

Aflatoxin : Prevalence and Control in Dairy Feeds and Milk



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November, 2019

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PART – I

PREVALENCE

1.0 Executive summary

Aflatoxins are the most widespread and the most studied group of all the mycotoxins, and they are prevalent in warm and humid climatic conditions; as exists in India and in many Asian countries. Aflatoxins are primarily produced by fungi of the genus *Aspergillus (Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius*), and are found in dairy feeds and human food products. Major forms of Aflatoxins found in feeds include Aflatoxins B1, B2, G1 and G2; with Aflatoxin B1 being the most common and toxic. Aflatoxin M1 is found in milk and milk products.

Strict control of Aflatoxin B1 level in feeds for lactating dairy cattle and buffaloes is required in order to minimize the level of Aflatoxin M1 in milk and milk products. The United States Food and Drug Administration (USFDA, 2018) and Food Safety and Standards Authority of India (FSSAI, FSS Regulation 2011) set a maximum permissible level for Aflatoxin M1 in milk at 0.5 μ g/kg (ppb; parts per billion) of milk, which means that one ton of milk should not contain more than 500 micrograms of Aflatoxin M1.

Aflatoxin B1 is readily transmitted from feed to milk; approximately 1.0 to 6.0% of Aflatoxin B1 present in feed is transferred to milk as Aflatoxin M1, depending on factors such as the genetics of animals, seasonal variation, the milking process and the environmental conditions. The occurrence of Aflatoxins in commercially available milk, and milk products is of concern, as milk is a key source of nutrients for humans.

2.0 Introduction

There are more than 10,000 known species of fungi. Fortunately, most of them are beneficial to man in the production of bread, cheese, antibiotics etc. There are about 50 fungi species harmful to livestock, poultry, and man known to produce toxins, which are collectively called mycotoxins. Mycotoxins are metabolites produced by fungi during metabolism of nutrients present in feeds and feed ingredients.

Fungi produce mycotoxins in the field ("field toxins": fusarium toxins) or in storage ("storage toxins": Aflatoxins, ochratoxins), or both. Mycotoxins are mainly produced by the fungi genera of *Aspergillus, Fusarium, Penicillia*, and *Claviceps*. Their formation may occur when the fungi grow on crops in the field, at harvest, in storage or during the processing of feed when conditions are favorable.

No region of the world escapes these silent killers, and their negative impact on animal productivity and human health is enormous. According to the United Nation's Food and Agriculture Organization (FAO), approximately 25% of World's grain supply is contaminated with mycotoxins. Recently, a 38 member scientific task-force from the Council for Agricultural Science and Technology (CAST, 2003) in the USA released a 200 page report entitled *"Mycotoxins: Risks in Plant, Animal, and Human systems"*. According to the report, in the United States alone the economic costs resulting from mycotoxins exceed \$ 1.5 billion annually.

3.0 Mycotoxins: An overview

3.1 Geographical distribution of mycotoxins

The threat of mycotoxins has been described as early as the Second World War when the soldiers from the Russian army suffered severe dermal necrosis, hemorrhages and destruction of bone marrow after eating mouldy grains (*Fusarium* contaminated, Figure 1). However, it was not until 1960, when the entire turkey population of Britain was decimated in a fatal liver disease called '**Turkey X Disease**', that the scientific community recognized the negative effects associated with mycotoxins. British agriculture officials later traced the source of the outbreak to Aflatoxin in a shipment of peanut (groundnut) meal that originated from Brazil.



Figure 1: Maize cobs contaminated with Fusarium fungus

The occurrence of mycotoxins in nature is considered a global problem. However, in certain geographical areas of the world, some mycotoxins are produced more readily than others. In colder, more temperate regions such as Canada, the Northern US and most of the European countries, Aflatoxins are not considered to be a major problem except in imported feedstuffs grown in warmer southern climates. Economically, in these regions the most important mycotoxins are deoxynivalenol (DON, Vomitoxin), Zearalenone (ZEA), T-2 toxin produced by *Fusarium* fungi (Figure 1).

