Mycotoxins

What are they?
Where do they occur?
What do they do?
Who makes them?
What is risk to food supply?
Important mycotoxins

Aflatoxins
Ergot alkaloids
Trichothecenes
Zearalenones
Fumonisins
Patulin
Ochratoxin

Mycotoxin producing fungi

Aspergillus - aflatoxin, ochratoxin
Penicillium - ochratoxin, patulin
Fusarium - trichothecenes, zearalenones
Stachybotrys - trichothecenes
Alternaria - alternariol, tenuazonic acid
Claviceps - ergotamine, ergovaline
Trichoderma - trichothecenes
Myrothecium - trichothecenes
Pithomyces - sporidesmin

Ascomycetes: Hypocreales, Eurotiales, Dothideales
**Mycotoxicosis**: poisoning caused by fungal toxins in foods

**Mycotoxins** are produced by some of the most common and widespread fungi in the environment. Function, presumed to deter fungivory by mammals, insects or other fungi, or to allow fungi to compete with animals and microbes for the substrate.

**Mycosis, mycotic infection**: disease caused by fungal infection. Diseases caused by fungal toxins will be dealt with separately from infectious diseases caused by fungi.
**Mycotoxin**: low molecular weight fungal metabolite capable of causing toxic responses in humans and animals. Chemistry varies.

**Mycotoxin effects**
- animal growth (weight gain)
- animal products (e.g. eggs, milk, meat)
- reproductive disorders
- mortality

**In Humans:**
- acute mycotoxicosis
- cancers
- diseases, nutritional disorders

**Mycotoxin symptoms**
- high concentrations: acute disease, death
  - organs: kidney, liver, stomach, skin, brain, lung
- low concentrations: carcinogenic, mutagenic, teratogenic; nutritional and reproductive disorders.
Mycotoxins in food supply

Estimated that 25% of world’s crops are contaminated with mycotoxins. Chronic exposures in populations of some developing countries.

In U.S. most common crops with mycotoxins are: peanuts, cornmeal, cottonseed, pecans, hay. Mycotoxins also may occur in meat, eggs, dairy products.

Aflatoxins are monitored in several commodities by USDA. Other mycotoxins are not regulated in the USA but some are regulated by the EU, so some US exports are monitored.

Allowed aflatoxin levels (USA) vary from 300 µg/kg in animal feed to 0.5 µg/kg in milk for human consumption.
Mycotoxins are a current, continuing, chronic problem for world food producers

Mycotoxins are monitored by regulatory agencies, but nobody is perfect

Dog Deaths Surpass 100 Despite Toxic Pet Food Recall

The Food and Drug Administration has reportedly determined that a pet food company improperly tested or failed to test corn shipments for a deadly fungus.

The State newspaper in Columbia, South Carolina, says the FDA found Diamond Pet Foods allowed the tainted corn into a plant and didn't properly test for the naturally occurring poison.

An FDA investigator says the agency started the investigation after the company recalled about one million pounds of dry dog food in December.

The newspaper says the FDA report due out this week doesn't penalize the company.
April 2013

Recalls of Aflatoxin-Contaminated Dog Food Have Begun

Reuters reports high levels of aflatoxins have been discovered in bags of dog food on store shelves in Iowa. And according to Michael Wright, the CEO of Pro-Pet, a pet food company in Ohio that recently learned some of its product was contaminated with aflatoxins, “Last year’s corn crop – it’s a huge issue. We test every load coming in. And we reject a lot of loads.”

During the last week of February, the Hy-Vee Inc. grocery chain was forced to recall five different products in its private dog food line due to high levels of aflatoxins in the corn used in the formulas. The dog food was produced at a Kansas City Pro-Pet plant and distributed across eight Midwestern states.

“This year the toxin was much more prevalent. According to crop insurance data from the U.S. Department of Agriculture, payouts for mycotoxins, of which aflatoxin is the most common, totaled nearly $75 million, triple the level of a year ago.

Nearly 85 percent of the claims were filed in six states: Arkansas, Illinois, Indiana, Kansas, Mississippi and Missouri.”

