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Environment

Aflatoxin M1 in milk and dairy products in Brazil: a review of characteristics, contamination, human exposure and health risks

Aflatoxina M1 em leite e derivados no Brasil: uma revisão das características, contaminação, exposição humana e riscos à saúde

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ABSTRACT

This paper aimed to present a review of the presence of aflatoxin M1 in milk and dairy products in Brazil, along with its characteristics and potential risks to human health. Overall, most studies analyzed during the defined period of this review (2013 to May 2023) reported average concentrations of AFM1 within the limits established by Brazilian legislation for milk, powdered milk, and cheese. However, considering the consumption of milk and dairy products by more vulnerable groups, such as children, two studies reported levels of AFM1 above the limits established for aflatoxins in different regions of Brazil. Thus, a more rigorous inspection by the responsible Brazilian authorities is necessary. Furthermore, since AFM1 is the only mycotoxin present in milk relevant to public health, it is important to continue conducting studies on the detection and determination of aflatoxins in milk and dairy products. This is necessary to monitor these contaminants and estimate the levels to which the population is exposed.

Keywords: Food safety; Emerging contaminant; Estimated daily intake

RESUMO

Este artigo objetivou apresentar uma revisão sobre a presença da aflatoxina M1 em leite e derivados lácteos no Brasil, além de suas características e potenciais riscos para a saúde humana. Em geral, a maioria dos estudos analisados no período definido nesta revisão (2013 a maio de 2023), relatou concentrações médias de AFM1 dentro dos limites estabelecidos pela legislação brasileira para leite,

leite em pó e queijo. No entanto, levando em consideração o consumo de leite e derivados por grupos mais vulneráveis, como crianças, dois estudos relataram níveis de AFM1 acima dos limites estabelecidos para aflatoxinas em diferentes regiões do Brasil. Portanto, é necessária uma inspeção mais rigorosa pelas autoridades brasileiras responsáveis. Além disso, considerando que a AFM1 é a única micotoxina presente no leite com relevância para a saúde pública, é de extrema importância a continuidade de estudos sobre a detecção e determinação de aflatoxinas em leite e derivados, a fim de monitorar esses contaminantes e estimar os níveis aos quais a população está exposta.

Palavras-chave: Segurança dos alimentos; Contaminante emergente; Ingestão diária estimada

1 INTRODUCTION

Milk is one of the most important agricultural commodities worldwide, consumed by billions of people in various forms on a daily basis. In Brazil, it holds significant economic importance as a source of income and livelihood for a large portion of the population (Nyokabi *et al*., 2021; EMBRAPA, 2019). Brazil ranks as the second largest milk producer globally, just behind Argentina (CONAB, 2023). The milk production chain plays a vital role in the country's economy, contributing positively to job creation and income generation (EMBRAPA, 2020).

However, as consumer demand for high-quality and safe food increases, food industries must implement robust and efficient food quality and safety management systems. The humid tropical climate in Brazil favors the growth of various mycotoxin-producing fungi (Kaur *et al*., 2021). *Aspergillus* spp. is the most widespread filamentous fungus known for its aflatoxin production. This fungus exhibits resilience to unfavorable environmental conditions, allowing it to thrive in a wide range of humidity and temperature conditions (Eltariki *et al*., 2018). Among mycotoxins, aflatoxins represent the most significant class (Silva *et al*., 2015).

Aflatoxins are highly toxic and carcinogenic, with aflatoxin M_1 (AFM_1) being a hepatocarcinogenic derivative of aflatoxin B_1 (AFB_1) excreted in milk after the ingestion of contaminated feed. Consequently, AFM_1 can be found in milk and its derivatives (Jakšić *et al*., 2021). Considering the consumption of milk and dairy products by vulnerable groups such as children and the elderly, contamination of these foods with AFM_1 concentrations above the tolerance limits established by regulatory bodies becomes a significant concern. In Brazil, the legislation sets a tolerance limit of 0.5 mcg/kg for fluid milk, 5 mcg/kg for powdered milk, and 2.5 mcg/kg for cheese (Ministry of Health, 2021).

Such contamination and daily exposure to AFM_1 can have long-term health effects on consumers, making it a relevant public health issue. AFM_1 has been associated with the development of hepatocellular carcinoma (HCC) and cancer, which have high mortality rates (Copetti *et al*., 2019; Conteçotto *et al*., 2021).

Considering the importance of food safety, the significant production and consumption of milk and dairy products in Brazil, and the toxicity of aflatoxins, this study aimed to present a review of the presence of aflatoxin M_1 in milk and dairy products in Brazil, as well as their characteristics and potential risks to human health.

2 METHODOLOGY

The articles used as the theoretical basis for this review were obtained through a search conducted in the Scopus databases, using the keywords “MILK” OR “DAIRY” AND “AFLATOXIN” AND “BRAZIL”. The search results were limited to the period from 2013 to 2023, and only experimental articles were considered (reviews, conference papers, books, and book chapters were excluded). The selection of articles was based on reading the abstracts, excluding works that did not fall within the scope of this review, resulting in a total of 25 articles.

The subsequent sections will address data on the production, consumption, and economic aspects of milk and dairy products in Brazil. The possible causes of contamination of these foods by aflatoxin M_1, along with its definition, characteristics and risks to human health. Additionally, key findings from studies reporting the detection of aflatoxin M_1 in milk and dairy samples from different regions of Brazil during the period from 2013 to May 2023 will be presented.

