Technews

National Dairy Development Board

For Efficient Dairying

July-August 2019 **Issue No. 98**

Aflatoxin: Reduction in Milk and Milk Products

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **"Aflatoxin: Reduction in Milk and Milk Products"** It may be understood that the information given here is by no means complete.

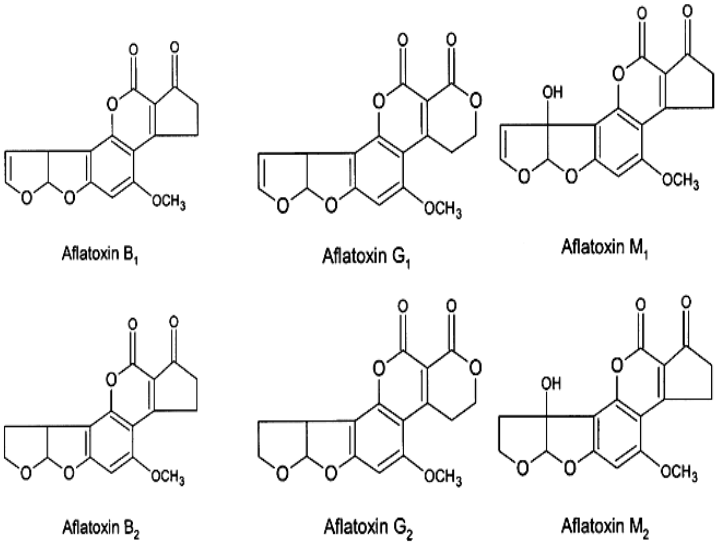
In this issue:

INTRODUCTION

Aflatoxins are one of many natural occurring mycotoxins that are found in soils, foods, humans, and animals. Aflatoxins are a group of secondary metabolites produced by three species of *Aspergillus: flavus, parasiticus* and *nomius*. Up until now, approximately 18 different toxic derivatives of aflatoxins have been reported. AFB1, AFB2, AFG1, AFG1, AFM1 and AFM2 are the most common type of aflatoxin. B1, B2, G1, and G2 are found in plant based food, while M1 (metabolite of B1) and M2 are found in milk. Among these, aflatoxin B1 (AFB1) is the most naturally occurring compound by toxigenic Aspergillus species. Chemically, aflatoxins (AFTs) are difuranocoumarin derivatives in which a bifuran group is attached at one side of the coumarin nucleus, while a pentanone ring is attached to the other side in the case of the AFTs and AFTs-B series, or a six-membered lactone ring is attached in the AFTs-G series. The physical, biological and chemical conditions of *Aspergillus* influence the production of toxins. Among the identified AFTs, AFT-B1, and AFT-B2 are produced by *A. flavus,* while AFT-G1 and AFT-G2 along with AFT-B1 and AFT-B2 are produced by *A. parasiticus.* AFTs- M1 and AFTs-M2 are derived from aflatoxin B types through different metabolic processes and expressed in animals and animal products.

The biosynthetic pathway of aflatoxins consists of 18 enzymatic steps for conversion from acetyl-CoA, and at least 25 genes encoding the enzymes and regulatory

pathways have been cloned and characterized. The gene comprises 70kb of the fungal genome and is regulated by the regulatory gene, *aflR*. Various genes and their enzymes are involved in the production of sterigmatocystin (ST) dihydrosterigmatocystin (DHST), which are the penultimate precursors of aflatoxins.



Chemical structure of aflatoxin B (AFB1 and AFB2), aflatoxin G (AFG1 and AFG2), and aflatoxin M (AFM1 and AFM2).

For basic information kindly refer our ***Technews 34*** on following link.

https://www.dairyknowledge.in/sites/default/files/technews_34.pdf

Health Risk: Human and Animal

Aflatoxin contamination of agricultural commodities poses considerable risk to human and livestock health and economic losses. In 1993, AFB1 and AFM1 were classified by the International Agency for Research of Cancer (IARC) of WHO as 1A (carcinogenic) and 2B (possible human carcinogen), respectively.

Humans

Exposure to aflatoxin leads to several health-related conditions including acute and chronic aflatoxicosis, immune suppression, and liver cancer (especially where hepatitis is prevalent), liver cirrhosis, stunted growth in children and many others. Exposure is associated with immune suppression and higher rates of illness. Aflatoxins in milk are of concern because milk consumption is often higher among infants and children, who are likely to be more vulnerable.

Animals

In animals, the effects of aflatoxins depend on various factors: dose, length of exposure, genetic (species and breed strain), physiological (age, nutrition, and exercise) and environmental (climatic and husbandry). Generally, calves are more susceptible than older animals' even low levels, and young and fast growing animals are more affected than adults. Males are more susceptible than females. There is considerable variation by species. A list of animals in order of decreasing sensitivity runs

rabbits>ducks>turkeys>chicken>fish>swine>cattle>sheep. Ruminants, if old enough to have a functioning rumen, are relatively resistant.