In Europe, the differences in climatic conditions among the northern, middle and southern parts favor the development of different fungal species. In the maize growing areas of southern and middle Europe (Sweden, Austria and Hungary) mainly fusariotoxins (DON, ZEA, T-2 toxin) cause illness and poor performance of dairy animals.

3.1.1 Deoxynivalenol (DON, Vomitoxin)

Deoxynivalenol is produced by several *Fusarium* species of mould primarily *Fusarium graminearum* and may co-occur with other mycotoxins in contaminated commodities. DON in cattle and buffaloes has been associated with reduced feed intake and lower milk production when fed with feed containing more than 5 parts per million (ppm).

3.1.2 Zearalenone

Zearalenone is produced primarily by *Fusarium graminearum* and *Fusarium roseum*. Zearalenone is responsible for reproductive disorders because of its estrogenic effect. In dairy animals, the clinical manifestations such as udder enlargement, decreased milk yield, vaginal discharge, continuous estrus, infertility and abortions are observed when the level of zearalenone in the feed is more than 0.5 ppm.

3.1.3 Fumonisins

Fumonisins are primarily produced by *Fusarium moniliforme* and *Fusarium proliferatum*, the most toxic being fumonisin B1. Fumonisin mycotoxins are found in a wide range of commodities from millets to grains to banana fruits. They are a major concern to food and feed producers since they affect human and animal health. The toxin causes liver damage and decreased milk production in dairy cattle at levels greater than 50 ppm in the ration. Usually, level of fumonisins exist between 5-20 ppm in feed.

4.0 Global scenario of Aflatoxins

Aflatoxins; the most widespread of all the mycotoxins, are common in warm and humid climatic conditions like those existing in India, Latin American, Asian and African countries, southern regions of US, and certain parts of Australia. Extensive surveys conducted in India, Pakistan, Egypt and South Africa suggested that Aflatoxins are often encountered in substantial levels in feeds and feed ingredients (Figure 2).

In Latin American countries including Brazil, Peru, Mexico, Columbia, Venezuela and Argentina, reports exist on the presence of Aflatoxins. Due to the increase in global trading of feedstuffs, mycotoxins are no longer solely found in certain geographical regions but are now more widely distributed than before (Devegowda *et al.*, 1998).

Aflatoxins B1, B2, G1, and G2 refer to toxins which fluoresce blue (B1, B2) or green (G1, G2) under ultraviolet light. Among all Aflatoxins, B1 is the more prevalent and toxic.



Figure 2: Maize naturally contaminated with Aspergillus fungus

5.0 Limits of Aflatoxins in feed and milk in other countries

Strictly speaking, there is **no** *safe level*. With reference to mycotoxins, the risk directly depends on the level of the major mycotoxins and also on the presence and levels of other mycotoxins in feeds. A mycotoxin level considered safe in one farm may not be safe in another farm because of differences in management and disease prevalence. Additionally, factors such as interaction of mycotoxins with pathogens, genetic variability, environmental conditions, and nutritional status etc. influence the severity of the manifestation of mycotoxicosis.

In order to reduce the toxic and economic impact of mycotoxins, several countries regulate the levels of some mycotoxins in foods and feeds. Worldwide, food and feed legislation safeguards the health of consumers and the economic interests of animal producers and traders. Virtually, all countries with fully developed market economies have regulations with the exception of some African countries.

Table 1:	Limits	for	Aflatoxin	B1	in	dairy feed
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Country	Aflatoxin B1 (µg/kg or ppb)
United States (FDA)	20
European Union (for milking animals)	5
European Union (for calves)	10
India (BIS)	20

Table 2: Limits for other mycotoxins in dairy feed

Mycotoxins (EU Guidelines)	Limits (mg/kg or ppm)
Deoxynivalenol: Adult animals	5
: Calves	2
Zearalenone: Adults and calves	0.5
Fumonisins B1 and B2: Adult animals	50
: Calves	20