3 DISCUSSION

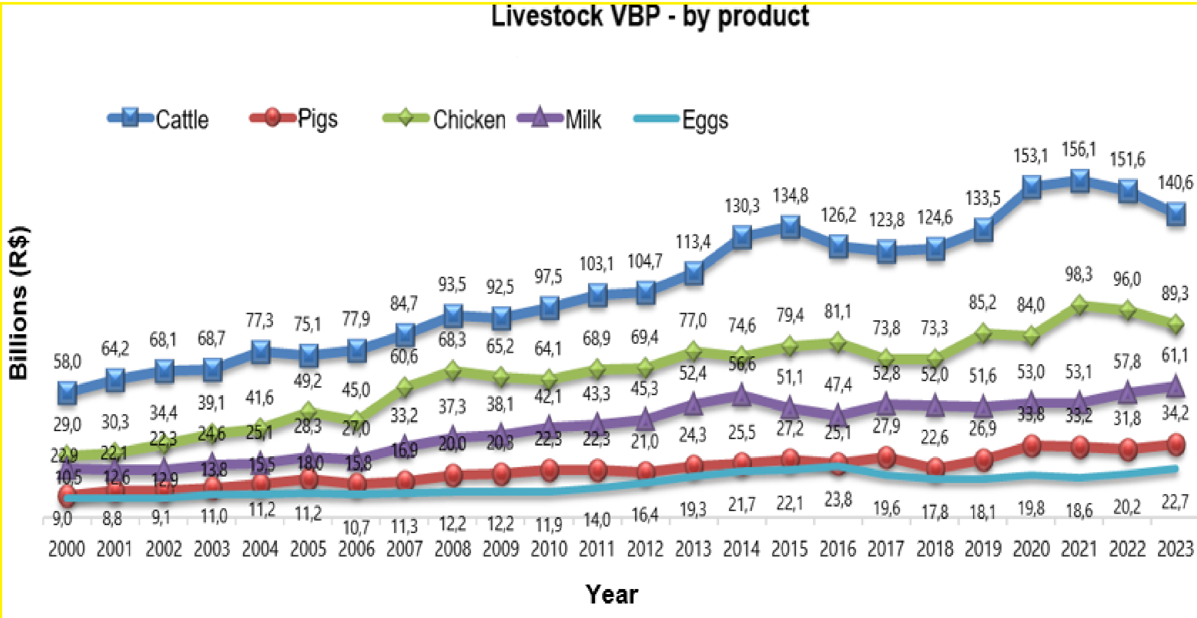
3.1 Milk and dairy products in Brazil: production, consumption and economic aspects

Over the last 20 years, the dairy sector has undergone several transformations and experienced different moments. Despite operating in different intervention environments, production has consistently grown. In the last 10 years alone, milk production in Brazil has increased by 55%. Milk processing companies are investing in the sector's growth by establishing facilities with capacities above the current processing volume. However, according to Vilela *et al*. (2017), this national production still falls short of meeting domestic consumption and export demands.

According to the Nutrient Rich Foods Index, a new concept developed by the American Food Guide and the United States Department of Agriculture (USDA) as part of their revised classification, where milk is classified as a food with high nutritional density. This means that it has a high concentration of nutrients in relation to its caloric content (USDA, 2010). Milk and its dairy products are considered highly nutritious foods as they provide high-quality proteins and contain essential vitamins and minerals such as calcium, which is essential for the development and maintenance of the body's bone structure (FAO, 2021). On average, cow's milk consists of 87% water and 13% solid components, which are further divided into approximately 4% to 5% carbohydrates, 3% protein, 3% to 4% lipids (mostly saturated), 0.8% minerals, and 0.1% vitamins (TACO, 2021).

In economic terms, the most recent estimates and projections indicate that the Gross Value of Agricultural Production (GPV) reached R$ 1,216.91 billion in the first months of 2023. Out of this total, R$ 868.96 billion belongs to the agricultural sector, while R$ 347.94 billion belongs to livestock. Specifically, dairy farming represented BRL 61.1 billion (Figure 1), showing an increase of BRL 7.5 million compared to the same period of the previous year (Brasil, 2023).

Figure 1 – Gross Value of Agricultural Production (GPV) for selected livestock products, covering the period from 2000 to 2023



*Values deflated by FGV's IGP-DI - April 2023; **Preliminary value based on January to April/2023; ***1st prognosis of the 2023 harvest (cotton, peanuts, rice, beans, castor beans, corn, soybeans, wheat, and other products repeated from the 2022 harvest)

Source: Extracted from Brazil (2023)

Regarding the per capita consumption of milk in Brazil in 2018, it was 166.4 liters per person, a value that is still below the consumption observed in other developed countries (around 250-300 liters per person), but significantly higher than the total consumed two decades ago. However, it still falls short when considering the consumption recommended by the World Health Organization (WHO) of 220 liters per inhabitant per year (FAO, 2021; EMBRAPA, 2019; Vilela, 2017).

In general, there has been an evolution in the consumption of dairy products over time (Table 1). Among the most consumed dairy products in the country are UHT milk, cheese, and powdered milk, which have shown higher consumption rates in recent years. Even with the pandemic, these products continue to experience growth, possibly due to the demand from a specific group of consumers (ABLV, 2021; EMBRAPA, 2019).

Table 1 – Evolution of consumption of dairy products in Brazil (in millions of liters)

Product	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Pasteurized milk	1.43	1.34	1.22	1.09	1.10	1.12	1.09	1.08	1.05	924
UHT Milk	6.13	6.38	6.6	6.73	6.83	7.02	6.88	6.85	6.97	6.74
Powdered milk	6.25	6.37	6.26	6.34	6.6	6.63	6.7	6.85	7.11	6.43
Cheese	7.25	7.76	8.17	8.19	8.24	8.4	8.58	8.77	9.03	8.84
Other products	2.36	2.57	2.72	2.28	1.95	2.22	2.25	2.41	2.54	2.15
Total	23.42	24.4	24.98	24.6	24.74	25.4	25.52	25.9	26.7	25.9

Source: ABLV (2021)

In parallel with the increasing consumption, ensuring milk quality control is of paramount importance. This control encompasses the entire production chain, from the animal's diet to the processing of milk and dairy products in the industries. The aim is to ensure the production of safe food, particularly in terms of preventing the presence of pathogenic microorganisms, residues and contaminants.

3.2 Aflatoxin M_1: Definitions, characteristics, contamination of milk and dairy products and human health risks

Ensuring food and nutrition security through the supply of safe food is essential for human development and a protective factor for improving population health conditions (Pereira *et al*., 2020). In this regard, consumers' concern about the potential hazards and risks associated with food consumption, particularly those of microbiological nature, is well-known, with particular attention given to animal products (Andrade *et al*., 2013).

Among the list of contaminants, fungi and their toxins have proven to be particularly challenging for food production chains. They have been the subject of investigation by researchers, nutritionists, and public health agencies due to the risks they pose to human and animal health, as well as their widespread distribution, frequency, and occurrence in various foods, especially in milk and dairy products (Variane, 2018; Franco; Landgraf, 2005).

