Original Article

A seasonal study for determination of aflatoxin M1 level in dairy products in Iranshahr, Iran

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Abstract

Background and Purpose: Aflatoxin M1, in milk and dairy products, is classified by the International Agency for Research on Cancer as human carcinogens (class 2B). The aim of this study was to evaluate aflatoxin M1 contamination level in dairy products from Iranshahr city, Iran, using ELISA technique .

Materials and Methods: In our study, 87 samples of milk, white cheese, yoghurt, and butter were collected in different seasons and after preparation, were analyzed by ELISA technique.

Results: The contamination level of aflatoxin M1 in pasteurized milk (n=40) yoghurt (n=15), white cheese (n=6), and butter (n=10) samples were determined to be 81.6%, 83.3%, 60%, and 100%, respectively. However, in 8.1% of the pasteurized milk samples, aflatoxin M1 concentration was above the legal accepted limit (>50 ng). The level of aflatoxin M1 was significantly higher in winter compared to summer ($P<0.05$). In addition, the concentration of aflatoxin M1 was higher in urban regions than rural ones ($P<0.05$).

Conclusion: The results of this study emphasized the importance of seasonal and regional effects on aflatoxin M1 contamination of dairy products.

Keywords: Aflatoxin M1, *Aspergillus*, Dairy product, ELISA

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Introduction

Mycotoxins are compounds and second metabolites produced by some mold fungi under specific conditions of humidity, temperature, and oxygen pressure [1]. They contaminate a broad spectrum of food and feed. One of the most important mycotoxins is aflatoxin produced by species of *Aspergillus* such as *Aspergillus flavus* and *Aspergillus parasiticus* [2].

Four major types of aflatoxin in agricultural commodities include B1, B2, G1, and G2. Aflatoxin M1 is a hydroxyl metabolite of aflatoxin B1, known as milk toxin, metabolized in the liver and kidneys and secreted in urine, feces, and milk of lactating animals [3, 4]. Humans may be exposed to aflatoxin M1 through the consumption of contaminated milk. This toxin, same as aflatoxin B1, could inhibit RNA transcription and protein synthesis. Moreover, it might cause unfavorable effects in the human body [1, 3]. However, aflatoxin M1 is less poisonous than aflatoxin B1, and according to the International Agency for Research on Cancer (IARC) classification, aflatoxin B1 and M1 were classified as group 1 and 2 of the human carcinogen, respectively [2, 4].

Although pasteurization and sterilization can reduce aflatoxin M1 activity, it is relatively stable during heating, boiling, autoclave, and processing of food production. This toxin cannot be inactivated in different dairy products [3, 5]. Given its complications for consumers and economical importance in milk and dairy products, numerous studies were performed in different regions for determining the levels of aflatoxin M1 in these samples [6-8]. Their results emphasized that the level of aflatoxin M1 is affected by various factors such as geographical conditions [7]. Similarly, the incidence of aflatoxin M1 in milk and related products was reported by several researchers in different locations of Iran [9-11].

However, there is no published study on aflatoxin M1 level in milk products from southeast of Iran. The aim of this study was to determine the level of aflatoxin M1 in some dairy samples produced in Sistan and Bluchestan provinces, located in the southeast of Iran. Furthermore, geographical and seasonal differences were investigated in this study.

Materials and Methods

This study was carried out during summer and winter of 2015. The samples were collected from seven rural and urban regions (coded by 1 to 7) of Iranshahr city, located in Sistan and Baluchestan provinces (southeast of Iran).

Sample collection

A total of 87 dairy product samples including pasteurized milk (n=49), yoghurt (n=18), white cheese (n=10), and butter (n=10) were collected from supermarkets. The samples were transferred immediately to laboratory and stored at -20°c before analysis.

Sample preparation

In milk samples, 10 ml was taken and centrifuged at 4°c for 10 min in 3000 g. The tap layer (fat layer) of the milk samples was completely removed by pastor pipette. The lower layer (skimmed milk) was kept for aflatoxin M1 analysis in a freezer at -70°c.

