Risk Analysis of Aflatoxin M1 Contamination in Raw Milk in China (Post-print)

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Abstract

This study aimed to systematically evaluate the contamination status of aflatoxin M1 (AFM1) in raw milk in China. A total of 5,080 raw milk samples were collected from major dairy-producing regions in China (Hebei, Henan, Heilongjiang, Shandong, and Inner Mongolia), and AFM1 concentrations were determined using enzyme-linked immunosorbent assay (ELISA). The results showed that AFM1 was detected in 234 of the 5,080 samples (incidence rate of AFM1 contamination in raw milk: 4.6%), none of which exceeded the maximum limits established by China and the United States (500 ng/L), with only 36 (0.7%) raw milk samples exceeding the regulatory limit of the European Union (50 ng/L). Comparative analysis of AFM1 contamination in raw milk indicates that the quality and safety of raw milk in China are continuously improving. Seasonal comparison revealed that the incidence rate of AFM1 contamination in raw milk during winter was 11.2%, substantially higher than those in spring, summer, and autumn (1.5%, 2.5%, and 4.5%, respectively). Therefore, focused attention on AFM1 contamination prevention and control during winter is warranted, along with rational standardization of silage and concentrate feed storage and utilization. This research will facilitate future risk analysis and management of AFM1 contamination in Chinese raw milk.

Full Text

Risk Analysis of Aflatoxin M1 Contamination in Raw Milk from China

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Abstract: This study systematically evaluated the contamination status of aflatoxin M1 (AFM1) in raw milk across China. A total of 5,080 raw milk samples were collected from major dairy-producing regions (Hebei, Henan, Heilongjiang, Shandong, and Inner Mongolia) and analyzed for AFM1 concentration using enzyme-linked immunosorbent assay (ELISA). The results revealed that 234 samples (4.6%) were positive for AFM1, with none exceeding the Chinese and American limit standards (500 ng/L). Only 36 samples (0.7%) exceeded the European Union limit standard (50 ng/L). Comparative analysis indicates continuous improvement in raw milk quality and safety in China. Seasonal comparison demonstrated that the incidence of AFM1 contamination was highest in winter (11.2%), significantly exceeding the rates in spring, summer, and autumn (1.5%, 2.5%, and 4.5%, respectively). Therefore, enhanced prevention and control measures for AFM1 contamination are necessary during winter, particularly regarding the proper storage and use of silage and concentrate feeds. This research provides valuable data for future risk analysis and management of AFM1 contamination in Chinese raw milk.

Keywords: aflatoxin M1; raw milk; season; China

Introduction

According to Food and Agriculture Organization (FAO) estimates, approximately 25% of global grain crops are contaminated by mycotoxins, posing a serious threat to food quality and safety [1]. The 2011 incident of AFM1 exceeding safety limits in Chinese milk drew significant attention from government authorities, researchers, and consumers regarding mycotoxin contamination in dairy products. AFM1 has been identified as a primary hazard factor in milk quality and safety risk assessments, exhibiting immunotoxicity, nephrotoxicity, and hepatotoxicity, and potentially causing teratogenic, carcinogenic, and mutagenic effects in humans [2]. Internationally, AFM1 contamination in milk and dairy products has been reported across Asia, the Americas, and Africa. Researchers detected AFM1 in buffalo and cow milk from a region in Pakistan with incidence rates of 34.5% and 37.5%, respectively [3]. In 2007, Alonso et al. [4] analyzed raw milk samples from Argentina and found that 11% exceeded the EU limit standard (50 ng/L), with concentrations ranging from 10.0 to 70.0 ng/L. In 2008, Motawee et al. [5] examined milk from various species in Egypt, revealing that 50% of buffalo milk and 30% of cow milk samples exceeded the EU limit standard (50 ng/L).

Risk assessment and monitoring of AFM1 in Chinese raw milk represent a long-

term endeavor, with analytical studies of the results helping both domestic and international stakeholders understand the current status of dairy product quality and safety in China. This study collected 5,080 raw milk samples from major dairy-producing regions to detect AFM1 levels, aiming to systematically evaluate the risk profile of AFM1 contamination in Chinese raw milk and provide support for improving national dairy quality and safety.

