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Optimum Solving ... Optimum Recovery

Control of Aflatoxin

Plan of Talk

- Introduction
- Aflatoxin production and biological action
- Control of aflatoxin production
  - Preventive measures
  - Fungal growth inhibition
- Detoxification of aflatoxin
  - Physical method
  - Chemical method
  - Biological method
  - Biotransformation
- Dietary manipulation

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Introduction

- Mycotoxins are the toxic products of fungal metabolism occurring in a wide variety of commodities like animal feeds and human food products.

- Mycotoxins, on ingestion can, cause health hazards in both of livestock and human beings and hence, there is a greater economic and public health implication.

Cont. …

- The severity of mycotoxin contamination is determined by environmental factors like:
  1. Excessive moisture in the field as well as in storage
  2. Hot and humid climate
  3. Insect infestation

Economic Losses

- Mycotoxin contamination of feed affects practically all livestock, but greater information is available on dairy cattle, poultry, and swine.
- Mycotoxins cause economic losses in term of:
  1. Reduced production efficiency
  2. Impaired resistance to infection
  3. Compromised reproduction
  4. Increased mortality
  5. Wasted contaminated feed

Cont. …

- On a global scale, it is estimated that around **25%** of the world's crops are affected by mycotoxins annually.
- In addition to the above losses, costs of monitoring level of mycotoxins should also be considered.
- The recent mycotoxin surveys have indicated that the percent contamination is much higher than the perceived 25%.

Cont. …

- The mycotoxins that are of significance in animal feed are:
  1. Aflatoxins
  2. Ochratoxins
  3. Fusarial toxins (Fumonisins, Zearalenone, Trichothecenes including Deoxynivalenol and T-2 toxin)

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Aflatoxins and Biological Action

- Aflatoxins are highly toxic and carcinogenic compounds produced by *Aspergillus* fungi.
- Aflatoxins are produced at:
  1. Temperature of 25-32 C
  2. Moisture of greater than 12-16%
  3. Relative humidity of 85%
- Commonly affected feeds are:
  1. Maize
  2. Groundnut cake
  3. Cottonseed cake
  4. Copra cake

Cont. …

- Aflatoxins cause toxicity in poultry, cattle, sheep and swine.
- Among all aflatoxins, B1 is the more prevalent, it is metabolized to Aflatoxin M1 in liver which is excreted in milk of dairy cattle and as residue in egg and/or meat.
- Animal consuming aflatoxin contaminated feed shows:
  1. Poor performance
  2. Reduced immunity
  3. Liver damage
  4. Kidney and intestinal haemorrhage
  5. Liver tumors

Limits of Aflatoxin

- The presence of **Aflatoxin M1** in food products meant for human consumption is not desirable and the residual concentration should not exceed 0.5 ppb as per FDA regulations.
- Such regulations are much more stringent in European Union where the level should not exceed 0.05ppb.

Cont. …

- **Aflatoxin limits in animal feed;**
  - Broiler chicken 20 ppb
  - Layer poultry 100 ppb
  - Cattle 20 ppb
  - Beef cattle 300 ppb

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Control of Aflatoxins

1. **Preventive Measures**
   - Toxin producing fungi may invade at pre-harvesting period, harvest-time, during post harvest handling and in storage.

Cont. …

According to the site and time of infestation, the fungi can be divided into three groups:

1. Field fungi
   - They are generally plant pathogenic fungi; namely *Fusarium.*
2. Storage fungi
   - Mainly, they are *Aspergillus* and *Penicillium.*
3. Advanced deterioration fungi
   - Normally, they do not infest intact grains but easily attack damaged grains and requires high moisture content, that include *Aspergillus clavatus, Aspergillus fumigatus.*

Cont. …

- Prevention and reduction of fungal growth and toxin production is very important.
- **Primarily**, the recommended practices include
  1. Development of fungal resistant varieties of plants
  2. Suitable pre-harvest, harvest and post harvest techniques
  3. Store commodities at low temperature as for as possible
  4. Use fungicides and preservatives against fungal growth
  5. Control of insect damage in grain storage with approved insecticides.

