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Review Article

Aflatoxin M1 contamination in milk: A serious issue to be tackled

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Abstract:

This comprehensive review addresses the critical issue of Aflatoxin M1 contamination in milk, emphasizing its presence, detection methods, permissible levels, and associated health risks. Aflatoxin M1, a carcinogenic byproduct of Aflatoxin B1, poses significant concerns for the safety of milk, a crucial source of essential nutrients. Regulatory standards from entities like the European Union, U.S. FDA, and FSSAI highlight the need for monitoring and controlling contamination to protect public health. The review extensively explores diverse detection techniques, with ELISA standing out for its speed and precision.

Preventive measures, including inhibiting contaminated feed intake, vaccination strategies, and judicious feed usage, are discussed as effective means to minimize Aflatoxin M1 in milk. However, complete elimination remains challenging, leading to the exploration of additional detoxification methods. The review covers various approaches during milk processing, such as pasteurization, sterilization, and the potential roles of probiotics. Absorption materials like bentonite and activated carbon show promise in binding and removing Aflatoxin M1, while chemical approaches with ammonia and hydrogen peroxide are examined with a cautious note on potential risks. Novel detoxification techniques involving plant extracts, including black cumin, garlic, and broccoli, is highlighted for their antioxidant properties. In conclusion, this review provides a foundational understanding of the multifaceted challenge of Aflatoxin M1 contamination in milk, offering insights for future research and the development of effective strategies to ensure the safety and quality of this dietary staple.

Key words: aflatoxin; milk; detoxification; detection; preventive measures

Introduction

Milk is consumed by individuals of all age groups and a vital source of micro and macro nutrients necessary for growth and development (Iqbal et al., 2015; Flores-Flores et al., 2015). However, it's important to note that milk can contain carcinogenic compounds such as Aflatoxin M1, a metabolite of Aflatoxin B1 (Sumon et al., 2021). This toxin is typically found in the milk produced by livestock that have consumed feed contaminated with Aflatoxin B1 (Creppy, 2002). Studies have shown that livestock converts approximately 0.3–6.2% of ingested Aflatoxin B1 (AFB1) into Aflatoxin M1 (AFM1) in. If the intake of AFB1 is halted, the concentration of AFM1 in the milk drops to an undetectable level after about 72 hours (Chin Lin et al., 2004; Ardic, 2009; Ardic et al., 2009; Fallah, 2010; Martins & Martins, 2004; Van Egmond, 1982). Aflatoxin M1 presence in milk is undesirable thus should be eliminated at all costs. There should be appropriate measures for lowering Aflatoxin M1 contamination in milk in every milk facility. The methods for detecting and safely removing Aflatoxin M1 will be explored in greater detail in the subsequent sections of this article.

2. Permissible level of Aflatoxins

Permissible levels of aflatoxin in milk vary globally, reflecting economic stability and regulatory requirements (Table 1). According to the standards of "Worldwide regulations for mycotoxins in food and feed in 2003" by (FAO, 2004), around 60 countries had established set limits of milk contaminated by Aflatoxin M1 by the end of 2003. Among these, 34 countries, including those in the European Union, set the upper limit at 0.05 μg kg^{-1} (FAO, 2004). In contrast, 22 countries, such as Brazil, the USA, and various Asian nations, adopted a limit of 0.5 μg kg^{-1}, as highlighted in the study by (Gonçalves et al., 2018). The substantial tenfold variation in these commonly adopted limits emphasizes the need for a comprehensive reevaluation of the potential risks associated with AFM1 to human health (FAO, 2004). This discrepancy in permissible levels emphasizes the importance of harmonizing global regulations to ensure consistent food safety standards.

