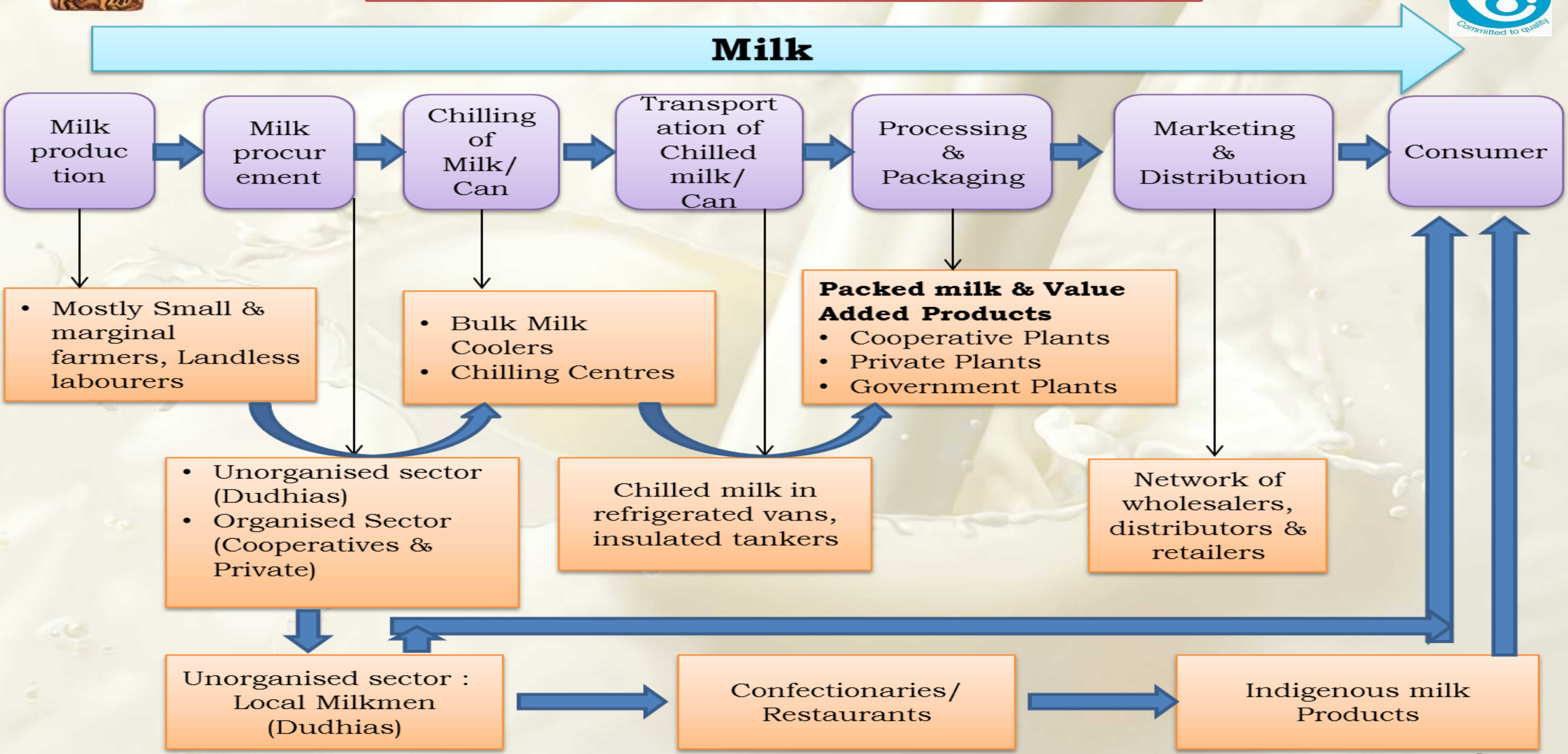
# Processing interventions to reduce Aflatoxins

