Aflatoxins

Sources

- Aflatoxins are secondary mold metabolites produced by some strains of *Aspergillus flavus* and *A. parasiticus*.
- Chemically they are polycyclic furan compounds possessing intense blue or green fluorescence under long wave UV light.
- Although at least 13 aflatoxins have been identified, 4 (B1, B2, G and G2) are most common.
- Aflatoxin tends to be produced in highly nutritious foodstuffs at high moisture concentrations and warm environmental temperatures. Aflatoxin can be produced in conditions of 85% relative humidity (e.g., corn moisture content of 15 to 28%) and temperatures over 55º that persist for over 48 hours. Most problems result with sustained high temperatures of 78 ºF or more.
- Under these conditions, peanuts, corn, walnuts, pecans, almonds, cottonseed and grain sorghum are more susceptible to *A. flavus* contamination than soybeans, wheat, rye and oats.
- *A. flavus* may grow on corn in the field as well as in storage. Corn is dried in order to create an internal environment of less than 85% relative humidity. This degree of relative humidity is the minimum for significant aflatoxin production. Of course relative humidity is dependent upon evaporation which is a variable greatly affected by increases in temperature. For these reasons, it is sometimes possible to store corn in the mid to late fall and early winter at somewhat higher moisture concentrations than could be tolerated in September. At usual seasonal temperatures the 85% relative humidity figure will not be exceeded if corn moisture concentrations in September, October and November do not exceed 17, 18 and 19%. However, just blowing air over corn at these or higher moisture concentrations will be inadequate if higher than normal temperatures prevail.
- Most problems in the Midwest occur as a result of a drought and insect damage, which allow the mold to get started, followed by prolonged hot weather. Thereafter improper drying and warmth after harvest are major contributing factors resulting in toxin production in corn.
- Findings in biased samples sent for aflatoxin analysis in 1983 (a drought year).
  - 1982 (a normal year) **Suspect** corn - less than 1% of that Illinois corn tested contained detectable concentrations of aflatoxin.
  - 1983 **Suspect** corn - approximately 70% were black light positive - and of these most had at least a little aflatoxin.
  - Of 90 samples of 1983 **Suspect** corn tested by Centralia:
    - Eight percent contained over 250 ppb aflatoxin.
    - Forty percent contained 50 to 250 ppb.
    - Sixty percent contained less than 50 ppb.
    - One sample contained 12,000 ppb (12 ppm).
  - If an unbiased sample was collected a lesser percentage would have been found.
  - In 1988 (another drought year), aflatoxin at up to 3,500 ppb was reported.
  - In 1995, Texas corn samples were found to contain aflatoxin at up to 5,000 ppb.
- Sometimes "high moisture" corn may not be high enough in moisture to ensile. Consequently, fungal growth and toxin production
Factors Affecting Sensitivity

- The sensitivity of animals varies according to age and species as shown.
- Young animals are generally more sensitive than adults.
- Sensitivity to aflatoxin.
  - Most sensitive ----------------------------- Least sensitive.
- Among domestic animals: ducklings, trout, dogs, pigs, cattle, sheep, chickens.
- Among livestock: piglets, pregnant sows, feeder pigs-calves-horses, finishing hogs, lambs, dairy cows (residues in milk possible), feeder cattle, mature sheep.

FDA Limits Set for 1988 Corn Crops (Pertains to Corn Rather than Feed)

- In corn, a maximum of 20 ppb aflatoxin is allowed in interstate shipments unless special FDA permission has been obtained for a higher aflatoxin level. This is ordinarily done when drought conditions cause widespread contamination.
- Set for 1988 corn crop.
  - 20 ppb maximum allowable for corn destined for human consumption, for interstate transport, or to be fed to dairy cattle or immature animals.
  - 100 ppb - Approved for corn destined for consumption by breeding cattle, breeding swine, and mature poultry.
  - 200 ppb - Approved for corn destined for finishing swine (greater than 100 lb).
  - 300 ppb - Approved for corn destined for finishing cattle (feedlot).
  - Individual states can act at levels below the above levels if they deem it necessary.
  - Blending - Contaminated corn can be blended with clean corn for animal feed use only and must be approved by the FDA regional office.
  - FDA limit in milk.
    - 0.5 ppb of aflatoxin M₁ = the actionable level in milk.
    - Milk is routinely monitored for aflatoxin M₁ by spot checking followed by trace back to the source farm(s).