It exerts carcinogenic, teratogenic, hepatotoxic and mutagenic effects and suppresses the immune system of cattle. Aflatoxins exert acute and chronic effects in animals. Animal studies provide ample evidence that high levels of aflatoxins in animal feeds have adverse effects for animal health, growth, and productivity, reproduction, immune functioning and ability to metabolize vaccines. When lactating animals such as cows, goats and humans are fed with feedstuffs contaminated with AFB1, this metabolite can be transferred to milk as aflatoxinM1 (AFM1) in the range of 0.3–6.3%. Higher-yielding animals consuming large amounts of concentrates typically have higher levels in their milk. The presence of mastitis may increase the secretion of aflatoxins. Aflatoxin is excreted into milk within12 hours in the form of aflatoxin M1 with residues of the dietary aflatoxin level. The FDA limits for for aflatoxin B1 should not be more than 20 ppb in feed.

OUTBREAKS DUE TO AFLATOXINS

Occurrence of aflatoxicosis in poultry in Mysore state was first recognized in 1966 where 2219 chicks died in one week. Subsequently, several sporadic incidences were found in various poultry farms in Karnataka.

In 1974, a major outbreak of hepatitis due to aflatoxin was reported in the states of Gujarat and Rajasthan in India,

resulting in an estimated 106 deaths. The outbreak lasted for 2 months and was confined to tribal people whose main staple food, maize, was later confirmed to contain aflatoxin. Another outbreak of aflatoxin affecting both humans and dogs was reported in North West India. A major aflatoxin exposure outbreak was subsequently documented in Kenya in 1981. Preliminary testing of food from affected areas revealed the presence of aflatoxin. Heavy mortality in chicks in Chittoor district of Andhra Pradesh was reported in 1982 due to aflatoxicosis. Another outbreak of aflatoxicosis in commercial poultry farms was also reported in the same district with hundred percent mortality. Since 2004, multiple aflatoxicosis outbreaks have been reported worldwide, resulting in 500 acute illness and 200 deaths. Most outbreaks have been reported from rural areas of the East Province of Kenya in 2004 and occurred because of consumption of home grown maize contaminated with molds.

In 2013, countries in Europe including Romania, Serbia, and Croatia reported the nationwide contamination of milk with aflatoxin. Indian childhood cirrhosis, a clinical condition mainly confined to the Indian subcontinent has been attributed to aflatoxin contamination. They also found a correlation between aflatoxin contamination and fungal load on the one hand and hepatomegaly in children on the other in south Canara district of Karnataka. A case of aflatoxicosis in Murrah buffaloes from Andhra Pradesh was also reported.

Regulations in Various country

Milk has the greatest demonstrated potential for introducing aflatoxin residues from edible animal tissues into human diet. Aflatoxin is the most important contaminant and alone were responsible for almost 30% of all the notifications (902 notifications) on the Rapid Alert System for Food and Feed (RASFF) of the European Union in in 2008. According to US regulations the level of AFM1in milk should not be higher than 500 ng/kg. Even the low regulatory limits set by countries could not prevent chronic effects of aflatoxins, due to continued exposure to subacute levels of aflatoxins. Because of the following reasons, it seems that monitoring and preventive program are the most effective strategies to decrease the risk of exposure to both human and animals:

1. Evaluation of human exposure levels and health risk based on animal toxicological research
2. Difficulties in assessing dietary intake
3. Decontamination and remove mycotoxins from human and animal diets

Most countries have adopted severe regulations (See Table 1) to limit the exposure to mycotoxins, having strong impact on food and animal crop trade.

Table 1. Regulation on Aflatoxin M1

Region	Maximum acceptable level (ng/l)	Type
India	500 (0.5ppb)	Milk
European Union	50 (0.05ppb)	Milk
Australia	50 (0.05ppb)	Milk

	20 (0.02ppb)	Children's milk
Argentina	50 (0.05ppb)	Milk
Bulgaria	500 (0.5ppb)	Milk
Germany	50 (0.05ppb)	Milk
Sweden	50 (0.05ppb)	Liquid milk products
Netherland	20 (0.02ppb)	Butter
Switzerland	50 (0.05ppb)	Milk and milk products
	250 (0.25ppb)	Cheese
Belgium	50 (0.05ppb)	Milk
USA	50 (0.05ppb)	Milk
Czech Republic	100 (0.1ppb)	Children's milk
	500 (0.5ppb)	Adult's milk
Serbia	500 (0.5ppb)	Milk
Iran	50 (0.05ppb)	Raw, pasteurized, and UHT
	200 (0.2ppb)	Cheese
	20 (0.02ppb)	Butter
France	30 (0.03ppb)	Children's milk < 3 years
	50 (0.05ppb)	Adult's milk
Turkey	50 (0.05ppb)	Milk and milk products
	250 (0.25ppb)	Cheese
Brazil	500 (0.5ppb)	Milk

Climatic condition and Aflatoxin

Aflatoxins are especially problematic in hot, dry climates and their prevalence is exacerbated by drought, pests, delayed harvest, insufficient drying and poor postharvest handling**.** The production of mycotoxins within the fungus depends on food sources, the enzymes of the fungus and other environmental factors (See figure 1). The primary factors influencing fungal growth in stored food products are the moisture content (more precisely, the water activity), relative humidity in the air and the temperature of the commodity.