Dr. DK Sharma
National Dairy Development Board
Anand-388001

# Value chain

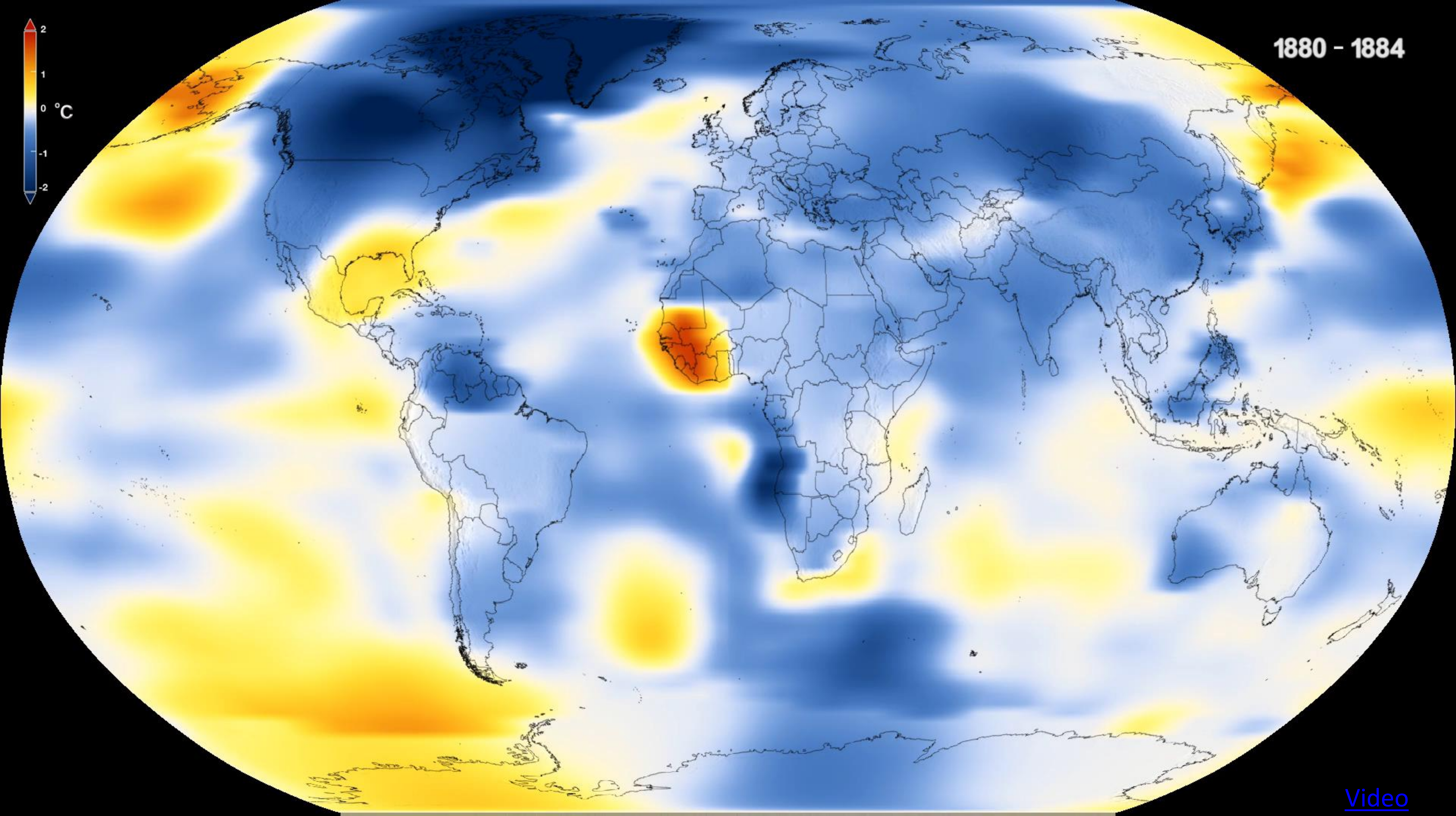

Milk
Milk production
Milk procurement
Chilling of Milk/ Can
Transportation of Chilled milk/ Can
Processing & Packaging
Marketing & Distribution
Consumer
• Mostly Small & marginal farmers, Landless labourers
• Bulk Milk Coolers
• Chilling Centres
Packed milk & Value Added Products
• Cooperative Plants
• Private Plants
• Government Plants
• Unorganised sector (Dudhias)
• Organised Sector (Cooperatives & Private)
Chilled milk in refrigerated vans, insulated tankers
Network of wholesalers, distributors & retailers
Unorganised sector : Local Milkmen (Dudhias)
Confectionaries/ Restaurants
Indigenous milk Products

# Challenges

- The findings from the recent survey by the FSSAI in 2018 of 6432 samples (2607 - 41% and 3825-59% of processed and raw milk, respectively).
- The samples were analysed for
  - Qualitative-Fat, SNF and proteins
  - 13 adulterants
  - Antibiotics residues
  - Pesticides residues
  - Aflatoxin M1-with MRL of 0.5µg/kg
- The major contaminants of safety concern found were **aflatoxin and antibiotic** carry over into milk.
  - **Aflatoxin M1** $\geq$ MRL in 368 (out of 6,432) that's **about 5.7%.**

# Aflatoxin

- Aspergillus is an opportunistic pathogen
- Fungi native to warm arid and tropical regions
- Toxin production influenced by climate
  - the increase in temperature,
  - carbon dioxide and
  - Humidity
- Fungal contamination occurs in two distinct phases
  - the infection of the developing crop
  - contamination after maturation in second phase




Video

# Public Health Significance of Aflatoxins

- Poses both acute and chronic health risk

- At high levels aflatoxins causes
  - acute toxicosis,
  - leads to liver damage,
  - liver cancer, gastrointestinal dysfunction,
  - decreased immunity and death

- Affects the health, Growth, Productivity in human & animals


- Factors aiding growth of *A flavus*
  - Stress situation - High temperature & long dry periods
  - Damage due to insects/mechanical bruising
  - Poor harvesting
  - Insufficient drying
  - Storage conditions

# Genetic trigger

Increased expression of structural aflD and regulatory aflR – under different stress treatments.

Two key regulatory genes (aflR/aflS) control $AFB_1$ production – related to temperature and $a_w$ interactions.

The higher the ratio, the higher the relative $AFB_1$ production.

# Possible control of fungal growth

1. Gamma irradiation of commodities

2. Chemical treatment

3. Fumigation with ammonia, phosphine, Hydrogen peroxide and Ethylene oxide

4. Use of organic acids – acetic acid, propionic acid, butyric acid, malonic acid, benzoic acid, sorbic acid, lactic acid, citric acid and their sodium salts

http://www.fao.org/3/x5036e/x5036E0q.htm

# Controlling Aflatoxin levels in Feed

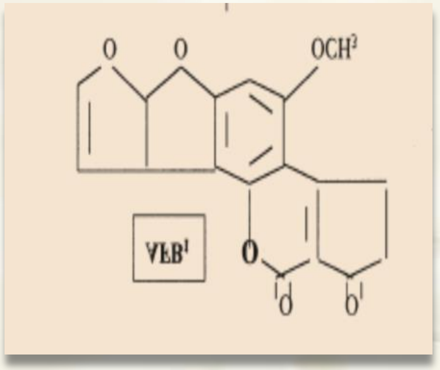
- **Decontamination/destruction** – Ammoniation as a decontaminant and destructing agent.

- **Nixtamilization** – Alkaline treatment especially of maize

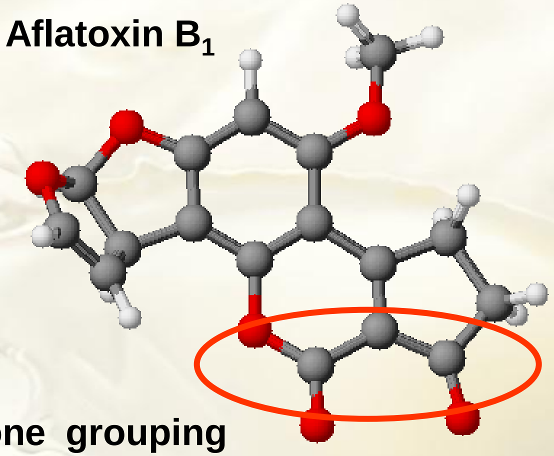
- **Binders & Sorbents** – addition of binding agents (zeolite clays and aluminosilicates) to effectively reduce toxin level. Sorbent (clay materials like Hydrated ammonium silicates of Sodium and calcium, Sodium bentonite, Esterified Glucomannan, Sodium montmorillonite, Diatomceous earth, activated charcoal) addition to animal feed can reduce aflatoxin in milk.

