

Toxic Mold and Mycotoxins

Food Toxicology

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Learning Objectives

- Understand the relationship between mold growth, their potential mycotoxins, and disease.
- Explore the environmental conditions for mold growth.
- Understand the major species of toxic molds and their disease endpoints.
- Review the route of exposure of mycotoxins, general pharmacologic effects and clinical disease.
- Discuss some recent mycotoxin outbreaks.

Fungi and Mycotoxins

- > 100,000 species of fungi
- Mycotoxins: Substances produced by fungi that are harmful to animals and humans
- > 300 mycotoxins isolated
 - ~ 30 well-characterized and considered harmful to animals and humans (more?)

Mold Growth → Mycotoxin Production

- Substrate (plant) specific
- Environmental:
(field and storage)
 - Temperature
 - Humidity
 - Moisture
 - Oxygen
- Crop damage:
 - Parasites
 - Drought
 - Pesticides

Mycotoxin Observations

- Not all moldy feeds/foods contain mycotoxins
- Not all feeds/foods containing mycotoxins are 'toxic'
- Feed/food does not have to look moldy to be contaminated
- May not be uniformly distributed

Toxic Mold Disease Endpoints

Toxic Molds

Mycotoxins

Stachybotrys

Stachybotrys

Stachybotrys

Stachybotryotoxicosis

Stachybotryotoxicosis

Toxins from *S. chartarum*

Stachybotryotoxicosis

Coccidioides

Coccidioidomycosis

Coccidioidomycosis: The Americas

Coccidioidomycosis - Symptoms

Claviceps

- *Claviceps purpurea, paspalli*
- Grows in wet and overwintered grains: rye, barley, wheat
- Sclerotia or "ergots"
 - Hard-packed mycelium
- "Ergotism"
 - Gangrene and/or convulsions and gastrointestinal symptoms
 - Livestock: decreased weight gains, milk production, reproductive efficiency

Ergotamine

How Ergot Exposure Occurs

- *Claviceps purpurea* – soil
- Spores released when grain flowers

- Land on stigma – germinate – hyphae extend into the ovary
- Replaces the ovary – hardens ergot body or sclerotium; recycle

How Ergot Exposure Occurs

- Seeds and sclerotia harvested together
- Screening techniques to remove the ergot based on size and weight
- Exposure occurs by ingesting grain / food

Ergot Toxins

- Ergot alkaloids - sclerotia
 - Ergonovine
 - Ergovaline
 - Ergosine
 - Ergocristine
 - Ergotamine
 - Medicinally for vascular migraine, postpartum uterine hemorrhage in abortions
- Types and concentrations of alkaloids vary

Ergot Pharmacological Effects

- Vasoconstriction
 - Gangrene
- Serotonin agonist
 - Neurological effects
- Dopaminergic agonist
 - Agalactia

Ergot Clinical Diseases

- Vasoconstriction → gangrene (toes, fingers, ears, worse) – ‘frostbite’
 - Epidemics of gangrenous limbs / 40,000 people in France died (944 AD)
 - Pain, swelling, numbness
 - Necrotic tissue
 - Death
 - Abortion

Ergot Clinical Diseases

- Action levels set for ergot in grains
- Neurological / tremorgenic / convulsive form
 - Tingling (ants crawling under the skin)

- Itching
- Numbness
- Twitching
- Spasms
- Seizures

Fusarium

- *F. sporotrichioides* and *graminearum*
- Corn, wheat, barley
- Veterinary and public health concerns
- Major toxins:
 - Tricothecenes, zearalenone, fumonisin

Fusarium Mycotoxins

- Tricothecenes, Zearalenone, Fumonisin

Tricothecenes

- Sesquiterpenoid tetracyclic compounds

Tricothecenes Metabolism of T-2 Toxin T-2 Toxin: Animal Toxicity

- Digestive disorders:
 - Feed refusal, vomiting, bloody diarrhea, intestinal inflammation
- Hemorrhage
 - Stomach, heart, intestines, lung, bladder, kidney
- Edema
- Oral lesions

- Blood disorders
- Immunotoxic
- The oral LD₅₀ of T-2 toxin in animals ranges from 3 to 5 mg/kg, and the DRC is very steep.

T-2 Dermal Toxicity

T-2 Toxin: Human Toxicity

- Dermal exposure: local cutaneous necrosis and inflammation
- Oral exposure: lesions to the upper gastrointestinal tract (ATA)
 - Because of the lipophilic nature of trichothecenes, they are rapidly and completely absorbed from the GIT and quickly distributed to all major organs.
 - The mechanism by which T-2 toxin causes cell death is ribosomal binding and inhibition of protein synthesis.
- Ocular exposure: corneal injury.

