Original Article

Occurrence of Aflatoxin M_1 in Raw and Pasteurized Milk Produced in Rafsanjan, Iran

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Abstract

Introduction: Aflatoxins, known as causative factors of hepatic and extra-hepatic carcinogenesis within humans, are extremely teratogenic, mutagenic, toxic, and carcinogenic compounds.

Materials & Methods: This study was undertaken to determine the occurrence of aflatoxin M_1 (AFM_1) in 40 raw milk and 47 pasteurized milk samples collected during spring and winter. In order to analyze the samples, the Enzyme-linked Immunosorbent Assay (ELISA) procedure was used. The statistical methods used in this study were based on normal confidence intervals and analysis of variance (ANOVA).

Results: Aflatoxin M_1 was detected in 97.5% of the raw milk ranging from 6.52 to 68.17 ng/l and 95.7% of the pasteurized milk, ranging from 0.8 to 58.13 ng/l. Toxin levels in 10% of the raw milk and 2.1% of the pasteurized milk samples exceeded the Iranian national standard limit i.e. 50 ng/l. Due to seasonal variations, mean concentration of AFM_1 in the samples collected in winter was significantly ($P < 0.03$) higher than those collected in the summer.

Conclusion: Large amount of AFM_1 in milk samples might be a potential hazard for the public health. Reducing the levels of AFB_1 in animal feedstuffs can be regarded as the initial step to control the transfer of AFM_1 to humans.

Keywords: Aflatoxin M_1; ELISA; Iran; Pasteurized milk; Raw milk

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Introduction

Aflatoxins can be produced by toxigenic strains of Aspergillus flavus, Aspergillus parasiticus and rarely by Aspergillus nomius in various agricultural products under appropriate conditions of temperature and humidity [1, 2]. These are regarded as extremely toxic, mutagenic, carcinogenic and teratogenic compounds that have been implicated as causative agents in human hepatic and extra-hepatic carcinogenesis [3]. Aflatoxin M_1 (AFM_1) can be detected in the milk obtained from livestock ingested feeds that have been improperly dried [1]. Dairy cattle eating contaminated food with AFB1. may be secreted AFM_1 in their milk [4, 5]. Aflatoxin M_1 is less toxic compared to its parent compound, aflatoxin B_1 (AFB_1 categorized as class 2B human carcinogenic by the International Agency for Research on Cancer). Owing to the importance of mycotoxins, specifically aflatoxins, several countries have proposed legal regulations for these toxins in various food products in order to reduce the hazards. These regulations are influenced by the conditions of the country , that may vary from one country to another [6]. The permissible level of AFM1 declared by Institute of Standards and Industrial Research of Iran (ISIRI, 2002) is 50 ng/l, which is Equal to the acceptable level of European Commission.[7]. Thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) are introduced as a common analytical method in regard with AFM_1 measurement in dairy products. ELISA is the most useful technique due to its velocity, sensitivity, ease of application and cheapness [8]. In the present study, the natural occurrence of AFM_1 in raw and pasteurized milk produced in Rafsanjan, Iran during summer and winter was determined for the first time.

Material and Methods

During the winter and spring 2012, a total of 40 samples of raw milk and 47 samples of pasteurized milk were cluster sampled out of supermarkets and retail outlets in Rafsanjan city of Iran. Rafsanjan is a county in Kerman Province located in southeast of Iran with dry and hot climate. The samples were carried to the laboratory inside an insulated container at about 4 ˚C and stored at -20 ˚C in order to analyze AFM_1.

A competitive enzyme immunoassay by RIDASCREEN® Aflatoxin M1 30/15 (R-Biopharm, Darmstadt, Germany) test kit was used in order to design AFM1 in the samples. Most of the applied reagents were provided by the kit manufacturer. Aflatoxin M_1 standard solutions used for creating the calibration curve were at levels of 0, 5, 10, 20, 40 and 80 pg/ml, which all were included in the test kit. ELISA test procedures were performed according to the method described by R- Biopharm, Darmstadt, Germany [9]. In order to analyze

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the study data, SPSS software (version 18) was utilized based on normal confidence intervals and analysis of variance (ANOVA).

The milk samples were centrifuged at 3500g for 10 min at 10 °C by aspirating via a Pasteur pipette. The top creamy layer of milk was removed, and the fat-free supernatant was directly used in the test.