*Aflatoxin: finding solutions for improved food safety, IFPRI 2013*

# Why does aflatoxin bind so strongly compared to other mycotoxins?

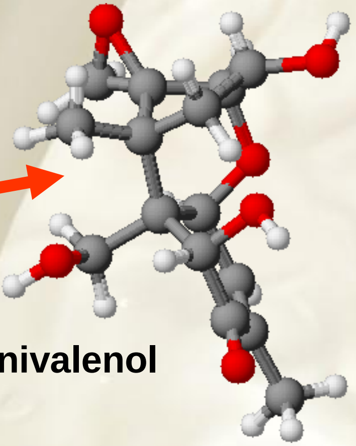


Aflatoxin $B_1$

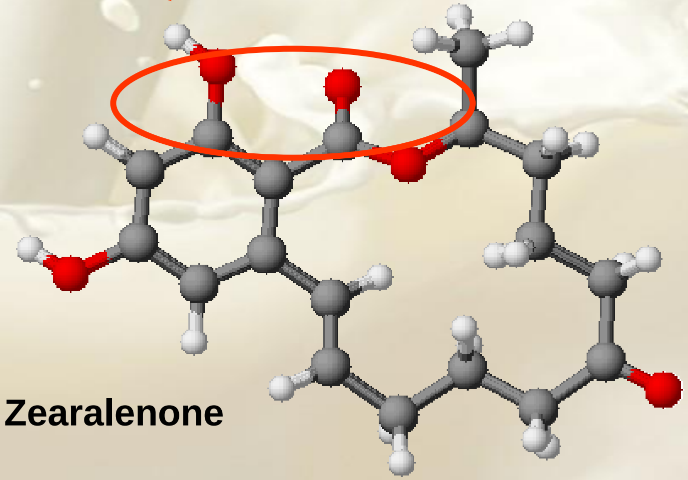


1,3-diketone grouping causes strong binding

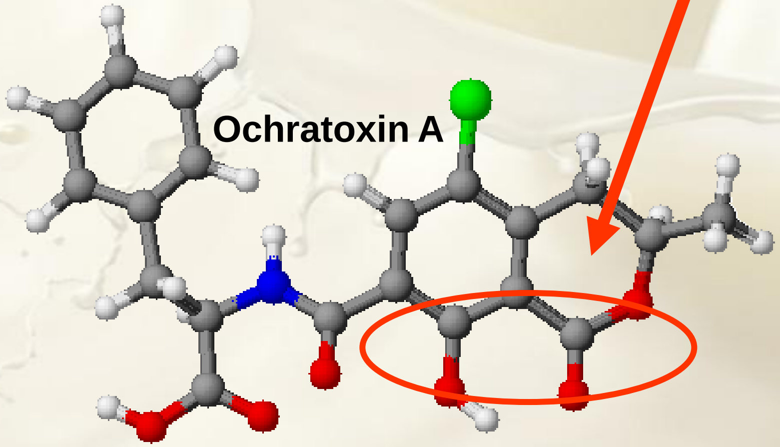
Some similarity
between these
and these
…but none here



Deoxynivalenol

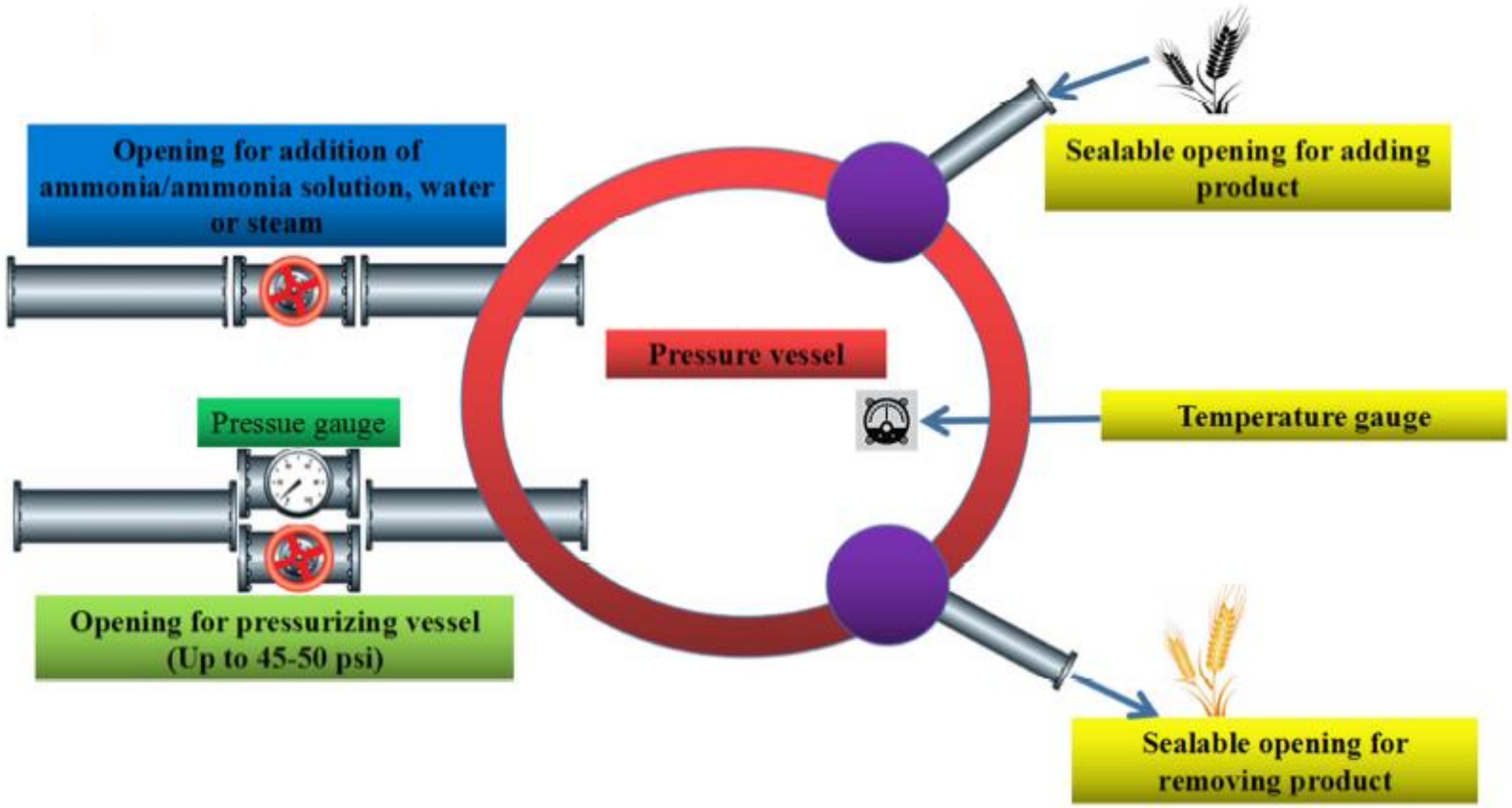


Zearalenone



Ochratoxin A

# Ammoniation Process



# Biological methods

- **Bio control technology** using carefully selected non toxigenic strains that safely outcompete and virtually eliminate their toxic relative effectively reducing contamination

- Application of bio control agents to the crop such as Aflasafe is based on the competition between two spp of *Asperigillus* ie toxigenic and non-toxigenic forms.