Materials and Methods

1.2 Sample Collection and Pretreatment A total of 5,080 raw milk samples were collected during spring (April), summer (August), autumn (October), and winter (February) of 2016 from five major producing provinces with dairy herds exceeding 500,000 head: Hebei, Henan, Heilongjiang, Shandong, and Inner Mongolia. The distribution of samples is shown in . All samples were obtained from milk tankers at dairy stations. To ensure representativeness, random sampling was employed [6], with sampling protocols developed based on the distribution of dairy farming and raw milk production across the five regions to guarantee uniform geographic coverage.

Collected samples were stored at 4°C and analyzed within the 保质期限 (within 5 days). Prior to analysis, samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to remove milk fat, and the supernatant was collected for testing.

1.3 Detection of AFM1 Content in Raw Milk AFM1 concentration was determined using enzyme-linked immunosorbent assay (ELISA) with a commercial test kit (RIDASCREEN Aflatoxin M1 Test Kit, R-Biopharm AG, Germany). The kit' s limit of detection (LOD) was 5 ng/L, with standard concentrations of 0, 5, 10, 20, 40, and 80 ng/L. Analytical procedures followed the manufacturer' s instructions. Samples with AFM1 concentrations below the LOD (5 ng/L) were classified as negative. Samples exceeding the kit' s maximum standard concentration (80 ng/L) were diluted and reanalyzed. Kit validation parameters included: LOD = 5 ng/L, limit of quantification (LOQ) = 8.5 ng/L, recovery rate = 86%–121%, and relative standard deviation (RSDr) < 10%, ensuring analytical accuracy.

1.4 Statistical Analysis All samples were analyzed in duplicate, with results expressed as mean ± standard deviation (mean±SD). Statistical analysis was performed using Mann-Whitney U non-parametric tests in SPSS 19.0.

Results and Analysis

presents the AFM1 contamination status in major dairy-producing regions of China in 2016. In Hebei province, only 4 samples (0.40%) were positive for AFM1, all below both EU (50 ng/L) and Chinese (500 ng/L) limit standards. No AFM1 contamination was detected in Heilongjiang province. Inner Mongolia and Shandong provinces had 3 (0.28%) and 7 (0.67%) positive samples,

respectively. Henan province exhibited a notably higher contamination rate, with 220 positive samples (27.5%). Overall, 234 of the 5,080 raw milk samples (4.6%) were positive for AFM1. The incidence rates by province, from lowest to highest, were: Heilongjiang (0%), Inner Mongolia (0.28%), Hebei (0.40%), Shandong (0.67%), and Henan (27.5%).

The maximum AFM1 concentration detected was 273 ng/L in a sample from Henan province. No samples exceeded the Chinese limit standard (500 ng/L). However, 34 samples from Henan and 2 from Shandong exceeded the EU limit standard (50 ng/L), rendering these 36 samples (0.7%) unsuitable for export to EU markets. All other provincial samples remained below the EU limit standard.

Seasonal analysis revealed that winter had the highest AFM1 contamination incidence (11.2%) among the four seasons, followed by autumn (4.5%), summer (2.5%), and spring (1.5%). Winter accounted for 49.6% of all quarterly AFM1-positive samples [Figure 1: see original paper].

Discussion

Previous studies by our research group in 2010, including Zheng et al. [7] and Han et al. [8], analyzed 360 and 200 raw milk samples, respectively, reporting AFM1 detection rates of 78.1% and 32.5%. None of these samples exceeded Chinese or American limit standards (500 ng/L). Additionally, our team conducted continuous risk assessments from 2013–2015 across southern, northern, northeastern, and western China [9]. This study collected 1,550 raw milk samples from southern regions (Chongqing, Fujian, Guangdong, Jiangsu, Shanghai, Sichuan), northern regions (Beijing, Tianjin, Hebei, Shandong), northeastern regions (Heilongjiang, Inner Mongolia), and western regions (Gansu, Shaanxi, Xinjiang), analyzing AFM1 concentrations via high-performance liquid chromatography (LOD = 10 ng/L) . In 2013, 366 samples showed a 21.0% contamination incidence (range: 10–240 ng/L), with 11.7% exceeding the EU limit standard (50 ng/L). In 2014, 624 samples had a 28.5% incidence (range: 10–250 ng/L), with 7.7% exceeding the EU standard. In 2015, 560 samples showed a 14.1% incidence (range: 10–144 ng/L), with 1.8% exceeding the EU standard. No samples from 2013–2015 exceeded Chinese or American limits (500 ng/L), demonstrating a year-by-year decreasing trend. Regional analysis indicated that southern China had more pronounced AFM1 contamination, with excess rates of 32.5%, 10.8%, and 4.0% from 2013–2015, respectively—significantly higher than other regions. Northeastern China showed no AFM1 contamination during this period.