Mycotoxins are significant food contaminants. They are metabolites produced by specific species of fungi when exposed to favorable environmental conditions (Prandini *et al*., 2009). Out of the 200 known species of toxin-producing fungi, approximately 15% are estimated to possess mycotoxicological properties. This implies that approximately 25% of all food produced may be contaminated with these metabolites (Mollayusefian *et al*., 2021; Pour *et al*., 2020).

Aflatoxins are secondary metabolites produced by certain species of *Aspergillus*, such as *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius*. They are primarily produced by the fungus during the logarithmic growth phase, resulting in a majority production of aflatoxin B_1 (AFB_1) (Conteçotto *et al*., 2021). Aflatoxins comprise a group of approximately 20 related fungal metabolites and can be found in a wide range of products, including cereals, nuts, spices, figs, and dried fruits (Prandini *et al*., 2009).

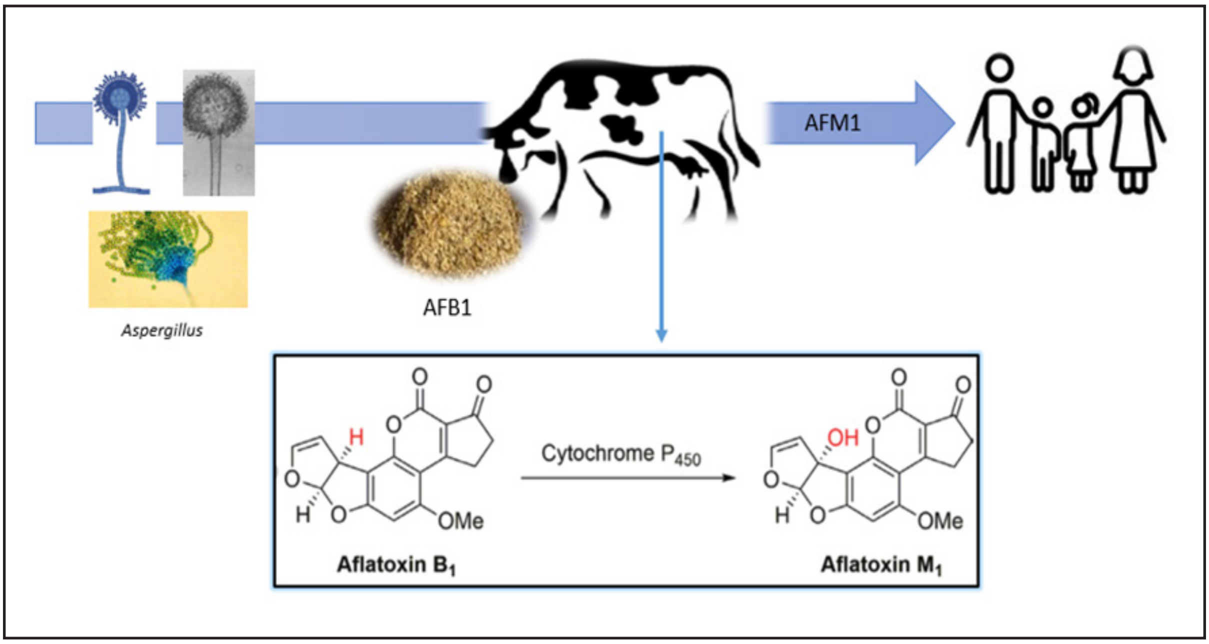
Exposure of humans and animals to aflatoxin occurs through the ingestion of contaminated food and/or feed. Furthermore, aflatoxins pose a significant risk to animals and can cause severe illnesses in humans (Safari *et al*., 2020; Oliveira *et al*., 2010). Once ingested by dairy cows, a small portion of AFB_1 is eliminated during rumination (<10%), while the remaining portion is rapidly absorbed through passive diffusion in the gastrointestinal tract and transported through the bloodstream to the liver, where it undergoes biotransformation (Yiannikouris; Jouany, 2002).

First, through epoxidation, the reduction process occurs, converting AFB_1 into aflatoxicol, another highly toxic metabolite for humans and animals. Subsequently, AFB_1 is transformed into AFB_1-8-9-epoxide, and finally, through hydroxylation, AFB_1 is converted into AFM_1 (with a molecular formula of $C_{17}H_{12}O_7$ and a molecular weight of 328.27 g/mol), by microsomal enzymes related to cytochrome P_{450}, along with other less toxic metabolites. AFM_1 is excreted in cow's milk and subsequently present in its by-products (Nguyen *et al*., 2020; Bbosa *et al*., 2013; Yiannikouris; Jouany, 2002).

The conversion of AFB_1 to AFM_1 is considered a detoxification process as the *in vivo* carcinogenicity of AFM_1 is approximately 10% relative to AFB_1. Factors such as exposure time and dosage of aflatoxins need to be taken into account (Copetti *et al*., 2019; Iqbal *et al*., 2015). Figure 2 illustrates the biotransformation cycle from AFB_1 to AFM_1.

Exposure to aflatoxin can lead to liver failure, encephalopathy, and Reye's syndrome, as well as affecting the health and development of fetuses and neonates. Acute exposure may result in acute hepatotoxicity, with a mortality rate of approximately 25%. Chronic exposure is another consequence of prolonged consumption, even at low doses of these mycotoxins (Copetti *et al*., 2019; Bennett; Klich, 2003).

Figure 2 – Biotransformation cycle from AFB_1 to AFM_1



Source: Elaborated by the authors

The main source of contamination occurs when lactating animals consume AFB_1-contaminated feed, which is subsequently transformed into AFM_1 and excreted in milk. Additionally, AFM_1 can also be present in powdered milk used in the production of certain types of cheese, and the growth of *Aspergillus* spp. (particularly *Aspergillus flavus* and *Aspergillus parasiticus*) in cheese can lead to the synthesis of various aflatoxins, including B_1, B_2, G_1, and G_2 (Khaneghah *et al*., 2021).