Toxicity

- Swine.
  - LD₅₀ aflatoxin B₁ = 0.8 mg/kg BW.
  - At 400 to 600 ppb in feed, severe clinical disease in feeder pigs.
  - Adults - 450 ppb in feed of sows and boars, had no effect on spermatogenesis, litter size, piglet quality or lactation.
  - Chronic toxicity, feeders 300 to 400 ppb in feed.
  - Less than 223 ppb, no effect.

ADME and Residue Considerations (See FDA Limits Above)

- Aflatoxin M₁ is the principal aflatoxin residue in meat and milk. It retains only a limited amount of the toxic potential of the parent compound.
- In exposed animals, residues in meat products may sometimes be of concern. Rations with less than 100 ppb aflatoxin will yield muscle tissues containing less than 0.5 ppb aflatoxin, the actionable concentration in human foods although kidney may be considerably higher. The actionable concentration in human foods although kidney may be considerably higher. Generally even at somewhat higher concentrations, 5 days on clean feed will allow depletion of residues from muscle to acceptable concentrations.
- Metabolite (epoxide) produced in the animal is the actual carcinogen.
Mechanisms of Action

- Aflatoxins are potent hepatotoxins and hepatocarcinogens.
- May react with and modify the DNA template to interfere with RNA transcription or may specifically inhibit RNA polymerase which would also impair transcription.
- Also increase the rate of RNA breakdown by alteration of lysosomal membranes resulting in the release of ribonuclease from lysosomes.
- May stimulate the alkylation of RNA by stimulation of RNA methylase.
- As a result of a reduction in the active forms of RNA, protein synthesis is secondarily impaired.
- Effects include hemorrhage, immunosuppression, stunting and liver damage.

Signs

- The early clinical signs of aflatoxicosis may be indistinguishable from signs of deoxynivalenol, T-2 toxin and a host of other disease syndromes. Thereafter the signs, clinical pathologic changes and lesions of aflatoxicosis often help in suggesting a diagnosis.
- Decreased feed efficiency is generally the first sign recognized at low concentrations.
- Reduced feed intake.
- Decreased growth rate.
- General unthriftiness.
- Rough, dull haircoat.
- May manifest as increase in prevalence, severity of or failure of therapy or vaccinations for infectious diseases such as:
  - Bloody dysentery.
  - Erysipelas.
  - Salmonellosis.
  - Pneumonias.
  - Failure to test feed, and antibiotic therapy after a (partial) diagnosis of an infectious disease, may result in a "partial" resolution of the clinical problem.
- Ascites.
- Hemorrhage and icterus.

Aflatoxin B₁ binds to DNA at the guanine base in liver cells, corrupting the genetic code that regulates cell growth. Out-of-control cells grow into tumors that eventually become cancerous. Drawing by G. Hedberg, USDA, ARS, National Animal Disease Center, Ames, Iowa.
Clinical Pathology

- Feeder pigs become anemic (appear pale) or may appear icteric.
- Abnormally elevated SGOT, SGPT, alkaline phosphatase, ornithine carbamyl transferase, isocitric dehydrogenase.
- Decreased albumin and A/G ratio.
- Increased prothrombin time (acute syndromes).