Aflatoxin production in the grain can happen in the field in the storage conditions between 20 and 40°C with 10-20% of humidity and 70-90% relative humidity in the air. *A. flavus* has relatively high moisture requirements among storage fungi. Hence, aflatoxin contamination of grains is aggravated by high seed moisture. Aflatoxin contamination is a perennial risk between 40°N and 40°S of the equator.

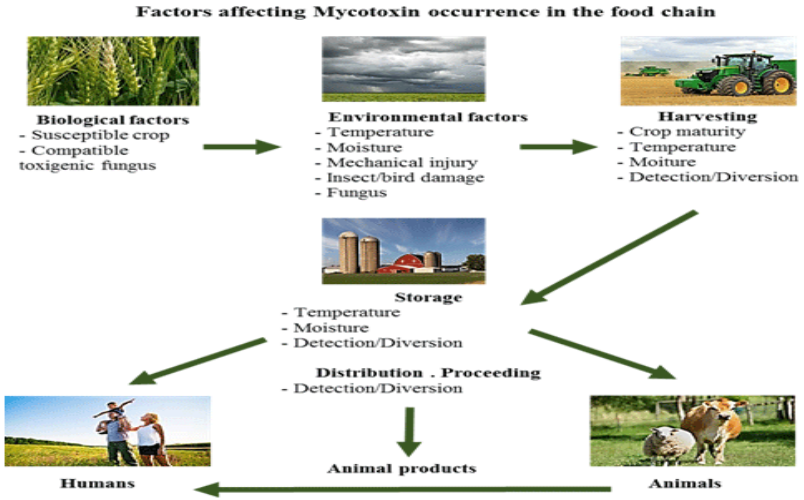


Fig 1:Factors affecting mycotoxin occurrence in the food and feed chain.

Climate change affects the interactions between different mycotoxigenic species and the toxins produced by them in foods and feeds. Changes in environmental temperature influence the expression levels of regulatory genes (aflR and aflS) and aflatoxin production in *A. flavus* and *A.*

parasiticus. Temperature interacts with water activity (aw) and influences the ratio of regulatory genes (aflR/aflS), which is directly proportional to the production of AFB1. The interactions between water activity and temperature have prominent effect on *Aspergillus* spp. and aflatoxin production. Increasing the temperature to 37°C and water stress significantly reduces the production of AFB1 produced, despite the growth of *A. flavus* under these conditions. The addition of CO_2 under the same temperature and water activity enhances AFB1 production.

Reduction of Aflatoxin in Feed and Food

FAO has estimated that up to 25% of the world's food crops and a higher percentage of the world's animal feedstuffs are significantly contaminated by mycotoxins. Completely to eliminate the aflatoxin toxicity or reduce its content in foods and feedstuffs to significant levels, increasing knowledge and awareness on aflatoxins as a potent source of health hazard to both human and animals is a great deal of effort will necessary. Although prevention is the most effective intervention physical, chemical and biological methods have been investigated to inactivate aflatoxins or reduce their content in foodstuffs. Of many approaches investigated to manage aflatoxin contamination, biological control method has shown great promise. Numerous organisms, including bacteria, yeasts and nontoxigenic fungal strains of *A. flavus and A.*

parasiticus, have been tested for their ability in controlling aflatoxin contamination.

If aflatoxicosis is suspected, feed should be analyzed immediately. If aflatoxins are occurred, the source should be eliminated immediately. Levels of protein in feed and vitamins A, D, E, K and B should be increased as the toxin binds vitamins and affects protein synthesis. Good management practices to alleviate stress are essential to reduce the risk of secondary infections which must receive immediate attention and treatment. The presence of molds in foodstuffs causes the appearance of flavors and odors that reduce palatability and affect feed consumption by animals as well as reduce the nutritional value of foods.
Detoxification treatments should be technically and economically reliable, and should meet the criteria. These criteria's are:

a) destroys or inactivates the toxin,
b) does not produce toxic or carcinogenic products in the finished product,
c) destroys fungal spores and mycelia that could proliferate and produce the toxin,
d) preserves the nutritive value and acceptability of the product, and
e) Does not significantly alter important technological properties of the product.

