Mycotoxins in animal feed: Challenges, prevention and treatment

IGP feed short course
March-2012

Lakshmikantha Channaiah, Ph.D
The Grain & Feed Microbiology and Toxicology Research Laboratory
Department of Grain Science and Industry
Kansas State University
Manhattan, Kansas
The issue of mycotoxins with feed industries
Mycotoxins in feed products

- World population growth demanding more meat means more grain and feed
- Federal agencies and global customers demanding safe and better quality feed products
- Mycotoxins are a severe problem in the animal feed industry
- **Raw-ingredients** (cereal grains) are the #1 source of contamination
- Other major source of contaminants are air and insects
- Poor sanitary condition in feed mills may favor mold growth
- Storage conditions of raw-ingredients and finished feed products may favor mold growth and subsequent mycotoxin synthesis
Why are molds and mycotoxins important?

- Molds are important as spoilage organisms
- Cause diseases in humans, livestock animals, crops, fruits and vegetables
- Molds spoil grain, feed & food products
- Molds can produce mycotoxins
Molds and mycotoxins are ubiquitous in nature
The life cycle of fungi

- Fungal spore
- Germination of spore
- Fruiting body with spores

Image source: http://www.visualsunlimited.com
Importance of mycotoxins

- Most molds do not produce mycotoxins
- Over 400 identified mycotoxins
- 30 well-characterized mycotoxins are considered harmful to animals and humans
- Usually ingested in contaminated food
- Most of these are not destroyed in normal cooking conditions
- Usually no treatment for mycotoxin poisoning (mycotoxicosis)
Mycotoxins: Important facts

- Mycotoxins are not visible to the eye
- Not all moldy grains/feed/foods contain mycotoxins
- Grain/feed/food does not have to look moldy to be contaminated
- Not all grains/foods containing mycotoxins are ‘toxic’
- Mycotoxins may not be uniformly distributed
- Molds grow over a temperature range of 10 to 40 °C (50 to 104 °F), a pH range of 4 to 8, and above 0.7a_w (water activity)
Importance of mycotoxins

- Mycotoxins are secondary metabolites produced by molds that are capable of causing disease and death in humans and other livestock animals.
- In 1960 approximately 100,000 turkeys died as a result of eating aflatoxin contaminated peanut.
- Worldwide, approximately 25% of crops are affected by mycotoxins with loss of billions of dollars annually.
- Annual economic costs of mycotoxins to the U.S. agricultural economy is estimated to average $1.4 billion.
- Economically important mycotoxins: aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, zearalenone.

http://www.ces.ncsu.edu/disaster/drought/Mycotoxin-Review.pdf
Important mold species and mycotoxins

- **Aflatoxins B1, B2, G1, G2 and cyclopiazonic acid**
  - *Aspergillus falvus* (B1 & B2), *A. parasiticus* (B1, G1 & G2)

- **Trichotheccenes:** Group A (T-2 toxin, HT-2 toxin) and Group B (DON, Nivalenol)
  - *Fusarium/Stachybotrys* spp. and many others

- **Ochratoxin A**
  - *Penicillium/Aspergillus* spp.

- **Fumonisins**
  - *Fusarium verticillioides.*

- **Zearalenone**
  - *Fusarium* spp. and others
FDA action levels for Aflatoxin

Corn; oilseeds; spices; nuts

- **20 ppb**
  - corn, peanut products, cottonseed meal, other animal feeds and feed ingredients
  - dairy animals and when intended use unknown

- **20 ppb**
  - corn, peanut products, other animal feeds and feed ingredients; excluding cottonseed meal
  - immature animals

- **100 ppb**
  - corn, peanut products
  - breeding beef cattle, breeding swine, mature poultry (e.g., laying hens)

Must be reported to FDA and lots are subject to FDA seizure
FDA action levels for Aflatoxin

- **200 ppb**
  - corn, peanut products
  - finishing swine (>100 lb)

- **300 ppb**
  - cottonseed meal
  - beef cattle, swine or poultry (any age and breeding status)

- **300 ppb**
  - corn, peanut products
  - finishing beef cattle (e.g., feedlot cattle)
FDA advisory levels for Deoxynivalenol (Vomitoxin)

- **5 ppm**
  grain and grain byproducts for swine
  not more than 20% of ration

- **10 ppm**
  grain and grain byproducts for cattle and chicken
  not more than 50% of ration