# Exploiting LAB to bind Aflatoxin in Feed

- Encourage Lactic Acid Bacteria (LAB) inoculated silage
- *Lactobacillus* spp are found to remove 25 to 77% $AFB_1$.
- Heat killed *LAB* to bind aflatoxin

**Select specific LAB & "*probiotics*" as novel approach**

# Effects of Processing on $AFM_1$

Reduction in aflatoxin levels are suggested to be achieved through minor interventions

- **Heating @ 62°C/30 minutes, 72°C/45 seconds and 80°C/45 seconds obtained reductions in aflatoxin levels by 32.5%, 45.5% and 63.6% in $AFM_1$ levels respectively**
- Heating at **115°C/45 seconds** reduced 81.3% of $AFM_1$ levels.
- **Drying:** Reduction upto 75.6% and 86.5% by roller drying and spray drying respectively.

# Manufacturing of products

- **Dahi/Yoghurt:** $AFM_1$ level decreases by 36.5% and 34.6% because of reduction of **pH & LAB quench the aflatoxin.**
  - Bacterial cell wall and cell components bind the toxin protein
  - LAB in fermented foods inhibit fungal growth and extend the shelf life of the product.
  - Antifungal and antibacterial compounds produced by LAB are assumed to reduce the toxin production.
- Bacterial cell wall binding could be exploited to inactivate harmful dietary compounds and alter their mutagenic and carcinogenic potential.

# Exploiting LAB to bind Aflatoxin in food

- The interaction mechanism between LAB and mycotoxins is thought to be similar to the interactions involved in adsorption by GMA (glucomannan) .
- The polysaccharide components (glucans and mannans) are common sites for binding, with different toxins having different binding sites.