To further characterize current AFM1 contamination, this study collected 5,080 seasonal samples from major dairy-producing regions (Hebei, Heilongjiang, Henan, Inner Mongolia, and Shandong) with herd sizes exceeding 500,000 head. The 2016 results revealed a 4.6% contamination incidence, with only 0.7% of samples exceeding the EU limit standard (50 ng/L) and none exceeding Chinese

or American standards (500 ng/L). These 2016 results are substantially lower than the 2010 findings (78.1% [7] and 32.5% [8]), demonstrating continuous improvement in Chinese raw milk quality and safety. This progress reflects the Chinese government' s strong commitment to dairy quality and safety, implementing comprehensive monitoring systems including Good Manufacturing Practices (GMPs), Sanitation Standard Operating Procedures (SSOPs), and Hazard Analysis and Critical Control Points (HACCP) management. These measures significantly reduce AFM1 contamination while improving quality, safety, and economic benefits [10,11]. The Dairy Innovation Team conducted three consecutive years of risk assessment from 2013–2015, producing China' s first "Report on Quality and Safety of Chinese Dairy Products," which data-driven analysis confirmed as representing the highest historical quality level for Chinese dairy products. Compared with other domestic food sectors, China' s dairy products show substantially lower non-compliance rates. National Food and Drug Administration data revealed that in 2015 national food safety inspections, 166,769 batches were qualified (96.8% pass rate) while 5,541 batches failed (3.2% failure rate). In contrast, dairy products showed 9,306 qualified batches (99.5% pass rate) with only 44 failures (0.5% failure rate). Internationally, Chinese dairy quality and safety have reached advanced levels. The EU' s Rapid Alert System for Food and Feed (RASFF) 2013 annual report documented 3,137 food non-compliance notifications, with 43 (1.4%) related to dairy products; in 2014, 3,097 notifications included 66 (2.1%) dairy-related cases. In 2015, China' s national report showed 5,541 failed food batches, with only 44 (0.8%) being dairy products. This confirms that Chinese milk quality and safety have essentially reached international advanced levels.

Comparison with international data reveals that although AFM1 contamination incidence varies globally, it represents a universal challenge. China' s AFM1 incidence (4.6%) is higher than New Zealand (0) [12] and the United Kingdom (3.0%) [13] but lower than Morocco (27.1%) [14], Egypt (38.0%) [15], Croatia (>46.1%) [16], Indonesia (57.5%) [17], Pakistan (71.0%) [18], Lebanon (73.7%) [19], Nigeria (75.0%) [20], Sudan (95.5%) [21], Brazil (100.0%) [22], Iran (100.0%) [23], and Italy (100.0%) [24]. Only 1.1% of Chinese samples exceeded the EU limit standard (50 ng/L), comparable to the European Food Safety Authority' s findings (0.4% excess rate) [25] and superior to Morocco (8.3%) [14], Brazil (14.0%) [22], Egypt (20.0%) [15], Croatia (27.8%) [16], Italy (44.0%) [24], Lebanon (44.7%) [19], Nigeria (48.0%) [20], Pakistan (58.0%) [18], Iran (80.6%) [23], and Sudan (83.3%) [21]. These results provide comprehensive, accurate assessment of AFM1 contamination in Chinese raw milk and support data-driven management and risk analysis. As a global issue, AFM1 contamination warrants heightened attention in countries with high incidence and excess rates (relative to EU standards).