Cont. …

- **Secondarily**, prevention of fungal growth include limiting the growth of infested fungi by
  1. Re-drying the product
  2. Removal of contaminated seeds

Cont. …

- **Thirdly**, measures to prevent transfer of fungi and their health hazardous toxins to feed and to environment, this include:
  1. Complete destruction of the contaminated product
  2. Diversion for fermentation to produce ethanol
  3. Detoxification or destruction of mycotoxins to the minimum level

Control of Aflatoxins

2. **Fungal Growth Inhibition**
   - The inhibition of fungal growth can be achieved by:
     1. Physical treatment
     2. Chemical treatment
     3. Biological treatment

Cont. …

1- Physical treatment

1) Drying seeds and commodities to the safe moisture level (< 9-11%).
2) Maintenance of the container or store house at low temperature and humidity.
3) Keep out insects and pests from the storage.
4) Gamma-irradiation of large-scale commodities.
5) Dilution of the contaminated feed with safe feed.

Cont. …

2- Chemical treatment

1. Use of fungicides:
   - Acetic acid, propionic acid, benzoic acid, citric acid and their sodium salts, copper sulfate at rate of 0.2–0.4 % in feed.
2. Use of fumigants
   - Ammonia at rate of 0.2-0.4%
3. Addition of herbal extracts
   - Garlic, onion, clove oil, turmeric powder, thyme at rate of 0.25-0.5%

Cont. …

3- Biological treatment

a) Enzymes

- Chitin and glucan, as constituents of fungal cell wall, could be enzymatically hydrolyzed resulting in killing of mycelia or spore of fungi.
- **Anti-fungal enzymes,** chitinase and Beta -1,3 glucanase:
  - They are found in plant seeds
  - They act as defense against pathogenic fungi
  - Plant seeds rich in these anti-fungal enzymes likely to resist the infestation of fungi.

Cont. …

b) Fungal bio-competition

- Application of **non-toxigenic strains** of *Aspergillus flavus* and *Aspergillus parasiticus* to soil in maize plots, lead to reduction in colonization of toxigenic fungi in subsequent years.
- The **non-toxigenic biocompetitive** *Aspergillus* strains out-compete the toxigenic isolates, resulting in reducing pre-harvest contamination with aflatoxin in peanut and cotton.

Cont. …

c) Lactic acid bacterial metabolites

› Some bacterial cultures may produce certain **metabolites** having anti-fungal activity as;

1. Cyclic dipeptides
2. Phenylactic acid
3. Caproic acid
4. Reuterin
5. Lactic acid
6. Acetic acid
7. Fungicin

Cont. …

- *Streptomyces Spp,.* MRI 142 produce Aflastin A, an anti-microbial compound known to inhibit aflatoxin production by *Aspergillus parasiticus.*
- *Bacillus subtilis* produces Iturin, an anti-fungal peptide that inhibits *Aspergillus Parasiticus* toxin production.

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Detoxification of Aflatoxins

- Aflatoxins in foods and feeds can be removed, inactivated or detoxified by means of:
  1. Physical
  2. Chemical
  3. Biological
  4. Biotransformation
- The treated feed should be:
  - Health safe from the chemicals
  - Keeping its essential nutritive value

1- Physical Methods

1) Contaminated seeds can be removed by hand picking or photoelectric detecting machines
   - This is labor intense and expensive
2) Heating and cooking under pressure can destroy nearly 70% aflatoxin.
3) Dry roasting can reduce about 50-70% of aflatoxin
4) Sunlight drying of aflatoxin contaminated feed could reduce the toxin level by more than 70%.

Cont. …

5) The addition of binding agents can reduce the bioavailability of these compounds in animals, and limit the presence of toxin residues in animal products.
   a) In case of aflatoxin B1 (AFB1), **HSCAS** and phyllosilicates derived from natural **zeolites** have a high affinity, both i*n vitro and in vivo.*
   b) Zeolites*,* which are hydrated aluminosilicates of alkaline cations are able to adsorb AFB1.
   c) Bentonites have been shown to be effective for the adsorption of AFB1.
   d) Other clays, such as kaolin, sepiolite and montmorillonite, bind AFB1 but less effectively than HSCAS and bentonite.
   e) Activated charcoal has mixed results against AFB1.

