Aflatoxin Control: Experience from Poultry Industry

Poultry Industry in India is a vibrant Agribusiness with an annual turnover of over Rs.1,00,000 crore

Prof. G. Devegowda
devegowdag@gmail.com
### Poultry feed production in million tons/year

<table>
<thead>
<tr>
<th>Category</th>
<th>Production (million tons/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>12.0</td>
</tr>
<tr>
<td>Layers</td>
<td>10.0</td>
</tr>
<tr>
<td>Breeders</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25.0</strong></td>
</tr>
</tbody>
</table>

![Corn kernels](image1.png)  ![Poultry feed](image2.png)

[Note: Images of corn kernels and poultry feed are included for visual context.]
Aflatoxins B1, B2, G1,G2 are produced by

Aspergillus Flavus: Storage Fungi
Aspergillus Parasiticus:

Aflatoxin B1 is the most prevalent and toxic Aflatoxin
Aspergillus Flavus on Rice

Murthy and Devegowda, 2004
Aflatoxin reference standards

Murthy and Devegowda, 2004
Molds and Aflatoxins Production...

- High moisture
- High humidity
- Temperature
- Improper drying
- Improper storage

Aflatoxins
Regulations for Aflatoxins for Dairy and Poultry feeds
Food Safety....

Aflatoxin $M_1$ in Milk and Milk Products and in Eggs and Chicken Meat
1 to 6% of Aflatoxin B1 present in the Dairy feed is transferred to milk as Aflatoxin M₁.

0.1 to 0.2% of Aflatoxin B1 present in Poultry feed is transferred to eggs and meat as Aflatoxin M1.
BIS Regulations for Aflatoxin B1 both in Dairy and Poultry feeds

20 mcg / kg (20ppb)

Source : BIS 2052 (2009) Compounded feeds for Cattle,

BIS 1374 (2007) Poultry feeds
U.S. FDA Regulations for Aflatoxin B1 both in Dairy and Poultry feeds

20 mcg / kg (20ppb)

Source: FDA (U.S), Ministry of Agriculture (Canada)
European regulations for Aflatoxin B1 in Feeds

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Limit (µg / kg, ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cows</td>
<td>5</td>
</tr>
<tr>
<td>Calves</td>
<td>10</td>
</tr>
<tr>
<td>Poultry</td>
<td>20</td>
</tr>
</tbody>
</table>
If the world adopts EU standard for Aflatoxins what will be the economic impact on Dairy Industry and raw material exporters?

- US and BIS 20 mcg/kg (20 ppb) in Dairy feeds
- EU 5 mcg/kg (5 ppb) in Dairy feeds
Good Manufacturing Practices and Storage of Feeds
Stop Fungal growth in Feeds by good Storage Practices

Source: Life line Poultry Feeds, Karnataka
Source: Sriya Poultry Feed plant, Bengaluru
Moisture Optimization Program (MOP) to control mold growth in feeds

Contains Mold Inhibitor and Surfactant

- Optimize the moisture in the final feed to 11-11.5%
- Convert free form of moisture into bound form
- Spraying of mold inhibitor and water varies depending on raw materials moisture content
Silos for Maize Storage with Aeration:
Pre-cleaned and Dried Maize is stored for 3 to 4 months
Maize pre-cleaner

- Removes dust *(contains Aflatoxins)*
- Removes all waste materials
Storage Conditions of grains…..

- Moisture content less than 13 %
- Avoid damaged and broken grains (susceptible for fungal growth)
- Avoid Insect damaged grains (susceptible for fungal growth)
KARIMNAGAR FEED PLANT WITH 800 TPD
New maize having high moisture more susceptible for fungal growth

https://en.wikipedia.org/wiki/maize

Use it immediately and use mold inhibitors to prevent fungal growth in feeds
 Dilution of new maize with old maize
 Dilution of contaminated maize with good quality maize
Good Quality Raw Materials: Energy Sources
Good Quality Raw Materials: Protein sources
Avoid using Distillers dried grains (DDGS) (It contains Aflatoxins 3 times more than the grain)

Avoid using Brewery waste (high level of Aflatoxins because of poor quality grains used)

Avoid Self mixing of feeds
The levels of Aflatoxins (ppb) in feedstuffs

<table>
<thead>
<tr>
<th></th>
<th>Aflatoxins</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 -50 ppb</td>
<td>50-100 ppb</td>
<td></td>
</tr>
<tr>
<td>Cereals, samples</td>
<td>88</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cereal byproducts, samples</td>
<td>48</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Oilseed meals, samples</td>
<td>152</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Source: G. Devegowda, K.L Aravind and C.K Girish
Prevention and Control of Aflatoxins
Prevention and Control

An integrated approach is needed

- Use of new technology
  Good Agricultural Practices (GAP)
  Good Manufacturing Practices (GMP)

- Drying in Sunlight

- Inorganic adsorbents:
  Clay materials

- Biotechnological:
  Organic adsorbents - Yeast cell wall, Algae
Inorganic adsorbents: Clay materials

✓ Zeolites (1:1) Sodium Aluminosilicates

✓ Bentonites (2:1)

Sodium and Calcium Bentonite (2:1)
Montmorillonites (Na, Ca) x (Al, Mg) $2\text{Si}_4\text{O}_{10} \text{(OH)} 2 \cdot n\text{H}_2\text{O}$
Hydrated Sodium Calcium Aluminosilicates (HSCAS)
Sodium and Calcium Bentonite (2:1)
Hydrated Sodium Calcium Aluminosilicates (HSCAS)
90% of aflatoxins are adsorbed between the interlaminar surfaces, the remainder, in the basal ends.

