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A survey of aflatoxin M1 of raw cow milk in China during the four seasons from 2013 to 2015

N. Zheng [a, b, c, 1], S.L. Li [a, b, c, 1], H. Zhang [a, b, c], L. Min [a, b, c], Y.N. Gao [a, b, c], J.Q. Wang [a, b, c, *]

[a] Laboratory of Quality and Safety Risk Assessment for Dairy Products of the Ministry of Agriculture (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China
[b] State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China
[c] Ministry of Agriculture – Milk and Dairy Product Inspection Center (Beijing), Beijing, 100193, PR China

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ABSTRACT

In the present study, a total of 1550 raw cow milk samples were collected from Southern, Northern, Northeast, and Western regions of China during the four seasons from 2013 to 2015. Samples were analyzed for aflatoxin M1 (AFM1) using high performance liquid chromatography (HPLC). In 2013, AFM1 was detected in 21% of 366 raw cow milk samples with levels ranging between 0.01 and 0.24 μg/L. In 11.7% of samples, AFM1 levels were >0.05 μg/L, which is the legal limit in the European Union. The mean and median of positive samples were 0.069 ± 0.052 μg/L and 0.056 μg/L, respectively. In 2014, AFM1 was detected in 28.5% of 624 raw cow milk samples, with levels ranging from 0.01 to 0.25 μg/L. Of these samples, 7.7% had AFM1 levels exceeding 0.05 μg/L, with a mean of 0.042 ± 0.039 μg/L and median of 0.028 μg/L. AFMI was detected in 14.1% of 560 raw cow milk samples in 2015, with levels ranging from 0.01 to 0.144 μg/L. In 1.8% of these samples, AFM1 levels were above 0.05 μg/L, with a mean of 0.026 ± 0.024 μg/L and median of 0.017 μg/L. Our results demonstrate that AFM1 levels of the samples did not exceed the legal limit of 0.5 μg/L in China, the United States, and Codex Alimentarius Commission. Geographically, AFM1 contamination was more predominant in raw cow milk samples from Southern China than in those from other regions, with a higher number of samples containing AFM1 levels above 0.05 μg/L in 2013, 2014, and 2015. AFM1 levels were higher in autumn than in the other seasons during the entire study period. According to our survey, AFM1 contamination has been well-controlled in China during recent years; however, some samples still exceeded the European Union (EU) legal limit. Better prevention and management of aflatoxins in both feed and milk should be considered especially in Southern regions of China and in autumn.

1. Introduction

Aflatoxins (AFs), a major class of mycotoxin, are primarily produced by molds as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Creppy, 2002). To date, 18 AFs have been identified, including AFB1, the most important toxic group, which is often found in animal feed (Bahrami, Shahbazi, & Nikousefat, 2016; Decastelli et al., 2007). AFM1, the hydroxylated AFB1 metabolite, is formed by the microsomal cytochrome P450-associated pathway and secreted into milk through the mammary glands of lactating animals (Fallah, 2010; Mohammadi, Shokrzadeh, Aliabadi, & Riahi-Zanjani, 2016). AFM1 is thermally resistant and not significantly inactivated by processing such as pasteurization, ultra-high temperature treatment, and autoclaving, which are generally used to process milk products (Tajkarimi et al., 2008; Wu & Khlangwiset, 2010).

AFM1 can cause DNA damage, chromosomal abnormalities, gene mutations, and cell transformation depending on the level of exposure (Michlig et al., 2016; Van Egmond, 1989). AFM1 has been designated a group 1 toxin, indicating that it is possibly carcinogenic to humans, by the International Agency for Research on Cancer (Sugiyama, Hiraokai, & Sugita-Konishi, 2008; IARC, 2012). Milk is a nutrient containing proteins, fatty acids, minerals, and vitamins, which are necessary for human health. Milk and milk

* Corresponding author. Institute of Animal Science, Chinese Academy of Agricultural Sciences, No. 2, Yuan Ming Yuan West Road, Haidian District, Beijing, 100193, PR China.
E-mail address: jiaqiwang@vip.163.com (J.Q. Wang).
[1] Both authors contributed equally to this work.

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Table 1
Prevalence of aflatoxin M1 in raw milk in China in previous study.

Year	Location	Positive[a]/total samples no.	Limit of quantitation (μg/L)	Concentration range (μg/L)	Ref
2010	Ten provinces[b]	45/200 (32.5%)	0.005	0.005–0.060	Han et al., 2013
2011 to 2012	Yangtze River Delta region	43/72 (59.7%)	0.01	0.010–0.42	Xiong et al., 2013
2012	Tangshan region	165/188 (87.8%)	0.01	0.010–0.16	Guo et al., 2016
2013	Tangshan region	46/154 (29.9%)	0.01	0.010–0.19	Guo et al., 2016
2014	Tangshan region	69/188 (36.7%)	0.01	0.0120–0.111	Guo et al., 2016

[a] Positive samples mean the AFM1 concentration in milk samples exceeding the quantitation limit.
[b] Ten provinces covered Heilongjiang, Inner Mongolia, Hebei, Shandong, Ningxia, Shanxi, Beijing, Tianjin, Shanghai and Guangdong.

products are consumed in large quantities worldwide. Hence, the presence of AFM1 in milk and milk products poses a health risk, particularly in children.