Aflatoxin Reduction in Feed

These are the most discussed and promised ways of decontamination and detoxification of aflatoxins in feed and feed ingredients.

1. **Purchase and Storage** of grain at appropriate moisture content (below 13%), inspection of grain regularly for temperature, insects and wet spots will limit the possibility of fungal development.
2. **Supplier quality assurance**: Raw material suppliers must understand the potential mycotoxin risks associated with materials they purchase, store, and later sell for feeds or further processing. This includes a solid understanding of regulatory requirements and customer food safety standards to ensure appropriate levels of monitoring, correct storage, and adequate control procedures. A clear specification is essential. Supplier quality assurance works with the raw material supply base to audit and verify the effectiveness of mycotoxin control programs to ensure that potential food safety risks are appropriately managed before the materials are shipped and subsequently received at production facilities. All of these activities should be audited to ensure compliance. In order to prevent material rejected by one manufacturer from re-entering another's supply chain, we must create a standardized approach to mycotoxins and ultimately to food safety management.

3. **Good Manufacturing Practices**: Develop a systematic inspection and clean-up program to keep bins, delivery trucks and other equipment free of adhering or caked feed ingredients. Minimize dust accumulation in milling and mixing areas. Check feed storage bins for leaks. Implement effective rodent and insect control programs in grain storage areas.
4. **Legislation in feed and feed ingredients:** Regulatory values or recommendations are mainly built on available knowledge on toxicity and potential carryover of these molecules in animal. Therefore, by limiting animal exposure through feed ingestion, one can guarantee against the presence of residues of mycotoxins in animal-derived products.
5. **Market incentives for aflatoxins free feeds:** commercial markets can provide incentives for reduced aflatoxins to encourage the ingredients producers and feed manufacturers.
6. **Use of binders/ Adsorbents/mold inhibitors:** The addition of mycotoxin binders to contaminated diets has been considered the most promising dietary approach to reduce effects of mycotoxins. There are many data available on binders, the most common additives used in animal diets are aluminosilicates, produced synthetically or extracted from clay mines. When binding agents are included in feed at a ratio of 200 parts feed to 1 part binding agent, they reduce most of the harmful effects of aflatoxins at levels of 1,000 ppb for pigs and 7,000 ppb for poultry. The cost is around ₹ 20 per ton of feed. Complex indigestible

carbohydrate polymers derived from yeast cell walls are shown effective in binding aflatoxin and restoring performance to animals consuming multiple mycotoxins (generally Fusarium produced). Bacterial cell walls also have potential to bind mycotoxins, but limited research has been conducted.

7. **Decontamination:** Ammoniation is a safe and effective way to decontaminate aflatoxins; it has been used with success in many countries, yet is not legal in others. The average costs are 5–20 percent of the value of the commodity. Nixtamilization, the traditional alkaline treatment of maize in Latin America, can reduce toxicity and has potential for wider applications. Other chemical and biological agents have been effective in experiments but are not yet commercially developed.
8. **Factory gate and finished product verification:** Mycotoxin risk management at the factory level starts with inbound inspection, sampling, and testing as a means of verifying that deliveries meet quality and food safety requirements. Finished product verification testing must also be risk based, whereby finished products manufactured from higher-risk materials may be evaluated lot for lot, placed on positive release, and subjected to final verification testing prior to market release. Conversely, finished products manufactured from lower-risk materials may not require positive release and can be evaluated at reduced frequency to verify effectiveness of up-front controls.
9. **Rapid screening of grains:** The presence of mycotoxins is very frequent. Therefore, testing of raw

materials and products is required to keep our food and feed safe. A portable screening tool was adapted for rapid assessment of aflatoxin contamination in maize in the rural village setting.

Please find the below link for additional information on Strategies for Reduction and Finding Solutions.
https://www.dairyknowledge.in/section/national-seminar-aflatoxin-strategies-reduction-and-finding-solutions

Aflatoxin Reduction in Milk & Milk Products

Techniques to reduce aflatoxins concentration in liquid foods include prevention strategies to reduce the fungal contamination before harvest, decontamination methods to select only the uncontaminated commodities and detoxification procedures aiming to deplete/completely eliminate the mycotoxin content of foods to guarantee the food safety and health concerns of consumers by means of physical, chemical or biological treatments. The main drawback of decontamination processes is related to the intrinsic complexity of recognizing and separating the contaminated crops from the uncontaminated ones.

Levels in milk are generally less than 100 ppb. Aflatoxin levels are around three times higher in soft cheese and five times higher in hard cheese than the milk of origin. But because cheese is more concentrated, using aflatoxin-contaminated milk for cheese production is risk mitigating (for example, if ten liters of milk makes one kilogram of

cheese and aflatoxins are five times higher in hard cheese, then the exposure of humans from consuming one kilogram of cheese is half as much as the exposure from consuming ten liters of milk).

