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Preventive Measures for Residual Aflatoxins in Milk and Dairy Products

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Abstract: *Potential threats to human health related to dairy products and dairy farming include errors in pasteurization, consumption of raw milk products, contamination of milk products by emerging heat-resistant pathogens, emergence of antimicrobial resistance in zoonotic pathogens, chemical adulteration of milk etc. Mammals that ingest aflatoxin B1 (AFB1)-contaminated diets excrete amounts of the principal 4-hydroxylated metabolite known as aflatoxin M1 into milk. According to Official Journal of European Union, the maximum level of aflatoxin M1 (µg/Kg) is 0.05 in milk (raw milk, milk for the manufacture of milk-based products and heat-treated milk). Determination of aflatoxins concentration in food stuff and feeds is very important. However, due to their low concentration in foods and feedstuff, analytical methods for detection and quantification of aflatoxins have to be specific, sensitive, and simple to carry out. The addition of sequestering or binding agents to aflatoxin contaminated feedstuffs is one of the most used methods worldwide as an approach to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins and reducing their intestinal absorption.*

I. INTRODUCTION

It is well established that foodborne diseases cause significant economic and social losses. Well-publicized foodborne disease outbreaks have created widespread consumer awareness of potential threats to human health from food. Consumer confidence in existing food handling and processing systems has been reduced. They are increasingly concerned about the safety of their food and uncertain about food production practices. Potential threats to human health related to dairy products and dairy farming include errors in pasteurization, consumption of raw milk products, contamination of milk products by emerging heat-resistant pathogens, emergence of antimicrobial resistance in zoonotic pathogens, chemical adulteration of milk, transmission of zoonotic pathogens to humans through animal contact, and foodborne disease related to cull dairy cows, etc. [**1**].

Aflatoxins are mycotoxins produced by molds, specifically A. *parasiticus* and *A. flavus.*, different types of aflatoxins have been identified with aflatoxins B1, B2, G1, G2, M1, and M2 being the most common [**2**] (**Fig. 1**). Although the highest concentrations are formed in food crops grown and stored in the warmer areas of the world. Mammals that ingest aflatoxin B1 (AFB1)-contaminated diets excrete amounts of the principal 4-hydroxylated metabolite known as aflatoxin M1 into milk. Milk is a highly nutritious food, and it is a source of necessary macro- and micronutrients for the growth, development and maintenance of human health. However, it may also be a source of natural food contaminants like aflatoxin M1 is important problem worldwide

especially for developing countries for the last ten to twenty years. Paneer is popular throughout India especially in the state of Haryana. An estimated 5 percent of milk produced in India is converted to paneer. Although exact figures are not available, considering the significant per capita availability of milk, it can be assumed that the people consume significant amount of paneer. Aflatoxin M1 (AFM1), the hydroxylated metabolite of aflatoxin B1 (AFB1), is an important but neglected food safety issue concern due to their ability to cause cancer, and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs. The toxin is heat stable and gets transferred from milk to paneer. The presence of AFM1 in milk and milk products is considered to be undesirable [**3**, **4**, **5**].

Levels of milk contamination do not only reflect peculiarities of the dairy species but also of the feed administered and the length of fodder storage, where the environmental conditions (humidity, temperature etc) foster fungal growth. In this context, some authors have reported that milk produced during the winter presents much higher levels of AFM_1 than those present in the summer. It can therefore be assumed that a lesser consumption of these feeds and the chance to allow the animals to graze freely could contribute to reduce the level of contamination in milk [**6**].