Results

The incidence and levels of AFM_1 contamination in raw and pasteurized milk samples are displayed in Table 1. Aflatoxin M_1 was detected above detectable level in 97.5% (39/40) of raw milk samples, ranging from 6.52–68.17 ng/l; and 95.7% (45/47) of pasteurized milk samples, ranging from 0.8– 58.13 ng/l. The mean concentration of AFM_1 was significantly higher in raw milk (24.86 ng/l; $P < 0.001$) than in pasteurized milk (13.47 ng/l). The t oxin level in 4 (10%) raw milk samples, and 1(2.1%) pasteurized milk sample exceeded the Iranian national standard limit i.e. 50 ng/l. Considering the seasonal variability, the levels of AFM_1 in pasteurized milk and raw milk samples collected in the winter were significantly higher ($P < 0.05$) than those obtained in the spring (Table 2).

Table1. Occurrence of aflatoxin M1 in raw and pasteurized milk

Type	Sample tested (n)	Positive samples, n (%)	Min-max (ng/l)	Mean±SD (ng/l)	Exceeded regulation, n (%)
Raw milk	40	39(97.5)	6.52-68.17	24.86±16.36 [x]	4(10)
pasteurized milk	47	45(95.7)	0.8- 58.13	13.47± 14.54[y]	1(2.1)
Total	87	84(96.5)	0.14- 68.17	18.71 ±16.54	5(5.74)

[a] The ISIRI limit for [a] The ISIRI limit for AFM_1 in milk is 50ng/l.

[x,y] Means ± SD with different letters are significantly different ($P < 0.05$).

Table2. Occurrence of aflatoxin M1 in raw and pasteurized milk. Comparison between samples obtained in spring and winter

Milk type	Winter		Spring	
	Samples tested, n	Mean ± SD(ng/l)	Samples tested, n	Mean ± SD (ng/l)
Raw milk	20	32.59±18.25[a]	20	17± 9.6 [b]
Pasteurized milk	25	22± 16	22	3.7±2.8

[a,b] Means ± SD in the same row with different letters are significantly different ($P < 0.05$).

Discussion

Raw milk is mainly produced by traditional industrial dairy farms in Iran. The cows of industrial farms are normally kept in an intensive or semi-intensive system fed with cultivated fodder, supplements and imported feed. These farms are located in the nearby cities where the marketing, veterinary services and sanitation can be

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accessed easily. The milk produced in industrial farms is transferred to the dairy factories under appropriate hygienic conditions. After examining its quality by laboratory of dairy factory, the milk is delivered and used for production of pasteurized milk, cheese, yoghourt and other dairy products. Traditional dairy farming is a common system in Iran, done mostly by farmers in a system of mixed farming, with animals in support of crop production. Crop residues, weeds, wheat, dry bread and barley stubble are regarded as the alternate sources of animal feed. The produced milk in the traditional system is usually sold to the retail outlets, which were sampled in the present study. In a previous survey conducted in Iran (Tajkarimi et al., 2008), the levels of AFM_1 contamination in milk samples were reported to be equal getting obtained from industrial and traditional dairy farms. In another study, no significant difference was detected in AFM_1 regard with contamination between raw and pasteurized milk [10]. The disagreement between the present study results and the findings of mentioned studies might be due to the differences in the level of AFB_1 contamination in the consumed feedstuffs. In the area of the current study, dry bread is normally applied as a feedstuff in small and traditional dairy farms. During the storage, the crop is disposed to mould growth and further contamination with aflatoxins.

Considering the seasonal variability, the levels of AFM_1 in pasteurized milk and raw milk samples collected in winter were significantly higher ($P < 0.05$) than those obtained in spring (Table 2). In comparison with the aflatoxins rate during spring, when the green pasture composed the major part of their feeds, the residual level of flatoxin in milk during winter was decreased. As a result, poor feed quality might be relevant to the residual level of aflatoxin in milk. The results of the current study are consistent with those of several studies demonstrating higher levels of AFM_1 contamination in cold seasons comparing to hot ones [4, 11-13].

Conclusion

Contamination of milk samples with AFM_1 could be regarded as a potential public health problem. Reducing the levels of AFB_1 in animal feedstuffs can be mentioned as the initial approach in order to control the AFM_1 transfer to humans. To this purpose, it is necessary to set stringent regulations on AFB_1 contamination in the animal feed. At the same time, supervision programs should be continuous and widespread in both feed and milk. Ultimately, further studies seem to be needed to estimate the representative intake of AFM_1 in Iran in regard with the occurrence of AFM_1in various dairy products in Rafsanjan as well as other parts of the country.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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