Lesions

- Acute form in swine.
  - Icterus.
  - Ascites + fibrin.
  - Intestinal hemorrhage.
  - Subcutaneous and muscular hemorrhage.
- Subacute or chronic (300 to 400 ppb of aflatoxin in young swine feed).
  - Generally debilitation.
  - Pallor or icterus.
- Decreased thymus size in very young.
- Variable ascites.
- Gross hepatic lesions-subacute.
  - Pale yellow to orange.
  - Friable or firm.
  - Granular or sometimes nodular.
  - Gallbladder edema in some swine.
- Gross hepatic lesions-chronic.
  - Fibrosis.
  - Regenerative nodules.
- Histopathologic hepatic lesions.
  - Biliary hyperplasia.
  - Hepatocellular swelling.
  - Hepatic lipidosis proceeding to vacuolation.
- Thymic involution.

Diagnosis

- When conditions are favorable for the production of aflatoxin grain elevators often utilize black light screening of corn in order to assess the possibility of aflatoxin occurrence in the grain. Several considerations must be understood when interpreting black light tests of corn. As shown in this table, all positives should be followed up by a more specific test to identify and quantitate any aflatoxin actually present.
- Black light test: factors.
  - Significant number of false positives but few false negatives.
  - Indicates the present of a derivative of kojic acid, a product that signals the growth of *Aspergillus*, not aflatoxin.
  - Remains positive only temporarily. Drying and storage can degrade the compound so that negative tests are likely when sampled thereafter.

### Aflatoxin - Effects in Swine According to FDA*

<table>
<thead>
<tr>
<th>Parts per Billion</th>
<th>Clinical Signs</th>
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</thead>
<tbody>
<tr>
<td>500</td>
<td>Reduced growth, liver impairment, appetite loss</td>
</tr>
<tr>
<td>1,000</td>
<td>Marked growth reduction; liver and kidney damage; petechial hemorrhages in muscles, viscera</td>
</tr>
<tr>
<td>2,000</td>
<td>Severe hemorrhage, bloody diarrhea, death in 1 to 3 days</td>
</tr>
<tr>
<td>Other Studies</td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>Decreased rate of gain and feed efficiency</td>
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</tbody>
</table>

*Note - Animal ages not stated.
- Samples with only one glowing kernel have had up to 4,000 ppb aflatoxin.
- Others - glowing kernels all through have had 30, 40, or 50 ppb.
- Crack corn (do not grind) before doing black light test.
- Follow up positives with laboratory analysis for aflatoxins.

- In Illinois, at least, a lot of elevators do black light screening in drought years and sometimes reject black light positive corn. Whether this succeeds in preventing storage of aflatoxin containing corn depends upon the diligence of the elevator personnel. However, some if not many elevators will not reject anyone as to do so means a loss of customers in future years. Sometimes, if purchased and black light positive, the elevator operators are aware of the risk and rapidly dry the grain to avoid further toxin production. However, this remains speculative.

- Methods of sampling feed for mycotoxin analysis.
  - 10-lb sample.
  - Sample all of bin, probe to get a representative sample.
  - Hot-spots, halfway down - or where ever you get greatest amount of fines may also be sampled.
  - Ship (for analysis) only.
    - At moisture concentrations of less than 14% -or-
    - Frozen -or-
    - Hand deliver.
  - Bank-frozen-labelled samples before all fed.
  - Samples sent for analysis should include a complete history in addition to labelling, detailing the sampling method.
  - ELISA test (Quik-card®, E-Z-Screen®, Signal®, Agri-Screen Aflatoxin®) gives a + or - with the "cut-off" being at 20 ppb. These are now widely used at elevators and appear to be relatively accurate (all assays dependent upon how representative the samples are).

Treatment and Control

- Change to aflatoxin-free ration.
- Increase dietary protein.
- Vitamin B12 and Vitamin K.
- Selenium supplementation.