Table 3: Limits for Aflatoxin M1 in milk

Country	(µg/kg or ppb)
United States (USFDA)	0.5
European Union	0.05
European Union, for baby foods/ infants	0.025
Australia (FSANZ)	0.05
Australia, for infants 0.02	
India (FSSAI)	0.5

6.0 Overview of Aflatoxins in feed and feed raw materials in India

Aflatoxins are primarily produced by fungi of the genus *Aspergillus (Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius*; Figure 3) and are found in dairy feed and human food products. Major forms of Aflatoxins found in feeds include Aflatoxins B1, B2, G1 and G2; with **Aflatoxin B1 being the most common and toxic.**



Figure 3: Growth of Aspergillus flavus on rice

Extensive surveys conducted in India suggest that Aflatoxins are often encountered in substantial levels in feeds and feed ingredients. Aflatoxins are rarely found in forages. To study the incidence of Aflatoxin B1 in different raw materials, a survey was conducted by the Division of Animal Sciences, University of Agricultural Sciences, Bengaluru. The results of the survey showed that out of 246 samples analyzed by Thin Layer Chromatography (TLC), 206 samples were found to be positive for the presence of either Aflatoxin.

The cereal and cereal byproducts analyzed were maize, deoiled rice bran, rice polish and wheat bran. The samples were found 88% positive for Aflatoxins. The oilseed meals analyzed were soybean meal, full-fat soy, sunflower meal, groundnut cake/ extract/ expeller, rapeseed meal and til cake. The oilseed meals contain higher incidence of Aflatoxin (96%) contamination as compared to cereals and cereal byproducts.

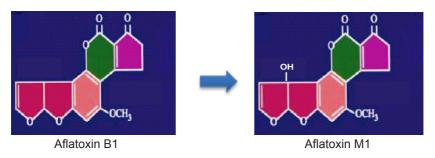
Aflatoxin B1 content was analysed in some samples of cattle feed (n=254) suspected to be contaminated with Aflatoxins at NDDB, Anand (NDDB, 2018). The results found that the average level of Aflatoxin B1 was 68 ppb in these samples.

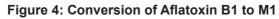
7.0 Metabolism of Aflatoxin in ruminants and conversion of Aflatoxin B1 to Aflatoxin M1

In milk, Aflatoxin appears as Aflatoxin M1, one of its metabolites. Aflatoxin B1 is metabolized by enzymes found primarily in the liver (Cytochrome P450) to Aflatoxin M1. Strict control of Aflatoxin B1 level in feeds for dairy animals is required in order to minimize the level of Aflatoxin M1 in milk and milk products.

In the US (FDA) and in India (FSSAI), it is required by the law that Aflatoxin M1 in milk should be less than **0.5 ppb**; which means that one ton of milk should not contain more than 500 micrograms of Aflatoxin M1. In European Union, the regulations are much more stringent, and maximum levels are set

at **0.05 ppb** (EC, 2002). Aflatoxin is readily transmitted from feed to milk; approximately **1.0 to 6.0% of Aflatoxin B1** present in feed is transferred to milk as Aflatoxin M1 (Figure 4). After Aflatoxin M1 is formed, it is excreted in milk and urine of the dairy animals.





Diaz *et al.* (2004) have indicated that Aflatoxins appear in milk within 12 hours following oral administration of Aflatoxin B1. The peak concentration in milk occurs after 24 hrs. Clearance is also very rapid, since Aflatoxin disappears from the milk four days after cessation of oral administration (Diaz *et al.*, 2004). These results confirm the rapid absorption and metabolism of Aflatoxins in ruminants.

The USFDA and BIS (India) stipulate a maximum level of 20 ppb of Aflatoxin in dairy feed, which means that one ton of feed should not contain more than 20 milligrams of Aflatoxin B1. The European Union regulations are much more stringent and the maximum level set at 5.0 ppb. Like Aflatoxin B1, Aflatoxin M1 is toxic, although toxicity of Aflatoxin M1 is somewhat lower than that of Aflatoxin B1.

