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The Evaluation of Aflatoxin M_1 Level in Collected Raw Milk for Pasteurized Dairy Factories of Kermanshah

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Abstract

***Background*:** Aflatoxins are fungal toxins that have carcinogenic, cellular mutations and malformation effects. Aflatoxin M_1 resists pasteurization, autoclave and the other methods that make foodstuff healthy. This study aims to determine the contents of aflatoxin M_1 in raw milk of milk factories in Kermanshah province.

***Materials and Methods*:** This research is carried out through the descriptive-cross sectional method. Among the raw milk received by four pasteurized milk factories in Kermanshah, coded by (A, B, C, D) labels, six samples, totally 320 samples (80 samples from each factory), were taken within four seasons. The concentration of aflatoxin M_1 was examined by Enzyme-Linked Immunosorbent Assay (ELISA). The mean difference was analyzed statistically through t-test using SPSS software.

***Results*:** The content of aflatoxin was higher than Codex standard (0.5 µg/l) in 295 samples. The total mean was 1.21, which exceeds two times the Codex standard. The highest and lowest contents of aflatoxin M_1 were observed in "Factory D" in spring and in "Factory A" in autumn, respectively. There was a significant difference between contamination of aflatoxin M_1 and different seasons ($p< 0.05$).

***Conclusion*:** High content of aflatoxin M_1 in raw milk is worrying. Measuring the content of aflatoxin M_1 is essential to reduce the toxin entering the daily food of animals and the other related factors. The considerable difference of aflatoxin M_1 content between Factory D and Factory A can be attributed to the amount of the local milk and the industrial milk received by the factories.

Introduction

Aflatoxins which are fungal products will threaten the health of consumers [1-5] if found in milk and its products. This hazardous substance has carcinogenic, cellular mutations and malformation effects on biota. Hydroxyl metabolites of aflatoxin B_1 and B_2 were named aflatoxin M_1 and M_2, respectively, which are found in milk and dairy products. This study aims to determine the content of aflatoxin M_1 in the raw milk collected from milk factories of Kermanshah province to reconsider feeding livestock and other principles and bases of production.

Aflatoxin causes acute and chronic toxicity in human. Studies have proved the unfavorable effects of this toxin on central nervous system (CNS), liver, kidneys, brain damage and death [5-7]. The long-term effects of little intake of mycotoxins are different. The major effects of the chronic toxicity of mycotoxins, especially aflatoxins, are different kinds of cancers, especially liver cancer [8]. Aflatoxins cause acute toxicity and they have immunocopromissed, mutagenesis, malformation, and carcinogenesis effects [9]. Milk and dairy products are the main foods for human, especially children. This would be an alarm because children show more sensitivity to the effects of aflatoxin and their ability for biological change of carcinogenic compounds is slower than adults. However, these products may be contaminated and they are dangerous for human. Therefore, most countries have regulations to control the content of aflatoxin B_1 in livestock diets and the allowed content of aflatoxin M_1 in milk in order to reduce such risks [10]. Hydroxyl metabolites of aflatoxin B_1 and B_2 were named aflatoxin M_1 and M_2, respectively that are found in milk and dairy products [11].

The Iranian Institute of Standards announced the allowed content of aflatoxin M_1 in raw milk as 0.5 microgram per liter. It would be ideal if aflatoxin content in raw milk is less than 0.3 ppb. Although there are no fully reliable methods to prevent completely from contamination of agricultural products to aflatoxin, detoxification processes, such as reducing toxin,

damaging toxin structure or in other words, activation of aflatoxin toxins [6]. Aflatoxin M_1 resists the heat of pasteurization, autoclave and other foodstuff sanitation methods and such measures have no effects on its reduction [13]. Various methods, such as chemical absorbents [8], high-performance liquid chromatography, and ELISA can be used to measure aflatoxin. In studies carried out by Nakajima, et al. on 2008 samples of pasteurized milk in Japan, the contamination of milk to aflatoxin in 11 states under study were reported to be about 0.029 to 0.001 microgram per kilogram with the average of 0.009 microgram per kilogram. Here, the suitable method of validation of laboratory test has also been used [15]. Measurement of aflatoxin M_1 using ELISA on raw milk of Babol husbandry indicated that the aflatoxin content was 1.5 to 2 times more than the allowed amount determined by Codex European Committee on Neutrition [16]. As aflatoxin is transferred to dairy products, according to the studies conducted in Mazandaran province on aflatoxin M_1 content in yoghurt, the content of this toxin exceeded the permissible content specified by the European standard [17].