Source: Phillips, 2010
Clay Materials

- High inclusion rates (10 to 12 kg/ton)
- Reduced mineral utilization: Manganese, Zinc, Copper, Phosphorous and Magnesium
- Reduced Vitamin utilization
- Contamination by Dioxins and heavy metals
Modified Organoclay

- Modified organoclay is synthesized from the bentonite / zeolite (natural clays) through substitution of inorganic cations for organic cations (Redding et al., 2002).

Quaternary amines
Risks to the Environment

• **Due to health and environmental risks related to quaternary amines, modified clays are difficult to produce.**

Biotechnological Solutions: It’s based on yeast cell wall
The cell wall contains **three key components**

- Glucan
- Mannan
- Chitin
Yeast cell wall

\[ \Rightarrow \text{Glucans} \]

- \( \beta_1,3 \text{ Glucans} \)
- \( \beta_1,6 \text{ Glucans} \)
- Mannoproteins
- GPI Anchor
- Chitin

Intracellular Space

Extracellular Space

Nucleus

Secretory organel

Bud
Adsorption of Aflatoxin B1 into the $\beta$-(1,3)-D-glucan chain

A. Yiannikouris et al. 2004. Biomacromolecules
β-D-glucans binding Aflatoxin B1

[Aflatoxin B1 + β-(1,3)-D-glucans]

High geometrical similarity between AFB1 and β-(1,3)-D-glucans

A. Yiannikouris et.al., 2012
Adsorbing aflatoxin: an efficient approach

Contaminated feeds

Mycotoxins chemically bond to the adsorbent

- Mycotoxins pass through the digestive tract without absorption
- Harmful effects of mycotoxins strongly decreased

Mycotoxin intrinsic toxic effects

Adsorbing aflatoxin: an efficient approach

MYCOSORB®

Gastro-intestinal tract

Ingestion

Activation

Detoxification

Recycling

Transfer

Excretion

faeces

milk

eggs

meat

urine

Taylor-Pickard et al., 2009
Scientific collaborations

**A. Yiannikouris,** UR1213, Unité de Recherches sur les Herbivores, INRA, Centre de Clermont-Theix, France.

**Dr. J François PhD,** UMR-CNRS 5504, UR-INRA792, INSA, Toulouse, France.

**Dr X Cameleyre Ph.D.,** Toulouse, France.

**Dr L Poughon & Pr. CG Dussap PhD** Laboratoire Chimique et Biologique, Clermont-Ferrand, France.

**Dr G Bertin PhD,** Regulatory Affairs Manager, Alltech-France, Goussainville, France.

**Dr G André PhD,** Unité de Biochimie Structurale, INRA-Institut Pasteur, Paris, France.

**Dr A Buléon PhD & Dr B Pontoire,** UR783, Laboratoire de Physico-Chimie des Macromolécules, Nantes, France.

**Dr P Galtier PhD,** UR66, Laboratoire de Pharmacologie et Toxicologie, Toulouse, France.

**INRA/Alltech PhD Student**

Yeast cell wall isolation + extraction

**NMR analyses**

Molecular modeling

**Mycotoxin analyses**

**X-ray diffraction analyses**

**Mathematical modeling**

**Scientific support**
Dr. G. Devegowda showed the ability of Mycosorb to act within 30 minutes.

The beginning of extensive research on Mycosorb took place in the lab of Dr. T.K. Merrill and co-workers unveiled the efficacy of Mycosorb in new circumstances.

Dr. A. Yiannikouris described the physical and chemical mechanisms involved in Mycosorb mode of action.

Mycosorb was created through the modifications to the glucomannan portion of the inner cell wall of yeast.

Mycosorb was awarded a patent by the US patent and trademark office.

Dr. Karl Dawson discovered conditions for the maximum efficacy of Mycosorb.

Mycosorb continue to be the leading technology worldwide with 13 PhDs and more than 50 peer reviewed papers.

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Evolution of Mycosorb
Reduction of aflatoxin M₁ in milk with Yeast Glucans

Aflatoxin M₁, ppb

<table>
<thead>
<tr>
<th></th>
<th>Without Glucans</th>
<th>Glucans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin M₁, ppb</td>
<td>1.354&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.506&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Aravind and Devegowda, 2004
Mycosorb® modulated improvement in milk yield and reduction in milk residue of aflatoxin M₁ in dairy cows

G. Devegowda¹ and T. N. K. Murthy² and A. Ramesh³

¹University of Agricultural Sciences, Bangalore, India
³Veterinary and Fishery sciences University, Bangalore, India
Effects of Binding Agents on Milk Aflatoxin Residues

Diaz et al, N.C.S.U., 1999

<table>
<thead>
<tr>
<th>Binding Agent</th>
<th>% Reduction (Kg/ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na Bentonite</td>
<td>70</td>
</tr>
<tr>
<td>Ca Bentonite</td>
<td>50</td>
</tr>
<tr>
<td>Glucan</td>
<td>30</td>
</tr>
<tr>
<td>Activated Charcoal</td>
<td>10</td>
</tr>
</tbody>
</table>

Kg/ton: 12.5  12.5  0.5  2.5
Efficacy of Glucans (1kg) and HSCAS (10kg) to alleviate the Aflatoxin toxicity in broilers on body wt

(Girish and Devegowda, 2004)
Efficacy of Glucans (1kg) and HSCAS (10kg) to alleviate the aflatoxin toxicity in broilers on liver wt

( Girish and Devegowda, 2004)
% Aflatoxin adsorbed by Glucans in broilers at different time intervals

In minutes

Krishnamurthy & Devegowda, 2002
Problems with Sampling for Aflatoxins

• Mycotoxins are not evenly distributed
• Mycotoxins are present in very small amounts (ppb)
Conclusions

“Safe feed Safe Milk and eggs”