Postprint: Research Advances on Aflatoxin M1 in Milk and Dairy Products

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Abstract

With the development of China' s economy and the improvement of people' s living standards, milk and dairy products, which are rich in nutritional value, have gradually become essential consumer goods in daily life. At the same time, people' s expectations for milk and dairy products are no longer limited to quantitative improvements, but have placed higher demands on their quality and safety. Due to the 2011 incident of excessive aflatoxin M1 (AFM1) levels in milk, and because infants and young children are more vulnerable to damage from AFM1 in milk compared to adults, there has been increasing attention to the issue of AFM1 contamination in milk and dairy products. Based on existing literature reports both domestically and internationally, this paper provides a comprehensive review of the source and biological properties, contamination and limit standards, detection technologies, and prevention and control measures for AFM1 in milk and dairy products.

Full Text

1.1 Physicochemical Properties and Production of AFM1

To date, more than 20 types of aflatoxins and their derivatives have been discovered in nature, with the chemical structures of over 10 varieties clearly identified. All aflatoxins share similar chemical structures, composed of carbon (C), hydrogen (H), and oxygen (O) elements as derivatives of dihydrofuranoxanthone, containing one difuran ring and one oxanthone (coumarin). The former constitutes the basic toxic structure, while the latter is associated with the carcinogenicity of mycotoxins [2]. The chemical structural formula of AFM1 is shown in [Figure 1: see original paper].

AFM1 has a molecular formula of $C_{17}H_{12}O_7$, a molecular weight of 328, and a melting point of 299 °C. It appears as rectangular, colorless crystals that produce blue-violet fluorescence under 365 nm ultraviolet light [2]. AFM1 is soluble in

various organic solvents such as methanol, acetone, acetonitrile, and chloroform, but insoluble in non-polar solvents like n-hexane, diethyl ether, and petroleum ether. It remains relatively stable in neutral and acidic solutions, with slight decomposition in strong acidic conditions. In strong alkaline solutions, AFM1 rapidly decomposes into essentially non-toxic salts; however, this reaction is reversible, and the original structure can be restored under acidic conditions. Due to its stable chemical structure, AFM1 cannot be inactivated by either pasteurization or ultra-high temperature sterilization processes [3-6].

AFM1 was first discovered by Alleroft in 1963 and named in 1965. It belongs to the aflatoxin family, which consists of toxic secondary metabolites secreted primarily by *Aspergillus flavus* and *Aspergillus parasiticus* [7-8]. When mammals ingest food or feed contaminated with aflatoxin B1 (AFB1), the terminal furan ring C-10 is hydroxylated under the catalysis of hepatic microsomal monooxygenase and the regulation of cytochrome P450, converting AFB1 into AFM1 [9]. AFM1 is excreted in animal milk and urine [1,10], with milk being the primary medium. Studies have shown that the conversion rate of AFB1 to AFM1 in milk ranges from 0.3% to 6.1% when humans and dairy cows ingest AFB1 [11-12]. Research indicates that 3.45% to 11.39% of ingested AFB1 is converted to AFM1 in humans, primarily distributed in the breast milk of lactating women, with the excreted amount positively correlated with intake [13]. The conversion reaction from AFB1 to AFM1 is illustrated in [Figure 2: see original paper]. Although AFM1 can also be produced directly by *Aspergillus flavus* and *Aspergillus parasiticus*, its proportion is significantly lower compared to AFB1, AFB2, AFG1, and AFG2 [2].

1.2 Toxicity of AFM1

Following ingestion, aflatoxins are absorbed through the intestinal tract and distributed throughout the body, with metabolism occurring primarily in the liver [14]. As the liver is the main target organ, aflatoxins can be considered hepatotoxins that cause symptoms such as cirrhosis and hepatomegaly.