1. Effect of processing technologies on Aflatoxin

The fate of aflatoxin varies with type of heat treatment (e.g., cooking, drying, pasteurization, sterilization, and spray drying) Aflatoxins decompose at temperatures of 237–306°C. Some researchers believe that heat does not cause significant changes in amount of AFM-1. While others have reported different levels of AFs detoxification through these techniques (See table 2). In a study, it was shown that pasteurization at 62°C for 30 min could reduce the AFM-1 content in milk by 32%. Another study showed that heating might decrease AFs-content by 12%–35% (depending on the conditions). It is also reported that AFM-1 may be relatively stable during milk pasteurization process. Sterilization of the milk at 121˚C and 80˚C under the same condition of time (15-20) min showed significant reduction of up to 58.8% when compared with the fresh untreated cow milk of the same source. 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. Reduction up to 75.6% and 86.5% by roller drying and spray drying respectively.

Research showed that AFMs are mainly present in the milk serum (~46.5%) and in the casein (~48.5%) while only a minor portion is contained in the fat fraction (~5%). The complete degradation of AFM1 (100%) was occurred by using sterilization at 121°C for 5 min. with 0.1 % H_2O_2 concentration.

Table 2. Effect of Heat Treatment on the Presence of AFM1 in Liquid milk

Heat Treatments	Remaining amount of AFM1 (ppb)	Degradation of AFM1 (%)
63°C for 30 min.	1.39	24.86
62°C for 30 min	-	32.50
72°C for 45 Sec	-	45.50
75°C for 40 sec	1.62	12.43
72°C for 2 min	1.50	12.00
	3.50	9.00
80°C for 45 Sec	-	63.60
90°C for 10 min	1.53	17.30
95°C for 5 min	1.50	17.90
	3.00	16.10
Boiling for 5 min	1.85	0.00
Sterilization (121°C for 5 min)	1.40	24.32
Sterilization (121°C for 10 min)	1.17	36.76
Sterilization (121°C for 15 min)	0.9	51.35

Motawee, M.M. et al.,2006, Sanli et al., 2012,Purchase et al., 1972, Devece 2007,

2. Lactic Acid Bacteria (LAB) & Yeast cells: Aflatoxin Binding

Biological detoxification of mycotoxins works mainly via two major processes, sorption and enzymatic degradation,

both of which can be achieved by biological systems. Microorganism detoxification can be performed in many different ways.

1. The entire organism can be used as a starter culture/lactic acid fermentation of milk.
2. The purified enzyme can be used in soluble or immobilized (biofilter) forms;
3. The gene encoding the enzymatic activity can be transferred and overexpressed in a heterologous system; interesting candidates for this application include yeasts, probiotics.

Yeast and LAB cells are known to bind different molecules such as killer toxins and metal ions on complex binding structures on the cell wall surface. Live microorganisms can absorb either by attaching the mycotoxin to their cell wall components or by active internalization and accumulation. Dead/heat killed microorganisms too can absorb mycotoxins, and this phenomenon can be exploited in the creation of biofilters for fluid decontamination or probiotics (which have proven binding capacity) to bind and remove the mycotoxin from the intestine.

LAB and Probiotic are able to bind dietary mutagens and carcinogens. LAB strains from different origin can be used as starter cultures to reduce or remove the AFM1 (See table 3). In brief, LAB cell wall consists of the peptidoglycan matrix forming major structural component of cell wall housing other components such as teichoic and lipoteichoic acid, proteinatious S layer and neutral polysaccharides. These components play various functions

including adhesion and macromolecular binding, especially fibrillar network of teichoic acids and neutral polysaccharides. Available experimental supports suggest the role of both peptidoglycon and polysaccharides in toxin binding properties.

Among bacteria, Lactobacillus rhamnosus L60 and Lactobacillus fermentum L23 have a high ability to inhibit mycelium growth of aflatoxigenic Aspergillus strains and reduce the AFB-1. AFM1 removal by LAB has a potential application to reduce toxin concentrations until safe levels in yoghurt. The application of this phenomenon in the removal of mycotoxins from contaminated food and feed is urgently needed to improve the safety of food and feed. *L. plantarum MON03* in PBS spiked with 50 µg/ml of AFM1, after 0, 12, and 24 h at 37 °C. They found that AFM1 binding by *L. plantarum MON03* increased in a time-dependent manner. *L. plantarum MON03* bounded 25.9 and 93.1% of AFM1 after 0 and 24 h of incubation in PBS. Researchers investigated the binding ability of AFM1 by Lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophiles* and found that they were effective in reducing the extent of free AFM1 content in liquid culture medium and during yogurt processing.