- **5 ppm**
  grain and grain byproducts for all other animal species
  not more than 40% of ration
FDA guidance for Fumonisins (FB₁+FB₂+FB₃) – Human Food

Mostly in corn (maize)

- 2 ppm
degermed dry milled corn products
- 3 ppm
cleaned popcorn
- 4 ppm
cleaned corn for masa
whole or partially degermed dry milled corn products
dry milled corn bran
FDA guidance for Fumonisins (FB₁+FB₂+FB₃) – Animal Feed

Mostly in corn (maize)

- 5 ppm
equids, rabbits
not more than 20% of diet

- 20 ppm
swine, catfish
not more than 50% of diet

- 30 ppm
breeding ruminants, lactating dairy cattle, breeding poultry, breeding minks
not more than 50% of diet
FDA guidance for Fumonisins (FB₁+FB₂+FB₃) – Animal Feed

Mostly in corn (maize)

- **60 ppm**
  - ruminants raised for slaughter, mink for pelts
  - not more than 50% of diet

- **100 ppm**
  - poultry raised for slaughter
  - not more than 50% of diet

- **10 ppm**
  - all other species or classes of livestock and pet animals
  - not more than 50% of diet
Ochratoxin A and zearalenone

- No action, guidance or advisory levels for ochratoxin A or zearalenone have been established by the FDA in animal feeds.

- These two mycotoxins are handled on a case-by-case basis.
Mycotoxins in feed products: CHALLENGES
Risks of mycotoxin contaminated feed

- **Farmers and grain elevators**
  - Loss in yield due to mold infection in field, and during storage
  - Low price for the product
  - Management of moldy and mycotoxin contaminated grain
  - Export (EU has strictest mycotoxin standards in the world!)

- **Feed Industry:**
  - Direct loss of feed products/ production loss
  - Recalls
  - Loosing market
  - Export (EU has strictest mycotoxin standards in the world!)

- **Livestock Industry**
  - Feed refusal/ reduced feed intake/ weight loss
  - Affects the total nutrition intake and low performance
  - Reproductive effects - abortion, reduced lactation etc.
  - Mortality

- **For consumers**
  - Vomiting,
  - Fever-Jaundice
  - Cancer and
  - Death
Soybean meal in animal feed

- Soybean meal (SBM) is one of the major protein of choices for feed manufacturers
  - High in crude protein (~50%) content
  - Year round availability
- In the US it contributes to about $15 billion annually
- Soybeans are grown on >73 million acres with an average of 40 bushes per acre and a total production of ~3 billion bushels
- Mycotoxin contamination of soybeans is not considered a significant problem as compared to commodities such as corn, cottonseed, peanuts, barley and other grains
- Common contaminants are T-toxins, DON, zearalenone by Fusarium spp. during storage
Corn in animal feed

- Corn (bran, germ, DG) is one of the predominant source of ingredient in feed products (~50% of US production, ~6 billion bushels)
- Corn is often contaminated with mycotoxins
- High corn price may lead to use of poor quality corn for ethanol conversion
- Corn-based ethanol byproduct: DDGS
- 90% of distillers grains (DG) are used for domestic animal feed (FDA 2006)
- Mycotoxins concentrates up to 3X in DG
- DG is directly sold to livestock producers and feed manufacturing facilities
- Concern of mycotoxins (aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, zearalenone) directly entering the feed chain
Wheat in animal feed

- Wheat middlings (endosperm, bran, germ) and wheat red-dog are an important source of energy, protein, vitamins and minerals.

- *Fusarium* Head Blight causes vomitoxin in wheat: Wet weather occurs during the flowering and grain filling stages of wheat crop development.

- Wheat by-products are used extensively in feeds for a variety of species.