In addition, the research conducted by Khaksar et al. on the distribution method of aflatoxin M_1 in producing the Iranian White Cheese specified that the concentration of aflatoxin in curd are 3.12 and 3.65 time more than its concentration in whey and the original milk, respectively [18]. Studies conducted on raw milk in Albania indicate that the aflatoxin M_1 content in winter is more than summer [19]. A research conducted by Tajkarimi et al. in five regions of Iran indicated that with respect to the geographical and seasonal conditions, aflatoxin content in raw milk is different, as the dietary type of milch animal, hunger time, temperature and relative humidity can affect the production of this toxin [11]. To prevent the aflatoxin M_1 entering into the human food chain through milk, first of all, we should prevent the entrance of its precursor, i.e. aflatoxin B_1 into milch livestock food. This is very difficult and nearly impossible to do at the moment.

An earlier measure would be the measurement of aflatoxin M_1 in milk and its products to prevent the distribution and consumption of dairy contaminated to the contents higher than the allowed ones. Due to the high importance of this toxin, its role in human health, determining its content in the consuming foodstuff, using musty bread in livestock dietary during recent years (2010-11), and with respect to this issue on which no study has been conducted in Kermanshah province, the researchers decided to study the aflatoxin M_1 in raw milk in Kermanshah province.

Materials and Methods

In this descriptive/cross-sectional study, 320 samples of raw milk, transported to Kermanshah Pasteurized Dairy Factories, were collected. From the raw milk received by four pasteurized dairy factories in Kermanshah, coded by (A, B, C, D) labels, 6-7 samples per week, totally 320 samples (80 samples from each factory) were selected in four seasons in 2010. Although the volume of the raw sample was 250 ml, 10 ml was taken for test in order to be centrifuged in laboratory at 10ºC for 10 min in 3000 xg RPM. Then the top fat (skim) was completely removed by pastor pipette and the lower liquid was kept in a freezer at -70ºC for AFM1 test. Aflatoxin ELISA kit, made by Tecna Company in Italy, was used to detect aflatoxin M_1. The ELISA is an immunoassay competitive enzyme method based on antibody-antigen reaction. Microtiter wells (coated) with antibody were covered against aflatoxin M_1. Then, sample absorption was read in the wavelength of 450 nm in an ELISA Reader and aflatoxin M_1 concentration was calculated after drawing a curve. The data were entered SPSS-16.5 software environment and were analyzed statistically using the descriptive statistics formula and a paired t-test.

The study was conducted through observing, interviewing, providing suppliers and milk factories with detailed information of the research, and visiting the factories. A sample was received from each milk supplier and the sampling was repeated from the same supplier. In addition, Research and Development Laboratory of Factories A and B were also used to prepare the samples. Due to the ethical considerations, the names of milk suppliers and factories are stated anonymously, using codes. They are only submitted to the Food and Medicine Department of Kermanshah University of Medical Sciences and the factories participated in this research upon their request. The statistical method was measured in terms of validity and reliability.

Result

The results of the research revealed that all the 320 samples had measurable contents of aflatoxin M_1. According to the Codex standard, 25 samples (7.82%) of all the samples had aflatoxin contents lower than the permissible contents of the Codex standard. 295 samples (92.18%) contained aflatoxin M_1 exceeding the permissible contents of the Codex Standard. The mean of aflatoxin M_1 of the collected samples in spring, summer, autumn and winter were 1.42±0.478, 1.08±0.461, 1.373±0.406, and 1.299±0.442 respectively. Out of 320 samples, 295 samples (92.18%) had more than 0.5 µg/l of aflatoxin M_1 and only 25 (7.82%) samples contained 0.5 µg/l of aflatoxin or lower.

The overall mean during a year was 1.21, which exceeded the double permissible contents of the Codex standard. The maximum measurable contents belonged to the samples collected in spring (with the average of 1.426) and the minimum contamination belonged to the samples collected in autumn (with the average of 1.04). The minimum content of contamination was 0.04 microgram per liter, which belonged to summer and the maximum contamination of 2.49 was related to the samples collected in spring (Fig. 1). The samples have been collected from four milk factories (A, B, C and D) in Kermanshah. Eighty samples were taken from each factory. According to the results obtained from the tests,

the maximum contamination relates to Factory D as 1.3 microgram per liter and the minimum amount of aflatoxin M_1 contamination was related to Factory A as 1.1 microgram per liter (Table 1).