Recommendations

- Interpreting the significance of aflatoxin residues in feeds can be done on the basis of past field experiences, which are of course variable, and/or on the basis of experimentation. Therefore, recommendations as to the risk of given concentrations of aflatoxin feeds are dependent upon the interpretation of the toxicologist. The following are recommendations of the NAPCC.
- Feeds containing concentrations less than 50 ppb of aflatoxins can generally be fed safely to all animals.
- Feeds containing over 50 ppb aflatoxins should not be fed to weanling pigs.
- Concentrations greater than 100 ppb should not be fed to feeder pigs or breeding stock.
- Concentrations up to 300 ppb can generally be fed to finishing swine provided the animals are getting slightly increased protein supplementation, are having no other disease problems, and are brought onto the feed gradually.
- The feed should be free of detectable aflatoxins for several days before marketing animals.
- Due to sample variation, it is probably best to avoid giving aflatoxin contaminated feed to dairy cattle altogether. Although one can usually avoid exceeding the 0.5 ppb of aflatoxin M1 level set by FDA by feeding less than 50 ppb of aflatoxins in the finished ration (dry weight basis). If the feed must be used, however, I would favor a maximum of 20 ppb aflatoxins in grain dry matter fed to dairy cattle.
- Do not feed over 50 ppb to calves less than 4 months old or over 100 ppb to calves 4 to 6 months old. Older calves and adult, nonbreeding cattle must be fed up to 300 ppb provided they are on a good plane of nutrition.
- Horses and dogs are comparatively sensitive, and it would usually be unwise to divert aflatoxin contaminated feed to their rations.
- Mature sheep are relatively resistant to aflatoxins.
- NovaSil® (hydrated calcium aluminum silicates - HSCAS) has been approved by FDA only as an anticaking agent at up to 2% of a ration. Although not currently approved for a binding agent, this product has this added benefit. Research has shown that NovaSil® binds aflatoxin and decreases its absorption. At concentrations up to 3,000 ppb Aflatoxin B, in the presence of NovaSil®, at 0.5% of the ration, swine grew at rates similar to the controls, and the animals had no aflatoxin-associated lesions; however, aflatoxin-positive controls given 3,000 ppb B1 all died. Poultry seem to benefit in a similar fashion; however, the efficacy of NovaSil® in preventing residues of aflatoxin M1 in the milk of dairy cows is considerably less than desirable.

Use of Contaminated Feedstuffs

- Feed affected foodstuff to less susceptible species and/or ages.
- Dilute with clean feed.
- Screen out broken kernels.
Ammoniation of corn to reduce aflatoxin.
- Ammoniation of corn is not done routinely in Illinois, however, for persons possessing large amounts of corn which is highly contaminated by aflatoxin, this alternative may be reasonable, provided several key considerations are made as illustrated.
- Ammoniation is a last resort only:
  - Corn turns brown.
  - Can be used only for animal feed.
  - Treated corn cannot be shipped across state lines.
  - Ammonia may corrode steel, galvanized metal, copper and brass.
- Hazards include:
  - Inhalation of mold spores and aflatoxin.
  - Ammonia lung-eye-skin irritation.
  - Ammonia vapor combustible.
  - Treated corn may cake up in equipment and not flow. Persons should not enter ammonia treatment bins "to help".
- Ammoniation:
  - May require rewetting of corn.
  - Slower process (longer ammonia contact necessary) in cool weather.

Avoidance of Mycotoxins (and Bacterial Spoilage)