Some nutritional factors, such as the type and amount of feed, digestion speed, and physiological factors of the animals, can influence the conversion of AFB_1 into AFM_1. Breed, welfare, liver detoxification capacity, lactation period, milk production, and environmental factors including season, climate, geographic location, and regional development level are also taken into account (Mollayusefian *et al*., 2021). Typically, AFM_1 has higher concentrations in winter compared to summer. This means that cooler regions also tend to have higher levels of AFM_1 in milk samples. The reason for this is that during winter, there is limited availability of pasture, grass, and green forage, and the feed is primarily composed of concentrated foods (feeds), made with grains potentially contaminated with aflatoxins (Asi *et al*., 2012).

The concentration of AFM_1 in milk depends on the amount present in the contaminated feed ingested by the animal, with a conversion rate ranging from approximately 0.3% to 6.2%. Whey proteins play a role in the detoxification process, with some binding more strongly to AFM_1. AFM_1 excretion in milk begins 12 to 24 hours after the ingestion of contaminated feed, reaching high levels within a few days, and disappearing approximately 24 hours after it is eliminated from the diet (Chavarría *et al*., 2015; Nachtmann *et al*., 2007; Creppy, 2002).

Due to the high toxicity of AFM_1, its stability to heat, and the significant consumption of milk and dairy products, several countries have established maximum levels of AFM_1 in milk, which vary based on the economic status and development of them (Mollayusefian *et al*., 2021; Min *et al*., 2021; ANVISA, 2011).

According to a study conducted by Prandini *et al*. (2009), when quantities of AFB_1 below 40 μg/cow/day are ingested, the production of milk with AFM_1 content remains below 0.5 μg/kg, thereby complying with the standards established by Brazilian regulation. In Brazil, the legislation adopts a limit of 0.5 μg/kg for aflatoxin in fluid milk, which is in accordance with the limit established by MERCOSUL (Brasil, 2021), and it follows a similar tolerance level as determined by the FDA (Food and Drug Administration of the United States), which establishes a limit of 0.5 ppb (parts per billion) for aflatoxin in whole, skim, and low-fat milk (FDA, 2005; FDA, 2012). On the other hand, the European Union has a stricter limit of 0.05 μg/L of aflatoxin in fluid milk, as well as in heat-treated milk and milk used for dairy product production (EU, 2006). In contrast, Egypt and Singapore have a zero-tolerance policy regarding the presence of aflatoxin in milk and dairy products, meaning that no aflatoxins are allowed in those countries (Farias *et al*., 2005).

Coppeti *et al*. (2019) point out that the occurrence of aflatoxin contamination in milk can be modified through various methods, such as heat treatment, cold storage, milk fermentation for yogurt and kefir production, and water removal, as presented in Table 2.

Table 2 – Type of processing and its observed effect on AFM_1 levels

Processing type	Effect on AFM_1 levels	
By heating	**Pasteurization** - Reduction of up to 8%.	**Boil** - Reduction of up to 15%.
By cooling	**Refrigeration** - Reduction between 11-25% (5° C for 3 days); - Reduction of up to 80% (0°C for 6 days).	**Freezing** - 14% reduction (-18°C for 30 days).
Fermentation (yogurt and kefir)	**Fermentation** - Reduction between 10-20%; - Greater stability of AFM_1 in yogurts at pH 4.6 than at pH 4.0.	
Water removal	Spray drying - There is a higher level of contamination in powdered milk due to the loss of water and the consequent increase in concentration, with aflatoxin remaining attached with the milk solids.	

Source: Copetti *et al*. (2019)

It is worth noting that studies investigating the effects of heat treatments on the destruction of AFM_1 have yielded contradictory and inconclusive results. Deveci and Sezgin (2006) studied cow's milk inoculated with AFM_1 (1.5 and 3.5 μg/L) and obtained reductions of this contaminant ranging from 12% to 68% after pasteurization at 72°C/10 min, followed by concentration and drying. They also reported that the reduction of AFM_1 in milk depends on the initial contamination level. On the other hand, Quevedo-

Garza *et al*. (2018) conducted a study on the occurrence of aflatoxin in 84 samples of commercially fluid milk sold in Mexico. They observed that the samples of milk subjected to UHT heat treatment had a higher percentage (45%) of non-compliance with the limit set by regulations (0.5 μg/kg) compared to the samples pasteurized ones (55%).

When applying thermoultrasound (20kHz, for 10 to 15 min), Hernández-Falcón et al. (2018) found out the potential of this technology as an alternative to pasteurization (85 ºC for 15s). They observed a significant reduction in the quality of the emulsion base without significantly altering the physicochemical and microbiological stability of the milk emulsion.

Şanli, Deveci, and Sezgin (2012) conducted a study on pasteurization of milk at 95 °C/5 min, which was artificially contaminated. They obtained an average reduction of 17% in AFM_1 content. In addition, yogurts produced with this raw material had their contamination reduced by 35.5%.

In two other studies, contrary to previous findings, the use of pasteurization temperatures of 92-95 °C/3 min was found to be ineffective in reducing AFM_1 content in milk (Jasutiene *et al*., 2006; Roussi *et al*., 2002), particularly under non-standard conditions such as those encountered at the domestic level (Awasthi *et al*., 2012). However, Purchase *et al*. (1972) reported approximately 80% reduction in AFM_1 content when using temperatures above 110 °C for a short duration.

The variation in the results obtained may be attributed to the heterogeneity of milk in different regions of the world, where the concentration of AFM_1 can vary significantly. This variation can be correlated to the degree of milk contamination, storage conditions, and the analytical methods used for toxin quantification (Khaneghah *et al*., 2021).

In terms of risk assessment, aflatoxins have been identified as one of the most dangerous mycotoxins that adversely affect human and animal health (Chiewchan *et al*., 2015). In animal production systems, aflatoxins can cause significant economic losses due to reduced meat and milk quality, as well as decreased herd productivity (Custódio *et al*., 2019; Prestes *et al*., 2019).

For humans, the International Agency for Research on Cancer has classified aflatoxins as Group 1 (carcinogenic to humans) (WHO, 2022). The lethal dose ranges from 20 to 120 μg/kg body weight per day for 1-3 weeks, and the assessment of exposure is based on the calculation of the Estimated Daily Intake (JECFA, 2011; Nguyen *et al*., 2020).