8.0 Impact of feeding Aflatoxin contaminated feed in dairy animals

Milk producers may not be able to see visual symptoms of aflatoxicosis in the animals at low level Aflatoxin in the feed. However, high concentrations of Aflatoxins and/ or prolonged duration may cause visual symptoms in dairy animals. Feed refusal, reduced growth rate and decreased feed conversion efficiency are the predominant signs of chronic Aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat and mild diarrhea may occur. The disease may also impair reproductive efficiency, including abnormal estrous cycles (too short and too long) and abortions. Other symptoms include impaired immune system response, and increased susceptibility to diseases.

PART – II

CONTROL

9.0 Use of Mycotoxin binders for control of Aflatoxins in feed

9.1 Type of mycotoxin binders

Practical mycotoxin control involves following two main stages:

- Prevention of fungal growth and toxin production in feedstuffs, and
- Decontamination of existing mycotoxins in feeds.

Several strategies, to be adopted in a coordinated manner have been proposed to reduce the impact of mycotoxins, which include: plant breeding for mould resistance, efficient harvesting and storage practices to minimize contamination and decontamination process for the contaminated commodities (Devegowda and Aravind, 2002).

9.1.1 Nutritional modifications

Nutritional routes for protection against mycotoxins include higher levels of methionine, selenium and vitamin supplementation of affected diets. Some plant and herbal compounds including chlorophyll derivatives are also used for protection against mycotoxins. This method is partially beneficial but not cost effective.

9.1.2 Herbal mould inhibitors

Certain herbs and herbal extracts have been found to exert inhibitory effect on mould growth and thus toxin production. Aqueous extracts of garlic, onion, turmeric, neem etc., have been shown to exert anti-fungal activity and inhibit Aflatoxin production. This method, though beneficial to some extent, presents challenges pertaining to standardization, quality control etc.

9.1.3 Chemical detoxification

Among the chemicals tested for their ability to detoxify/ inactivate mycotoxins, ammonia, sodium bisulfite, peroxide, acids, bases and gases are effective. However, most of the chemical methods are not practical and they do not fulfill all the requirements, specifically those concerning the safety of reaction products and the palatability of the feed.

9.1.4 Biological methods

Advances in the field of biotechnology have opened a new avenue for tackling mycotoxicosis. A natural organic product, glucomannan-containing yeast product, a cell wall derivative of *Saccharomyces cerevisiae have* shown considerable binding ability with commonly occurring mycotoxins (Devegowda and Murthy, 2005) and is found beneficial in minimizing the adverse effects of mycotoxins in livestock and poultry (Raju and Devegowda, 2002).

9.1.5 Application of mineral clays

Many types of clay have been tested for counteracting mycotoxins. These include bentonites. zeolites and aluminosilicates. Several studies have demonstrated that sodium aluminosilicate, sodium calcium aluminosilicate, and sodium bentonites can adsorb Aflatoxins (Phillips et al., 1990; Mahesh and Devegowda, 1996). A hydrated sodium calcium aluminosilicate (HSCAS) is the most widely studied mycotoxin sequestering agent among the mineral clays. Interest in its potential as a mycotoxin sequestrant was based on the ability of the silicate mineral to sequester positively charged or cationic compounds (Ramos and Hernandez, 1997).

9.2 Characteristics of an ideal mycotoxin binder

The most effective method of neutralizing mycotoxins already in feed is by binding them to an inert compound before they can be absorbed from the intestines. The question that feed manufacturers, and milk producers frequently ask is: *"Which mycotoxin binder is most effective?"* The "ideal" features of good mycotoxin binder are:

- Ability to bind a wide range of mycotoxins;
- Low effective inclusion rate in feed;
- Rapid and uniform dispersion in the feed during mixing;
- Heat stability during pelleting, extrusion, and during storage;
- No affinity for vitamins, minerals or other nutrients.