- It is always best to avoid mycotoxicoses. Even corn initially toxin-free can become contaminated after it is delivered to the farm. Therefore, drying to below 14% moisture is essential. Screening can be used to remove broken kernels which are much more often moldy and will also reduce the amount of "fine material". "Fines" reportedly encourage mold and insect damage and inhibit aeration which delays drying.
- Low temperature drying of corn (by blowing air) that is field infected with molds is risky. Warm air temperatures can encourage mold growth. This method should, therefore, only be used when the temperature of the corn in the bin can be kept sufficiently cold to keep the humidity in the corn kernel below 85 º.
- Corn can often be stored at 15% H2O, and it has approximately 180 days before it goes bad, so if sold at 150 days, it has the potential to go bad in 30 days or so. The resultant bacterial spoilage can greatly reduce palatability.
- Avoiding mycotoxins/minimizing problems.
  - Dry corn to less than 14% moisture.
  - Aerate regularly during storage to prevent condensation.
  - Harvest no more than 1 week after maturity.
  - Adjust harvesting equipment to minimize cracking of kernels.
  - Clean and decontaminate storage bins with Clorox ®/water 1:9.
  - Repair storage bins to avoid leakage; moisture can cause hot spots.
  - Clean the feeding system-feeders, augers, mixers, etc.
  - Do determine the history of corn your clients are getting.
  - They can avoid rewetting of corn or blending to increase moisture by the following procedures.
    - Buy corn quantity wise on a dry matter basis to remove the incentive to rewat and blend.
    - Keep corn in good condition-if necessary, redry it.
    - **Note** - Excessively dry corn may become brittle so that an increased amount of "fines" may occur enhancing the likelihood of fungal problems, and fungi may also enter damaged kernels; so this, too, must be avoided.
  - Be sure that they:
    - Do submit appropriate samples for mycotoxin analyses when toxins are suspected.
    - Do have necropsies performed to establish that lesions compatible with specific mycotoxin poisonings are present.

**Note** - Reexamination of notes provided during the poultry course would be of value.
Hepatotoxic Mycotoxins: Sterigmatocystin, Rubratoxin, Sporodesmin, Penicillinic Acid
(Apart from Aflatoxins)

1. Sterigmatocystin

Sources

- Aspergillus versicolor
- A. rugulosus
- A. nodulans
- Bipolaris sp.
  - Has produced sterigmatocystin on corn meal in concentrations as high as 1.2 gm/kg.
  - Its racemate is produced by P. luteum

Structure of Sterigmatocystin

![Structure of Sterigmatocystin](image)

- Toxins containing same nucleus include:
  - Aflatoxins.
  - 5-methoxysterigmatocystin.
  - 0-methylsterigmatocystin.
  - Demethylsterigmatocystin.
  - Aspertoxins
  - Versicolorins.
  - Autocystins.
  - Sterigmatin.
  - Related toxins also include Bipolarin, Averufarin and Curvularin.

Toxicity

An LD<sub>50</sub> of sterigmatocystin administered IP was 60 to 65 mg/kg in albino rats.

Absorption, Distribution, Metabolism and Excretion (ADME)

- In vervet monkeys 50% was eliminated in urine as its glucuronic acid conjugate.
- Readily epoxidized to probable actual carcinogen.
- Sterigmatocystin is 1/100 or less as potent as aflatoxin.
Lesions

- Hepatocarcinogen, necrosis of kidney and liver cells.
- Experimental administration was associated with squamous cell carcinoma production (topical application to rats).

2. Rubratoxins

Sources

- Rubratoxins A and B are produced by \textit{P. rubrum} and \textit{P. purpureogenum}. Both are common soil fungi.
- Sources of exposure - Cereal grains-primarily corn. The natural distribution of rubratoxin is not well established.

Toxicity

- LD50 values of rubratoxin range from 0.27 to over 5 mg/kg when given parenterally but were 400 to 450 when given orally to rats. The LD50 in chicken feed was 83 ppm.
- Males and females (rats) are equally susceptible; but neonate rats are 49 times as susceptible as adults.
- At sufficient doses, chronic dosing causes same effects; but animals getting subclinical doses had no lesions suggesting effective detoxification.
- Chicks:
  - 500 ppm rubratoxins (80% B) in diet was required to affect growth rate.
  - Dose of over 1,000 ppm in diet caused atrophy of liver and bursa of \textit{Fabricius}.
  - (These levels not known to occur in nature.)

Mechanisms of Action

- Mutagen.
- Embryocidal.
- Teratogenic in mice.
- So far noncarcinogenic (rats).
- May potentiate the action of aflatoxin.

Absorption, Distribution, Metabolism and Excretion (ADME)

- Long plasma half-life (days); most excreted in urine as parent compound (rats). Slightly less excreted in feces.
- This is contradicted in other studies which suggested a great amount was metabolized and excreted as CO2.

