



Aflatoxin M₁ Contamination in Pasteurized Milk in Mashhad, Iran

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Abstract

The aim of this study was to evaluate aflatoxin M₁ (AFM₁) contamination in pasteurized milk samples in Mashhad, Iran. One hundred and ten milk samples from different supermarkets were collected during three months in spring and investigated by Enzyme Linked Immuno Sorbent Assay (ELISA). AFM₁ was found in 100% of the milk samples. About 5.4% of the samples contained AFM₁ greater than the maximum tolerance limit (0.05 µg/l) accepted by European Union. There was not a significant difference among the mean value of AFM₁ in three months.

Keywords: Aflatoxin; Contamination; Pasteurized milk.

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1. Introduction

Milk is a good source of many nutrients, and it is used extensively even as a main food in many countries. However, milk could also be a source of toxic substances such as aflatoxin M₁ (AFM₁). Aflatoxins are a group of naturally occurring toxins produced by moulds such as *Aspergillus flavus*. When some animals ingest aflatoxin B₁ (AFB₁)-contaminated feed, it is metabolized to AFM₁ and transferred to food materials such as milk and eggs [1, 2]. AFM₁ remain stable when milk is heat-treated; there is no evidence that cold storage, concentrating or drying changes the level of AFM₁ [3, 4].

Although the potency of AFM₁ is less than that of its parent compound, it is also known to be hepatotoxic and carcinogenic [5, 6].

More recently, aflatoxin exposure early in life has been associated with impaired growth, particularly stunting [7]. Therefore, the presence of AFM₁ in milk and dairy products may pose a threat, mainly towards children who are considered to be the major consumer of milk and dairy products in many countries [8]. This study was carried out to evaluate the prevalence of pasteurized milk contamination with AFM₁ in Mashhad, Iran and to compare the results with maximum tolerance limit (0.05 µg/l) accepted by some European countries [9].

For measurement of AFM₁, we used ELISA because immunochemical assays are reliable, rapid, simple, specific and sensitive methods for the routine analysis of mycotoxins in food and feed materials [10, 11].

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Table 1. Concentration of aflatoxin M₁ in milk samples collected in different months.

Month*	Samples	Minimum($\mu\text{g/l}$)	Maximum($\mu\text{g/l}$)	Mean \pm SD ($\mu\text{g/l}$)
March	29	0.008	0.034	0.016 \pm 0.009
April	42	0.009	0.039	0.017 \pm 0.011
May	39	0.013	0.089	0.022 \pm 0.015

*Samples were collected in 2006.

2. Materials and methods

2.1. Material

One hundred and ten pasteurized milk samples from different supermarkets in Mashhad were collected during spring (March, April, May, 2006).

2.2. Methods

The method is based on the modification of Alborzi *et al.* [12]. Milk samples were centrifuged in 20 °C for 10 min. with 3500 g. After discarding the upper cream layer, the lower phases were used for quantitative test. The AFM₁ quantity was determined by Ridascreen AFM₁ test (R-biopharm, Germany) which is a competitive enzyme immunoassay based on antigen-antibody reaction. Sample solutions of 100 μl were added to wells that coated antibodies to AFM₁ and after mixing, incubated for 60 min. at the room temperature in dark. Then the liquid was poured out of the wells and wells were washed with buffer solution. In the next state, 100 μl of enzyme conjugate washed with buffer and 50 μl of the enzyme substrate and 50 μl of chromogen were added to wells and incubated for 30 min. at the room temperature in the dark. Bond enzyme conjugate converted the chromogen to a blue product and then 100 μl of the stop solution was added to wells which lead to a yellow discoloration of the chromogen. The AFM₁ measurement was performed photometrically at 450 nm.

2.3. Statistical analysis

The results are expressed as mean \pm SD. Data were analyzed by ANOVA. Sequential differences among means were calculated at the level of $p < 0.05$, using Tukey contrast analysis or Dunn's test as needed.

3. Results and discussion

The results of our study are shown in Table 1. AFM₁ in March, April and May samples ranged from 0.008 to 0.034, 0.009 to 0.039 and 0.013 to 0.089 $\mu\text{g/l}$, respectively. There are differences in maximum permissible limit of AFM₁ in various countries, and many including Iran have no legal limit for AFM₁ in milk.

AFM₁ was found in 100% of milk samples. About 5.4% of the samples had AFM₁ greater than maximum tolerance limit (0.05 $\mu\text{g/l}$) accepted by European Union. There was not a significant difference among the mean value of AFM₁ in the three months.

There is little documented data about the occurrence of milk AFM₁ in Iran (Table 2). In Tehran, from 73 samples were analyzed. AFM₁ detected in 60 samples [13]. All contaminated samples had a level of AFM₁ above the European limit. In Sarab, a city in north west of Iran, from 111 samples, AFM₁ was observed in 85 samples. In 40% of positive samples, AFM₁ levels were higher than the European limit [14]. In Shiraz, 624 samples analyzed. 100% of the samples were contaminated and 17.8% of samples had AFM₁ greater than the European limit [12].

Table 2. The prevalence of milk contamination in cities of Iran.

Location	Time	Number of samples	Samples above 0.05 $\mu\text{g/l}$ (%)
Tehran	1998	73	82.2
Sarab	2001	111	40.0
Shiraz	2003	624	17.8
Tehran	2005	128	78.0
Mashhad	2006	110	5.4

Another study in Tehran showed that from 128 samples, 78% was higher than the European limit [15]. The changes in milk contamination in Iran may be due to differences in the time of sampling, method of determination and level of cowsheds sanitation in cities.

Our results showed that contamination with AFM₁ in our region is lower than other regions reported in Iran, most probably because of low contaminated food collections used by cows, industrialization of cowsheds, training the personnel and the season of our sampling (spring). Some studies indicated seasonal effect influences AFM₁ occurrence and a higher incidence of AFM₁ contamination during cold seasons than hot ones [16, 17]. Therefore, the results obtained by us were in agreement to prior studies.

According to results obtained in Iran, the incidence and contamination levels of AFM₁ in some cities of Iran seem to be a serious problem for public health, especially infants and children. So, to produce high quality milk, it is essential to keep feeds free from contamination to AFB₁. The concentration of AFB₁ in animal feed can be reduced by good manufacturing and good storage practices. If preventive measures fail, however, AFB₁ can be reduced in food by blending with feed that has lower concentrations or by chemical, physical or biological treatment [18]. Also, increasing the intake of antioxidants and vitamins with the diet in order to prevent carcinogenesis should be involved in the preventive strategies [19].

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