**Cell Wall**

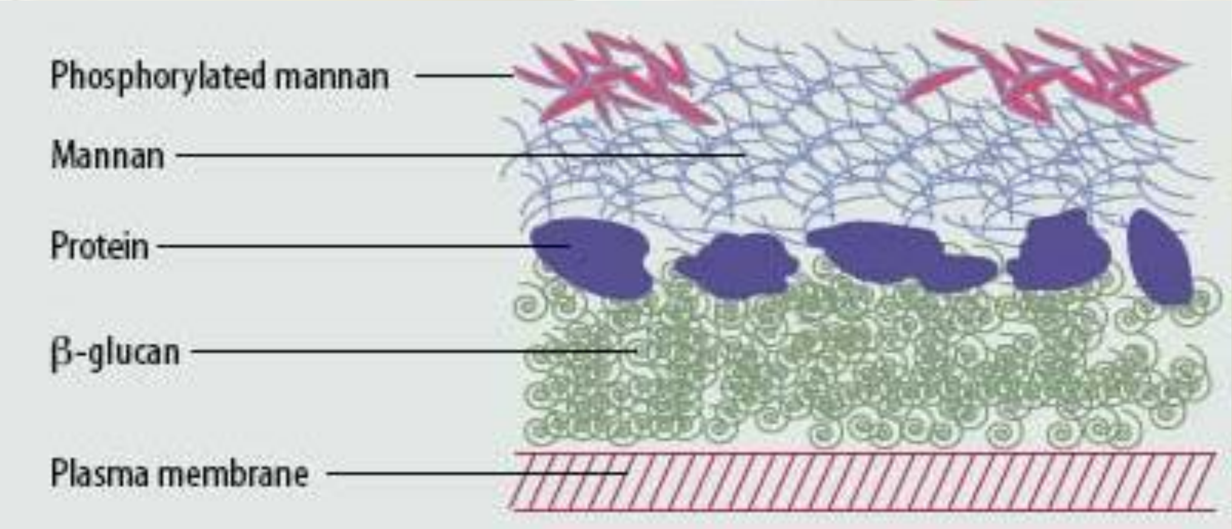


**Table 5** Binding of aflatoxins by viable bacteria *in vitro* conditions.

| Bacteria | Bacterial conc'n (CFU ml$^{-1}$) | AF conc'n (μg ml$^{-1}$) | % AF bound | Reference |
|---|---|---|---|---|
| *Lb. acidophilus* E-94507 | $1 \times 10^{10}$ | 5 $AFB_1$ | 18.2 | Peltonen et al. (2001)[273] |
| *Lb. acidophilus* CSCC 5361 | $1 \times 10^{10}$ | 5 $AFB_1$ | 20.7 | Peltonen et al. (2001)[273] |
| *Lb. acidophilus* ATCC 4356 | $1 \times 10^{10}$ | 5 $AFB_1$ | 48.4 | El-Nezami et al. (1998)[265] |
| *Lb. acidophilus* LA1 | $10^9$ | 0.15 $AFM_1$ | 18.3 | Pierides et al. (2000)[272] |
| *Lb. acidophilus* NCC 12 | $10^8$ | 0.1 $AFM_1$ | 30.5 | Kabak and Var (2004)[275] |
| *Lb. acidophilus* NCC 36 | $10^8$ | 0.1 $AFM_1$ | 28.0 | Kabak and Var (2004)[275] |
| *Lb. acidophilus* NCC 68 | $10^8$ | 0.1 $AFM_1$ | 25.7 | Kabak and Var (2004)[275] |
| *Lb. rhamnosus* E-97800 | $1 \times 10^{10}$ | 5 $AFB_1$ | 22.7 | Peltonen et al. (2001)[273] |
| *Lb. rhamnosus* CSCC 2420 | $1 \times 10^{10}$ | 5 $AFB_1$ | 33.1 | Peltonen et al. (2001)[273] |
| *Lb. rhamnosus* LBGG | $10^{10}$ | 5 $AFB_1$ | 75.3 | El-Nezami et al. (1998)[265] |
| *Lb. rhamnosus* LC705 | $10^{10}$ | 5 $AFB_1$ | 76.1 | El-Nezami et al. (1998)[265] |
| *Lb. rhamnosus* LBGG | $2 \times 10^{10}$ | 5 $AFB_1$ | 78.5 | Kankaanpaa et al. (2000)[264] |
| *Lb. rhamnosus* GG | $10^8$ | 0.15 $AFM_1$ | 50.7 | Pierides et al. (2000)[272] |
| *Lb. rhamnosus* LC705 | $10^8$ | 0.15 $AFM_1$ | 46.3 | Pierides et al. (2000)[272] |
| *Lb. rhamnosus* 1/3 | $10^8$ | 0.15 $AFM_1$ | 18.1 | Pierides et al. (2000)[272] |
| *Lb. rhamnosus* GG | $10^{10}$ | 5 $AFB_1$ | 76 | Haskard et al. (2000)[270] |
| *Lb. rhamnosus* LC705 | $10^{10}$ | 5 $AFB_1$ | 77 | Haskard et al. (2000)[270] |
| *Lb. plantarum* E-79098 | $1 \times 10^{10}$ | 5 $AFB_1$ | 28.4 | Peltonen et al. (2001)[273] |
| *Lb. paracasei* F19 | $10^{10}$ | 5 $AFB_1$ | 28 | Peltonen et al. (2000)[269] |
| *Lb. crispatus* M247 | $10^{10}$ | 5 $AFB_1$ | 6 | Peltonen et al. (2000)[269] |
| *Lb.crispatus* MU5 | $10^{10}$ | 5 $AFB_1$ | 20 | Peltonen et al. (2000)[269] |
| *Lb. fermentum* CSCC 5362 | $1 \times 10^{10}$ | 5 $AFB_1$ | 22.6 | Peltonen et al. (2001)[273] |
| *Lb. johnsonii* CSCC 5142 | $1 \times 10^{10}$ | 5 $AFB_1$ | 30.1 | Peltonen et al. (2001)[273] |
| *Lb. johnsonii* LJ-1 | $10^{10}$ | 5 $AFB_1$ | 31 | Peltonen et al. (2000)[269] |
| *B. lactis* CSCC 5094 | $1 \times 10^{10}$ | 5 $AFB_1$ | 34.7 | Peltonen et al. (2001)[273] |
| *B. lactis* Bb-12 | $10^{10}$ | 5 $AFB_1$ | 17 | Peltonen et al. (2000)[269] |
| *B. longum* CSCC 5304 | $1 \times 10^{10}$ | 5 $AFB_1$ | 37.5 | Peltonen et al. (2001)[273] |
| *B. longum* Bl 24 | $10^8$ | 0.1 $AFM_1$ | 26.7 | Kabak and Var (2004)[275] |
| *B. bifidum* Bb13 | $10^8$ | 0.1 $AFM_1$ | 32.5 | Kabak and Var (2004)[275] |
| *Propionibacterium freu.* ssp. *shermani* JS | $10^{10}$ | 5 $AFB_1$ | 34.1 | El-Nezami et al. (1998)[265] |
| *P. freu.* ssp. *shermani* JS | $10^{10}$ | 5 $AFB_1$ | 22 | Haskard et al. (2000)[270] |

**Table 6** Binding of aflatoxins by heat-killed bacteria *in vitro* conditions

| Bacteria | Bacterial conc'n (CFU ml$^{-1}$) | AF conc'n ($\mu$g ml$^{-1}$) | % AF bound | Reference |
|---|---|---|---|---|
| *Lb. rhamnosus* GG | $10^{10}$ | 5 $AFB_1$ | 30.5 | El-Nezami et al. (1998)[266] |
| *Lb. rhamnosus* GG | $10^{10}$ | 5 $AFB_1$ | 83 | Haskard et al. (2000)[270] |
| *Lb. rhamnosus* GG | $10^{8}$ | 0.15 $AFM_1$ | 57.8 | Pierides et al. (2000)[272] |
| *Lb. rhamnosus* LC 705 | $10^{10}$ | 5 $AFB_1$ | 28.5 | El-Nezami et al. (1998)[266] |
| *Lb. rhamnosus* LC 705 | $10^{8}$ | 0.15 $AFM_1$ | 51.6 | Pierides et al. (2000)[272] |
| *Lb. rhamnosus* 1/3 | $10^{8}$ | 0.15 $AFM_1$ | 39.9 | Pierides et al. (2000)[272] |
| *Lb. acidophilus* LA1 | $10^{9}$ | 0.15 $AFM_1$ | 25.5 | Pierides et al. (2000)[272] |
| *Lb. acidophilus* LC1 | $10^{10}$ | 5 $AFB_1$ | 74.7 | Haskard et al. (2001)[276] |
| *Lb. acidophilus* ATCC 4356 | $10^{10}$ | 5 $AFB_1$ | 69.7 | Haskard et al. (2001)[276] |
| *Lb. gasseri* ATCC 33233 | $10^{9}$ | 0.15 $AFM_1$ | 61.5 | Pierides et al. (2000)[272] |
| *Lc. lactis ssp cremoris* ARH74 | $10^{9}$ | 0.15 $AFM_1$ | 38.9 | Pierides et al. (2000)[272] |
| *Lc. lactis ssp cremoris* | $10^{10}$ | 5 $AFB_1$ | 40.1 | Haskard et al. (2001)[276] |
| *Lc. lactis ssp lactis* | $10^{10}$ | 5 $AFB_1$ | 58.1 | Haskard et al. (2001)[276] |
| *Bifidobacterium* spp. JO3 | $10^{10}$ | 10 $AFB_1$ | 41 | Oatley et al. (2000)[268] |
| *Bifidobacterium* spp. JR20 | $10^{10}$ | 10 $AFB_1$ | 37 | Oatley et al. (2000)[268] |
| *Bifidobacterium* spp. CH4 | $10^{10}$ | 10 $AFB_1$ | 37 | Oatley et al. (2000)[268] |
| *Bifidobacterium* spp. Bf 6 | $10^{10}$ | 10 $AFB_1$ | 25 | Oatley et al. (2000)[268] |
| *B. adolescentis* 14 | $10^{10}$ | 10 $AFB_1$ | 31 | Oatley et al. (2000)[268] |
| *B. bifidum* BGN4 | $10^{10}$ | 10 $AFB_1$ | 46 | Oatley et al. (2000)[268] |