For yoghurt, white cheese, and butter samples, 2 g of each sample was taken separately and mixed with 4 ml of HCl (0.1 m) and 8 ml of CH4Cl2, respectively. After centrifugation of the prepared samples for 15 min in 3000 g, the tap layer was removed. Supernatant of the samples was diluted and recentrifuged in 2500 rpm for 15 min at 25°c. Finally, 100 µl of the lower liquid was kept for ELISA assay in -70°c.

The level of aflatoxin M1 was measured by commercial aflatoxin M1 fast ELISA Kit, (EuroProxima, the Netherlands). The method was performed by competitive enzyme technique based on antigen-antibody reaction. For ELISA analysis, 100 µl of the samples or aflatoxin M1 standards were added to each well, which was coated with specific antibodies against aflatoxin M1. After incubation in 25°C for 30 min, the wells were washed and filled with horseradish peroxidase-conjugated anti-aflatoxin M1 antibodies. Following additional incubation (15 min in dark at 25°C), 100 µl of substrate was added and reaction was stopped after 15 min of incubation in dark at 25°C. Sample absorption was measured in 450 nm by ELISA reader (Biotek, USA). The level of aflatoxin M1 in samples has a reverse correlation with sample absorption, calculated based on drawing a standard curve.

Statistical analysis of the results in different groups was performed by Student's t-test and One way analysis of variance (ANOVA) using the SPSS, version 19.0. *P-value* less than 0.05 was considered statistically significant.

Results

In the current study, 71 (81.6%) of the 87 samples had measurable levels of aflatoxin M1. The range of contamination in milk samples was between 3.3 and 96.1 ng/l, with a mean value of 23.30 ng/l (Table 1). According to our results, 8.1% of the milk samples had higher levels of aflatoxin M1 than the maximum tolerance limit approved by Institute of Standards and Industrial Research of Iran (ISIRI; 50 ng/l) [12].

The frequency of aflatoxin M1 contamination in yoghurt samples was between 7.8 and 12.1 ng/l (mean=10.25 ng/l; Table 1). Based on the aflatoxin M1 level limits in yoghurt by ISIRI (25 ng/l), all the samples had lower levels than the maximum level (Table 1). As described in Table 1 , the level of aflatoxin M1 in white cheese is lower than the maximum tolerance limit and ranged from 5.8 to 21.2 ng/l (mean=15.86 ng/l).

The level of aflatoxin M1 contamination in butter samples was between 4.7 and 16.7 ng/l and the mean of aflatoxin M1 contamination was 14.28 ng/l. Similarly, all the butter samples had lower levels than the permissible content of aflatoxin M1 by ISIRI. For evaluation of seasonal effects, data of samples collected in winter were compared with those in summer. The results demonstrated that aflatoxin M1 level in each dairy product was significantly higher in winter than in summer (*$P<0.05$*; Table 2).

Also, the results of samples from rural regions were compared with urban regions. The frequency of aflatoxin M1 contamination in all the samples from urban regions was significantly (*$P<0.05$*) higher than the collected samples from rural regions (Table 3).

Table 1. The levels of aflatoxin M1 in dairy products in Iranshahr city, Iran

Dairy product	Samples tested, n	Positive samples, n (%)	Min-max (ng/l or ng/kg)[a]	Mean±SEM (ng/l or ng/kg)[b]	Exceed regulation (%)
Pasteurized milk	49	40 (81.6)	3.3-96.1	23.30±18.84	8.6
Yoghurt	18	15 (83.3)	7.8-12.1	10.25±18.84	0
White cheese	10	6 (60)	5.8-21.2	15.86±8.72	0
Butter	10	10 (100)	4.7-16.7	14.28±7.32	0

[a] The results represents as ng/l in pasteurized milk and ng/kg in yoghurt ,white cheese ,and butter

[b] The standard approved limits of aflatoxin M1 is 50 ng/l for pasteurized milk, 50 ng/kg for yoghurt, 250 ng/kg for white cheese, and 20 ng/kg for butter

Table 2. The comparison of aflatoxin M1 concentration in dairy products of Iranshahr city between winter and summer

