AFLATXINS: AWARENESS AND CONTROL

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AFLATOXIN

Most references to “mycotoxin”, unspecified, refer to Aflatoxin.
Why to deal with aflatoxin?

• Mycotoxins are present in all stages of food production, processing and storage.

• These natural toxins occur primarily in those cereal crops (e.g. wheat, maize) which serve as main food items for the human population and as important raw materials of animal feeds.

• The risk posed mycotoxins consumed in low amount but continuously is not yet explored, however the danger is very high (‘hidden killers’).
History of Aflatoxins

- 5,000 years ago in China.
- Early 1861, was reported in Russia.
- 1891, there was a report of mouldy rice in Japan.
- 1913, First describes but the toxin was not isolate.
- 1940, Aflatoxicosis in swine had been reported due to ingestion contaminated Maize.
- 1950, similar ingestion happened in Alabama, US.
- 1960, is the time of the discovery of aflatoxin in England (Turkey X Disease).
Sources of Aflatoxins

• Produced by 3 species of Aspergillus: A. flavus, A. parasiticus, A. nomius.

• Aflatoxin: A (Aspergillus) - fla (flavus) – toxin.

• Four toxins soon identified: Aflatoxin B$_1$, B$_2$, G$_1$, G$_2$ - blue or green florescence under UV-light.

• Aflatoxin B$_1$ most important - highly carcinogenic and widespread occurrence in foods

• (B$_1$ > M$_1$ > G$_1$ > B$_2$ > M$_2$ ~ G$_2$).

• Aflatoxin M$_1$ : hydroxylated product of B$_1$ appears in milk, urine, and feces as metabolic product.
Physiological Characteristics of Fungi

- **Growth temperature**
  - Opt. 25 ~ 30°C, Min. 4°C

- **Water activity (a_w)**
  - a_w = 0.62 ~ 1.0 (If substrate RH = 95%, aw = 0.95).

- **pH**
  - pH 2 ~ 8.5 (pH 5.0 ~ 5.5).

- **Aerobic**
  - Oxygen (1-2%)
Temperature Range for Growth of Toxigenic Moulds

- **Aspergillus**
  - Minimum: 10°C
  - Optimum: 30°C
  - Maximum: 50°C

- **Penicillium**
  - Minimum: 0°C
  - Optimum: 20°C
  - Maximum: 40°C
Aflatoxins

- aflatoxin B₁
- aflatoxin G₁
- aflatoxin M₁
- aflatoxin B₂
- aflatoxin G₂
- aflatoxin M₂
Chemical Properties

- AFs are **difuro-coumarin** (heterocyclic compounds), they are freely **soluble in chloroform and methanol**.
- Pure AFs are stable at High temp. and unstable when exposed to UV and air when dissolved in polar solvents.
- Chloroform and Benzene solution are stable for year if stored in dark cold place.

The important sites in AFB₁ molecule are:

- The 1\textsuperscript{st} site: Double bound in position 8,9 of furofuran ring, (DNA and protein)
- The 2\textsuperscript{nd} site: Lacton ring in coumarin moiety
- Lacton ring is easily hydrolyzed (alkaline hydrolysis)
Mycotoxins entering the food chain

Infection of plants by moulds

Contaminated feed

Toxin in the blood and organs

Contaminated food of plant origin

Contaminated food of animal origin

 aflatoxins
 ochratoxin-A
 zearalenone
 fumonisins

Meat
 Milk
 Egg
Occurrence of Mycotoxins in Foods

- Milk and milk products ($AFM_1$, OTA)
- Meat and meat products (OTA)
- Egg ($AFB_1$, DON, CPA)
- Cereals ($AFB_1$, Fusarium toxins, OTA)
- Oilseed ($AFB_1$)
- Wine, grape juice and grape products (OTA)
- Beer ($AFB_1$, OTA, Fusarium toxins)
- Fruits, juices, vegetables ($AFB_1$, OTA, citrinin, patulin, Fusarium toxins)
- Coffee and cocoa ($AFB_1$, OTA)

Galvano et al., 2005
HEALTH EFFECTS of AFLATOXINS

• The primary target of aflatoxins is hepatic system
• AFs induced Liver cancer and bile duct proliferation
• The chronic exposure to AFs lead to (on both humans and livestock):
  1- Impaired immunity  2- Reduce food intake
  3- Reduce growth rate  4- Reproductive disorders
  5- Increased susceptibility to infectious disease.
  6- Disorder of protein and lipid metabolism.
  7- Inhibits protein synthesis and stimulate lipid peroxidation
  8- Dysfunction of enzyme function and their synthesis.
  9- Depressed complement and interferon production.
Factors Influencing The Harmful Effects of AFLATOXINS