Reuters Feb 2013
River Run and Marksman Dog Food: Recalled Product List

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Cargill has announced a voluntary recall of the following products in 13 states and 2 territories: Kansas, Missouri, Northeast Oklahoma, Arkansas, Louisiana, Mississippi, Tennessee, Western Kentucky, Southeast Indiana, Southern Illinois, Hawaii, and limited areas of Florida, California Guam, and the Virgin Islands. The products may contain higher-than-acceptable levels of aflatoxin, a substance found widely in nature as a result of
More and more cattle producers are using fall and winter grazing to extend the grazing season, and for good reason. This management practice is a cost-effective option that reduces labour requirements and can keep livestock out of the corrals until just before calving season. Maximizing forage yield while maintaining forage quality is the goal for most producers. However, one area that is commonly overlooked is that of plant disease and the effects that mycotoxins can have on grazing livestock.

Crop disease is largely dependent on the weather conditions and for that reason it is very hard to manage. Cool and moist conditions when cereal crops were flowering has resulted in an increase of crop disease, particularly fusarium and ergot. Mycotoxins can be quite harmful to livestock, and while ruminants are generally more tolerant to mycotoxins than other livestock species, they are still at risk.
An elevated level of the mycotoxin, ochratoxin A, was detected in one batch of Girolomoni pasta. The batch is being recalled and point of sale notices are being displayed in the shops supplied. Consumers are advised not to eat the implicated pasta.
Eliminating Aflatoxin Goal Of Kenya Program

Jeff Caldwell
10/06/2014 @ 9:34am
Multimedia Editor for Agriculture.com and Successful Farming magazine.

Aflatoxin is a problem in some corn fields in the U.S., where infection of the fungus typically means either additional storage costs or occasional refusal of loads of the grain at the local elevator.

In Kenya, though, the crop disease means a lot more. Corn is grown more for human consumption there, and aflatoxin can cause serious illness and death if enough is consumed. The U.S. Centers for Disease Control (CDC) considers aflatoxin a "public health problem" in Kenya and when the disease started causing broader-scale health problems and fatalities about a decade ago, officials there said finding and constructing "culturally appropriate storage methods for dry maize" would be of utmost importance in stemming the disease and saving lives.
Mycotoxin contamination of food/grain

May develop/increase during storage but not necessarily caused by storage

Mycotoxins naturally occurring in various grain crops, develop during crop ripening

Not necessarily associated with visible mold
<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Products affected</th>
<th>Animals affected</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Corn, peanuts, cottonseed, tree nuts, dairy products</td>
<td>Swine, dogs, cats, cattle, sheep, birds, humans</td>
<td>Liver damage, intestinal bleeding, cancer</td>
</tr>
<tr>
<td>Ergot alkaloids</td>
<td>Rye, sorghum, pasture grasses</td>
<td>Cattle, sheep, humans</td>
<td>Hallucinations, gangrene, loss of limbs, hastening of birth</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>Corn, silage</td>
<td>Horses, swine, humans</td>
<td>Pulmonary edema, leukoencephalomalacia, esophageal cancer, neural tube defects, liver damage</td>
</tr>
<tr>
<td>Ochratoxins</td>
<td>Cereal grains, coffee, grapes</td>
<td>Swine, humans</td>
<td>Kidney and liver damage, cancer</td>
</tr>
<tr>
<td>Trichothecenes</td>
<td>Wheat, barley, oats, corn</td>
<td>Swine, dairy cattle, poultry, horses, humans</td>
<td>Feed refusal, diarrhea, vomiting, skin disorders, reduced growth</td>
</tr>
<tr>
<td>Zearalenones</td>
<td>Corn, hay</td>
<td>Swine, dairy cattle</td>
<td>Enlargement of uterus, abortion, reproductive issues</td>
</tr>
</tbody>
</table>
Ergotism, ergot alkaloids, *Claviceps purpurea*

The Beggars, Pieter Brueghel (the elder), 1568
Ergot alkaloids

Clavine alkaloids
Ergotamine, ergovaline
indole alkaloids, e.g LSD
peptide alkaloids

August 1951, Pont St Esprit, France
mass food poisoning linked to a single bakery
burning, chills, violent pain, hallucinations, seizures,
convulsions, due to grain contaminated by Claviceps purpurea

Ergotism
poisoning due to C. purpurea, “ergot”
numerous historic accounts thought to be related to ergotism
gangrenous ergotism, convulsive ergotism
REPORT ON AN OUTBREAK OF ALLEGED ERGOT POISONING BY RYE BREAD IN MANCHESTER.