Authority	Permissible Level in Milk (µg/kg)
European Union	0.05
U.S. FDA	0.5
FSSAI	0.5

Table 1: Representing the permissible levels of Aflatoxin M1 in milk (European Commission, 2006; FDA, 2011)

3. Detection of Aflatoxins by various method

The presence of aflatoxin in milk can be detected by various methods but choosing the appropriate detection method by analyzing the sample is important. Rapid methods can be used to detect the presence of analyte and quantative methods must be used to quantify the analyte. The reverse-phase HPLC is the most common technique used for the detection of aflatoxins. (Espinosa-Calderon, et al, 2011) The other common methods used are the enzyme linked immune-sorbent assay (ELISA) and thin-layer chromatography (TLC) (Magan and Olsen, 2004). These methods are affordable and easy to perform that's why they are used for AFM1 screening. (Manetta, 2011). The importance of immunoassays, mainly ELISA, has increased rapidly in the recent years. ELISA is not only suitable technique for easy, quick and sensitive analysis with larger sample intake. (Lee et al., 2009) but it is reliable, fast, inexpensive and this techniques needs only little amount of sample to perform test (Sherry, 1997). The following Table 2 explains the comparative analysis of these 3 detection methods.

Methods	Principle	Reliance	Affordability
HPLC	HPLC is based on column chromatography technique consisting of mobile and stationary phase. It works under high pressure where mobile phase is pumped through a packed column (Akash 2020)	Highly accurate with higher sensitivity and specificity.	Highly expensive.
TLC	TCL is based on adsorption chromatography. A thin plate seperates the components of mixture (Bele, 2011)	Accurate and reliable	Less expensive.
ELISA	It is based on antigen and abtibody, Ab are attached on plate against antigen, will bind to the antigen (Gaastra,1984)	Easy and reliable	Less expensive

Table 2: Detection Methods for Aflatoxin M1

4. Toxicity of Aflatoxin M1

The toxicity of Aflatoxin M1 (AFM1) poses significant health concerns, ranging from acute Aflatoxicosis to chronic conditions such as cancer and immunosuppression. Acute Aflatoxicosis, characterized by severe symptoms such as depression, blood in the stool, muscle tremors, and hyperthermia, has the potential to result in fatality within hours of consuming contaminated food (Murray et al., 2006). The liver, being the primary organ affected by aflatoxin B1 (AFB1), often manifests acute hepatitis. Chronic exposure to AFM1 results in more prolonged pathological alterations, with mutagenic and carcinogenic effects primarily attributed to the formation of adducts between AFB1–8,9 epoxide and DNA molecules, particularly in the p53 tumor suppressor gene (Hsieh and Atkinson, 1991). The activation of AFB1 into its toxic form, AFB1-epoxide, involves cytochrome P450 family enzymes, initiating a cascade of events leading to carcinogenesis (Ferreira et al., 2007).

The mutagenic process induced by aflatoxins leads to permanent genetic changes in affected cells, initiating the neoplasia process. Experimental studies indicate a direct correlation between AFB1-DNA adduct development and ingested doses of AFB1, with livestock exposed to aflatoxin exhibiting hepatic tumors (Ferreira et al., 2006). While the

carcinogenic potential of AFM1 is notably lower than AFB1, the risk of its presence in milk samples remains a public health concern, particularly for children and young individuals who are more susceptible due to higher milk consumption and varying metabolic rates (Farias et al., 2005).

Factors influencing AFM1 toxicity in humans include bioavailability, synergic effects among contaminants, daily mycotoxin intake, and individual factors such as weight, health status, physiological condition, and age (Farias et al., 2005). Although traditionally associated with hepatic cells, evidence suggests that AFB1 and AFM1 may also impact other organs, such as the intestinal tract, with potential cytotoxic and genotoxic effects on intestinal cells (Guerra et al., 2005; Zhang et al., 2015). Notably, AFM1 induces damage to DNA in intestinal cells, highlighting its broader impact beyond the liver (Caloni et al., 2006).

5. Biotransformation of AFB1 into AFM1

In farms where the low cost feed are used causes the Aflatoxin B1 to enter in gastrointestinal tract and gets in to the liver where the biotransformation takes place and converts Aflatoxin B1 into Aflatoxin

M1. Aflatoxin B1 goes through the complex pathways such as hydroxylation which leads to the production of less toxic water-soluble derivative such as AFM1 that can be secreted through milk (Oliveria and Germano, 1997). Figure: 1 shows the hepactic biotransformation of Aflatoxin M1 into Aflatoxin B1.