# Immobilization of *Saccharomyces cerevisiae* on Perlite Beads for the Decontamination of Aflatoxin M1 in Milk

Marjan Foroughi, Mahboobe Sarabi Jamab, Javad Keramat, and Mahsa Foroughi

**Abstract:** Aflatoxin M1 (AFM1) contamination presents one of the most serious concerns in milk safety. In this study, the immobilization of *Saccharomyces cerevisiae* was used to detoxify AFM1-contaminated milk. The yeasts were immobilized on perlite for 24 and 48 hr, and the best immobilization time was achieved at 48 hr. Microscopic examination confirmed successful immobilization. The milk samples with 0.08, 0.13, 0.18, and 0.23 ppb AFM1 contamination were passed through the biofilter for 20, 40, and 80 min. The results showed a significant reduction in AFM1 concentration for all the milk samples with various initial AFM1 contents. The contaminated milk with 0.08 ppb AFM1 was completely cleared after 40 min of circulation while for the milk solution with 0.23 ppb, the highest AFM1 reduction was obtained at about 81.3% after 80 min circulation. In addition, the biofilter was saturated after the third step of milk circulation, containing 0.23 ppb AF, in which each step duration was 20 min. This study showed the excellent capability of the immobilized cells on the perlite beads to detoxify the AFM1-contaminated milk without any side effects on its physicochemical properties.

**Keywords:** aflatoxin M1, decontamination, immobilization, perlite, *Saccharomyces cerevisiae*

**Practical Application:** The immobilization of *Saccharomyces cerevisiae* cells on perlite beads can be used to detoxify AFM1-contaminated milk. The perlite can provide a perfect support for immobilization. With respect to qualitative properties, 20 min, was suggested as the optimum time for milk decontamination. This study indicated that the detoxification of contaminated milk using immobilized *S. cerevisiae* cells on the perlite support did not affect the different properties of detoxified milk. This method can lead to a practical solution to address aflatoxin contamination in dairy products considered high-risk foods.

# Biotransformation

- **Biotransformation**

**Dual cultivation of:**

  - *Aspergillus niger*
  - *Mucor racemosus*
  - *Alternaria alternata*
  - *Rhizopus oryzae*
  - *Bacillus stearothermophilus*

**With**

- Toxigenic strain of *Aspergillus flavus*

**Results in**

- 70-80% degradation of aflatoxins

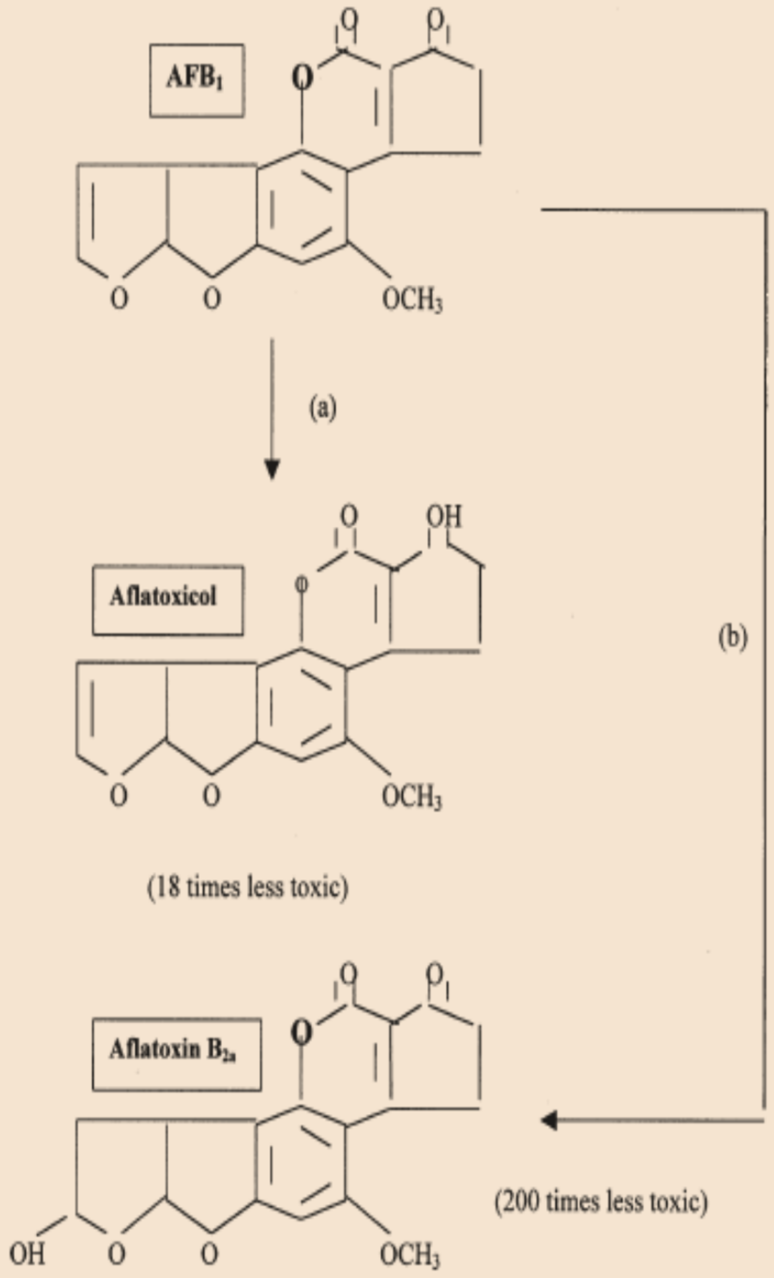



FIGURE 3. (a) Biologically reduced $AFB_1$ by *T. pyriformis* W. (Robertson et al., 1970) *Rhizopus* sp. (Nakazato et al, 1990). (b) Hydroxy derivative of $AFB_1$ by *Lactobacillus delbrueckii* (Maing et al., 1973).