By M. T. Morgan, M.C., M.D.

(Medical Officer of the Ministry of Health.)

Ashby and Robertson have recently given an account of an outbreak of illness in Manchester which had come under their notice and which they attributed to the consumption of rye bread made from rye meal contaminated with ergot.

Nature of the illness.

On reference to Robertson's and Ashby's article it appears that the first symptoms observed in the patients (all of whom were Jews) were coldness and numbness in the extremities; tailors, button-makers, etc., noticed their fingers felt numb and they experienced difficulty in keeping up with their work. Sensation was also impaired; they could prick their fingers without feeling anything. Formication was a very typical symptom in all well-defined cases, and many of the patients stated that they experienced a sensation as if an insect was creeping over or under their skin. Itching was also a common symptom. Nervousness, depression, headaches and abdominal pains were frequent, and in the more severe cases ataxia with a staggering gait occurred. In most cases of long standing, the blood pressure was definitely raised, for example, a woman aged 44 had a systolic blood pressure of 174 mm.
Sclerotia of *Claviceps purpurea* contain several alkaloid mycotoxins, probably as a deterrent to herbivory by rodents. Sclerotia easily contaminate grain at harvest, alkaloids contaminate flour produced from contaminated grain.

Sclerotia spur-like, ergot Fr. for spur

Affects major grain crops: rye, wheat, barley, oats
Sclerotia germinate to produce a stroma.
C. purpurea alkaloids

Symptoms of ergotism: gangrenous form, convulsive form

Ergotamine, vasoconstrictor, can limit blood circulation to digits, limbs. Gangrenous ergotism results. Blood starved limbs begin to decay and eventually drop off.

Sensation of intense heat, “St. Anthony’s fire”

Cases of gangrenous ergotism reported in late 1970s in Africa and India

Ergonovine, a second alkaloid in ergot, causes spontaneous abortions in humans and livestock that consume contaminated grain
C. purpurea alkaloids

Ergine, lysergic acid hydroxyethylamide (LSD)

Cause of “convulsive ergotism”: tremors, hallucination, sensation of ants crawling on skin (formication), seizures.

Much documented evidence of ergotism in central Europe during the middle ages, consumption of ergotized grain more prevalent among the poor.

Germany 857: “A great plague of swollen blisters…limbs were loosened and fell off before death.”

France 944: “A plague of invisible fire…cutting off limbs from the body…the stench of rotten flesh…”
Epidemics of ergotism from 800 – 1800 were preceded by weather conducive to infection of plants by *C. purpurea*, cool, moist spring caused prolonged flowering and increased infections. If harsh winter followed, grains supplies would be depleted and lead to use of contaminated grain.

Witchcraft associated with people suffering from ergotism. Accused witches in the Salem trials of 1692 exhibited classic symptoms of convulsive ergotism. Also contemporaneous symptoms in livestock. Geographic distribution of witchcraft trials in Europe 1500 – 1700 occurred in places where rye was a major food source and where conditions were favorable for *C. purpurea*. 
Pharmaceutical uses of ergot alkaloids
Extracts from boiled sclerotia administered to induce labor 1750-1950. Also administered postdelivery to reduce hemorrhaging.
Ergotamine used to treat migraine and cluster headache

LSD psychoactive drug discovered in 1933 by A. Hoffmann
amide derivative of ergot alkaloid lysergic acid
Investigated for potential therapeutic potential
Also for potential “mind control agent” by CIA and chemical warfare agent

The rest is history!
*Epichloe typhina* (“endophyte”) is a related fungus that causes “choke disease” of grasses. Infected grass plants contain various alkaloids that are suppressive to insect herbivores, also to mammalian herbivores.
symptoms of endophyte toxicosis