- Chemical structure (accumulation)
- Concentration
- Time of exposure
- Multitoxic effect (interaction, synergism, additive effect)
- Individual sensitivity (Age, Sex, Species, Breed)
- Health status (Diseases)
- Nutritional status
- Vitamin supply
- Stressors
Target Organs of Some Mycotoxins

Mycotoxin       Target
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Aflatoxin        Liver
Ochratoxin A     Kidney
Trichothecenes   Mucosa
Ergot alkaloids  Peripheral vascular system
Zearalenone      Uro-genital tract
Aflatoxin Levels

- Levels permissible in foods subject to legal limits in many countries
- Today, foods most frequently contaminated, routinely screened before processing or sale
- Permissible limits generally quite low (15-20 parts per billion)
- Some scientists feel that no detectable levels of aflatoxins should be permitted because of the carcinogenic effects
Factors to Consider in Setting Regulatory Limits

• Availability of data on incidence and occurrence
• Availability of toxicological data
• Availability of analytical methodology
• The need to maintain an adequate food supply at reasonable cost
• Knowledge of legislation in other countries involved in international trade
## Maximum Acceptable Levels for A Range of Aflatoxins In Foodstuffs for A Selection of Countries

<table>
<thead>
<tr>
<th>Aflatoxins</th>
<th>Country</th>
<th>Limit ($\mu$g per kg)</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin $B_1$</td>
<td>EU</td>
<td>2</td>
<td>Nuts or dried fruit intended for direct human consumption or as an ingredient in foodstuffs</td>
</tr>
<tr>
<td>Aflatoxin $B_1$, $B_2$, $G_1$, $G_2$</td>
<td>EU</td>
<td>4</td>
<td>Nuts or dried fruit intended for direct human consumption or as an ingredient in foodstuffs</td>
</tr>
<tr>
<td>Aflatoxin $B_1$, $B_2$, $G_1$, $G_2$</td>
<td>Canada</td>
<td>20</td>
<td>Feeding stuffs for animals</td>
</tr>
<tr>
<td>Aflatoxin $M_1$</td>
<td>EU</td>
<td>0.025</td>
<td>Infant formulae including infant milk. The maximum level for infant formulae refers to the product ready to use</td>
</tr>
<tr>
<td>Aflatoxin $M_1$</td>
<td>US</td>
<td>0.5</td>
<td>Milk for human consumption</td>
</tr>
</tbody>
</table>
**Action or recommended maximum concentrations of aflatoxins in animal feeds***

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Animal</th>
<th>Maximum conc. in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, peanut products, other products or feed ingredients</td>
<td>Finishing (feedlot) beef cattle</td>
<td>300 ppb</td>
</tr>
<tr>
<td></td>
<td>Breeding beef cattle, breeding swine, mature poultry</td>
<td>100 ppb</td>
</tr>
<tr>
<td></td>
<td>Finishing swine &gt; 100 lb</td>
<td>200 ppb</td>
</tr>
<tr>
<td>Corn, peanut products, other products or feed ingredients, excluding cottonseed meal</td>
<td>Immature animals</td>
<td>20 ppb</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>Beef, cattle, swine, poultry (regardless of age)</td>
<td>300 ppb</td>
</tr>
<tr>
<td>All feeds or feed Ingredients</td>
<td>Dairy animals, animal species not listed above, uses not listed above, intended use unknown</td>
<td>20 ppb</td>
</tr>
</tbody>
</table>

### Maximum Limits of Mycotoxins Permitted in Foods and Animal Feeds – Aflatoxins

**In United Arab Emirates (GSO 841 / 1997)**

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Maximum limits of Total aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foods</strong></td>
<td></td>
</tr>
<tr>
<td>Infants and children foods</td>
<td>0.05 µg/kg</td>
</tr>
<tr>
<td>Dried milk not used for infant foods.</td>
<td>0.5 µg/kg.</td>
</tr>
<tr>
<td>Liquid milk and its products. (except dried milk).</td>
<td>0.2 µg/kg.</td>
</tr>
<tr>
<td>Grains, nuts, oil seeds and their products (e.g. peanuts, peanuts butter, pistachio, almond, sesame, cotton seeds, sunflower seeds, corn, wheat, rice, soya bean).</td>
<td>20 µg/kg</td>
</tr>
<tr>
<td>Other foods</td>
<td>20 µg/kg</td>
</tr>
<tr>
<td><strong>Animal feeds</strong></td>
<td></td>
</tr>
<tr>
<td>Feeds for dairy cattles and small calves.</td>
<td>10 µg/kg.</td>
</tr>
<tr>
<td>Poultry feeds and other animal feeds.</td>
<td>20 µg/kg.</td>
</tr>
</tbody>
</table>
Strategies for Prevention & Control of Aflatoxins

• Use “clean” procedures.
• Prevent contamination
• Inhibit mold growth
  – Drying
  – Refrigeration
  – Mold inhibitors
Prevention of Aflatoxin Formation

• Pre-harvest
  Resistant varieties (if practicable).
  Crop rotation, irrigation
  Insect management
  Minimize kernel damage during harvesting

• Post-harvest
  Clean and dry corn to <9%, for oily crops, and 13.5%, for corn, moisture
  Facility with temperature-moisture control
  Insect and pest management
Decontamination Strategies

• Physical separation
  - Sorting, washing, crushing, dehulling, Remove contaminated portions.
  - Diluting contaminated food with uncontaminated food!!!!