Table 3. Binding of aflatoxins by viable bacteria in vitro

Bacteria	Bacterial concentration (CFU/ ml)	AFM1 concentration (µg ml /1)	%AF bound
Lb. acidophilus LA1	10^9	0.15	18.3

Lb. acidophilus NCC 12	10^8	0.1	30.5
Lb. acidophilus NCC 36	10^8	0.1	28.0
Lb. acidophilus NCC 68	10^8	0.1	25.7
Lb. rhamnosus GG	10^8	0.15	50.7
Lb. rhamnosus LC705	10^8	0.15	46.3
Lb. rhamnosus 1/3	10^8	0.15	18.1
B. longum Bl 24	10^8	0.1	26.7
B. bifidum Bb13	10^8	0.1	32.5
B. bifidum NCC 381	10^8	-	15.5-18.3
L. rhamnosus	10^8	-	20.4-22.2
Lactobacillus strains	-	-	64-80.5, 96 h, 37 °C , milk
Lactococcus strains	-	-	46.0-68.5 96 h, 37 °C , milk
Bifidobacterium strains	-	-	67.0-72.5, 96 h, 37 °C, milk
L. bulgaricus	-	-	80.5, 96 h, 37 °C , milk
B. adolescentes	-	-	73, 96 h, 37 °C , milk
L plantarum	10^{10}	-	80
L. lactis	-	-	76
Binding of aflatoxins by Heat Killed bacteria in vitro conditions.			
Lb. rhamnosus GG	10^8	0.15	57.8
Lb. rhamnosus LC705	10^8	0.15	51.6
Lb. rhamnosus 1/3	10^8	0.15	39.9
Lb. acidophilus LA1	10^9	0.15	25.5
Lb. gasseri ATCC 33233	10^9	0.15	61.5
Lc. lactis ssp cremoris ARH74	10^9	0.15	38.9
B. bifidum NCC 381	10^8	-	17.1-22.2
L. rhamnosus	10^8	-	22.9-26.3

Ref: 1. Bulent Kabak et al., Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review. 2006. 2. CAF Oliveira et al., Excerpts from Recent Trends in Microbiological Decontamination of Aflatoxins in Foodstuffs Chapter 4 from *Aflatoxins - Recent Advances and Future Prospects,*

The Saccharomyces cerevisiae (SC) is one of the yeasts that can bind to AFM-1 effectively. The components of SC cell wall, called oligomannanes, after esterification, are able to bind more than 95% AFB1. The possible binding mechanisms between yeast cell wall and mycotoxins were studied, and authors suggested that β-D-glucans are the components of the cell wall that are responsible for forming the complex with the toxin, and that the reticular organization of β-D-glucans and their distribution in β-(1,3)-D-glucans and β-(1,6)-D-glucans have an important role in the efficiency of the bond. When yeast cells were inactivated by heat, they were inefficient to bind the toxins, but when glucomannanes extracted from the cell wall of yeasts were used, there was an increase in the efficiency of the bond with AFB1, OTA and T-2 toxin. Several studies have demonstrated that the cell wall of SC and LAB and their components are responsible for binding with aflatoxins. However, the mechanisms by which this bond occurs remain unclear. Cell walls with glucomannanes and manno-oligosaccharides have been pointed out as the responsible elements for AF bond with yeasts. The cell wall of S. cerevisiae represents about 30% (w/w) of total weight of the cell and is a bi-layered structure, structural part of which is made up of b-1,3-glucan and b-1,6-glucan. Majority of the cell wall proteins (mannoproteins) are covalently linked to b-1,3-glucans

through b-1,6-glucan chains. The cell wall proteins (mannoproteins) consist of a very heterogeneous class of glycoproteins out of which 70 of them are identified. Products based on SC (cell wall from baker and brewer yeasts, inactivated baker yeast, or alcohol yeast) was studied, and it was observed that in pH 3, 37°C and 15 minutes of contact, AFB1 removal ranged from 2.5% to 49.3%, depending on the concentration of the toxin in the medium, and on the yeast-based products used.

Reduction of Aflatoxin by Adsorption techniques

2.1. Clay materials

Several types of natural and industrial sorbents have been identified for a reliable detoxification of the milk. Use of toxin absorbents is one of the main methods to reduce aflatoxin amount in milk. Absorbent soils such as bentonite, vermiculite, hydrated sodium calcium aluminosilicate (HSCAS) and active carbon are known as absorbent compounds for absorbing various toxins in aqueous environments (see table 4). For instance, bentonite has been known as an effective reducer of aflatoxin M1 in infected milk. Binding capacity and stability of compound formed between absorbent and toxin are highly variable and influenced by temperature and pH. Information about the effect of absorbents on milk constituents is scarce; however, it has been shown that these substances have slight effect on nutritional quality of milk. While selecting the clays, we shall consider the

possible effect on the nutritional properties of the milk (in terms of protein, fat and lactose).