- Concern with direct addition of DON/vomitoxin to animal feed
Cottonseed in animal feed

- Cottonseed meal is one of the important sources of protein used to balance livestock diet

- Often contaminated by aflatoxins

Sorghum by-productd in animal feed

- Milo hominy, or grain sorghum mill feed, is a mixture of grain sorghum bran and germ, and part of the starchy portion of grain sorghum kernels

- Concern with direct addition of aflatoxin and zearalenone to animal feed products
## Mycotoxins and toxin levels

### A Guide to Mycotoxin Levels in Feed: Mild to Severe Disease

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Toxins</th>
<th>No clinical effect</th>
<th>Toxic level</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus sp</td>
<td>Aflatoxins</td>
<td>&lt; 100ppb</td>
<td>300 - 2000ppb</td>
<td>Poor growth, Liver damage, Jaundice, Immunosuppression</td>
</tr>
<tr>
<td>Aspergillus sp and Penicillium sp</td>
<td>Ochratoxin &amp; Citrinin</td>
<td>&lt; 100ppb</td>
<td>200 - 4000ppb</td>
<td>Reduced growth, Thirst, Kidney damage</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>T2 DAS DON (Vomitoxin)</td>
<td>&lt; 2ppm</td>
<td>4 - 20ppm</td>
<td>Reduced feed intake, Immuno-suppression, Vomiting</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>Zearalenone (F2 toxin)</td>
<td>&lt; 0.05ppm</td>
<td>1 - 30ppm</td>
<td>Infertility, Anoestrus, Rectal prolapse, Pseudo pregnancy, Early embryo mortality, Delayed repeat matings</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>Fumonisin</td>
<td>&lt; 10ppm</td>
<td>20 - 175</td>
<td>Reduced feed intake, Respiratory symptoms, Fluid in lungs, Abortion</td>
</tr>
<tr>
<td>Ergot</td>
<td>Ergotoxin</td>
<td>&lt; 0.05%</td>
<td>0.1-1.0% Ergot bodies by weight (sclerotium)</td>
<td>Reduced feed intake, Gangrene of the extremities, Agalactia due to mammary gland failure.</td>
</tr>
</tbody>
</table>

**ppm** - parts per million  
**ppb** - parts per billion  
**sp** - species - each of these fungi have several species only some of which are toxic (Fig.13-4)
Mycotoxins in feed products: **HOW TO PREVENT ?**
Factors favoring mold incidence

- Harvesting
- Mycotoxin
- Biological
- Storage
- Environmental
Biological

- Hybrids (Bt. corn)
- Plant maturity
- Soil moisture
Environmental

- Temperature
- Relative humidity
- Soil moisture
- Nutrients
- Bird damage
- Insect damage
- Irrigation
- Rain
Environmental

- Cool, wet weather
  - *Fusarium* spp.
    - DON/Vomitoxin
    - T-2 toxin (T-2)
    - Zearalenone (F-2)
    - Fumonisin

- Hot, humid weather
  - *Aspergillus* spp.
    - Aflatoxin
Harvesting

- Grain moisture
- Service equipment & clean equipment
- Drying
- Bin cleaning
- Residual grain
Field damaged grain, regardless of reason, should not be mixed with good grain

Reduce grain moisture - it arrests fungal development and mycotoxin

Storage temperatures are critical

- **Winter months:** after drying, temperatures between 34 and 40°F keeps fungal metabolism reduced
- **Summer months:** grain temperature between 55 and 65°F acceptable

Maintain 1-2% lower moisture for field damaged grain (Ex: 13-14% corn instead of typical 15%)

Preparing for storage
- Storage molds grow at moistures of 13-20% or moistures in equilibrium with 65-90% RH.
- Members of the genera *Aspergillus* and *Penicillium*. 
- Check grain twice a week
- Look for crusted, colored, wet, slimy grain on top surface
- Poor flow out of grain bins
- A musty or moldy odor indicates the beginning of the storage problem (fermented smell = serious problem)
- Watch temperature: If temperature of the grain begins to raise (hotspot), the aeration fans should be turned on immediately
Mycotoxins in feed products: CONTROL
Control

- Physical
- Biological &
- Chemical
Physical Methods

- **Cleaning/separation**
  - Effective removal of fines (aflatoxins), broken kernels, chaffy seeds reduces (BKCS) the mycotoxin content in the stored grain

- **Heating/irradiation (ex: peanuts)**
  - Reduces 40-60% of the toxins

- **UV irradiation**
  - Exposure of toxin contaminated milk with UV light reduced AFM1 up to 100% depending on the exposure time
  - Toxicity in peanut was reduced by 75 to 100% after exposing it to gamma irradiation at dosages of 1 to 10 kg respectively.