• Thermals Treatment

• Ammoniation

• Nixtamalization

• Radiation processing

• Binding Agents
  - Sodium aluminosilicate, hydrated sodium calcium aluminosislicate, bentonits and activated carbons

• MUCH BETTER TO PREVENT FORMATION
Heating and Cooking

- Food not largely affected by routine cooking temperature.
- Heating and Cooking under pressure can destroy 70% of AFs in rice. Extrusion process in combination with 0.3% lime and H\textsubscript{2}O\textsubscript{2} reduced AFs level than lime and H\textsubscript{2}O\textsubscript{2} alone.
- Roasting: dry and oil rousing can reduce 50 - 70% of AFB\textsubscript{1}.
- AFs resists to higher temp. upto 260 °C.
- Over heating affected essential nutrients.
Nixtamalisation

• Maize cooked and steeped in alkaline solution of lime (0.3% CaOH).
• AFs are reduced during Nixtamalization by 90%.

Ammoniation

• Treatment with aqueous NH$_2$OH (2%) at 121 °C. and 17 psi reduced AFB$_1$ by 99% in naturally contaminated yellow corn.
• Gaseous NH$_3$ (2%) showed lower efficiency at lower moisture content.
Radiation Processing
Purposes of Food Irradiation

• **Low doses (< 1 kGy):**
  control of insects and
  delay of ripening

• **Medium doses (1 - 10 kGy):**
  reduction of foodborne pathogens
  extension of shelf life

• **High dose (> 25 kGy):**
  sterilization
Purposes of Food Irradiation

- Wheat flour – control of mold
- White potatoes – inhibit sprouting
- Pork – kill Trichinia parasites
- Fruit and Vegetables – insect control; increase shelf life
- Herbs and Spices - sterilization
- Poultry – bacterial pathogen reduction
- Meat – bacterial pathogen reduction
Radiation Processing

• Effective process, safe, and improve the safety of our food supply
• Inexpensive, approved by international organizations (WHO, FAO, IAEA, FDA and Others).
• Suppression and detoxified foodstuffs AFs in contaminated food.
  • 5 kGy significantly detoxified 44 - 48% of AFB₁.
  • 10 kGy detoxified AFB₁ by 62 – 88%.
  • 20 kGy was quite enough for complete detoxification.
150 + irradiation facilities 40+ countries
Dietary Modification

• The carcinogenic effect of AFB$_1$ is affected by dietary factor such as:
  • Vit. Am C and riboflavin reduce the binding of AFB$_1$ to DNA.
  • Sulfur containing AAS inhibit AFB$_1$ mutagenicity in microbial systems.
  • Essential oil extracted, from Nutmeg, celery, black pepper and cardumon, Inhibit AFB$_1$-DNA formation.
  • Micronutrient, such as copper, manganese, zinc and selenium, inhibit mutagenicity.
Dietary Modification

• Plant flavonoids, such as flavone, flavonol, isoflavon inhibit AFB$_1$-DNA formation.
• Butylated hydroxyanisole containd diet resulted in lower AFB$_1$-DNA formation.
• Butylated hydroxytoluene lower cancer incidence.
• Broccoli sprouts, green tea, medicinal herbs lower cancer incidence.
Control of Aflatoxins

Cooperation between:
Ministry of health
Ministry of Agriculture
Drug Administration
National Environment Committee board
Consumer Protection Committee Board
Legislative body
International organizations
Awareness Campaigns

Information Dissemination to:
Farmer
Food and feed factories
Exporter
Middlemen
Non Governmental Organization
Public service association
Health care providers
School
Early Warning System

EWS is intended to be safety net as supplemented to quality management system like ISO 9002, HACCP,GMP
EWS helps to prevent future outbreaks.
EWS is able to detect potential food contamination
Any EWS should involve:
• Monitoring AFs in food sources and individuals
• Response protocol to prevent further AF exposure
• Inclusion of key members from various government agencies, health care sector and non governmental organization
CONCLUSION

MUCH BETTER TO PREVENT FORMATION

- Selection of fungal resistant hybrids of crops
- Dry of commodities after post harvest is most effective means
- Gamma irradiation of commodities
- Raising awareness of AFs and disseminating information to formers, traders and consumers
- Collaboration between agricultural and public health communities and also between local, regional, national and international governing bodies.
Don’t forget who you heard this from!!!
Thank You