Aflatoxins are highly toxic substances, and AFM1, as one of them, exhibits strong pathogenicity encompassing both toxicity and carcinogenicity. Regarding toxicity, AFB1 is the most potent among known aflatoxins. Although AFM1' s toxicity is one order of magnitude lower than AFB1 [15-16], it remains an extremely toxic substance—40 times more toxic than arsenic and 5 times more toxic than potassium cyanide [17]. Concerning carcinogenicity, AFM1 is a potent carcinogen with carcinogenicity comparable to AFB1. The International Agency for Research on Cancer (IARC) has upgraded AFM1' s carcinogenic classification from Group 2 to Group 1 [17-18]. Physiological studies on carcinogenic mechanisms reveal that the distal furan epoxy structure of AFM1 covalently binds to DNA purine residues, causing DNA damage and altering DNA structure and function, thereby inducing carcinogenesis [19]. AFM1 has been shown to cause tumors in experimental animals; doses of 50 g/kg body weight can induce liver cancer and colon adenocarcinoma in rats, with reports

also documenting odontogenic tumors [20]. Asian disease research institutions have established a positive correlation between dietary aflatoxins and hepatocellular carcinoma [21]. Epidemiological studies by Sun et al. [22] and Qian [23] demonstrated that the incidence rate in high-risk areas for liver cancer is closely related to AFB1 intake and the conversion rate to urinary AFM1. However, as dietary patterns evolve, direct human exposure to AFB1 has decreased, while contamination of AFM1 in animal milk and dairy products poses a serious threat to human health.

2.1 AFM1 Contamination Overview

To prevent AFM1 poisoning incidents from milk and dairy products and safeguard consumer health and food safety, numerous countries have conducted surveillance of AFM1 levels in milk and dairy products. In Europe, Tsakiris et al. [24] used enzyme-linked immunosorbent assay (ELISA) to test 196 milk samples from Greece in 2010, finding 91 positive samples (46.5%), with 2 samples (1.0%) exceeding the EU limit of 50 ng/L. Santini et al. [25] employed fluorescence spectrophotometry (FL) to analyze···

2.2 AFM1 Limit Standards

Based on six factors including hazard analysis, exposure assessment, analytical methods, trade harmonization, sampling protocols, and domestic food supply [36], countries worldwide have established strict maximum limits for AFM1 in milk and dairy products. The limit regulations for major countries are summarized in . However, many countries have yet to establish maximum limits for AFM1 in milk and dairy products.

3.1 AFM1 Detection Technologies

Current detection methods for AFM1 in milk fall into two categories: physicochemical analysis based on chromatographic techniques, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC); and rapid immunochemical methods, including fluorescence spectrophotometry (FL), enzyme-linked immunosorbent assay (ELISA), and colloidal gold immunochromatography (GICT). The detection principles, advantages, and disadvantages of each method are presented in .

3.2 AFM1 Prevention and Control Technologies

Milk and dairy products are nutrient-rich foods that constitute a major component of infant diets and represent a significant proportion of daily human nutrition. Therefore, effective prevention and control of AFM1 contamination is critically important. Since AFM1 contamination in milk originates primarily from AFB1 in feed, strict feed control is key to managing AFM1 pollution. First, mold prevention and detoxification of feed are essential. Mold prevention

relies on maintaining dry, ventilated, and hygienic conditions during feed processing and storage to disrupt mold growth conditions [44]. For already moldy feed, detoxification measures can be implemented. Jiang et al. [45] found that physical sieving could effectively remove broken grains and impurities containing high mycotoxin levels, thereby reducing deoxynivalenol (DON), zearalenone (ZEN), and AFB1 content in corn. However, this method involves cumbersome operational steps that are impractical for large-scale animal feed processing.