Toxicity assessments of aflatoxins indicate that AFM_1 and AFB_1 act through similar mechanisms, causing subcellular changes in liver parenchyma cells (Pietri *et al*., 2016). The liver and kidneys are the primary target organs affected by AFM_1, leading to liver cancer and an increased risk of other cancers, particularly when associated with chronic hepatitis B. AFM_1 exposure can also induce tumor formation and have immunosuppressive, mutagenic, and teratogenic effects (Conteçotto *et al*., 2021). Additionally, AFM_1 exposure can result in liver failure, encephalopathy, and Reye's syndrome. It can also impact the health and development of fetuses and neonates, with long-term effects including birth defects such as stunted growth and immunosuppression in children (Nguyen et al., 2020).

Strict surveillance of AFB_1 levels is of utmost importance throughout the milk and dairy production chain due to the significant risk it poses, especially for infants who consume milk as a major component of their diet. AFB_1 contamination directly impacts human and animal health, making it a crucial concern in terms of food safety (Mollayusefian *et al*., 2021). Analyzing the risk factors associated with AFB_1 contamination in corn silage and grain production is essential for assessing the risk of AFM_1 contamination in milk and its derivatives (Prandini *et al*., 2009). In the Brazilian context, scientific studies consistently demonstrate the frequent presence of AFB_1 in animal feed and the occurrence of AFM_1 in milk from cows fed contaminated feed.

Oliveira *et al*. (2010) conducted a study on feeds and milk from properties in the State of São Paulo, Brazil, and detected the presence of AFB_1 (1.0 to 19.5 µg/kg) in 40% of the feeds. They also found the presence of AFM_1 in 36.7 % of milk samples (0.010 to 0.645 µg/L). The researchers emphasized the high frequency of aflatoxins in the analyzed samples, highlighting the need for continuous monitoring to prevent contamination of ingredients and feeds intended for dairy cattle.

In another study conducted on feeds offered to dairy cows in the same Brazilian state, Motta *et al*. (2015) detected the presence of 15 genera of filamentous fungi, with *Aspergillus* spp. (20.0%), *Fusarium* spp. (14.2%), and *Penicillium* spp. (11.5%) being the most prevalent. AFB_1 was found in approximately 31% of the samples analyzed. The authors suggested implementing good production, storage, and feed usage practices to reduce the occurrence of AFB_1 in the diets provided to lactating cows.

Custódio *et al*. (2019) conducted research on 30 feedlots of cattle intended for meat production and reported that 40% of the visited feedlots had visible fungi in the rations. They found that all feed were contaminated with at least one mycotoxin, with an average aflatoxin concentration of 10.5 µg/kg.

Matos *et al*. (2021) investigated fungal contamination in feeds offered to goats in northeastern Brazil and the AFM_1 content in the milk produced. The authors found filamentous fungi counts in the range of 3.1 to 4.2 log CFU/g in the diets, with *Aspergillus, Penicillium, Fusarium, Rhizopus*, and *Acremonium* being the five identified genera. The contamination of milk with AFM_1 ranged from 5.6 to 48.2 ng/L, with a mean of 21.9 ng/L.

Furthermore, a study conducted in southern Brazil collected silage and concentrate samples from 21 dairy farms and analyzed them for aflatoxigenic Aspergillus contamination. The researchers identified a high presence of *Aspergillus* spp. in both the silage samples and concentrated feed. Among the seven strains of *Aspergillus* spp. isolated, two in the silage samples and two in the concentrated feed samples produced aflatoxins B_1, B_2, G_1, and G_2 in culture media. These strains were identified as *Aspergillus parasiticus* and *Aspergillus nomius* (Variane *et al*., 2018).

Certain preconditions are crucial for mycotoxin production. The availability of toxigenic fungal strains, susceptible hosts, and favorable agroclimatic conditions are among the main factors. Interactions between fungi, hosts, and the environment during crop cultivation play a critical role in predisposing to mycotoxin contamination. Factors such as drought, temperature stress, agronomic practices, and insect infestations contribute to the intensification of mycotoxin-producing fungi (Bilgrami; Choudhary, 1998).

According to Martins *et al*. (2012), corn and soybean meal are the main inputs used in animal feed production. Corn is particularly susceptible to fungal problems, either during pre-harvest, when ear rot can be identified, or during post-harvest, resulting in moldy grains during storage, processing, and transport, depending on environmental conditions (EMBRAPA, 2006; Loy; Lundy, 2019).

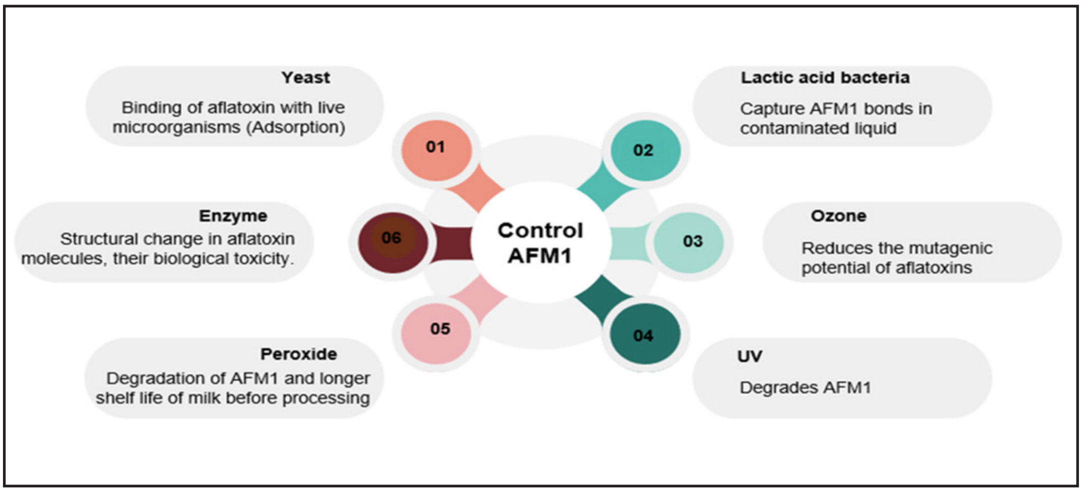
Spoilage can also be facilitated by water deficiency during the filling period and a high level of rainfall after the physiological maturity of the grain. Plants that are attacked by insects, damaged, or with poorly stuffed ears are more susceptible to the penetration of rainwater and fungal spores (Marcondes, 2012). Similarly, soybean crops in the field are susceptible to various fungal diseases, which can negatively impact both yield and seed quality. From a sanitary standpoint, the ideal seed would be free from any undesirable microorganisms. It has been observed that seeds harvested with high moisture contents, when subjected to a delay in the beginning of the drying process for a few days, can experience reduced quality due to fungal activity (EMBRAPA, 2018).