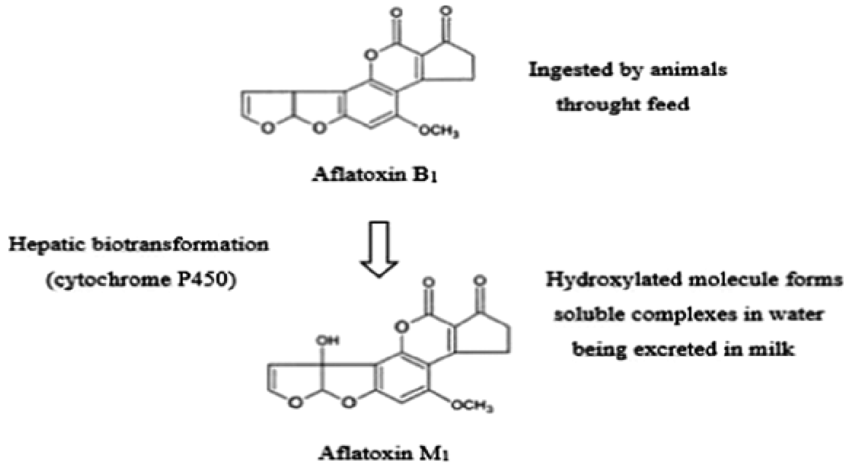

Figure 1: The hepactic biotransformation of Aflatoxin M1 into Aflatoxin B1.

6. Prevention and Detoxification techniques

Aflatoxin can cause acute or chronic Aflatoxicosis hence it is critical to prevent or decrease the growth of microbes. Preventing Food contamination by Aflatoxins is not only an expensive method but also very difficult. Prevention through pre and post harvesting technique can help in sufficient reduction of Aflatoxins However there are many techniques that are made to reduce Aflatoxin contaminated milk (Naeimipour, et al., 2018).

Prevention in Contamination of Aflatoxin M1 in milk

Studies have revealed that inhibition of AFB1 contaminated feed reduces the amount AF M1 in milk (Galvano, et al., 2001). Vaccinating the young Hiefers with anti-Aflatoxin B1 anti bodies can result in lowering the level of Aflatoxin M1 in milk (Abdul-Baki, et al., 1973). Another method for preventing the risk of contamination is to inhibit the intake of contaminated feed. Feeding the non-damaged and clean parts of feed to cow can help in reduction of Aflatoxin contamination more over storage places should be properly maintained to reduce any risk of fungal growth(Grenier and Applegate, 2013).

These Prevention methods are very critical in minimizing the amount of Aflatoxin M1 production in milk however the contamination of Aflatoxin M1 cannot be fully removed by these prevention methods. Not all farms are able to perform such actions to stop AF M1 contamination therefore some other methods should be used for the detoxification of AFM1.

7. Reducing Aflatoxin M1 content during milk processing

Milk processing techniques like pasteurization or sterilization can reduce microbial growth subsequently but are ineffective against AFM1 however some studies have revealed that pasteurization at 62oC for 30 minutes can reduce AFM1 by 32% (Jalili and Scotter, 2015). In conclusion heat processing of milk can reduce microbial contamination from the milk effectively but AFM1 is not reduced.

Role of probiotics in reduction of Aflatoxin M1

Probiotics are the significant bacteria that Aids gut microbiota of humans and animals. Several probiotics can be used to lessen the amount of AFM1 contamination in milk. Most important bacteria that help in controlling AFM1 contamination are Lactobacillus, Streptococcus, Pseudomonas, Bifidobacteria and Burkholderia. Also Saccharomyces species and non-toxicogenic Aspergillus species are considered as competitive biocontrol agents (Gratz et al., 2004). While in field during storage of feed such microorganism can be used as biocontrol agents that will compete against fungi and limits its growth (Kamkar, 2008). Saccharomyces Kefir and Lactobacillus casei where used to reduce the level of AFM1 which was considered effective in reducing high level of contamination in milk. Addition of bacteria that have high binding ability can result in the subsequent reduction in Aflatoxin M1 from the milk (Ayoub et al., 2011).

Reduction of Aflatoxin M1 by absorbing material

By using absorption material such as bentonite and activated carbon can help in removal AFM1 due to their ability to bind (Asi et al., 2012).

