

Presence of Aflatoxin M₁ in Pasteurized and UHT Milk Commercialized in Shiraz, Khuzestan and Yazd, Iran

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Abstract: Aflatoxin M₁ (AFM₁) is a hydroxylated metabolite of the carcinogen aflatoxin B₁ (AFB₁), and may be found in the milk of lactating animals that have ingested feedstuffs contaminated with AFB₁. Between Jun 2010 and March 2011, 149 samples of pasteurized (n=90) and UHT (n=59) milk were collected from supermarkets in the cities of Shiraz, Khuzestan and Yazd, Iran. All samples were analyzed for AFM₁ contamination by ELISA and 142 (95.3%) were positive with mean concentrations 41 ng.l⁻¹. These concentrations are lower than the standards of Codex Alimentarius and FDA (500 ng.l⁻¹), but 33 samples (22.1%) had higher concentrations than the maximum tolerance accepted by some European countries (50 ng.l⁻¹). Mean concentrations of AFM₁ in pasteurized and UHT milk were 39 and 46 ng.l⁻¹, respectively. Mean concentrations of AFM₁ in fall and winter samples were significantly higher (P<0.05) than those of summer but differences between AFM₁ concentrations of fall and winter samples were not significantly different. No significant differences in Concentrations of AFM₁ in milk samples between Shiraz, Khuzestan and Yazd. Considering the results, this survey revealed a high frequency of AFM₁ contamination in milk samples from central part of Iran.

Keywords: Aflatoxin M₁; Milk; ELISA

INTRODUCTION

Aflatoxins, a class of mycotoxins, are produced by filamentous fungi particularly by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. There is a widespread concern due to the toxic effects of aflatoxins on the health of human and animal consumers. The aflatoxin B₁ (AFB₁) is the most acutely toxic (IARC, 1993; Hussain et al. 2008) and hepatocarcinogenic (Creppy, 2002). Aflatoxin M₁ (AFM₁) is a hepatocarcinogen found in milk of animals that have consumed feeds contaminated with aflatoxin B₁ (AFB₁), the most important toxic metabolite produced by storage fungi of the genus *Aspergillus*, particularly *Aspergillus flavus* and *Aspergillus parasiticus* (Van Egmond, 1989). The conversion rate of ingested AFB₁ to AFM₁ present in milk is in the range of 0.3 to 6.1% for many species, including dairy cattle and human beings (Creppy, 2002).

The occurrence of AFM₁ in milk and milk products is a public health concern because the International Agency for Research on Cancer (IARC, 1993) has classified it in Group 2, a probable human carcinogen with a high risk of hepatotoxicity and mutagenicity (FAO/WHO,

1999) and high genotoxic activity (Lafont et al., 1989). As milk is a major nutrient for infants, children, convalescents and old people, the presence of AFM₁ in commercial milk and dairy products is a matter of concern. Infants in Iran usually consume pasteurized and sterilized milk after breast weaning and up to three years of age as the main source of food, so the problem is most important in this age group. AFM₁ is relatively stable during pasteurization, sterilization, preparation, and storage of various dairy products (Park, 2002). Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country. The European Community and Codex Alimentarius has prescribed that the maximum limit of AFM₁ in liquid milk and dried or processed milk products is 50 ng.l⁻¹ (Codex Alimentarius Commission, 2001; European Commission Regulation, 2001), whereas according to USA regulations the level of AFM₁ in milk should not be higher than 500 ng.l⁻¹ (Stoloff et al., 1991).

There is little information on the basic diet, including milk and milk products as well as aflatoxin M₁ concentrations in milk, in some

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Tajkarimi et al., 2007; Rahimi et al. 2009a; Rahimi et al. 2009b). The aim of this survey was therefore to determine concentrations of AFM₁ in pasteurized milk and UHT milk marketed in cities.

MATERIALS AND METHODS

Samples

from Jun 2010 to March 2011, 149 samples of pasteurized (n=90) and UHT (n=59) milk were collected from supermarkets in the cities of Shiraz, Khuzestan and Yazed, Iran.

Method of analysis

The quantitative analysis of AFM₁ was performed using enzyme immunoassay: Ridascreen[®] aflatoxin M₁ kit (R- Bipharm AG, Germany). The test is based on the antigen-antibody reaction. The wells in the microtitre strips are coated with specific antibodies to AFM₁. By adding AFM₁ standards or the sample solutions, the antibody binding sites are occupied proportionally to the AFM₁ concentration. Any remaining free binding sites are occupied in the next stage by enzyme labeled toxin (enzyme conjugate). Unbound enzyme conjugate is then removed in a washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethyl benzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow which is measured photometrically at 450 nm. Light absorption is inversely proportional to the AFM₁ concentration in the sample (Lopez et al., 2003). Milk samples were centrifuged for 10 min at 3500 g and 10 °C. After centrifugation, the upper cream layer was removed completely by aspiration with a Pasteur pipette and the skimmed milk used directly in the test (100 µl per well). The mean lower detection limit of the RIDASCREEN[®] AFM₁ test was 10 ng.l⁻¹.