Many countries have established a legal limit of AFM1 in milk and milk products to reduce exposure to AFM1. The United States (U.S.) and European Union (EU) have legal limits of 0.5 μg/L and 0.05 μg/L, respectively. These standards have been adopted by numerous countries (FAO, 2004; Commission Regulation [EC], 2006a, b). AFM1 levels in milk have been investigated in most counties to understand and control the safety of milk and milk products (Mohammadi et al., 2016; Gizachew, Szonyi, Tegegne, Hanson, & Grace, 2016). A large scale investigation in Serbia confirmed that AFM1 levels in milk and milk products increased in 2013 and suggested segregation of contaminated lots (Tomasevic et al., 2015).

In China, the national legal limit of AFM1 in milk is 0.5 μg/L and numerous studies have been conducted in recent years. In our previous study, AFM1 was detected in 32.5% of 200 raw milk samples from ten provinces in China, with a maximum level of 0.060 μg/L in 2010 (Han et al., 2013). Xiong, Wang, Ma, and Liu (2013) reported on the detection of AFM1 in 43 milk samples (59.7%), with concentrations ranging from 0.01 to 0.42 μg/L from the Yangtze River Delta region in China. Recently, Guo et al. (2016) reported the detection of AFM1 in raw milk in the Tangshan region of China during 2012–2014. In a previous study, the incidence of AFM1 was 87.8% (range, 0.01–0.16 μg/L) in 2012, 29.9% (range, 0.01–0.19 μg/L) in 2013, and 36.7% (range, 0.012–0.111 μg/L) in 2014 (Table 1). However, as an integral whole, the spatial distributions and seasonal changes in AFM1 levels in raw cow milk have not been reported in China, which limits our understanding of the health risks posed by AFM1 contaminated dairy products. The aim of the present study was to investigate differences in AFM1 levels in raw cow milk from different regions and seasons in successive years. From 2013 to 2015, AFM1 levels were analyzed in 1550 raw cow milk samples from 15 main milk production provinces in five geographical regions of China during all four seasons.

2. Materials and methods

2.1. Sample collection

During all four seasons between 2013 and 2015, 1550 raw cow milk samples were collected from the Southern (560), Northern (461), Northeast (265), and Western (264) regions of China, representing 15 provinces (Table 2). Raw milk was collected directly from milk-holding tanks at milk stations on the dairy farms. After stirring the milk-holding tank, 200 mL of milk was removed from the upper third of the tank, 200 mL from the middle, and 200 mL from the lower third. Six hundred milliliters of milk from each tank was mixed, and a 100 mL sample was removed and stored at −20 °C until analysis.

Table 2
Sample characteristics.

Regions (provinces)	Year	Sample no.				
		Spring	Summer	Autumn	Winter	Total
South (Chongqing, Fujian, Guangdong, Jiangsu, Shanghai, and Sichuan)	2013	20	20	40	40	120
	2014	60	60	60	60	240
	2015	50	50	50	50	200
	Total	*130*	*130*	*150*	*150*	*560*
North (Beijing, Hebei, Shandong, and Tianjin)	2013	30	30	41	40	141
	2014	40	40	40	40	160
	2015	40	40	40	40	160
	Total	*110*	*110*	*121*	*120*	*461*
Northeast (Heilongjiang and Inner Mongolia)	2013	26	26	28	25	105
	2014	20	20	20	20	80
	2015	20	20	20	20	80
	Total	66	*66*	*68*	*65*	*265*
West (Gansu, Shaanxi, and Xinjiang)	2013	–	–	–	–	–
	2014	30	30	43	41	144
	2015	30	30	30	30	120
	Total	*60*	*60*	*73*	*71*	*264*
	Total	**366**	**366**	**412**	**406**	**1550**

2.2. Determination of AFM1 levels

AFM1 levels in raw cow milk samples were determined using the official method of the Ministry of Health of China (MOH, 2010).

Sample preparation: Raw milk samples were incubated at 37 °C in a water bath and centrifuged at 7000 rpm for 15 min. At least 50 mL of supernatant was collected from each sample for extraction and purification.

Extraction and purification: Fifty milliliters of supernatant was placed in a syringe connected to an immunoaffinity column (AFLAPREPM, R-Biopharm Rhone Ltd., Glasgow, Scotland) and passed through the column at a flow rate of 2 mL/min. After capture of AFM1 by the antibodies in the column, the column was washed with 10 mL of Milli-Q water to remove extraneous nonspecific substances. Bound AFM1 was eluted from the column with 4 mL of acetonitrile. The eluate was evaporated to approximate dryness under a gentle stream of nitrogen at 30 °C and the resultant residue was diluted with 1 mL of Milli-Q water. Finally, the solution was forced through a PTFE syringe filter (pore size, 0.22 μm).

Quantitation: Determination of AFM1 levels was performed using a HPLC system (Waters, Milford, MA, USA) equipped with a 2695 separation module, a 2475 fluorescence detector, and Empower 2 professional software (Empower Software Solutions, Inc., Orlando, FL, USA). Separation of AFM1 was achieved with a mycotoxin analysis column (C18, 5 μm, 4.6 mm × 250 mm, Mycotox, Pickering laboratories, Mountain view, CA, USA). Acetonitrile water (1–3, V-V) was used as the mobile phase at a flow rate of 1.0 mL/min. AFM1 was monitored at an excitation wavelength of 360 nm and an emission wavelength of 450 nm, and quantified by co-chromatography with authentic AFM1 standards (Sigma Aldrich, Inc., St Louis, MO, USA). The limit of detection (LOD) and limit of quantification (LOQ) were set at 0.003 μg/L and 0.01 μg/L,