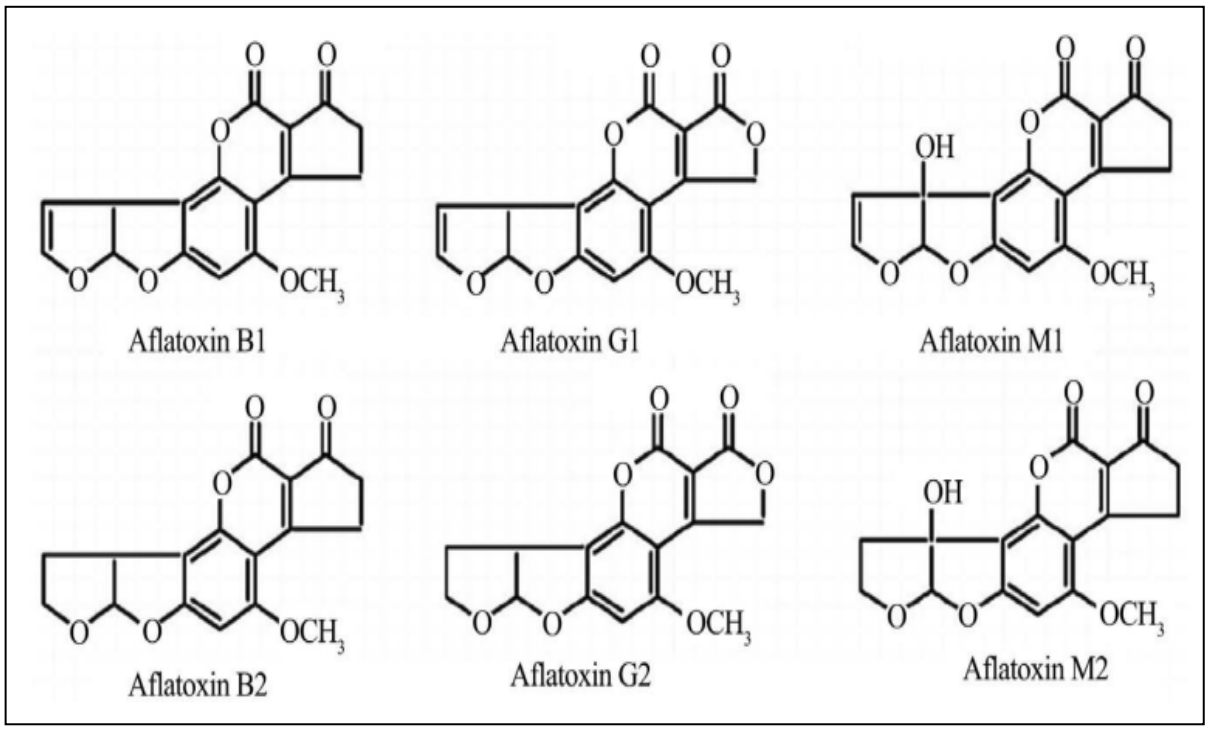

Figure 1: Structures of common aflatoxins [**16**]

II. METABOLISM OF AFB1

After ingestion by dairy cows, a portion of the AFB1 is transformed by some ruminal microbes to form aflatoxicol, while the remaining AFB1 reaches the intestine and is rapidly absorbed and transported via the portal blood stream to the liver. In the liver, AFB1 is subjected to reduction, epoxidation, hydroxylation, and demethylation with each transformation pathway leading to different metabolites: reduction to aflatoxicol (highly toxic), epoxidation to AFB1-8,9- epoxide (highly toxic, mutagenic, and carcinogenic), hydroxylation to AFM1 (highly toxic and excreted in milk) and aflatoxin Q1 (AFQ1, less toxic), and demethylation to aflatoxin P1 (AFP1, less toxic), **Fig. 2.** Aflatoxicol formation is catalyzed by a nicotinamide adenine dinucleotide phosphate (NADPH) reductase, while other reactions are primarily carried out by the cytochrome P450 enzyme [**7**].

