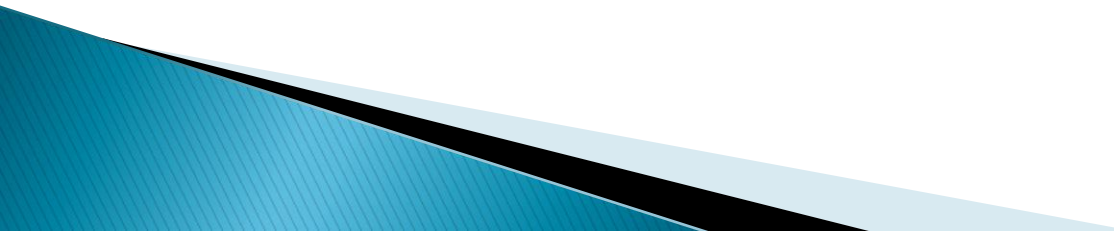
Diagnosis

- ▶ 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
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Fusarium Toxicoses

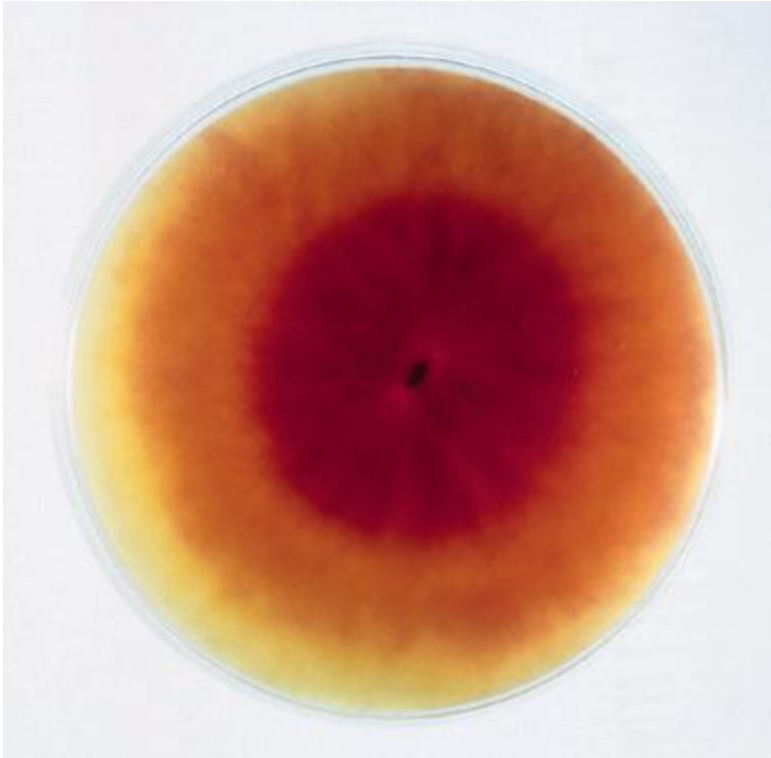
- ▶ The genus *Fusarium* is the largest single group of fungi with known toxigenic capability.
 - ▶ Because of their close association with plants and their relatively high water activity requirements for growth, fusaria are usually well established in a crop before harvesting and may cause many problems in cereals following a late harvest after a wet summer.
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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, $\times 400$)**

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.
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- ▶ 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.
- 