10.0 Test methods for Aflatoxin analysis at laboratory and field levels

10.1 Tests to detect Aflatoxin

There are simple, and fast tests which can be performed for testing of Aflatoxin B1 in finished feed and raw materials, and M1 in milk and milk products. Kits using ELISA (Enzyme-Linked Immunosorbent Assay) technology are available to test at the farm as well as at the cattle feed plant level. Other analytical methods are also available such as Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). Off-late, lateral flow based strips are available in the market for quick detection of Aflatoxins in feed and milk.

11.0 Measures to be taken during pre-harvest, harvest and storage under field conditions

The most practical approach is by good agricultural practice, which includes 'plant breeding' to develop mould resistance crops and the second approach is '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 bio-competitive** *Aspergillus* strains out-compete the toxigenic isolates, resulting in reducing pre-harvest contamination with Aflatoxin in groundnut and cotton.

11.1 Storage under field conditions to prevent fungal growth

- Dry the raw materials immediately after harvesting to moisture content less than 13%.
- Avoid damaged and broken grains (susceptible for fungal growth).
- Avoid insect damaged grains (susceptible for fungal growth).
- Pre-clean and dry the grains before storage in silos or in bags.
- Use mould inhibitors to prevent fungal growth, if the moisture in raw materials is more than 13%.
- Store the raw materials on wooden pallets or crates and away from the walls to prevent moisture migration from the floor and walls.

11.2 Preventive measures to be taken at Cattle Feed Plant level

- Pre-clean the maize to remove dust (contains high level of Aflatoxins) and all waste materials.
- Use mould inhibitors in finished feed in hot and humid conditions.
- Store the finished feeds on wooden pallets or crates to prevent moisture migration from the floor.
- Systematic inspection and clean-up program to keep bins, delivery trucks, and other equipment free of adhering or caked feed ingredients.
- > Remove dust (contains Aflatoxins).
- Remove all waste materials.

12.0 Common raw materials used in cattle feed that are susceptible to Aflatoxins

- a. Raw materials containing high levels of Aflatoxins: Groundnut cake, Cottonseed cake, Copra cake, Dried Distillers Grains with Solubles (DDGS), and maize gluten meal.
- **b.** Raw materials containing moderate levels of Aflatoxins: Maize, rice polish, de-oiled rice bran and wheat bran.
- c. Raw materials containing low levels of Aflatoxins: Rapeseed meal, soybean meal, straw and hay.

13.0 Standard sampling procedure for Aflatoxin analysis

Major problems associated with sampling for testing of mycotoxins are i) Mycotoxins are not evenly distributed, and ii) Mycotoxins are present in very small amounts (ppb).

To sample the feed, take at least 8 to 12 representative samples at each of 3 to 5 locations from the feed bin/ truck/ gunny bags etc. Mix the sub-samples, and take about 500 gram of composite sample. Divide the composite sample into 2 parts of 250 gram each. Store one sample in a cool, dry place, and use the other one for testing at the laboratory. The stored 250 gram sample may be used for possible confirmatory testing.

14.0 Conclusions

Under practical conditions, no dairy feed is virtually free from mycotoxins. Additionally, no feed can be expected to contain only one mycotoxin. A strong recommendation is made hereby to view the problem in its natural way i.e. co-contamination of feeds and feedstuffs by mycotoxins. It is therefore essential to take all the necessary precautions such as:

- Prevention of fungal growth on crops in the field, at harvest of crop, during storage of feedstuffs and processing of feed.
- Time to act is not after the dairy animals are affected with mycotoxins, but before the contaminated feed is consumed by the dairy animals.
- Application of appropriate mycotoxin binder in order to achieve good productivity, safe milk and milk products for human consumption and economy.

PART – III

FREQUENTLY ASKED QUESTIONS (FAQs)

Frequently Asked Questions (FAQs)

1. What can I do, if feed contains excessive level of Aflatoxin B1 concentrations?

The feed should be reformulated with ingredients that contain less than 20 ppb of Aflatoxin B1. Inclusion of Aflatoxin binder in the feed is another solution to reduce the Aflatoxin B1 level in feed to the permissible level. However, growing animals and dry dairy cows and buffaloes can be fed with a feed containing more than 20 ppb of Aflatoxin, but less than 50 ppb.