An early study by researchers pointed out that the adsorption on bentonite of AFMs from naturally contaminated milk allows a removal efficiency ranging from 65% to 89% by increasing solid loading from 5 to 20 g/kg. Batch experimental tests have been carried out at 25°C and shows that the protein content of the treated milk is around the 95% of the original material. Activated carbon and HSCAS reduced AFB-1 converting to AFM-1 in milk by 50% and 36%, respectively.

Literature shows that the activated carbons have by far the highest removal efficiency (n > 93% for AFMs concentration as high as 0.5 μg/kg), followed by bentonite (n ~ 80% at 0.5 μg/kg). The correlation between sorbent properties and adsorption efficiencies shows that AFM adsorption is relevant only if the average size of sorbent micropores is higher than a critical value (~10 Å). The capture of AFM mainly depends upon low energy van der Waals interactions involving the sorbent surface rather than specific superficial functional groups. This preliminary study points out that the adsorption is a promising, reliable, method to remove aflatoxins from milk whose application should be optimized in order to assure both the required detoxification and the preservation of milk properties within given acceptability limits that allow its reuse either as a food or as raw material for dairy products.

Table 4. In vitro adsorption of mycotoxins by different adsorbents		
Adsorbent	**Mycotoxin**	**Adsorption Index (%)**
Activated carbon	AFB1	>99.0
Activated carbon	AFB1	>90.0
Activated carbon	OTA	0.8-99.8
Activated carbon	DON	1.8-98.9
HSCAS	AFB1	>90.0
HSCAS	OTA	13.2
HSCAS	DON	3.9
Zeolite	AFB1	99.0
Zeolite	ZEN	5.0
Zeolite	OTA	40.0
Organozeolite	OTA	41-52
Sepiolite	OTA	10.50
Sepiolite	DON	4.50
Clinoptilolite	AFB1	6.0
Na-bentonite	AFB1	95-98.5
Ca-bentonite	AFB1	98.5
Esterified glucomannan	AFB1	96.6

2.2. Nanoparticles as a Solution for Eliminating the Risk of Mycotoxins

Clay binders, yeast cell walls, or antioxidant additives are the most widely used products for mycotoxin elimination to reduce their impact. Although conventional methods are constantly improving, current research trends are looking for innovative solutions. Nanotechnology approaches seem to be a promising, effective, and low-cost way to minimize the health effects of mycotoxins. The high expectations of

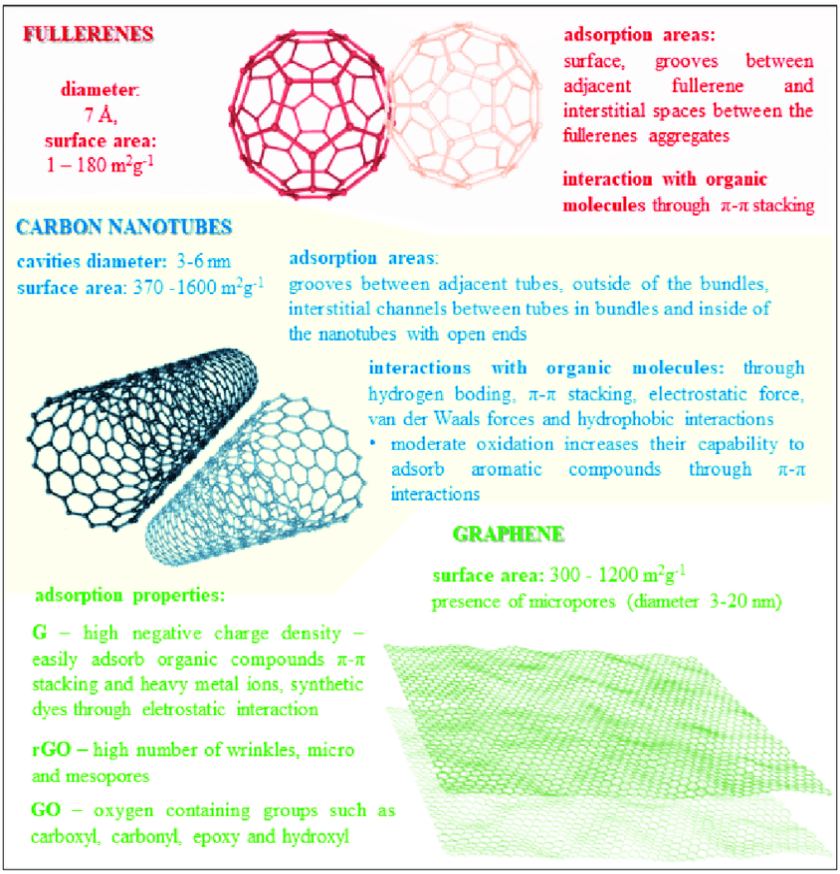
using nanomaterials as special adsorbents to remove pollutants relies not only on the high surface area and the high affinity to organic compounds (properties of conventional adsorbents such as those possessed by activated carbon) but also greatly on the fact that nanomaterials can be engineered or modified specifically to enhance selectivity to specific target pollutants. The following subsections describe the most promising nanomaterials for the elimination of mycotoxins.