“sleepy grass” (sw USA) and “dunken horse grass” (China) named for their narcotic effects on grazing animals, caused by fungal endophytes
Inhibition of herbivory by pests makes endophyte infected grass preferred for lawns and golf courses.

sod webworm

bluegrass billbug
Effects of endophyte alkaloids on mammals:
elevated body temperature
reduced feeding
reduced fertility
reduced lactation
spontaneous abortion
vasoconstriction
gangrene
tremors (ryegrass staggers)
death

Benefits to grass host
insect feeding deterrence
drought resistance
vigor
disease resistance
Mycotoxins produced by *Fusarium*

Fusarium wheat scab  F. graminearum
Mycotoxins produced by Fusarium species

Trichothecenes
150 structurally related compounds
Fusarium, Trichoderma, Stachybotrys, Cephalosporium,
Primary human food source: *Fusarium graminearum*
  deoxynivalenol (DON, vomitoxin)
  T-2 toxin
  nivalenol

Fumonisins
*Fusarium verticilloides* (*F. moniliforme*)
  equine leucoencephalomalacia
  esophageal cancer

Zearelenones
*Fusarium graminearum*
  Estrogen-mimic
  Swine estrogenic syndrome
Trichothecenes and Fumonisins

Alimentary toxic aleukia, T-2 toxin
1932, 1944 central Asia, Russia, Kazakhstan
Skin rash, severe GI irritation, often fatal
Symptoms similar to viral hemorrhagic fevers (ebola)
but not contagious
Multiple subcutaneous hemorrhage, bleeding nose
and throat
Inflamed throat causes death by strangulation
Correlated with consumption of grain (wheat, millet) left
in fields over winter and consumed in spring
Fusarium sporotrichoides, F. poae, F. tricinctum
disease reproduced by feeding infected grain to
animals
T-2 toxin effects

Found in corn, wheat, barley, oats, rice, millet, rye
Inhibits protein synthesis
Inhibits DNA/RNA synthesis
Affects actively dividing cells such as GI tract lining
Immunosuppressive

Not regulated by FDA or EU
Vomitoxin, Deoxynivalenol DON

Akakabi-byo (red mold disease)
1933, 1950 - 1970 Japan
Disease associated with grains (wheat, barley)
contaminated by red mold
Severe GI irritation, vomiting, hemorrhage, headache, hallucinations. Rarely fatal.
“drunken bread” made with contaminated grain
*F. graminearum*, *F. kyushuense* implicated
several toxins, deoxynivalanol (DON), nivalenol

DON is heat stable, survives cooking
Found in Canadian barley and malt
brewers use 0.5 ppm as general threshold, but may use up to 2-5 ppm

Also found in dry dog and cat food
Swine feed refusal

1928, 1972 Central USA. Linked to *F. graminearum* contaminated grain (barley)

1965, 1972 weather caused severe maize ear rot
contaminated maize used for swine feed

Swine vomited, refused feed, weight loss

Deoxynivalenol (DON), vomitoxin
Fusarium graminearum
Yellow rain
During Vietnam war, symptoms of chemical poisoning in Vietnam, Cambodia, Laos

Chemical warfare of natural contamination?
Controversial: evidence for both

1981 Secretary of State Alexander Haig alleged that the Soviet Union and its allies were using chemical weapons in Laos, Kampuchea, Afghanistan. Claimed mycotoxins found in samples smuggled out.

Trichothecenes are highly stable, toxic at low doses and ideal for weaponization

T-2 toxin, DON, nivalenol found in samples and also in blood and urine of victims, but:

Fusarium spp rare in SE Asia, natural source not known
Low amounts of mycotoxins found in environmental samples
Yellow spots contained various types of pollen from local plants and were deposited by cleansing flights of Asian honeybees
No cases of toxic ‘rain’ reported since 1980s
Generalized structure of the trichothecene ring, with epoxide at C-12