# Way forward

**Feed:**

- Application of **bio control techniques**
- **Process Modification:**
  - Binding agents and Sorbents: zeolite clays, Novasil clay & Organic acids
  - LAB & probiotic bacteria.
  - Use of enzymes
  - Gamma irradiation of commodities
  - Chemical treatment with fungicides
  - Fumigation with ammonia and phosphine
  - Ammoniation and Nixtamilization

**Food Processing:** Heating @ 72°C/**45 seconds** and 80°C/**45 seconds** for reductions by 45.5% and 63.6% in $AFM_1$ respectively.

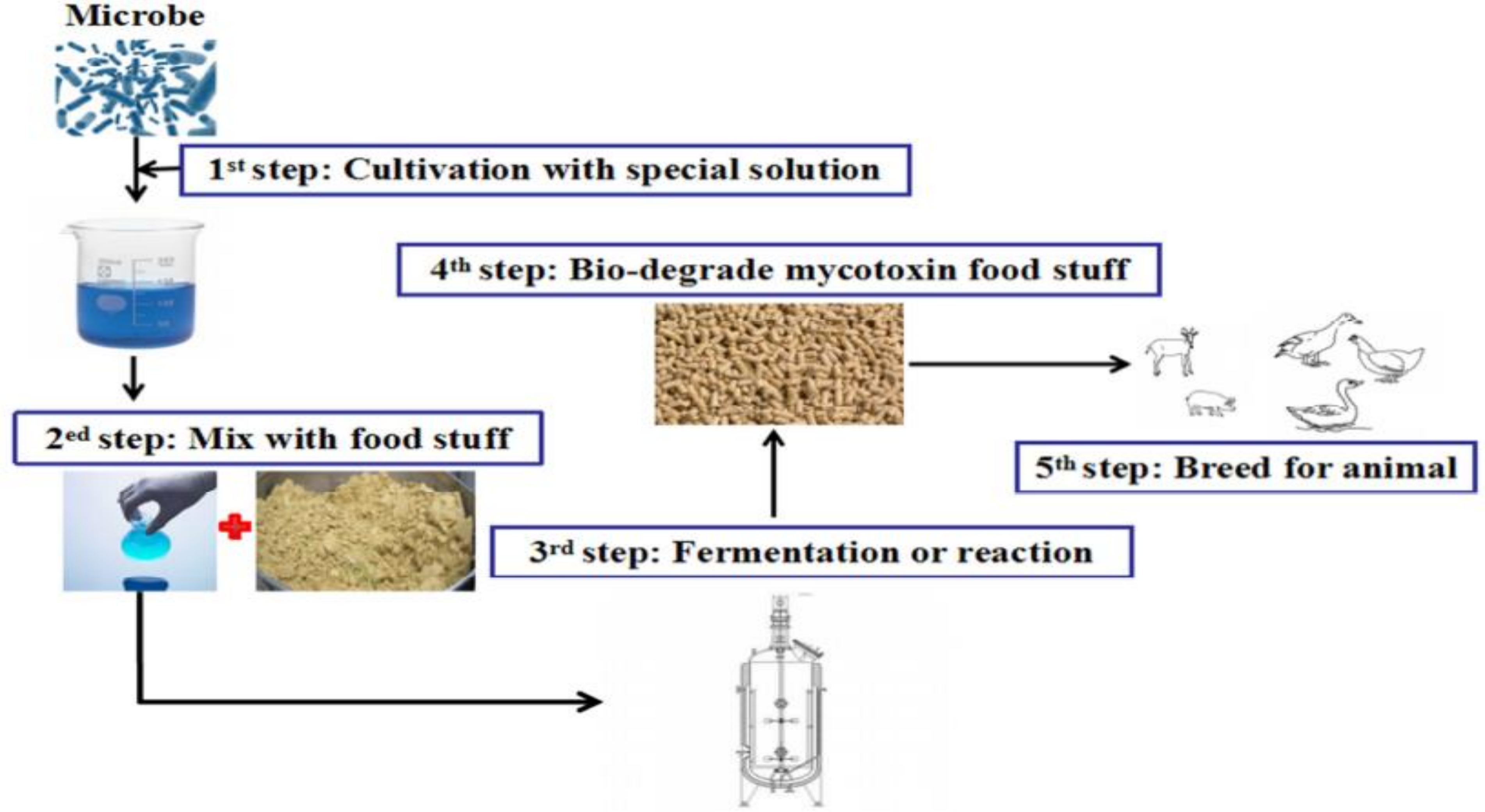
# Open question

1. Can **extending the holding time in pasteurization** be an intervention to reduce aflatoxin in milk ???

2. Can **impregnated adsorption** be an intervention technique ???


THANK YOU

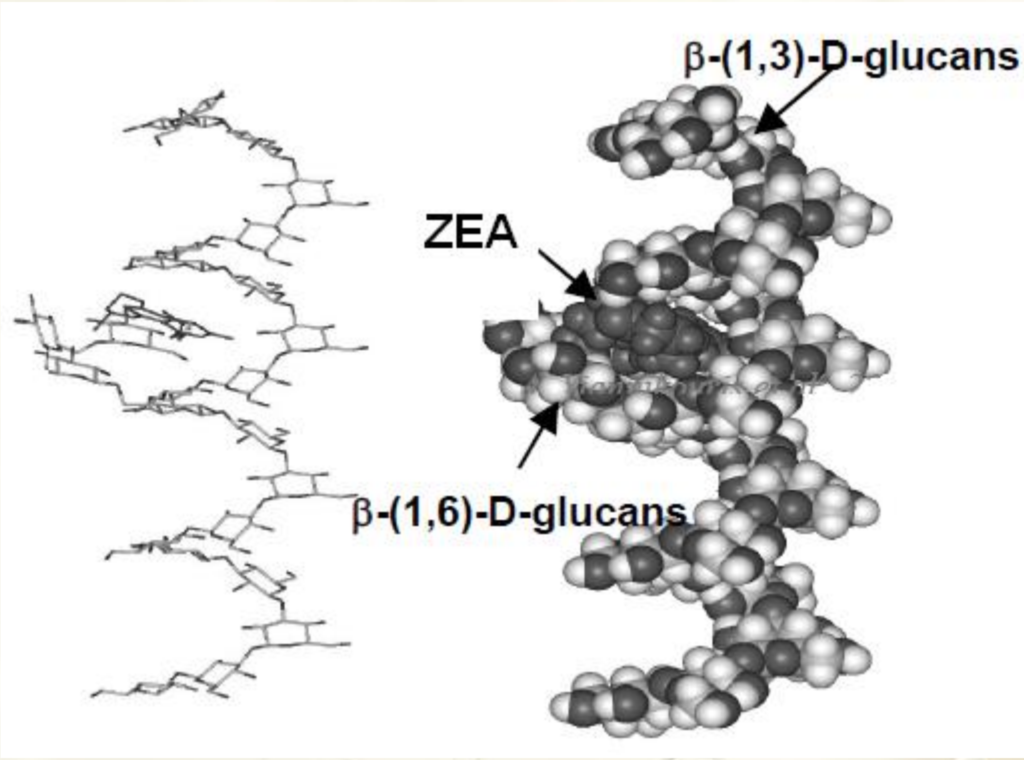
# microbiological deactivating mycotoxins (AFT) in animal food stuff.