Alimentary Toxic Aleukia Toxicosis (ATA)

- **First stage:** immediately or several days after consumption of grain products contaminated with trichothecene mycotoxins.
 - Inflammation of the gastric and intestinal mucosa causes vomiting, diarrhea, and abdominal pain. In most cases, excessive salivation, headache, dizziness, weakness, fatigue, and tachycardia accompany this stage, and fever and sweating may also be present.
- **Second stage:** the leukopenic or latent stage—which is characterized by leukopenia, granulopenia, and progressive lymphocytosis.
 - When the ingestion of the toxin-contaminated food is not interrupted or if large doses are consumed, the next stage develops.
- **Third stage:** Characterized by the appearance of a bright red, or dark cherry-red, petechial rash on the skin of the chest and other areas of the body.
 - At first, the petechiae are localized in small areas, but they then spread and become more numerous. In the most severe cases, intensive ulceration and gangrenous processes develop in the larynx, leading to aphonia and death by strangulation. At the same time, affected individuals have severe hemorrhagic diathesis of the nasal, oral, gastric, and intestinal mucosa.
- **Fourth stage:** The necrotic lesions heal and the body temperature falls; the recovery stage begins.
 - During this period, exposed patients are susceptible to various secondary infections, including pneumonia. Convalescence is prolonged and can last for several weeks. Usually, 2 months or more are required for the blood-forming capacity of the bone marrow to return to normal.

Zearalenone (ZEN)

- *F. graminearum* and *F. sporotrichioides*
- Corn, wheat, barley, oats, sorghum, hay
- High humidity, low temperature
 - Autumn harvest in upper Midwest US
- Often coincident with T-2

Zearalenone: Animal Toxicity

- Swine (>0.1 ppm): estrogenic effects

- Vulvovaginitis, swollen mammae
- Swine (50-100 ppm): decreased reproduction
 - Cycling, conception, ovulation, implantation
- Boars (>0.1 ppm): feminization
 - Testicular atrophy, enlarged nipples
- Cows: decreased conception rates

Zearalenone: Mechanism of Action

- Binds to estrogen receptor
- ZEN binding affinity
 - Equivalent with 17 β estradiol
 - Less than estradiol
- Estrogen receptor affinity
 - Swine > rat > chicken

Fumonisin

- *F. moniliforme* (universal in corn)
 - Corn, wheat, barley, oats, sorghum, hay
- High humidity, low temperature
 - Autumn harvest in upper Midwest US
- Often coincident with T-2
- Horses, pigs most susceptible
- FB₁ most toxic

Fumonisin B1: Animal Toxicity

- Neurotoxicity: Equine leukoencephalomalacia (ELEM)
 - “Moldy corn toxicosis”
 - Rapid onset (few hours)
 - Feed and water refusal, lameness, ataxia, paralysis
 - Severe cerebral edema, focal malacia (softening), liquefaction of white matter
- Pulmonary Edema: Porcine pulmonary edema syndrome (PPE)
 - Hydrothorax and lung edema
 - Usually fatal
- Liver cancer and liver toxicity

Fumonisin B1: Animal Toxicity

- Horses (1-126 ppm):
 - Fatal ELEM
 - Liver toxicity at higher doses
- Swine (<1 – 5 ppm) :
 - Low dose: hepatic toxicity

- High dose: acute pulmonary edema, hepatic toxicity
- Sheep:
 - Nephritis

Fumonisin Concern for Human Health

- Milk residues?
- Meat residues?
- 1996: 89% of corn grown in 3 areas of Costa Rica were contaminated with fumonisin

Fumonisin Carcinogenic Potential

- Carcinogen / promoter
- Esophageal cancer
 - South Africa, Italy, China, South Carolina
- Corn: staple, home brewed beer, moonshine, polenta

Aspergillus

- Dietary carcinogen

Aflatoxin B1

- From **Aspergillus flavus**
 - Universal food contaminant
 - Corn, peanuts, wheat, rice, etc.
- Animal carcinogen - 5 ppb
- FDA action level - 20 ppb

Aflatoxin B1

- Human liver carcinogen
 - Binds to N⁷ Gua; DNA-adduct
 - G → T in p53 codon 249
- Lung cancer risk
 - Respirable grain dusts > 4000 ppb AFB1
- Problem in food industry and grain handling
 - Harvesting
 - Transport
 - Storage
 - Processing
- Requires bioactivation

Cytochrome P450 (CYP) Reaction Sequence

AFB1 Activation: Enzymology

Aflatoxin B1 Metabolic Pathways

Aflatoxin: Etiological Role in Disease

- Indian childhood cirrhosis
- Hepatotoxicity
- Immunosuppression
- Kwashiorkor
- Carcinogenesis

Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
 - Outbreak of jaundice, liver failure
 - High fatality rate
 - 317 reported cases and 125 reported deaths
 - Consumption of maize – visibly discolored or moldy

Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
 - Range: 20 to 8,000 ppb
 - Widespread
 - Maize harvested when wet
 - Food shortage
 - Education needs
 - Harvesting, drying, storing