In this regard, traditional control measures focus on preventing fungal contamination in cattle feed, which is the raw material used to produce animal feeds, although complete avoidance of contamination can be challenging in some countries (Nguyen *et al*., 2020).

The most effective strategy to prevent and control aflatoxin contamination in agricultural commodities is primarily centered on pre-harvest measures. This includes the use of cultivars with genetic resistance, biocontrol methods, and the adoption of good farming practices. Post-harvest eradication of toxigenic fungi is also crucial, involving cleaning, sorting, segregation, improved drying, storage, and transportation practices, as well as the use of pesticides and preservatives. Additionally, it is essential to completely eliminate grains contaminated with aflatoxins that are harmful to human and animal health from food products intended for human consumption or animal feed (Ismail *et al*., 2021).

Furthermore, physicochemical and biological detoxification methods are alternative approaches, with apparent reduction rates ranging from 1.9% to 90% (Min *et al*., 2021). Figure 3 illustrates some control methodologies that are used to minimize AFM_1 contamination.

Figure 3 – Control strategies to reduce AFM_1



Source: Adapted from Nguyen *et al.* (2020)

However, physicochemical methods have certain disadvantages, including inefficient removal, high costs, and potential nutritional losses. On the other hand, biological decontamination methods can be a highly promising choice due to their efficiency, specificity, practicality, and cost-effectiveness (Campagnollo *et al*., 2016).

Nguyen *et al*. (2022) applied UV light treatment (254 nm) to skim milk and observed a reduction of up to 50% in AFM_1 levels after 20 min of treatment regardless of the initial AFM_1 contamination level. Additionally, the degradation of AFM_1 through UVC exposure is enhanced with increasing temperature, although it has also been demonstrated to be effective at refrigeration temperatures.

Another option that has not been addressed for this purpose is cold plasma. Although it has not been applied to degrade AFM_1 in milk specifically, it has been used to reduce AFB_1 in other food samples while maintaining their characteristics. Cold plasma, known for its environmentally friendly nature, is considered one of the most relevant methods for degrading AFM_1 in milk and dairy products (Nguyen *et al*., 2020).

Fakhrabadipour *et al*. (2023) observed that a combination of probiotic bacteria can be an efficient strategy for the detoxification of AFM_1 from skim milk. The authors reported that the percentage of AFM_1 removal by *Saccharomyces cereviseae* (66.8%) was higher than that by *Bifidobacterium bifidum* (51.3%) after 24 hours in contaminated milk spiked with 0.5 μg/mL and treated with 10^{10} CFU/mL at 37°C and 25 °C. However, the mixed strains demonstrated a remarkable AFM_1 removal rate of 90% at 37 °C.

Although these alternative technologies have shown promising results for reducing AFM_1 levels in milk, further studies are needed to understand the mechanisms that affect anti-aflatoxin activity, provide sensory evaluation results, and conduct *in vivo* model studies (Fakhrabadipour *et al*., 2023).

3.3 Detection of aflatoxin M1 in milk and dairy products in Brazil and human exposure

The characteristics of foods in each country, consumption frequency, and climatic conditions apparently influence the maximum limits adopted in each region (Jager *et al*., 2013).

AFM_1 can be present in both raw and heat-treated milk, as the toxin exhibits high heat resistance. To address its toxic effects, the European Union (EU) has established a maximum permissible level (MPL) of 0.05 μg/L for raw milk, heat-treated milk, and milk used in dairy product manufacturing (European Commission, 2020). In certain European countries like Austria and Switzerland, MPLs of 0.25 μg/kg have been adopted for cheese. However, in Brazil, the MPLs for milk and cheese are ten times higher than these levels, set at 0.5 μg/L and 2.5 μg/kg, respectively. For powdered milk, the MPL is 5 μg/kg (ANVISA, 2011; Gonçalves *et al*., 2021).

Table 3 presents studies published from 2012 to 2021, reporting the presence of AFM_1 in raw or heat-treated milk (pasteurized or UHT) and its derivatives in different regions of Brazil.

Most Maximum Permissible Limits (MPLs) found in the studies presented in Table 3 are below the limits established by Brazilian regulation (ANVISA, 2011). However, when evaluating these results from the perspective of EU legislation, which sets a maximum limit of 0.05 μg/L in fluid milk (EU, 2006), approximately 40% of the studies showed AFM_1 concentrations above the established MPL.

Gonçalves *et al*. (2021) reinforce the need to control AFM_1 not only on the farm but also in dairy products since the concentration levels in heat-treated milk can also contribute to general exposure to aflatoxins in the diet. Preventive measures, including good agricultural practices and adequate food storage conditions for dairy cows, should be improved to avoid AFM_1 in milk delivered to dairies (Gonçalves *et al*., 2021; Oliveira *et al*., 2010).

Contamination with mycotoxigenic species and the production of mycotoxins occur under favorable environmental conditions, which include specific climatic conditions, CO_2 availability, temperature, and water availability, as well as the interactions between them (Gonçalves *et al*., 2017; Georgiadou *et al*., 2012). In this context, Picinin *et al*. (2013) reported that the concentrations of AFM_1 detected in raw milk samples suggest that feed given to cows in dairy farms in Minas Gerais was probably contaminated with the toxin, especially in the dry period when rainfall did not exceed 8.0 mm and temperatures were moderate. Additionally, the estimated daily consumption in the dry period was 1.48 times greater than the expected value of food consumption in Latin America (JECFA, 2011; Picinin *et al*., 2013).

