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Aflatoxin M_1 Contamination in Raw Milk within the Central Region of Thailand

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Abstract Aflatoxin M_1 (AFM_1) was found in all of the 240 raw milk samples collected from milk tanks of 80 dairy farms at a collecting center in the central region of Thailand. Milk samples from individual farms were collected in three seasons. The average concentration of AFM_1 in milk samples collected in winter (0.089 ± 0.034 μg/L) was significantly higher than those in rainy season (0.071 ± 0.028 μg/L) and summer (0.050 ± 0.021 μg/L). The present study suggests that regulatory limits for AFM_1 are needed to regulate and ensure the quality of raw milk and milk products in Thailand.

Keywords Aflatoxin M_1 · Raw milk · Immunoaffinity column · HPLC

Aflatoxins are fungal toxins produced by certain species of *Aspergillus*. These molds grow on several kinds of plants and plant products. Aflatoxin B_1 (AFB_1) is considered to be the most potent hepatocarcinogen of this group of mycotoxins. Aflatoxin M_1 (AFM_1) is a metabolite of AFB_1 and is secreted in the milk of mammals that have eaten contaminated foods. AFM_1 is also a hepatocarcinogen and is classified in Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC 2002). Exposure to AFM_1 through milk products is a serious problem for public health. Several countries have established regulatory limits for AFM_1 in raw milk and milk products, which vary from country to country. The regulatory limit for AFM_1 in USA is 0.5 μg/L (van Egmond 1989). The European Community has set the maximum permitted level for AFM_1 in raw milk and heat-treated milk at 0.05 μg/L (EC 2006). However, several countries, including Thailand, have not yet established regulatory limits for AFM_1.

It has been reported that concentrations of AFM_1 in raw milk vary seasonally with the highest levels in winter and the lowest in summer (Hussain and Anwar 2008; Kamkar 2005). Thailand has three seasons, each approximately 4 months long: summer (March–June), rainy season (July–October), and winter (November–February). For administrative purposes, Thailand is divided into four regions: north, central, northeast, and south. The central region was selected for investigation of AFM_1 contamination in this study since this region has the highest production of raw milk and consumption of milk products in the country. In 2003, raw milk production and consumption of the central region comprised 66% and 70% of the total for Thailand, respectively (OAE 2003). Heat treatments used in the production of pasteurized and ultra-high temperature treated (UHT) milk, in general, do not cause an appreciable reduction in the AFM_1 levels (Galvano et al. 1996; Prandini et al. 2009). In Thailand, determination of AFM_1 in raw milk is very important as approximately 97% of raw milk is used for the production of pasteurized and UHT milk products (Chudam and Toros 2008).

The purposes of this study were to determine the level and incidence of AFM_1 in raw milk within the central region of Thailand, and to study the seasonal effect on the levels of AFM_1 in the raw milk.

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Materials and Methods

The reference standard of AFM_1 (from *Aspergillus flavus*) was purchased from Sigma–Aldrich. AflaM_1™ immunoaffinity columns were purchased from Vicam (USA). Solvents (HPLC grade): acetonitrile, methanol, and water were obtained from Merck.

Aliquots of 500 mL of raw milk samples were collected in amber glass bottles from milk tanks of 80 dairy farms at a collecting center in the central region of Thailand. Milk samples from individual farms were collected in all three seasons. The first period was in summer between April and May 2006; the second period was in the rainy season between September and October 2006; and the third period was in winter between January and February 2007. Milk sample bottles were kept in ice boxes and transported to the laboratory within 3 h after collection. Milk samples were frozen at −20°C until analysis. A total of 240 milk samples were collected and analyzed in this project.

AFM_1 was extracted from raw milk samples using immunoaffinity columns. The method was provided by the manufacturer and previously described by Ruangwises and Ruangwises (2009). In brief, an aliquot of 50 mL of milk sample was transferred to a 50-mL plastic centrifuge tube and defatted by centrifugation at 3,500*g* for 20 min. Fat was then separated from the skimmed milk. The resulting skimmed milk was applied to a 50-mL plastic syringe which was attached to an immunoaffinity column. The skimmed milk was allowed to flow onto the column at the rate of 1 mL/min by gravity. The column was washed with 20 mL of water. AFM_1 was eluted with 1.25 mL of acetonitrile:methanol (3:2) and followed by 1.25 mL of HPLC water. A total volume of 2.5 mL of eluate was filtered through a nylon filter (0.45 μm) and used for analysis of AFM_1 using HPLC. All milk samples were analyzed in duplicate.

A complete chromatographic system of Shimadzu Class-LC10 (Japan) consisted of HPLC pump model LC-10AD, an auto injector model SIL-10A, a column oven model CTO-10A, and a fluorescence detector model RF-10AXL. The HPLC conditions for analysis of AFM_1 were as follows: column: Spherisorb ODS-2 (5 μm ID, 4.6 × 250 mm), column temperature: 40°C, mobile phase: water:methanol:acetonitrile (57:23:20), flow rate: 1 mL/min, detector: fluorescence spectrophotometer (excitation 360 nm; emission 440 nm).

Limit of quantitation (LOQ) of the method was determined using the Q2B method of US FDA (1996). The LOQ was 0.01 μg/L and the overall recovery across the five concentrations of fortified AFM_1 (0.0125, 0.025, 0.05, 0.25, and 0.5 μg/L) was 85.6%. The precision of the method expressed as %CV (coefficient of variation) ranged from 2.8 to 5.6% as previously discussed by Ruangwises and Ruangwises (2009).