Signs

- Clinical syndrome is similar to acute aflatoxicosis with anorexia, dehydration, diarrhea and possibly hemorrhage.
- It has been implicated in abortions in swine.

Lesions

- Acute and chronic syndrome characterized by hepatotoxic insult, nephrosis and general bleeding tendency. Congestion, hemorrhage and damage to liver, kidney and spleen.
- Early midzonal hepatic necrosis.
- Later extending to massive necrosis and replacement with hemorrhage with chronic doses.

3. Sporodesmin (Cause of Facial Edema)

Source

- \textit{Pithomyces chartarum}, formerly called \textit{Sporodesmium bakeri} which has an almost worldwide distribution and normally grows on any dead material.
- Sporodesmin derivatives have also been isolated.
- Toxicosis usually seen in late fall and early summer.
- Source of exposure - Ryegrass pasture Australia and New Zealand (on north part of island).
Requires 100% humidity and 55 °F for spore germination and rapid perfuse growth.
Not known to be a big problem in USA.

**Susceptible Species**

- Bovine.
- Ovine. Sheep with resistance to facial edema have been bred in New Zealand.

**Toxicity**

- Toxic oral dose 0.4 to 0.7 mg sporodesmin per kg produces disease identical to field syndrome.
- Toxicity of hay decreases rapidly with proper storage.

**Mechanisms of Action**

- Hepatocellular damage - excreting of unconjugated sporodesmin in bile results in:
  - Occlusion of biliary system (acute cholangitis).
  - Terminates in fibrous obliteration of the ducts involved.
  - With resulting phylloerythrin accumulation which is normally secreted in the bile.
  - Photosensitivity results.
  - Get mild disorganization of organelles and triglyceride accumulation.

**Signs**

- Affected areas occur in light colored or sunlight exposed areas of the skin (ears and face).
- Edema of the skin, especially about the head.
- Serous exudation.
- Blistering, scab formation.
- Icterus.
- Anorexia, cystitis (early sign).
- Affected animals seek shade, and the apparent intense irritation causes the animals to rub the photosensitized areas on trees and posts; sheep may traumatize their eyes.
- Over 10 to 14 days there are stages of serous exudation, skin necrosis, and if animal recovers, sloughing of the necrotic tissue.
- The skin of the mammary glands on dairy cattle may slough in large pieces.
- Sheep's ears may become so edematous they droop from their own weight.
- Sheep may secondarily develop myiasis.
- Sheep and cattle may become chronically ill and die close to parturition.

**Lesions**

- Hepatocellular degeneration and fibrosis.
- Liver damage is more prevalent than clinical signs.
- Thickening of extrahepatic bile ducts-necrosis of all bile ducts.
- May proceed to biliary cirrhosis with recanalization with new bile ducts.
- Survivors develop large nodules of regeneration and persistent areas of atrophy and fibrosis in liver.

**Diagnosis**

- Appropriate clinical signs and lesions.
- Common in fall but does occur year round.
- Associated with ryegrass pasture.
- Check with lab as to possibility of analysis.

**4. Penicillic Acid**

**Sources**

- Implicated in hepatic cirrhosis in swine.
- Probably not a very significant problem.
- First known mycotoxin-1911.
- Many *Penicillium* spp. produce it.
- Many *Aspergillus* spp. produce it.

**Toxicity**

- Low toxicity.
  - LD₅₀ mice 110 mg/kg SQ.
  - LD₅₀ mice 250 mg/kg IV.
  - LD₅₀ mice 600 mg/kg Oral.
  - But other reports of LD₅₀ as low as 2 mg/kg IP mice.
  - Carcinogen (SQ administration).

**References**

**Hepatotoxic Mycotoxins (Apart from Aflatoxins) Sterigmatocystin, Rubratoxin, Sporodesmin, Penicillnic Acid**


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