Chemical agents such as calcium hydroxide, monoethylamine, ozone, or ammonia can be used to destroy mycotoxins in feed, but these chemicals may leave residues that affect feed palatability and safety, potentially harming animals [46]. Furthermore, chemical methods face challenges including environmental pollution, high processing costs, and time consumption, making large-scale industrial application difficult. Currently, the most practical and extensively studied detoxification technology involves adding non-nutritive mycotoxin adsorbents to feed [47]. These adsorbents prevent or limit toxin absorption in the animal gut by forming chelates with mycotoxins for direct excretion. Importantly, adsorbents are not absorbed by dairy cows, do not produce harmful substances, and do not contaminate milk [48]. Commonly used mycotoxin adsorbents include hydrated sodium calcium aluminosilicate (HSCAS), esterified glucomannan (EGM), and montmorillonite.

HSCAS, derived from natural zeolite, is the most widely studied mycotoxin adsorbent. In vitro screening tests demonstrate that HSCAS exhibits strong affinity for AFB1, forming stable complexes that prevent gastrointestinal absorption [49]. Kutz et al. [50] reported that adding HSCAS adsorbent to diets reduced AFM1 levels in milk by 50%. EGM, a functional carbohydrate extracted from brewer' s yeast, is considered the active component of mycotoxin adsorbents. Diaz et al. [51] found that adding 0.05% EGM to diets containing 55 g/kg AFB1 reduced AFM1 content in milk by 59%. However, some studies indicate that EGM did not reduce AFM1 levels in cow' s milk [50] or goat' s milk [52], possibly due to variations in dietary AFB1 concentrations and adsorbent dosages. Montmorillonite, the main component of bentonite, is a layered silicate clay mineral with a sheet-like crystalline structure. Queiroz et al. [53] observed a 19.3% reduction in milk AFM1 concentration when 1% modified montmorillonite was added to diets containing 75 g/kg AFB1.

In addition to reducing AFB1 contamination in feed, researchers worldwide have been actively investigating effective methods to treat milk directly to reduce AFM1 content and toxicity. El Khoury et al. [54] reported that traditional Lebanese industrial lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains) could reduce free AFM1 levels in liquid cultures. Elsanhoty et al. [55] utilized different lactic acid bacterial strains to reduce AFM1 content in yogurt, finding the most significant reduction in a medium containing 50% yogurt culture (*S. thermophilus* and *L. bulgaricus*) and 50% *Lactobacillus plantarum* culture. Further research on AFM1 detoxification methods in milk is warranted.

4 Conclusion

AFM1 exhibits strong pathogenicity, with toxicity second only to AFB1 and carcinogenicity comparable to AFB1. The liver is its primary target organ, with hepatocarcinoma being the main risk. Consequently, countries and international organizations worldwide prioritize monitoring AFM1 in milk and dairy products, with varying degrees of contamination detected globally. Enhanced surveillance provides a theoretical foundation for developing and revising food safety regulations. Current detection methods for AFM1 include TLC, HPLC, LC-MS/MS, ELISA, FL, and GICT, with HPLC being the most widely applied. AFM1 contamination in milk and dairy products poses a serious threat to human health, primarily originating from AFB1-contaminated feed consumed by animals. Therefore, the key to AFM1 control lies in strict feed management, particularly mold prevention and detoxification. Current detoxification technologies involve high production costs and various limitations, emphasizing the importance of proper feed storage, mold prevention, and promotion of green feeding practices. Future research should focus on developing methods with low detection and quantification limits capable of simultaneously detecting multiple mycotoxins in milk and dairy products, with limits established based on actual milk consumption and contamination levels to better protect human health.

References

[1] PRANDINI A, TANSINI G, SIGOLO S, et al. On the occurrence of aflatoxin M1 in milk and dairy products[J]. Food Chemical Toxicology, 2009, 47(5): 984-991.

[2] WANG Lei. Establishment of Detection Methods for Aflatoxin M1 in Milk and Effects of Aflatoxins in Feed on Milk Quality[D]. Master's thesis. Zhengzhou: Henan Agricultural University, 2008.

[3] FALLAH A A. Aflatoxin M1 contamination in dairy products marketed in Iran during winter and summer[J]. Food Control, 2010, 21(11): 1478-1481.