- **Roasting**
  - Roasting may reduce mycotoxin contamination by burning surface contaminants and removing volatile, heat labile toxins and other mold metabolites
  - Roasting coffee beans at 200°C for 5 minutes reduces the ochratoxin A by 78.6%
Extrusion

- Extruding contaminated cottonseed at 160°C as compared to 104 °C reduced 33% of aflatoxins in the feed product.

- Multi-pass-extrusion (four times than once) reduced another 55% of aflatoxins in the feed product.

- Extrusion with 0.3% lime and 1.5% hydrogen peroxide was the most effective process in reducing aflatoxins in corn tortillas.

- However, high lime and hydrogen peroxide affected the taste and aroma of final tortillas.

- Extrusion was also found effective against DON/vomitoxin.
Microbial degradation:
- De-epoxidases enzyme (from the genus Eubacteria) can be used in degradation of tricothecenes
- Yeast strain (*Trichosporon mycotoxinivorans*) is capable of degrading ochratoxin A

Bt. Corn
- Bt. corn reduces the insect damage (thus reducing plant stress and exposure to mold spore)
- It is estimated that a total benefit of Bt corn’s reduction of fumonisin and aflatoxin in the US is around $23 million/yr

Non-toxic strains:
- Competition with toxic strains of *Aspergillus* spp.

Bacterial strains:
- Several bacterial species, such as *Bacillus*, *Lactobacilli*, *Pseudomonas*, *Ralstonia* and *Burkholderia* spp., have shown the ability to inhibit Aspergillus spp growth and subsequent aflatoxin synthesis
Chemical Methods

Ammoniation (ammonia)

- Effective against aflatoxin and fumonisin contaminated corn and cotton seed
- Ammoniation reduced aflatoxins in the range of 30-45%
- Effective against aflatoxin contaminated feed
- Approved in several countries
- Less effective against other mycotoxins
- Found effective in reducing aflatoxins in rice (up to 90-100%)
- May end up as a residue in feed causing deterioration of animal health performance
Chlorine Dioxide

- ClO₂ gas was effective in reducing tricothecene (verrucarin and roridin) on paper, glass and cloth @1000ppm with long exposure times
- Variation in the data with ClO₂ treatment

Ozone

- Ozone treatment resulted in reduced sporulation, hyphal growth and mycotoxin synthesis

Citric acid

- Use of 1 N aqueous citric acid reduced aflatoxins in corn by 96% to 100%
Practical applicability of detoxification methods

- Efficacy depends on type and distribution of mycotoxin throughout the lot
- Often results in high product loss
- Expensive
- Time consuming
- Availability of technology
- May destroy the nutrients in feed products
- May not be legal
Binders

They are chemicals that binds to mycotoxins through binding site and avoid toxins to react with GIT or animal body, and are usually excreted
Ideal binder qualities

- Reduces mycotoxin activity
- Effective adsorption of mycotoxins from GIT of livestock animals
- Reduce residues
- Will not affect the animal
- Resistant to feed processing
- Economic
- Easily available in the market
Types of Binders

- **Organic products**
  - Dioctatin A, aflastatin A, dillapiol, indigestible carbohydrates (cellulose, glucomannas, peptidoglycans), etc.
  - Dioctatin A, Dillapiol, aflastatin-A, and Apiol as specific inhibitors of biosynthesis of aflatoxin G1 in Aspergillus parasitics

- **Inorganic products**
  - Activated carbon, aluminosilicates (clay, bentonite, montmorillonite, seolite, phyllosilicate etc.), Polyvinylpyrrolididine (PVP), montmorillonite, bentonite etc.

- **Hydrated sodium calcium aluminosilicate (HSCAS)**
  - Mostly studied one, “aflatoxin-selective clay”
Binders

- Binders can provide solution to manage mycotoxins in animal feed
- However, results vary with type of mycotoxins, their amounts, animal species and feed type
- Currently no product meets all the characteristics for a desirable binder
- No binder-products are approved by the FDA for such claims
- Most of the binders are generally regarded as safe (GRAS) to use in the feed
- The use of sodium aluminosilicate and hydrated sodium calcium aluminosilicate (HSCAS) as binders for mycotoxins is not considered to be generally recognized as safe (GRAS) according to FDA's Center for Veterinary Medicine (CVM)
- Furthermore, any mycotoxins which are bound might not remain bound when the feed is consumed and exposed to the acid environment of the gut
Blending