2. Which raw materials contain high level of Aflatoxin B1?

Groundnut cake, Cottonseed cake, Copra cake, DDGS, and maize gluten meal contain high level of Aflatoxin B1. Whereas, maize, rice polish, de-oiled rice bran and wheat bran contain moderate level of Aflatoxin B1.

- Which raw materials contain low level of Aflatoxin B1? Rapeseed meal, soybean meal, and rice brokens contain low level of Aflatoxin.
- 4. What about Aflatoxins content in straw, hay and silage? Straw, hay and silage contain very low level of Aflatoxins.

5. What is the permissible level of Aflatoxin in the feed of milking cows and buffaloes?

Bureau of Indian Standards (BIS) in India has set a maximum permissible level of 20 µg/kg or ppb for Aflatoxin B1 in dairy feeds. The Food and Drug Administration (FDA) has also set the similar limit in USA.

6. What is the permissible level of Aflatoxin M1 level in the milk as per Indian, American and European Standards?

In the US (FDA) and in India (FSSAI) it is required by the law that Aflatoxin M1 in milk should be less than 0.5 ppb (μ g/kg). In Europe, the regulations are much more stringent, and maximum levels are set at 0.05 ppb.

7. How Aflatoxin B1 get into milk as Aflatoxin M1?

Aflatoxin B1 is metabolized by enzymes found primarily in the liver (Cytochrome P450) to Aflatoxin M1. Strict control of Aflatoxin B1 level in feeds for dairy animals is required in order to minimize the level of Aflatoxin M1 in milk and milk products.

8. How many days it takes for Aflatoxin M1 to enter into milk after feeding Aflatoxin contaminated feed?

It has been reported that Aflatoxins appear in milk within 12 hours following oral administration of Aflatoxin B1. The peak concentration in milk occurs after 24 hrs. Clearance is also very rapid, since Aflatoxin disappears from the milk four days after cessation of oral administration. The results confirm the rapid absorption and metabolism of Aflatoxins in ruminants.

9. How many days it is required to bring Aflatoxin M1 within the permissible limit by feeding the feed containing less than 20 ppb?

It takes about four days to bring Aflatoxin M1 within the permissible limit by feeding the feed containing less than 20 ppb.

10. What to do with feed containing more than 20 ppb of Aflatoxin B1.

May be fed to growing animals and dry (non-milking) cows and buffaloes with feed containing more than 20 ppb, but less than 50 ppb.

11. What is the maximum level of moisture for storage of grains?

The moisture content should be less than 13% for storage of grains for about 3 to 4 months with good ventilation and storage practices. Avoid broken and insect damaged grains.

12. How to store the raw materials in the feed godown?

Store the raw materials on wooden pallets or crates and away from the walls to prevent moisture migration from the floor and walls.

13. Does pelleting of feed destroy Aflatoxin B1?

Aflatoxins are heat stable, therefore pelleting of feed will not destroy Aflatoxin B1.

Abbreviations

BIS	:	Bureau of Indian Standards
CAST	:	Council for Agricultural Science and Technology
DDGS	:	Dried Distillers Grains with Solubles
DON	:	Deoxynivalenol
EC	:	European Commission
ELISA	:	Enzyme Linked Immunosorbent Assay
EU	:	European Union
FAO	:	Food and Agriculture Organization
FAQs	:	Frequently Asked Questions
FDA	:	Food and Drug Administration
FSANZ	:	Food Standards Australia New Zealand
FSSAI	:	Food Safety and Standards Authority of India
HPLC	:	High Performance Liquid Chromatography
NDDB	:	National Dairy Development Board
PMU	:	Project Management Unit
ppb	:	Parts per billion
ppm	:	Parts per million
TLC	:	Thin Layer Chromatography
USA	:	United States of America
ZEA	:	Zearalenone

Acknowledgement

Financial and technical supports provided by Project Management Unit (PMU), National Dairy Development Board, Anand, Gujarat under the National Dairy Plan-I are gratefully acknowledged.

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