Cont. …

- **Precautions:**
  1. Although clays are effective against aflatoxins, caution should be exercised to make sure that:
     - Their inclusion level is not too high
     - They are free from impurities such as dioxin
  2. When the level of inclusion is very high, which is actually required for them to be effective, there are chances that these compounds can bind minerals and antibiotics like monensin.
  3. Some of the binders are not biodegradable and could pose environmental problem.

2- Chemical Methods

- A variety of chemical agents have been used to degrade mycotoxins in contaminated feeds, particularly aflatoxins; they are;
  1. Acids
  2. Bases (ammonia, caustic soda)
  3. Oxidants (hydrogen peroxide, ozone, sodium hypochlorite)
  4. Reducing agents (Bisulphites)
  5. Chlorinated agents
  6. Formaldehyde

Cont. …

- These techniques are
  1. Not totally safe
  2. Expensive
  3. **Not well accepted by consumers**

3- Biological Methods

- The biological decontamination of mycotoxins may be achieved using:
  1. Yast *Saccharomyces cerevisiae*
  2. Lactic acid bacteria

- Yeast and lactic acid bacterial cell wall structures can bind with different toxins which would be of great value in reducing the mycotoxin hazards.
- Yeast and lactic acid bacteria strains that have effective binding with mycotoxins could eventually be used to minimize aflatoxin exposure and improving overall health in animals.

Cont. …

Yast *Saccharomyces cerevisiae*

- Beta-glucans (esterified glucomannans), specific sugars present in the inner cell wall of yeast, can bind aflatoxins.

Yeast compared to clay (Inclusion level)

- 500 gm of glucomannans from yeast cell-wall have the same adsorption capacity as 8 kg of clay.
- This binder reduces the AFM1 content of milk by 58% in cows given a diet contaminated with AFB1 at a concentration of 0.05% of dry mater.

Cont. …

Lactic acid bacteria

- Probiotic strain of *Lactobacillus acidophilus CU028* has shown to bind aflatoxin.
- Probiotic fermented milk containing *Lactobacillus casei and Lactobacillus rhamnosus* strains alone or in combination with chlorophyllin exhibited protective effect against aflatoxin B1- induced hepatic damage.
- Acid treated lactic acid bacteria were able to bind high dosage of aflatoxin in gut conditions.

4- Biotransformation

Dual cultivation of:

1. *Aspergillus niger*
2. *Mucor racemosus*
3. *Alternaria alternata*
4. *Rhizopus oryzae*
5. *Bacillus stearothermophilus*

With

Toxigenic strain of *Aspergillus flavus*

Results in

70-80% degradation of aflatoxins

Cont. …

- **Certain microbes are also able to:**
  1. Metabolize mycotoxins (*Corynebacterium rubrum)* in contaminated feed
  2. Biotransform mycotoxins *(Rhizopius, Trichosporon mycotoxinivorans, Rhodotorula rubra, Geotrichum fermentans).*

- **These biological processes are generally slow and have a varied efficiency.**

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Dietary Manipulations
Hepatotropic nutrients and anti-oxidants

› Various nutritional strategies have been employed to alleviate the adverse effects of aflatoxins.

Cont. …

Amino acids

› Addition of specific amino acids like methionine in excess of their requirement protect the chicks from growth depressing effects of AFB1, possibly through an increased rate of detoxification by glutathione, a sulfur amino acid metabolite.

Phenyl alanine

› Alleviates toxicity of ochratoxin.

Vegetable oil

› Adding vegetable oils such as safflower oil and olive oil, to aflatoxin contaminated feed, improves the performance of chicks.

Cont. …

Antioxidants

- Aflatoxins cause toxicity through release of free radicals and lipid peroxidation.
- Antioxidants could aid in the overall detoxification process in liver and hence may help in alleviation of aflatoxicosis.
- Vitamin E and Selenium supplementation also has shown to overcome negative effects of aflatoxin.

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