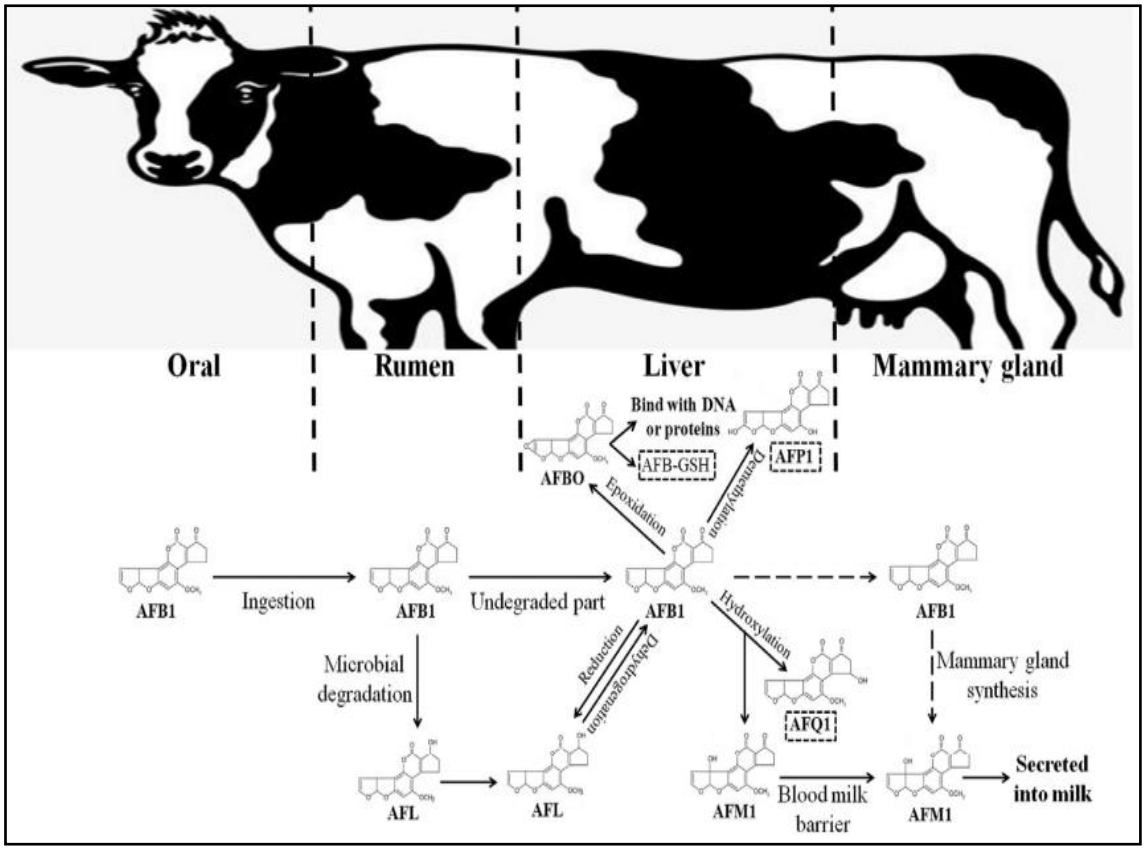

Figure 2: General metabolism and biotransformation pathways of AFB1 [**7**]

Determination of aflatoxins concentration in food stuff and feeds is very important. However, due to their low concentration in foods and feedstuff, analytical methods for detection and quantification of aflatoxins have to be specific, sensitive, and simple to carry out. Several methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectroscopy, enzyme-linked immune-sorbent assay (ELISA), and electrochemical immunosensor, among others, are used for detecting and quantifying aflatoxins. Each of these methods has advantages and limitations in aflatoxins analysis [**8**]. As per satisfactory percentage recovery, no interference in peaks, liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method (LC/MS/MS) is a valuable confirmation technique for aflatoxins [**9**].

The total consumption at all India level (including household and non-residential consumption) is 162.4 million metric tons for milk and milk products. An estimated 5 percent of milk produced in India is converted to paneer and as mycotoxins are heat stable compounds, hence AFM1 contamination of milk can be considered as milk food safety problem. According to Official Journal of European Union, the maximum level of aflatoxin M1 (µg/Kg) is 0.05 in milk (raw milk, milk for the manufacture of milk-based products and heat-treated milk) [**3**, **10**]. On a worldwide basis, at least 99 countries had mycotoxin regulations for food and/or feed in 2003. Compared to other regions of the world, Europe has the most extensive and detailed regulations for mycotoxins. In the EU, harmonized regulations exist for aflatoxins in various foodstuffs, aflatoxin M1 in milk and for aflatoxin B1 in various feedstuffs. It is again the European countries that contribute in major part with 0.05 µg/kg, but some other countries in Africa, Asia and Latin America also apply this limit. This is understandable and logical from the point of view that it is aflatoxin M1, the metabolite of aflatoxin B1, which causes health concern. Consequently, limiting aflatoxin B1 in animal feeds is the most effective means of controlling aflatoxin M1 in milk. A limit of 5 µg/kg dominates the distribution pattern of aflatoxin B1 regulations. Generally, the