AFM1 in raw milk primarily originates from aflatoxin B1 (AFB1) contamination in feed. When dairy cows consume AFB1-contaminated feed, AFB1 is metabolized in the liver to AFM1, which is excreted in milk. Researchers note that in developed countries, proper storage practices and strict regulations minimize

AFM1 contamination [26]. Therefore, implementing rigorous storage measures is essential: feed ingredients and finished products should be stored in ventilated, cool, dry, clean warehouses without mold accumulation; forage should be kept in haylofts with rain protection, ventilation, moisture control, and sun protection. Regular feed testing, warehouse cleaning, and disinfection are critical, with moldy feed requiring decontamination or safe disposal to eliminate contamination sources. Minimizing inventory time, using silage immediately after opening, and properly sealing silage pits or bags prevent secondary fermentation. In cases of severe AFB1 contamination, appropriate mycotoxin adsorbents should be used to prevent gastrointestinal absorption and reduce AFB1 concentration at the source [27]. Concurrently, strict implementation of dairy quality and safety monitoring systems ensures product safety [26,28].

Notably, seasonal analysis revealed winter contamination incidence (11.2%) far exceeding other seasons (spring 1.5%, summer 2.5%, autumn 4.5%). Our previous research demonstrated similar seasonal patterns in the Yangtze River Delta region [29], and monitoring in Tangshan, Hebei, also identified elevated winter AFM1 risk [30]. These findings align with international studies from Croatia [16], Pakistan [22], Iran [31], Turkey [32], Thailand [33], and Serbia [34]. Winter's lack of fresh green fodder increases reliance on silage and concentrates (corn, peanut meal, cottonseed meal) that are susceptible to aflatoxin contamination [35]. Improper storage of these feeds during winter exacerbates contamination risk [36]. Therefore, targeted AFM1 prevention and control measures during winter, with standardized storage and usage protocols for silage and concentrates, are particularly crucial.

Conclusion

1. Analysis of AFM1 contamination in major Chinese dairy-producing regions in 2016 demonstrates continuous improvement in raw milk quality and safety, with no samples exceeding Chinese or American limit standards (500 ng/L).
2. Seasonal analysis reveals the necessity for enhanced AFM1 prevention and control during winter, emphasizing proper storage and usage protocols for silage and concentrate feeds.

References:

[1] FAO. Worldwide regulations for mycotoxins in food and feed in 2003[S]. Rome: Food and Agriculture Organization of the United Nations, 2004.
[2] PRANDINI A, TANSINI G, SIGOLO S, et al. On the occurrence of aflatoxin M1 in milk and dairy products[J]. Food and Chemical Toxicology, 2009, 47(5): 984-991.
[3] HUSSAIN I, ANWAR J, ASI M R, et al. Aflatoxin M1 contamination in milk from five dairy species in Pakistan[J]. Food Control, 2010, 21(2): 122-124.

[4] 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.
[5] MOTAWEE M M, BAUER J, MCMAHON D J. Survey of aflatoxin M1 in cow, goat, buffalo and camel milks in Ismailia-Egypt[J]. Bulletin of Environmental Contamination and Toxicology, 2009, 83(5): 766-769.
[6] CARRILLO E, VARELA P, FISZMAN S. Influence of nutritional knowledge on the use and interpretation of Spanish nutritional food labels[J]. Journal of Food Science, 2012, 77(1): H1-H8.
[7] ZHENG N, WANG J Q, HAN R W, et al. Survey of aflatoxin M1 in raw milk in the five provinces of China[J]. Food Additives & Contaminants: Part B, 2013, 6(2): 110-115.
[8] 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.
[9] ZHENG N, LI S L, ZHANG H, et al. A survey of aflatoxin M1 of raw cow milk in China during the four seasons from 2013 to 2015[J]. Food Control, 2017, 78: 176-182.
[10] CUSATO S, GAMEIRO A H, SANT' ANA A S, et al. Assessing the costs involved in the implementation of GMP and HACCP in a small dairy factory[J]. Quality Assurance and Safety of Crops & Foods, 2014, 6(2): 135-139.
[11] CUSATO S, GAMEIRO A H, CORASSIN C H, et al. Food safety systems in a small dairy factory: implementation, major challenges, and assessment of systems' performances[J]. Foodborne Pathogens and Disease, 2013, 10(1): 6-12.
[12] NZFSA. Dairy national chemical contaminants programme 2009/10 full year results[EB/OL]. 2012. www.foodsafety.govt.nz/elibrary/industry/.
[13] UKFSA. Survey of milk for mycotoxins[EB/OL]. 2001. http://www.food.gov.uk/science/surveillance/fsis20 myco.
[14] EL MARNISSI B, BELKHOU R, MORGAVI D P, et al. Occurrence of aflatoxin M1 in raw milk collected from traditional dairies in Morocco[J]. Food and Chemical Toxicology, 2012, 50(8): 2819-2821.
[15] AMER A A, IBRAHIM M A E. Determination of aflatoxin M1 in raw milk and traditional cheeses retailed in Egyptian markets[J]. Journal of Toxicology and Environmental Health Sciences, 2010, 2(4): 50-53.
[16] BILANDŽIĆ N, BOŽIĆ Đ, ĐOKIĆ M, et al. Seasonal effect on aflatoxin M1 contamination in raw and UHT milk from Croatia[J]. Food Control, 2014, 40: 260-264.
[17] NURYONO N, AGUS A, WEDHASTRI S, et al. A limited survey of aflatoxin M1 in milk from Indonesia by ELISA[J]. Food Control, 2009, 20(8): 721-724.
[18] IQBAL S Z, ASI M R. Assessment of aflatoxin M1 in milk and milk products from Punjab, Pakistan[J]. Food Control, 2013, 30(1): 235-239.
[19] ASSEM E, MOHAMAD A, OULA E A. A survey on the occurrence of aflatoxin M1 in raw and processed milk samples marketed in Lebanon[J]. Food Control, 2011, 22(12): 1856-1858.
[20] OLUWAFEMI F, BADMOS A O, KAREEM S O, et al. Survey of aflatoxin