Bentonite

Bentonite is an effective toxin bounding absorptive material. Bentonite acts as an electromagnetic agent that binds with poison when activated with carbon. It works as ion exchange method removes positively charged toxins and replaces it with minerals (Jaynes et al., 2007).

Sodium bentonite clay pellets are used to lower the amounts of AFM1 below European standards without lowering the nutritional properties of milk (Fowler et al., 2015).

Activated Carbon

Activated carbon is prepared from the carbonaceous source like coal, wood or any other carbon rich material that can be used to purify gasses and liquids. Sodium bentonite with combination of activated carbon (by 1%) has been reported in efficient reduction in Aflatoxin M1 without changing milk composition (Abdel-Wahhab and Kholif, 2010).

Chemical approaches for the reduction of Aflatoxins M1

Chemical techniques can be useful for lowering, inactivating or destructing the AFs in milk. Chemical processes include ammonia treatment, acidic treatment and reducing techniques. But in chemical processes there is risk associated with it. Chemical treatment reduces nutritive values and palatability. Moreover, chemical processes conditions should be accurately measured because it can produce toxic byproducts

Ammonia and Hydrogen peroxide

Acids, basses and oxidizing agents can be used to reduce or inactivate Aflatoxins. Aflatoxins ammonification can reduce 79-90% of AFM1 from milk. Ammonia reacts with AFs molecule by breaking its oxygen bonds and forms compound with lower toxicity (Phillips, 1999).

Hydrogen peroxide is a strong oxidizing agent that can be used for detoxification of Aflatoxin contamination in milk. Report has shown that using Hydrogen peroxide at 36 degree Celsius for 30 minutes can help in the reduction by 27.8% of Aflatoxins. Another study has revealed that addition of hydrogen peroxide and sodium hypochlorite in diet for 7 days can result in reduction of 62% and 82% of AFB1 content (Jouany, 2007; Abdul-Baki and Anderson, 1973).

Using plant extracts for reducing AFM1 contamination in milk

Most plants have natural antioxidants that help in the effective removal of Toxins from the body. The plants i.e. black cumin, curcumin and garlic are found effective against aflatoxins because these are rich in antioxidants, antifungal and anti-inflammatory agents. Most plants have the ability to inhibit infection and inflammatory molecules (Kazemi and Mohammad, 2017).

Reduction of Aflatoxins M1 through black cumin, garlic and broccoli

Plant extracts are the novel techniques used for the reduction of AFM1 from milk due to their richness in antioxidants. Water extracts like cumin, garlic and carrots were used for the detoxification of Aflatoxin contamination.

Antioxidants from plant extract have shown anti-inflammatory, anti-carcinogenic and antimicrobial effects that help in boosting the immunity. In a study done by the turmeric concentrate was used to decrease the level of AF significantly moreover it can inhibit spore count and AFs production by Aspergillus Flavus.

Conclusion

In conclusion, the pervasive issue of Aflatoxin M1 (AFM1) contamination in milk demands a comprehensive understanding and strategic interventions to safeguard public health. The carcinogenic nature of AFM1, a metabolite of Aflatoxin B1, underscores the importance of regulatory standards set by entities such as the European Union, U.S. FDA, and FSSAI to mitigate associated health risks. This review delves into the multifaceted challenge, encompassing detection methods, permissible levels, and preventive measures against AFM1. Notably, ELISA emerges as a key detection technique for its speed and precision. Preventive strategies, including controlling feed intake, vaccination, and judicious feed usage, are discussed, emphasizing the difficulty in complete elimination. The exploration of detoxification methods during milk processing introduces various approaches, from heat treatments to the potential roles of probiotics. Absorption materials like bentonite and activated carbon show promise, while chemical approaches with ammonia and hydrogen peroxide are cautiously examined due to associated risks. Novel detoxification techniques involving plant extracts, such as black cumin, garlic, and broccoli, offer promising avenues with their antioxidant properties. Overall, this review not only highlights the gravity of AFM1 contamination in milk but also provides insights for future research and the development of effective strategies to ensure the safety and quality of this dietary staple. The challenge lies in adopting a holistic approach that combines prevention, detection, and detoxification methods to address this critical food safety concern.

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