limit of Aflatoxin B1 in feed materials is 20 ppb, 10 ppb for complementary & complete feed, (based on a moisture content of 12%). These developments reflect the general concerns that governments have regarding the potential effects of mycotoxins on the health of humans and animals. At the same time, harmonization of tolerance levels is taking place in some free trade zones. The hazard of mycotoxins to individuals is probably more or less the same all over the world, hence there is requirement for establishment of regulatory norms in India as well [**11, 12**].

III. SUGGESTED CONTROL MEASURES

To date, partial solutions such as selective breeding of crops for greater resistance, modified growing conditions, and biological controls have shown positive effects. However, post-harvest storage is the most overlooked stage for effectively preventing aflatoxin growth. In hot, humid climates, long term conventional storage can produce exponential growth of aflatoxins, shows that restricting the increase in aflatoxin levels during both drying and long-term storage is a major challenge, particularly in hot and humid conditions. Several strategies, to be adopted in a coordinated manner have been proposed to reduce the impact of mycotoxins [**13**] which includes:

1.1 Pre-harvest, harvest and storage under field conditions: The most practical approach is by good agricultural practice, 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.

1.1.1 Dry the raw materials immediately after harvesting to moisture content less than 13%.

1.1.2 Avoid damaged and broken grains (susceptible for fungal growth).

1.1.3 Avoid insect damaged grains (susceptible for fungal growth).

1.1.4 Pre-clean and dry the grains before storage in bags.

1.1.5 Use mould inhibitors to prevent fungal growth, if the moisture in raw materials is more than 13%.

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

1.1.7 Systematic inspection and clean-up program to keep bins, delivery trucks, and other equipment free of adhering or caked feed ingredients.

1.1.8 Remove dust (contains Aflatoxins) and all waste materials [**14**].

1.2 Use of mycotoxins binders: The addition of sequestering or binding agents to aflatoxin contaminated feedstuffs is one of the most used methods worldwide. Detoxification and inactivation methods include the use of binders or sequestering agents added to feed as an approach to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins and reducing their intestinal absorption. A binder must be effective at sequestering the mycotoxin(s) of interest, physically usable in commercial feed manufacturing situations, use and efficacy should be verifiable.

Some toxin adsorbents are: silicate products (montmorillonite, bentonite and hydrated sodium calcium aluminosilicate, zeolites and clinoptilolite), carbon products (activated or super activated charcoal), inorganic polymers (cholestyramine, polyvinylpyrrolidone). Among all these adsorbents, hydrated sodium calcium aluminosilicate (HSCAS) has been the most extensively studied *in vitro* and was selected for extensive *in vivo* application in a varied number of farm animals. HSCAS adsorb and retain 95% of aflatoxins. HSCAS are activated by heat drying process. These inorganic clays are thought to work by ion exchange interactions between free radicals on the clays and potentially charged groups on the toxins. That is why the clay binders are most effective against the polar toxins such as aflatoxins. 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. [**14, 15**].

IV. CONCLUSION

Milk, a highly nutritious food, may also be a source of natural food contaminants like aflatoxin M1 is important problem worldwide especially for developing countries. Aflatoxin M1 (AFM1), the hydroxylated metabolite of aflatoxin B1 (AFB1), is an important but neglected food safety issue concern due to their ability to cause cancer, and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs. Europe has the most extensive and detailed regulations for mycotoxins. Consequently, limiting aflatoxin B1 in animal feeds is the most effective means of controlling aflatoxin M1 in milk. The hazard of mycotoxins to individuals is probably more or less the same all over the world, hence there is requirement for establishment of regulatory norms in India as well. Determination of aflatoxins concentration in food stuff and feeds is very important. However, due to their low concentration in foods and feedstuff, analytical methods for detection and quantification of aflatoxins have to be specific, sensitive, and simple to carry out. Partial solutions such as selective breeding of crops for greater resistance, modified growing conditions, proper pre-harvest, harvest and storage of crops, biological controls and use of mycotoxins binders have shown positive effects.

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