M1 in cows' milk from free-grazing cows in Abeokuta, Nigeria[J]. Mycotoxin Research, 2014, 30(4): 207-211.
[21] 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.
[22] PICININI L C A, CERQUEIRA M M O P, VARGAS E A, et al. Influence of climate conditions on aflatoxin M1 contamination in raw milk from Minas Gerais State, Brazil[J]. Food Control, 2013, 31(2): 419-424.
[23] MOHAMADI SANI A, NIKPOOYAN H, MOSHIRI R. Aflatoxin M1 contamination and antibiotic residue in milk in Khorasan province, Iran[J]. Food and Chemical Toxicology, 2010, 48(8/9): 2130-2132.
[24] MANETTA A C, GIAMMARCO M, DI GIUSEPPE L, et al. Distribution of aflatoxin M1 during Grana Padano cheese production from naturally contaminated milk[J]. Food Chemistry, 2009, 113(2): 595-599.
[25] EFSA. Report on the implementation of national residue monitoring plans in the member states in 2013[EB/OL]. 2015. http://ec.europa.eu/food/safety/docs/cs_vet_med_residues-workdoc_2013_en.
[26] IQBAL S Z, JINAP S, PIROUZ A A, et al. Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: a Review[J]. Trends in Food Science & Technology, 2015, 46(1): 110-119.
[27] 王丽芳, 杨健, 姚一萍, 等. 影响生鲜乳质量安全的风险因子——霉菌毒素 [J]. 畜牧与饲料科学, 2017, 38(9): 41-44.
[28] CAMPAGNOLLO F B, GANEV K C, KHANEGHAH A M, et al. The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: a review[J]. Food Control, 2016, 68: 310-329.
[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] FALLAH A A, RAHNAMA M, JAFARI T, et al. Seasonal variation of aflatoxin M1 contamination in industrial and traditional Iranian dairy products[J]. Food Control, 2011, 22(10): 1653-1656.
[32] 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.
[33] RUANGWISES S, RUANGWISES N. Occurrence of aflatoxin M1 in pasteurized milk of the school milk project in Thailand[J]. Journal of Food Protection, 2009, 72(8): 1761-1763.
[34] 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.
[35] 熊江林. 日粮黄曲霉毒素 B1 向牛奶的转化及其对奶牛生产性能与健康的影响 [D]. 博士学位论文. 杭州: 浙江大学, 2014.
[36] BILANDŽIĆ N, VARENINA I, KOLANOVIĆ B S, et al. Monitoring of aflatoxin M1 in raw milk during four seasons in Croatia[J]. Food Control, 2015, 54: 331-337.