- Deliberate mixing of adulterated food with good food renders the finished product adulterated under FDA act (Sec. 555.200 Adulterated food mixed with good food)
- Blending mycotoxin contaminated grain is generally not permitted
- States may have to request for a special permission explaining extraordinary situations
- Blending, toxin content in the final product, shipping information and final usage should be recorded strictly
- To date, blending of corn with > 500 ppb total aflatoxins has not been permitted
Sampling and sampling errors during mycotoxin analysis

- Sampling is one of the most critical factor in determining the mycotoxin content in the product
- For a representative average of contaminated product the sample sizes should be as large as possible
When to test for molds/mycotoxins

- All other possible causes of symptoms rejected
- Symptoms for moldy feeds/fermentation smell
- Significant changes in production and health
- Similar symptoms in large number of animals
Types of tests for mycotoxins

- **Quick Test (Qualitative/Quantitative)**
  - Immunochromatographic strip
  - Thin Layer Chromatography (TLC)
  - Immunoassays (ELISA)

  **Use:** *Detects Specific Mycotoxin*
  *Limited range*

- **Confirmatory Tests (Quantitative)**
  - High Pressure Liquid Chromatography (HPLC)
    (Requires well trained technicians and equipment)

  **Use:** *Determines level of mycotoxins*
  *Detect several mycotoxins*
Monitoring stored grain quality

- Traditional methods for spoilage detection in bins involve manual walking, smelling, and sampling the grain inside the storage structure and temperature monitoring.

- Human sensory exposure for mold spoilage and other quality parameters is biased and it varies from person-to-person.

- Unfortunately, a temperature cable will not detect the mold growth several feet away from the cable until the size of the spoiling grain mass is large enough to raise the temperature around the volume of the cable.

- These limitations are overcome with CO₂ sensors which help in monitoring stored grain quality.
The CO$_2$ sensor is inserted through the grain storage bin’s roof and suspended into the headspace. The CO$_2$ sensor is connected to the communication box through a cable. The CO$_2$ sensor automatically records the CO$_2$ concentration hourly, and depending on the settings sends the data to a server via the wireless telephone network. The user can log into the BinTech website and view a bin’s CO$_2$ trend data over the last few days, weeks or months.
Sniffing Out Bugs Automatically

Carbon dioxide detector monitors insect infestations in stored grain.

Compiled by staff

Published: Oct 20, 2010

Grain storage issues bugged many farmers during 2009 and into 2010, but GSI’s Binspect offers some real relief.

The system detects the slightest release of carbon dioxide within grain storage, so bugs better learn to hold their breath. GSI says this technology comes with several years of testing at Kansas State University.

Binspect is in limited release for 2010. Suggested list price is $10,500. Call 888-GSI-BINS, or visit www.gsiag.com.

As a result of our successful research, this technology was introduced at the 2010 Farm Progress Show!

Sniff out pests in your grain storage

**PAM’S CHOICE:** Grain storage issues bugged many farmers during 2009 and into 2010. A visit with Gary Woodruff regarding GSI’s Binspect was like the rainbow after the storm. The Binspect detects the slightest release of carbon dioxide. Bugs better learn to hold their breath. Messages go to the grower by text. It has been tested by Kansas State University and Purdue University. Binspect is in limited release for 2010. Suggested list price is $10,500. Call 888-GSI-BINS, or visit www.gsiag.com.
Summary

- Mycotoxins are of potential risk to both humans and livestock.
- No animal species is immune to the acute toxic effect of aflatoxins including humans.
- Diagnosing mold/mycotoxin problems is a complex process.
- Effects differs among species, age, gender and by duration of exposure.
- Sampling procedures are critical for accurate analysis.
- Variation in test results is a major dispute.
Summary

- No single treatment to take care of mycotoxin problem
- Store grain/feed/food products at safe moisture content
- Avoid insect and pest in feed mill area
- No binder-products are approved by the FDA for such claims
- Requires an integrated approach to manage mycotoxins in food chain
- Regular inspection, sampling and testing of raw-ingredients and finished feed products are must to avoid mycotoxins entering the food chain
- Prevention is not better, it’s the BEST option than control
References

http://www.allaboutfeed.net/weblog/from-feed-to-food-/mycotoxins-underestimated%3F-4905.html

http://www.sickhousecenter.com/mold-pictures/
Thanks

Questions?