[4] FALLAH A A. Assessment of aflatoxin M1 contamination in pasteurized and UHT milk marketed in central part of Iran[J]. Food Chemical Toxicology, 2010, 48(3): 988-991.

[5] IQBAL S Z, PATERSON R R M, BHATTI I A, et al. Survey of aflatoxins in chillies from Pakistan produced rural, semi-rural and urban environments[J]. Food Additives & Contaminants: Part B, 2010, 3(4): 268-274.

[6] BOUDRA H, BARNOUIN J, DRAGACCI S, et al. Aflatoxin M1 and ochratoxin A in raw bulk milk from French dairy herds[J]. Journal of Dairy Science, 2007, 90(7): 3197-3201.

[7] LANCASTER M C, JENKINS F P, PHILP J M. Toxicity associated with certain samples of groundnuts[J]. Nature, 1961, 192(4807): 1095-1096.

[8] ALLCROFT R, CARNAGHAN R B A. Groundnut toxicity: an examination for toxin in human food products from animals fed toxic groundnut meal[J]. Veterinary Record, 1963, 75: 259-263.

[9] WANG H F, DICK R, YIN H Q, et al. Structure-function relationships of human liver cytochromes probe[J]. Biochemistry, 1998, 37(36): 12536-12545.

[10] OVEISI M R, JANNAT B, SADEGHI N, et al. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran[J]. Food Control, 2007, 18(10): 1216-1218.

[11] NIE Jing, LIU Xingjie. Research Progress on Aflatoxin M1[J]. Foreign Medicine: Hygiene Volume, 1992(3): 159-161.

[12] RASTOGI S, DWIVEDI P D, KHANNA S K, et al. Detection of Aflatoxin M1 contamination in infant milk ELISA[J]. Food Control, 2004, 15(4): 287-290.

[13] GALVANO F, GALOFARO V, RITIENI A, et al. Survey of the occurrence of aflatoxin M1 in dairy products marketed Italy: second year of observation[J]. Food Additives & Contaminants, 2001, 18(7): 644-646.

[14] SZAKÁCS G, VÁRADI A, ÖZVEGY-LACZKA C, et al. The role of ABC transporters in drug (ADME-Tox)[J]. Drug Discovery absorption, distribution, metabolism, excretion and Today, 2008, 13(9/10): 379-393.

[15] SHYU R H, SHYU H F, LIU H W, et al. Colloidal gold-based immunochromatographic assay for detection of ricin[J]. Toxicon, 2002, 40(3): 255-258.

[16] SUN X L, ZHAO X L, TANG J, et al. Development of an immunochromatographic assay for detection of aflatoxin B1 in foods[J]. Food Control, 2006, 17(4): 256-262.

[17] IARC. IARC monographs on the evaluation of cancinogenic risk to humans[M]. Lyon: World Health Oranisation, 2002: 53-64.

[18] IARC. IARC monographs on the evaluation of carcinogenic risk to humans[M]. Lyon: World Health Organization, 1993: 56.

[19] LUTZ W, JAGGI W, LÜTHY J, et al. In vivo covalent binding of aflatoxin B1 and aflatoxin M1 to liver DNA of rat, mouse and pig[J]. Chemico-Biological Interactions, 1980, 32(3): 249-256.

[20] SUN Xingrong. Development of Immunoaffinity Chromatography Column for Aflatoxin M1[D]. Master' s thesis. Daqing: Heilongjiang Bayi Agricultural University, 2011.

[21] YANG Qiming. Detection of Aflatoxins B1 and M1 in Liver, Kidney, and Lean Meat of Swill-Fed Pigs[D]. Master' s thesis. Lanzhou: Gansu Agricultural University, 2004.

[22] SUN Guiju, QIAN Gengsun, JIN Xipeng, et al. Assessment of Aflatoxin Exposure Levels in Populations from High-Incidence Areas of Liver Cancer[J]. Journal of Southeast University: Medical Edition, 2002, 21(1): 118-122.