2.2.1. Carbon Nanostructures

Activated charcoal has been used for mycotoxin elimination for a long time. From this established practice proceeds the use of carbon nanoforms as a promising successor to activated carbon. The advantages of carbon nanomaterials are excellent stability, inertness, high adsorptive properties, large surface area per weight, and colloidal stability upon various pH. Chemically, the carbon–carbon covalent bonds and crystalline structure provide specific properties such as strength, elasticity, and great conductivity. Graphene, graphene oxide, nanodiamonds, fullerenes, fiber, and nanotubes have a great potential to become novel adsorbents of mycotoxins. Nanocarbon structures are amphoteric and their surface could be protonated or deprotonated, which results in the binding capacity of polar or nonpolar compounds.

Generally, carbon nanotube adsorption affinity poorly correlates with hydrophobicity but increases in the order nonpolar aliphatic<nonpolar aromaticas<nitroaromatic

functional groups. Fullerene adsorption behavior was found to be higher than that of activated carbon. Nanodiamonds show all the benefits of carbon nanomaterials and their large-scale production is considered to be inexpensive. Their chemical structure allows surface functionalization, including carboxylation, hydrogenation, and hydroxylation, which could provide binding affinity to various types of the mycotoxins. The adsorption capacity was estimated for AFLB1 as approximately 10 μg per mg of nanodiamonds and for OTA around 15 μg per mg of nanodiamonds. These results indicate greater adsorption capacities than commercially used yeast cell walls and clay minerals. Magnetic graphene (MGO) synthesized from iron oxide nanostructures and graphene oxide is inexpensive and easily accessible.



Key properties of carbon nanoparticles such as fullerenes, carbon nanotubes, and grapheme (native graphene (G), reduced graphene (rGO), and graphene oxide (GO)). Mycotoxins can be bound to the surface, bundles, grooves, or channels between nanoparticles via different binding interactions.

2.2.2. Chitosan Polymeric Nanoparticles

Chitosan (CS) is a natural cationic polysaccharide produced from chitin, which is the structural element found in the exoskeleton of crustaceans. CS is nontoxic, biodegradable, and possesses low immunogenicity. Therefore, CS has shown promising results for mycotoxin elimination from different raw materials. In 1990s, CS began to be considered as a suitable mycotoxin adsorbent with approximately 70% efficacy. CS is easily subjected to nanoparticles via the gelatation process using aldehydes (e.g., glutaraldehyde) and acids (e.g., thioglycolic, acrylic, and oxalic acids). Another way for nanoparticle formation is ionic cross links based on electrostatic interaction with phosphoric acid derivatives such as sodium tripolyphosphate (TPP). Chitosan's ability to quickly gel relies on the formation of inter- and intramolecular cross linkages between TPP phosphates and chitosan amino groups. The properties of prepared CS nanoparticles depends on physicochemical conditions such as pH, temperature, time, and functionalization or modification by specific ligands. CS NPs are able to encapsulate various compounds. Glutaraldehyde crosslinked chitosan showed promising adsorption ability for AFL B1 (73%)

2.2.3. Nanoclay Binders

This group includes minerals that are used for the detoxification of mycotoxins from food and feed, such as montmorillonite, bentonite, zeolite, or hydrated sodium (calcium) aluminosilicate. Their specificity is the

willingness to form multiphase solid materials where one of the phases has one, two, or three dimensions of less than 100 nm, e.g., montmorillonite. Montmorillonite nanocomposite (MN) has been introduced as a perspective sorptive additive possessing sizable surface area, higher porosity, strong cation exchange activities, and more active sites, which enable its interaction with mycotoxins. The in vitro obtained adsorption capacity of MN for AFL was estimated to be 66.67 μg/mg MN.

Analytics on Aflatoxin

The detection and quantification of aflatoxin in food and feed is a very important aspect for the safety concerns. Aflatoxins are usually detected and identified according to their absorption and emission spectra, with peak absorbance occurring at 360 nm. B toxins exhibit blue fluorescence at 425 nm, while G toxins show green fluorescence at 540 nm under UV irradiation. This florescence phenomenon is widely accepted for aflatoxins. Thin layer chromatography (TLC) is among one of the oldest techniques used for aflatoxin detection, while high performance liquid chromatography (HPLC), liquid chromatography mass spectroscopy (LCMS), and enzyme linked immune-sorbent assay (ELISA) are the methods most frequently used for its detection.

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