A randomized block experiment was used to assess the differences between AFM_1 concentrations of milk samples. Duncan multiple comparison test was applied to obtain significance levels between sampling periods.

Results and Discussion

All of the 240 raw milk samples collected from milk tanks of 80 dairy farms at a milk collecting center in the central region of Thailand were contaminated with AFM_1. Table 1 summarizes the concentration and incidence of AFM_1 found in raw milk samples collected in three seasons. Concentrations of AFM_1 were 0.014–0.102 μg/L in summer, 0.022–0.128 μg/L in rainy season, and 0.028–0.197 μg/L in winter. A high incidence of AFM_1 contamination in raw milk has been reported in several studies in other countries (Table 2). Thirty-five (71%) of 49 raw milk samples collected from 20 private dairy factories in four provinces of Libya during July–August 2002 were contaminated with AFM_1 ranging from 0.03 to 3.13 μg/L (Elgerbi et al. 2004). Kamkar (2005) reported that 85 (77%) of 111 raw milk samples collected from dairy plants in Sarab city, Iran, between January and December 2001 were contaminated with AFM_1 ranging between 0.015 and 0.280 μg/L. Seventy (95%) of 74 raw milk samples purchased from markets in the eastern, northern, and southern regions of Syria during April 2005–April 2006 presented high levels of AFM_1 ranging from 0.020 to 0.690 μg/L (Ghanem and Orfi 2009). All of the 168 raw milk samples collected throughout 2005 from 14 districts of Punjab province, Pakistan were found contaminated with AFM_1 ranging between 0.01 and 0.70 μg/L (Hussain and Anwar 2008).

In the present study, statistical analysis showed that the average concentration of AFM_1 in raw milk samples collected in winter (0.089 ± 0.034 μg/L) was significantly higher than those in rainy season (0.071 ± 0.028 μg/L) and summer (0.050 ± 0.021 μg/L). The higher contamination of AFM_1 in raw milk is the result of ingestion of feedstuffs containing high AFB_1 levels by dairy cows. Mahosotanand (2002) reported that feedstuffs collected from 24 dairy farms in the central region of Thailand had the highest AFB_1 levels in winter, 126 μg/kg compared to 41 μg/kg in rainy season and 30 μg/kg in summer. In Thailand, the marked higher contamination of AFB_1 in feedstuffs during winter is presumably as a result of toxin accumulation during storage under humid conditions from the preceding rainy season.

Several studies have also shown greater levels of AFM_1 during the winter when compared to summer. Hussain and Anwar (2008) reported that the average AFM_1 concentration in raw milk samples collected from dairy farms in the Punjab

Table 1 The concentration and incidence of AFM_1 in 240 raw milk samples

Season	n	AFM_1 concentration (μg/L)				AFM_1 incidence			
		Min	Max	Mean	SD	≤0.05 μg/L	0.051–0.075 μg/L	0.075–0.100 μg/L	≥0.101 μg/L
Summer	80	0.014	0.102	0.050[a]	0.021	42 (53)	27 (34)	10 (12)	1 (1)
Rainy season	80	0.022	0.128	0.071[b]	0.028	27 (34)	13 (16)	30 (38)	10 (12)
Winter	80	0.028	0.197	0.089[c]	0.034	16 (20)	12 (15)	31 (39)	21 (26)

Different letters (a, b, c) denote significant differences between means of each season ($p < 0.05$)

Numbers in parentheses are percent of each season

Table 2 High incidence of AFM_1 in raw milk samples of various countries

Country	Year	Analyzed	Positive (%)	Concentration (μg/L)	Reference
Libya	Jul–Aug 2002	49	35 (71)	0.03–3.13	Elgerbi et al. (2004)
Iran	Jan–Dec 2001	111	85 (77)	0.015–0.280	Kamkar (2005)
Syria	Apr 2005–Apr 2006	74	70 (95)	0.020–0.690	Ghanem and Orfi (2009)
Pakistan	Jan–Dec, 2005	168	168 (100)	0.01–0.70	Hussain and Anwar (2008)
Thailand	Apr 2006–Feb 2007	240	240 (100)	0.014–0.197	Present study

of Pakistan from January to December 2007 was the highest in winter and the lowest in summer. Kamkar (2005) showed that the mean AFM_1 concentration in raw milk samples in Sarab City of Iran was the highest in winter (0.086 μg/L) and the lowest in summer (0.032 μg/L).

In this study, 42 (53%) samples in summer, 27 (34%) in rainy season, and 16 (20%) in winter were found contaminated with AFM_1 below the EU limit of 0.05 μg/L. All concentrations of AFM_1 in 240 milk samples were within the US regulatory limit of 0.5 μg/L. Our previous study showed that AFM_1 was found in all of the 150 pasteurized milk samples collected from 50 schools in the central region of Thailand in three seasons between May 2006 and January 2007 (Ruangwises and Ruangwises 2009). The average concentration of AFM_1 in pasteurized milk samples collected in winter (0.084 ± 0.024 μg/L) was also significantly higher than those in rainy season (0.073 ± 0.026 μg/L), and summer (0.053 ± 0.020 μg/L). Thailand is one of several countries that have not yet established regulatory limits for AFM_1 in raw milk and milk products. The present study and our previous report suggest a regular monitoring of raw milk and milk products, especially in winter which raw milk is contaminated with highest levels of AFM_1, and regulatory limits for AFM_1 for regulating and ensuring the quality of raw milk and milk products in Thailand.

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