[23] QIAN Gengsun. Recent Research Progress on the Relationship Between Aflatoxins and Human Liver Cancer[J]. China Cancer, 2002, 11(11): 633-636.

[24] TSAKIRIS I N, TZATZARAKIS M N, ALEGAKIS A K, et al. Risk assessment scenarios of children' s exposure to aflatoxin M1 residues in different milk types from the Greek market[J]. Food and Chemical Toxicology, 2013, 56: 261-265.

[25] SANTINI A, RAIOLA A, FERRANTELLI V, et al. Aflatoxin M1 in raw, UHT milk and dairy products in Sicily (Italy)[J]. Food Additives & Contaminants: Part B, 2013, 6(3): 181-186.

[26] EL-TRAS W F, EL-KADY N N, TAYEL A A. Infants exposure to aflatoxin M1 as a novel foodborne zoonosis[J]. Food and Chemical Toxicology, 2011, 49(11): 2816-2819.

[27] ELZUPIR A O, ELHUSSEIN A M. Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan[J]. Food Control, 2010, 21(6): 945-946.

[28] HAN R W, ZHENG N, WANG J Q, et al. Survey of aflatoxin in dairy cow feed and raw milk in China[J]. Food Control, 2013, 34(1): 35-39.

[29] XIONG J L, WANG Y M, MA M R, et al. Seasonal variation of aflatoxin M1 in raw milk from the Yangtze River Delta region of China[J]. Food Control, 2013, 34(2): 703-706.

[30] GUO L Y, ZHENG N, ZHANG Y D, et al. A survey of seasonal variations of aflatoxin M1 in raw milk in Tangshan region of China during 2012-2014[J]. Food Control, 2016, 69: 30-35.

[31] ALONSO V A, MONGE M P, LARRIESTRA A, et al. Naturally occurring aflatoxin M1 in raw bulk milk from farm cooling tanks in Argentina[J]. Food Additives & Contaminants: Part A, 2010, 27(3): 373-379.

[32] IHA M H, BARBOSA C B, OKADA I A, et al. Aflatoxin M1 in milk and distribution and stability of aflatoxin M1 during production and storage of yoghurt and cheese[J]. Food Control, 2013, 29(1): 1-6.

[33] ASI M R, IQBAL S Z, ARIÑO A, et al. Effect of seasonal variations and lactation times on aflatoxin M1 contamination in milk of different species from Punjab, Pakistan[J]. Food Control, 2012, 25(1): 34-38.

[34] GOLGE O. A survey on the occurrence of aflatoxin M1 in raw milk produced in Adana province of Turkey[J]. Food Control, 2014, 45: 150-155.

[35] ŠKRBIĆ B, ŽIVANČEV J, ANTIĆ I, et al. Levels of aflatoxin M1 in different types of milk collected in Serbia: assessment of human and animal exposure[J]. Food Control, 2014, 40: 113-119.

[36] HUANG Liangce, ZHENG Nan, HAN Rongwei, et al. Research Progress on Detection Methods for Aflatoxin M1 in Milk[J]. Analysis and Testing, 2012(3): 39-41.

[37] FALLAH A A, RAHNAMA M, JAFARI T, et al. Seasonal variation of aflatoxin M1 contamination products[J]. Food industrial Control, 2011, 22(10): 1653-1656.

[38] CHIAVARO E, CACCHIOLI C, BERNI E, et al. Immunoaffinity clean-up and direct fluorescence measurement of aflatoxins B1 and M1 in pig liver: comparison with high-performance liquid chromatography determination[J]. Food Additives & Contaminants, 2005, 22(11): 1154-1161.

[39] Ministry of Health of the People' s Republic of China. GB 5413.37—2010 National Food Safety Standard: Determination of Aflatoxin M1 in Milk and Dairy Products[S]. Beijing: China Standards Press, 2010.