Toxicity due to inhibition of protein synthesis, bind directly to ribosomes

<table>
<thead>
<tr>
<th>Trichothecene</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
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<tbody>
<tr>
<td>T-2 Toxin</td>
<td>-OH</td>
<td>-OCOCH₃</td>
<td>-OCOCH₃</td>
<td>-H</td>
<td>-OCOCH₂CH(CH₃)₂</td>
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<tr>
<td>HT-2 Toxin</td>
<td>-OH</td>
<td>-OH</td>
<td>-OCOCH₃</td>
<td>-H</td>
<td>-OCOCH₂CH(CH₃)₂</td>
</tr>
<tr>
<td>4,15-Diacetoxyseconidol (DAS, also called anguidine)</td>
<td>-OH</td>
<td>-OCOCH₃</td>
<td>-OCOCH₃</td>
<td>-H</td>
<td>-H</td>
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<tr>
<td>Nivalenol</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
<td>=O</td>
</tr>
<tr>
<td>Deoxynivalenol (DON)</td>
<td>-OH</td>
<td>-H</td>
<td>-OH</td>
<td>-OH</td>
<td>=O</td>
</tr>
<tr>
<td>Macro cyclic</td>
<td>-H</td>
<td>-O-R’-O-</td>
<td>-H</td>
<td>-H</td>
<td></td>
</tr>
</tbody>
</table>

Occurrence of trichothecenes in food

DON can contaminate barley and malt, and survive the brewing process
1 ug per kg body weight considered the max tolerable daily intake
70kg person consume 3.4L of beer with 0.05 mg/ml
1994 survey of Canadian beer > 1 ug/L in over 50% of samples

Trichothecene mycotoxins occur in cereal grains worldwide.
wheat, barley, sorhum, millet
DON most common trichothecene worldwide
T-2 toxin, nivalenol more common in Europe than NA

FDA advisory levels for DON:
1 ppm (1 mg/L) in finished products for human consumption
5 ppm for swine feed, <20% of diet
10 ppm for cattle, chicken, < 50% of diet
Fumonisins, *Fusarium verticilloides* (*moniliforme*)

Equine leucoencephalomalacia, “hole in the head”
- 1900 USA, disease associated with moldy grain (corn, maize)
- horses particularly affected, liquified brain matter
- *Fusarium verticilloides* (*moniliforme*) implicated (1904)
- 1981 disease reproduced by feeding *F. verticilliodes*
- 1989 fumonisin B1 characterized, shown to reproduce symptoms

consumption of contaminated feed causes liquification of the brain in horses

Swine pulmonary edema
- 1989 USA, hot dry summer, epidemic death of hogs
- fluid in lungs
- associated with feed of maize “screenings”, low grade grain
- disease reproduced by feeding fumonisin B1
Esophageal cancer clusters
1955-1990, Transkei region of S Africa
maize consumption by poor, rural population
epidemiological evidence links consumption of *F. verticilliodes* contaminated maize
Higher incidence of contamination correlated with
greater incidence of esophageal cancer
Evidence circumstantial at this point

Neural tube defects
1989 Texas-Mexico border region, Rio Grande valley
hot summer, high fumonisin levels in maize
also concurrent unusually high incidence of equine
leucoencephalomalacia
High incidence of neural tube defects in infants, folic
acid deficiency, cluster in Mexican-Americans, maize diet
fumonisins block folate uptake
Fumonisin metabolism and effects

Interferes with sphingolipid metabolism
Interferes with folate (vitamin B9) uptake
Accumulate in kidney and liver tissue
Different effects in different animals (e.g. equine leucoencephalay in horses, pulmonary edema in swine)
Carcinogenic (liver), esophageal cancer in humans (?)
Neural tube defects in humans (?)

Fumonisins are only produced by Fusarium species \textit{F. verticillioides}, very common and abundant in maize

FDA has ‘guidelines’:
  2 ppm in cornmeal, 3 ppm in popcorn
Zearalenones, Fusarium graminearum

Swine estrogenic syndrome
1950-1960 USA central states
moldy grain used as feed for swine
vomiting, feed refusal (DON), also reproductive system disorder in females
enlarged mammary glands, atrophy of ovaries, infertility, reduced litters, reduced weight
Also in males enlarged mammary, atrophy of testes
symptoms reproduced in mice fed F. graminearum Zearalenone identified as an estrogenic substance in contaminated maize
Since reported in S. Africa, Europe, N. America, S. America
cattle, swine affected
contaminated barley and malt, survives brewing