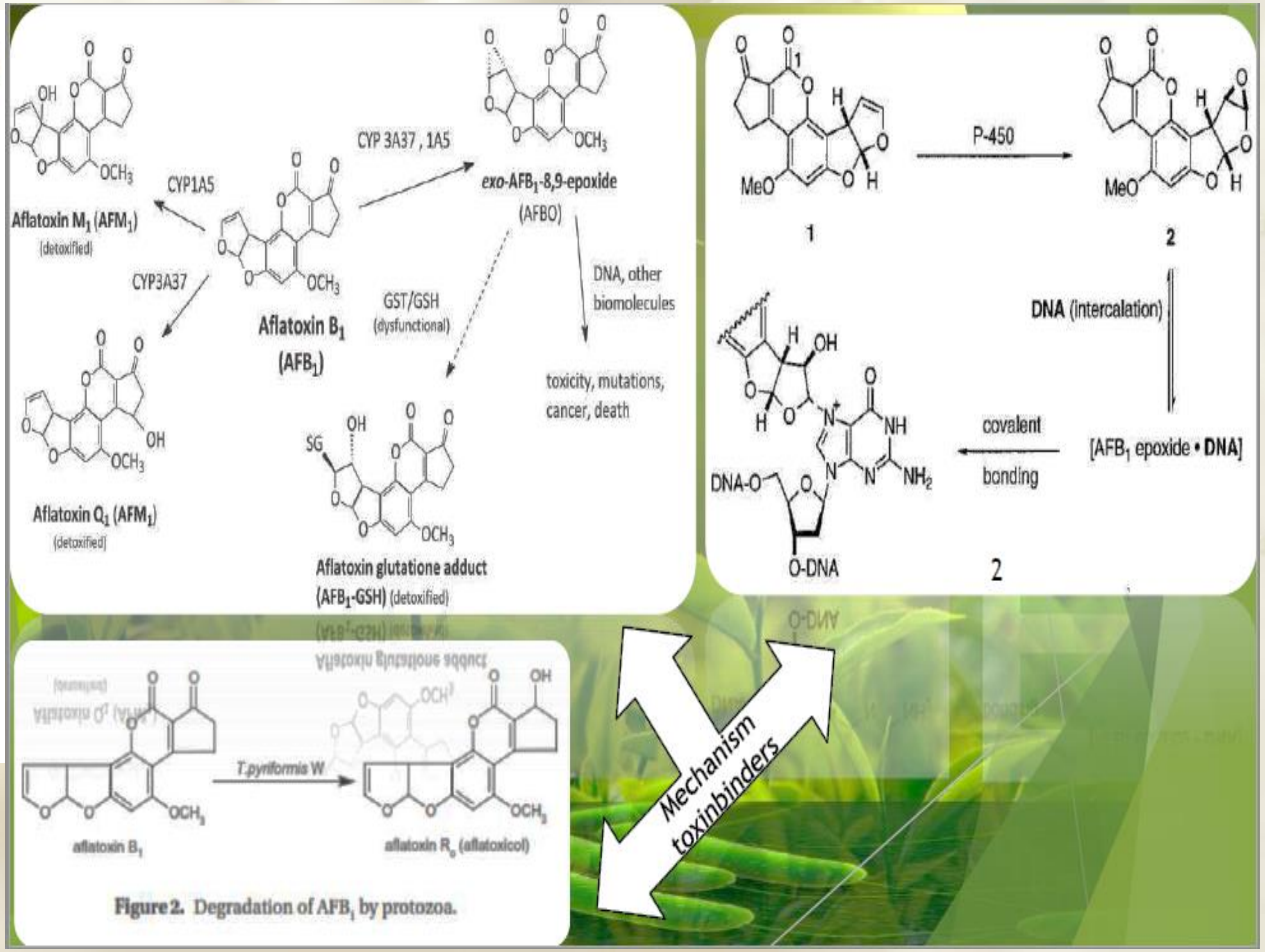



Microbe mix with food stuff or food raw materials in fermentation instruments to degrade mycotoxins. Finally, the final food or food stuff can maintain original profile or nutrients for feeding animal.

# Public Health Significance

- The use of antimicrobials to treat dairy animals has the potential affect to human health through:
  - Increasing the risk of antimicrobial residues, and
  - Influencing the generation or selection of antimicrobial resistant foodborne pathogens.
  - Allergic reactions, toxicity, carcinogenic effects, disruption of human normal flora, provoke immunological response.


β-(1,3)-D-glucans
ZEA
β-(1,6)-D-glucans


CYP1A5
CYP 3A37 , 1A5
CYP3A37
Aflatoxin M1 (AFM1)
(detoxified)
exo-AFB1-8,9-epoxide
(AFBO)
Aflatoxin B1
(AFB1)
GST/GSH
(dysfunctional)
DNA, other
biomolecules
toxicity, mutations,
cancer, death
Aflatoxin Q1 (AFM1)
(detoxified)
Aflatoxin glutatione adduct
(AFB1-GSH) (detoxified)
P-450
DNA (intercalation)
covalent
bonding
[AFB1 epoxide • DNA]
DNA-O
O-DNA
T.pyriformis W
aflatoxin B1
aflatoxin R0 (aflatoxicol)
Figure 2. Degradation of AFB1 by protozoa.
Mechanism
toxinbinders

## Impacts of aflatoxins in animal source foods

- Aflatoxin B1 is metabolized to aflatoxin M1 in liver
- Rumen microflora degrade aflatoxin
- Excreted into milk
- 1-7% of ingested aflatoxin B1 get secreted in to milk