[40] DING Jian, LI Peiwu, LI Guangming, et al. Rapid Determination of Aflatoxin M1 in Milk by Online Solid Phase Extraction Enrichment-High Performance Liquid Chromatography[J]. Food Science, 2013, 34(10): 289-293.

[41] DONG Bin, YANG Lixin, LI Bin, et al. Determination of Aflatoxin M1 in Milk by Multi-Functional Column Purification Liquid Chromatography-Tandem Mass Spectrometry[J]. Modern Agrochemicals, 2011, 10(4): 38-40.

[42] ANFOSSI L, BAGGIANI C, GIOVANNOLI C, et al. Occurrence of aflatoxin M1 in Italian cheese: results of a survey conducted in 2010 and correlation with manufacturing, production season, milking animals, and maturation of cheese[J]. Food Control, 2012, 25(1): 125-130.

[43] WANG J J, LIU B H, HSU Y T, et al. Sensitive competitive direct enzyme-linked immunosorbent assay and gold nanoparticle immunochromatographic strip for detecting aflatoxin M1 in milk[J]. Food Control, 2011, 22(6): 964-969.

[44] LI Peng, WANG Wenjie. Common Mycotoxins in Feed and Their Prevention and Control[J]. Feed Research, 2009(4): 13-17.

[45] JIANG Miao, WU Bencheng, WANG Gaiqin, et al. Effect of Physical Sieving on Removal of Mycotoxins from Corn[J]. Feed Industry, 2013, 34(6): 45-48.

[46] FANG Xiangjun, ZOU Xiaoting. Hazards of Mycotoxin Contamination in Feed and Its Prevention and Control[J]. China Feed, 2006(11): 34-37.

[47] JI Shaoli, MOORE D, JIN Lizhi. Residues and Control Measures of Aflatoxins in Milk[J]. China Dairy Cattle, 2012(24): 36-40.

[48] TIAN Weirong, DAI Xinghong, HU Ping. Hazards of Aflatoxins in Milk and Quality Control[J]. Animal Husbandry and Feed Science, 2013, 34(2): 36-38.

[49] PHILLIPS T D, KUBENA L F, HARVEY R B, et al. Mycotoxin hazards in agriculture: new approach to control[J]. Journal of the American Veterinary Medical Association, 1987, 12: 1617.

[50] KUTZ R E, SAMPSON J D, POMPEU L B, et al. Efficacy of solis, novasil plus, and MTB-100 to reduce aflatoxin M1 levels in milk of early to mid lactation dairy cows fed aflatoxin B1[J]. Journal of Dairy Science, 2009, 92(8): 3959–3963.

[51] DIAZ D E, HAGLER W M, Jr, BLACKWELDER J T, et al. Aflatoxin binders : reduction of aflatoxin M1 in milk by feed[J]. Mycopathologia, 2004, 157(2): 233–241.

[52] FIRMIN S, MORGAVI D P, YIANNIKOURIS A, et al. Effectiveness of modified yeast cell wall extracts in dairy ewes[J]. Journal of Dairy Science, 2011, 94(11): 5611–5619.

[53] QUEIROZ O C M, HAN J H, STAPLES C R, et al. Effect of adding a mycotoxin-sequestering agent on milk aflatoxin M1 concentration and the performance and immune response of dairy cattle fed an aflatoxin B1-contaminated diet[J]. Journal of Dairy Science, 2012, 95(10): 5901–5908.

[54] EL KHOURY A, ATOUI A, YAGHI J. Analysis of aflatoxin M1 in milk and yogurt and AFM1 industry[J]. Food reduction Control, 2011, 22(10): 1695–1699.

[55] ELSANHOTY R M, SALAM S A, RAMADAN M F, et al. Detoxification of aflatoxin M1 in yoghurt using probiotics and lactic acid bacteria[J]. Food Control, 2014, 43: 129–134.

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