Table 3 – Aflatoxin M_1 in milk and dairy products in Brazil (2013 to May 2023)

(Continue)

Study location	Product	Concentration (Mean or Range) (µg/L)[a] or (µg/kg)[b]	Estimated Daily Intake (ng/kg b. w./day)	Reference
Supermarkets and grocery stores in Brazil	Cow powder milk	0.39[b]	Not evaluated	Londoño et al. (2013)
Dairy farms in Minas Gerais State	Raw milk	0.0195[a]	2.8–5.2	Picinin et al. (2013)
Different cities of Minas Gerais state	UHT milk	1.0–4.1[a]	Not evaluated	Oliveira et al. (2013)
Residences of employees of the University of São Paulo in Pirassununga/SP	Liquid milk	0.03[b]	0.10	Jager et al. (2013)
	Powder milk	0.65[b]	0.042	
	Cheese	0.16[b]	0.040	
	Yoghurt	–	Not evaluated	
Supermarkets in Ribeirão Preto/SP	UHT milk	0.08–0.215[a]	Not evaluated	Iha et al. (2013)
	Pasteurized milk	0.09–0.437[a]		
	Powdered milk	0.02–0.76[b]		
	Fluid milk with additives	0.009–0.061[a]		
	Infant formula	<0.03[a]		
	Powdered milk	1.02[b]		
	Infant formula	0.32[b]		
Supermarkets in the Metropolitan Region of Rio de Janeiro/RJ	Grated parmesan cheese	0.16[b]	Not evaluated	Trombete et al. (2014)
11 cities in the Paraná State	Pasteurized milk	–	Not evaluated	Santos et al. (2014)
Dairy farms in Bauru/SP	Raw milk	0.038[b]	0.120–0.358	Santili et al. (2015)
Dairy farms in Araçatuba/SP		0.017[b]		
Dairy farms in Vale do Paraíba/SP		<0.01[b]		
Local supermarkets in Rio de Janeiro/RJ	UHT milk	0.005–0.042[b]	Not evaluated	Sartori et al. (2015)
	Powered milk	0.08–1.19[b]		
Supermarkets in Maringá/PR	UHT milk	0.0196[a]	0.07	Silva et al. (2015)
Supermarkets in Londrina/PR	Pasteurized milk	0.02[b]	0.384–0.559	Santos et al. (2015)
	UHT milk	0.04[b]		
	Powder milk	0.61[b]	Not evaluated	

(–) Not detected or below quantification limit (LOQ).

Source: Elaborated by the authors

Table 3 – Aflatoxin M_1 in milk and dairy products in Brazil (2013 to May 2023)

(Continuation)

Study location	**Product**	**Concentration (Mean or Range) (μg/L)[a] or (μg/kg)[b]**	**Estimated Daily Intake (ng/kg b. w./day)**	**Reference**
Different regions of Brazil	Infant milk powdered	46.0[b]	0.078–0.306	Ishikawa et al. (2016)
Dairy production systems from three regions in Paraná's State	Raw milk	0.010–1.237[b]	Not evaluated	Ramos et al. (2016)
Organic and conventional farms in South Brazil	Raw milk (Organic)	0.023[b]	0.009–0.013	Santos et al. (2016)
	Pasteurized milk (Organic)	0.015[b]		
	Raw milk (Conventional)	0.017[b]	0.007–0.011	
	Pasteurized milk (Conventional)	0.021[b]		
Small farms in Concórdia/SC	Fresh milk	0.09–3.385[a]	Not evaluated	Gonçalves et al. (2017)
Farms with subtropical and temperate climates in Brazil	Refrigerated raw milk	0.0166[a]	0.0072–0.0107	Venâncio et al. (2018)
Supermarkets or drugstores in southern Brazil	Infant formula	15.0[a]	Not evaluated	Tonon et al. (2018)
	Follow-on formula	35.0[a]		
	Powdered milk-based products	26.0[a]		
Commercial establishments in Rio Grande/RS	Whole milk	0.06–3.67[a]	1.70	Gonçalves et al. (2018)
	Skimmed milk	0.04–1.05[a]		
	Semi-skimmed milk	0.09–1.4[a]		
	Powder milk	0.088–2.8[a]		
	Infant formula	–		
Dairy farms in Paraná State	Cow's milk	0.045–0.442[a]	Not evaluated	Navarro et al. (2020)
Dairy plants in northeast region of São Paulo State	Raw milk	0.028[a]	Not evaluated	Gonçalves et al. (2021)
	Pasteurized milk	0.023[a]		
	Minas Frescal cheese	0.113[b]		
Child Education Center in Maringá/PR	UHT milk	0.17–0.56[b]	0.828–2.523	Conteçotto et al. (2021)
	Powdered milk	1.02[b]	0–2.113	
	Infant formula	0.32[b]	0.029–0.833	

(–) Not detected or below quantification limit (LOQ).

Source: Elaborated by the authors

Table 3 – Aflatoxin M_1 in milk and dairy products in Brazil (2013 to May 2023)

(Conclusion)

Study location	Product	Concentration (Mean or Range) (µg/L)[a] or (µg/kg)[b]	Estimated Daily Intake (ng/kg b. w./day)	Reference
Supermarkets in Pirassununga/SP	Type A milk Pasteurized milk UHT milk	0.19[a] 0.227[a] 0.093[a]	0.029–0.128	Frey et al. (2021)
Experimental Animal Station from UFPB in Bananeiras/PB	Goat milk	0.0056–0.0482[a]	0.028–2.355	Matos et al. (2021)
Northeast region of São Paulo State	Raw milk Pasteurized milk UHT milk Minas cheese Yogurt	0.114[a] 0.032[a] 0.080[a] 0.122[b] 0.050[b]	Not evaluated	Corassim et al. (2022)
Processing plants from northeastern region of São Paulo State	Yogurt	0.071[b]	Not evaluated	Pires et al. (2022)
Araripe Sertão and Agreste regions of Pernambuco State	Artisanal mozzarella cheese Manufactured mozzarella cheese Artisanal coalho cheese Manufactured coalho cheese	0.07[b] 0.06[b] 0.04[b] 0.04[b]	Not evaluated	Silva et al. (2023)
Municipalities of Autazes and Apuí in the Amazonas State	Raw bovine milk	0.093–0.320	0.536–0.626	Diogenes et al. (2023)

(–) Not detected or below quantification limit (LOQ).