Dairy product	Winter		Summer	
	Samples tested, n	Mean±SEM (ng/l or ng/kg)	Samples tested, n	Mean±SEM (ng/l or ng/kg)
Pasteurized milk	27	32.97±29.17	13	9.78±4.50
Yoghurt	7	15.9±3.7	8	10.27±2.05
White cheese	4	19.2±2.51	2	7.5±2.4
Butter	5	14.2±2.8	5	10.65±6.4

Table 3. The comparison of aflatoxin M1 concentration in dairy products of Iranshahr city between urban and rural regions

Dairy product	Urban regions		Rural regions	
	Samples tested, n	Mean±SEM (ng/l or ng/kg)	Samples tested, n	Mean±SEM (ng/l or ng/kg)
Pasteurized milk	26	25.58±20.0	14	19.07±6.79
Yoghurt	9	12.1±0.5	6	8.4±0.69
White cheese	3	15.3±6.43	3	9.5±2.4
Butter	6	10.65±6.4	4	6.8±0.75

Discussion

The presence of aflatoxin M1 in milk and dairy products is a major health problem. This toxin has dangerous effects on humans, especially children and young children, and could have mutagenic, carcinogenic, and immunosuppressive effects [5].

The results of this study demonstrated high level of aflatoxin M1 in 81.6% of the pasteurized milk samples, but only 8.1% of the samples exceeded the maximum limits of aflatoxin M1 approved by ISIRI (50 ng/l). In previous studies performed in different countries, such as Brazil [13], Syria [14], Turkey [15], and Greece [16], high levels of aflatoxin M1 (mean>50 ng/l) in milk samples were reported. This is in agreement with our results. In addition, similar studies in different regions of Iran mentioned that milk samples have high levels of aflatoxin M1 in comparison with the standard limits. These values ranged from 36% [17] and 33% [18] to 12.5% [19] and 4.4% [20].

Based on our results, 60% of the white cheese samples were contaminated by aflatoxin M1, which is lower than milk samples. In previous studies, different results were observed regarding contamination of white cheese samples with aflatoxin M1. For example, the level of aflatoxin M1 in imported cheese was below the standard limit in Japan [21]. However, another study indicated a high rate of positive samples in Turkey [22].

Considering the hydrophilic structure of aflatoxin M1, it has high affinity to milk casein and it seems that the level of aflatoxin M1 must be higher in white cheese. However, different factors, including type and level of milk contamination, differences in milk quality, and extraction methods, could alter this condition [23].

As compared to other dairy products, the level of aflatoxin M1 in yoghurt was lower. The significant decrease in aflatoxin M1 level of yoghurt samples was probably related to low pH, production of organic acids, or presence of lactic acid bacteria [24]. All the butter samples had measurable levels of aflatoxin M1, which were lower than the legal limit approved by ISIRI. Other studies indicated the low levels of aflatoxin M1 contamination in butter samples [25, 26]. This could be due to breaking of protein membrane around fat globules and separation of protein phase from fat during butter processing or high affinity of aflatoxin M1 to milk casein [26]. Levels of the toxin were also higher in winter than summer. This is in accordance with previous reports, which emphasized on the high milk contamination levels in winter [27-29]. This may be related to improper feed storage and suitable condition for growth of fungi and production of aflatoxin M1.

Similarly, the environmental humidity and temperature conditions could affect the production of aflatoxin M1 in animal feed [30]. Iranshahr has suitable moisture and temperature in winter season, which could be a risk factor for accelerating fungal growth and aflatoxin production. Another reason foe low level of aflatoxin M1 in summer may be associated with out–pasturing of milking cattle.

The present study showed that regional effect is another important factor for aflatoxin M1 contamination. Here, we showed that rural regions have lower levels of contamination, compared to urban ones, which is probably due to the traditional ways of animal feeding, that is, in low volume and proper storage.

However, there is no information on the feedstuff given to lactating cattle in the sampling regions. According to the importance of presence of aflatoxin M1 in milk for consumers and public health, educating suitable agricultural procedures for feed storage and production of silage could be useful for diminishing aflatoxin M1 contamination in milk and dairy products.

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Author's contribution

N.S. designed and supervised the study. H.G. performed sample collection and examinations. The final version of the manuscript was revised by N.S.

Conflicts of interest

The authors declare no conflicts of interest.

Financial disclosure

There was no financial interest related to the materials of the manuscript.

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