Statistical analysis

Statistical analysis of results was performed with SPSS (version 16) software (SPSS Chicago, IL, USA). The mean AFM₁ concentration in raw milk, pasteurized milk, UHT milk and the mean of the AFM₁ concentration of different seasons in samples milk was compared by one way analysis of variance (ANOVA) and Tukey's (HSD) tests. Student's t-test was used to find the differences between the results from the different regions.

RESULTS AND DISCUSSION

In our study, the recovery percentage of AFM₁ in spiked milk samples at concentration of 50 ng.l⁻¹ was found to be 94.7% as mean, and 1.98% as coefficient of variation (CV). All experiments were made in triplicate. Table 1 summarizes the number of samples analyzed and the number of samples found to contain detectable levels of AFM₁ contamination in Shiraz, Khuzestan and Yazed.

Milk and dairy products play an important role in a healthy human diet since they are good sources of bioavailable calcium and proteins. From 149 samples, 142 (95.3%) contained AFM₁ in concentrations ranging from 17 to > 100 ng.l⁻¹ (mean = 41 ng.l⁻¹). All positive samples were within the tolerance limit (500 ng.l⁻¹) determined by USA regulations (Stoloff et al., 1991). However, 33 samples (22.1% of the positive samples) contained concentrations above 50 ng.l⁻¹ which is the tolerance limit adopted by the European Community and Codex Alimentarius Commission for liquid milk and processed milk products (Codex Alimentarius Commission, 2001; Creppy, 2002). The number of positive samples for AFM₁ in pasteurized and UHT milk were 88 (97.8%), and 54 (91.5%), respectively. Mean concentrations observed in pasteurized and UHT milk samples were 39, and 46 ng.l⁻¹, respectively.

Table 1. Occurrence of AFM₁ in pasteurized milk and UHT milk and raw milk in Shiraz, Khuzestan and Yazed, Iran

Sample milk	Analysed samples	Positive n (%)	Distribution of samples (ng.l ⁻¹)				
			<10 ^a	10-30	31-50	51-100	> 100
Pasteurized	90	88(97.8)	2 (2.2)	25 (27.8)	43 (47.8)	18(20.0)	2 (2.2)
UHT	59	54(91.5)	5 (8.5)	18 (30.6)	23 (39.0)	10(16.9)	3 (5.1)
Total	149	142(95.3)	7 (4.7)	43(28.9)	68(28.8)	28(18.8)	5(3.4)

^a < 10 ng.l⁻¹ – negative for aflatoxin M₁

Our results show that the contamination of pasteurized and UHT milk with AFM₁ are in agreement with those of Bakirci (2001), Alborzi et al. (2006), Rahimi et al. (2009b), and Fallah (2010), however, higher the contamination of pasteurized and UHT milk with AFM₁ have also been reported from other countries. In Thailand,

all of the examined pasteurized and sterilized milk samples had AFM₁ content greater than 50 ng.l⁻¹ (Saitanu, 1997). In Syria, AFM₁ was detected in all pasteurized milk samples with a mean value of 492 ng.l⁻¹; of which 80% and 60% exceeded the European Community and US FDA recommended limits, respectively (Ghanem and

Orfi, 2009). In another study in Tabriz, Iran, AFM₁ was detected in all of UHT milk samples which 83.7% exceeded the EC recommended limits (Movassagh, 2011). The occurrence of AFM₁ in 129 commercial UHT milk samples from Central Anatolia, Turkey, has been reported by Unusan (2006). These differences may be attributable to the fact milk to be processed may contain different levels of AFM₁ according to the seasonal changes and different analytical methods and processing technique (Galvano et al., 1996). In addition, the AFM₁ level in the milk was significantly affected by the geographical region (Galvano et al., 1996).

AFM₁ contamination of milk is the result of cows feeding on material containing aflatoxin B₁. The concentration of this mycotoxin in animal feedstuffs is influenced by the type, the time and method of harvest and temperature and relative humidity of storage facilities, all of which vary among different regions of Iran (Tajkarimi et al., 2007). Concentrations of AFM₁ varied among seasons, being 24 to >100, 17 to >100, and 17 to 48 ng.l⁻¹ for summer, autumn and winter samples, respectively. AFM₁ concentrations in milk samples were significantly higher in the colder seasons in agreement with the results of other authors. For example Tajkarimi et al. (2008) reported that AFM₁ concentrations in milk samples in winter were significantly higher than summer (P<0.05), 30% of samples in winter were >50 ng.l⁻¹; however, in summer 16% of samples were >50 ng.l⁻¹. One reason for this is that milking animals are fed with compound feeds in winter that are prone to aflatoxin B₁ concentration (Galvano et al., 1996; Kamkar, 2005; Hussain and Anwar, 2008).

Concentrations of AFM₁ in milk found in the present study are similar to those reported in other countries, especially those in Asian and African although higher than those in many European countries (Turcksess, 1999). They were, nevertheless, below the limits established by Codex Alimentarius and the FDA (500 ng.l⁻¹) and only just over half had higher concentrations than the maximum tolerance accepted by some European countries (50 ng.l⁻¹).

AFM₁ concentration of milk and milk products is potentially a serious public health problem as all age groups, including infants and children, consume these products world wide. For this reason, it is important to inspect and control dairy products and animal feed for presence of aflatoxins in a regular manner to evaluate the hygienic managements.

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