Source: Elaborated by the authors

Regarding the presence of AFM_1 in dairy products, based on milk samples collected in the city of Ribeirão Preto/SP, Iha *et al.* (2013) found that the toxin was stable during storage and maturation of Minas Frescal cheese, in which the concentrations of AFM_1 in cheese and whey were 1.9- and 0.4-fold of that for the milk used for cheese production. Also, the fermentation process in the production of yogurt had no effect on AFM_1.

A major concern regarding the presence of aflatoxin in powdered milk is its impact on infant formulas. Conteçotto *et al*. (2021) reported that in Brazil, powdered milk is the main food for children aged 1 to <2 years, while UHT milk is the most consumed for children aged 2 to <6 years. As children reach 5 to 6 years of age, their exposure to AFM_1 decreases as their consumption of milk decreases and they start eating other types of food.

Risk assessment involves estimating human exposure to xenobiotic compounds through food consumption and provides a connection between potential hazards in the food chain and the risks to human health (Ishikawa *et al*., 2016). The degree of exposure can be indirectly estimated based on consumption data of contaminated food and the average occurrence of the toxin. In this estimation, the degree of exposure is measured as the estimated daily intake (EDI) per unit of body weight and is usually expressed in ng per kg body weight (BW) per day. In the risk analysis, the EDI is compared to the tolerable daily intake (TDI) determined in toxicological studies (Jager *et al*., 2013).

Due to the carcinogenic potential of aflatoxins, the Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committees on Food Additives (JECFA, 2011) did not specify a TDI for aflatoxins and concluded that even a daily exposure of <1 ng/kg b.w./day contributes to the risk of liver cancer. Therefore, the levels should be reduced to the lowest extent possible.

In this context, Kuiper-Goodman (1990) proposed a TDI for AFM_1, determined by dividing the median toxic dose (TD_{50}) by an uncertainty factor of 5000. The proposed value is 0.2 ng/kg body weight, which corresponds to a risk level of 1:100,000.

Santos *et al*. (2015) estimated the EDI of AFM_1 in Londrina/PR, and the averages intake were 0.468, 0.384, and 0.559 ng/kg b.w./day for adolescents, adults, and the elderly, respectively. In another study by Santos *et al*. (2016), similar EDI values for AFM_1 were observed in organic and conventional milk. The range for organic milk consumption was 0.009 ng/kg b.w./day for adults to 0.013 ng/kg b.w./day for adolescents. For conventional milk consumption, the range was 0.007 ng/kg b.w./day for adults to 0.011 ng/kg b.w./day for adolescents.

Conteçotto *et al*. (2021) presented recent and concerning data. The EDI for AFM_1 in the population aged 0–5 years was found to be 0.828–2.523, 0–2.113, and 0.029–0.833 ng/kg b.w./day for UHT milk, powdered milk, and infant formula, respectively. The authors found that the number of cases of Hepatocellular Carcinoma associated with exposure to AFM_1 (ranging from 0.0015 to 0.0045) exceeded the limit of 0.001 case/100,000. Additionally, Margin of Exposure (MOE) values below 10,000 indicate a potential risk to public health (EFSA, 2005). The MOE values obtained for AFM_1 ranged from 728 to 239, significantly below the safety margin of 10,000. According to the authors, these results indicate a potential health risk for children in the population of Maringá/PR exposed to AFM_1 in dairy products.

Matos *et al*. (2021) estimated the daily intake of AFM_1 from goat milk consumption in one-year-old children and adults. For adults, the average EDI of AFM_1 was 0.10998 ng/kg b.w./day when considering goat milk as the only source of milk, and 0.00005 ng/kg b.w./day when considering the estimated per capita consumption of goat milk. These results showed that the EDIs for the calculated AFM_1 concentrations for one-year-old children exceeded the TDI suggested by Kuiper-Goodman (1990).

Regarding aflatoxin M_1 intake in different seasons of the year, Silva *et al*. (2015) reported that the highest EDI value (0.07 mg/kg b.w./day) was observed in autumn. In contrast, in the State of Minas Gerais, Picinnin *et al*. (2013) reported that the highest levels of AFM_1 were detected in the dry period, with an estimated intake of 0.0052 mg/person/day. Meanwhile, Venâncio *et al*. (2018) found no significant differences in AFM_1 levels between properties located in two different climate zones, both in summer and winter. The estimated daily intake of AFM_1 based on the analyzed milk was 0.0107 ng/kg b.w./day for adolescents, 0.0072 ng/kg b.w./day for adults, and 0.0098 ng/kg b.w./day for the elderly.

Sibaja *et al*. (2022) recently published a similar study that compiled publications from 2003 to 2018 reporting the occurrence of AFM_1 in milk and dairy products from Latin America. The authors highlight that the highest EDIs of AFM_1 were obtained for Brazil, Costa Rica, Colombia, and Mexico, with respective values of 2.4, 1.0, 1.2, and 20.9 ng/kg b.w./day.

Considering the presented data, Frey *et al*. (2021) emphasize the need for further studies to assess the presence of mycotoxins in milk, adopting a comprehensive sampling approach in Brazil. This is particularly important to understand the potential adverse effects, especially in relation to children who consume milk or powdered milk more frequently.

4 CONCLUSIONS

This article presented a review on the presence of aflatoxin M_1 in milk and dairy products in Brazil, focusing on the risks to consumer health, the limits established by current regulation, as well as the concentrations detected and estimated daily intake by the Brazilian population.

Overall, most studies analyzed in the defined period of this review reported average concentrations of AFM_1 within the limits established by Brazilian legislation for milk, powdered milk, and cheese. However, considering the consumption of milk and dairy products by more vulnerable groups, such as children, two studies reported AFM_1 values above the limit established for aflatoxins in infant formula in different regions of Brazil.

Considering that AFM_1 is the only mycotoxin present in milk that is relevant to public health, it is important to conduct studies on the detection and concentration of aflatoxins in milk and dairy products to monitor these contaminants and estimate the levels to which the population is exposed. Therefore, investing in programs of good agricultural practices for the storage of feed and good manufacturing practices for milk and diaries in Brazil can be interesting strategies to mitigate the risks associated with aflatoxin contamination.

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