Table 1. Average and standard deviation of aflatoxin M_1 contamination of factories (A, B, C, and D) with respect to seasons as per microgram in liter

	Spring	Summer	Autumn	Winter
Factory A	1.28 ±0.45	0.99 ± 0.41	0.97 ± 0.61	1.16 ± 0.46
Factory B	1.38 ± 0.33	1.10 ± 0.33	1.02 ± 0.32	1.27 ± 0.3
Factory C	1.44 ± 0.43	1.11 ± 0.38	1.05 ± 0.38	1.29 ± 0.4
Factory D	1.60 ± 0.46	1.14 ± 0.46	1.04 ± 0.47	1.41 ± 0.4

Table 2. The minimum and maximum contents of aflatoxin M_1 in terms of microgram per liter for each factory and season

Season / Factory		Spring	Summer	Autumn	Winter
A	Min	0.46	0.49	0.41	0.5
	Max	2.1	1.99	1.72	2.16
B	Min	0.51	0.4	0.41	0.51
	Max	2	1.71	1.69	1.72
C	Min	0.82	0.48	0.4	0.77
	Max	2.29	1.93	1.84	2.07
D	Min	0.44	0.49	0.43	0.46
	Max	2.42	2.13	1.72	2

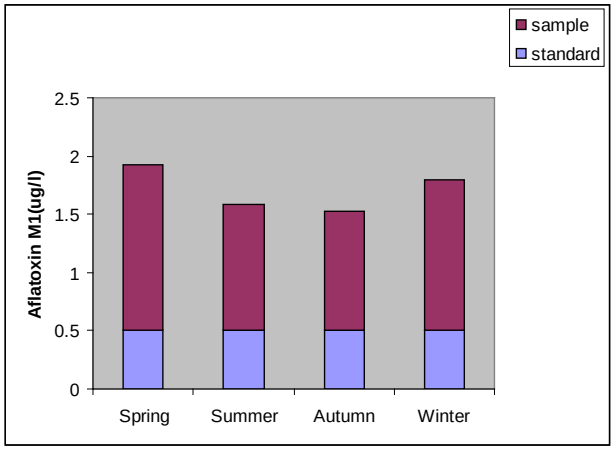

Figure 1. Average of contamination in four seasons of 2010

Discussion

The samples of raw milk were collected in four seasons and 92.18% of the samples collected from Kermanshah Milk Collection Center indicated contamination exceeding 0.5 microgram per liter (Codex standard). If aflatoxin exists in raw milk, improvement processes such as sterilization and pasteurization will be unable to remove it. In fact, these processes play no role in reducing their contents. If the pasteurized products produced in Kermanshah milk factories, including cheese, yoghurt and milk are supplied from regions with lower level of contamination, they will be consistent with the world and Codex standards.

The significant difference of aflatoxin in winter and spring as well as summer and autumn indicates its relationship with livestock feed. It seems that lack of a significant difference in the milk transferred to the factories under study can be related to the existence of aflatoxin under study with the feed of milch livestock, which almost follows the same procedure in this province. In addition, with the other studies carried out, as the one conducted in Babol, contamination of all the samples was reported to be more than the permissible amounts [16]. However, they are inconsistent with the results of the other study conducted in Kurdistan province within the first three months of 2008. A study conducted in Kurdistan indicated that the mean of aflatoxin M_1 contamination was less than one-twentieth of the maximum permissible contents specified in Codex standards [8].

The study carried out by Mokhtarian and Mohsenzadeh in Gonabad suggested that a high percentage of milk samples does not consistent with the available standards in terms of having aflatoxin; on average, the aflatoxin contamination in 58 percent of the samples, exceeds the standard [23]. The study conducted by Shipra et al. in India stated that aflatoxin contaminations in milk products and pasteurized milk were between 65-102 ng/lit and 28-164 ng/lit respectively, while the aflatoxin content in 4 percent of the samples exceeded the permissible contents [24]. In a study carried out in Mexico City, Carvajal et al. concluded that the concentration of aflatoxin in 40 percent of samples exceeded 0.05 µg/l and in 60 percent of the samples less or equal to 0.05 µg/l [25]. Since the intake of aflatoxin is not done only through milk and its products and most of the different foodstuff such as dried fruit, wheat and its products may contain large amounts of aflatoxin and due to the fact that aflatoxin M1 cannot be removed by heating processes and it continues to exist in milk products, the existence of this dangerous and highly toxic substance in raw milk is of paramount importance. That is why most countries consider contamination of dairy products to this toxic substance as a very important problem [10].

The Department of Supervision on Food mostly controls the industrially produced foods, whereas, at present, 85 percent of people's foods are those which are not produced by food industries. Unfortunately, in our country, milk is almost produced without any hygienic control and it is placed within the production line of the dairy products. In the scientific centers and factories producing dairy products in Iran, studies on the dairy contaminants, including pharmaceuticals, pesticides and toxins residues are very limited. Among them, aflatoxins are of paramount importance. Although aflatoxins are created due to unfavorable diet with musty bread and stale meal, its figures are not significant as compared with its status. Contamination of the warehouses in this region is beyond the permissible value. Therefore, on the milk received from Kermanshah province, it is believed that the maximum contamination is caused by aflatoxin.

It is recommended to the relevant organizations to find a suitable solution in order to reduce the contamination of aflatoxin in milk collection centers such as controlling livestock food, forage and warehousing condition, storing forage and livestock feed.

Thus, it seems to be essential to have a continuous and accurate supervision on controlling and storing the forage and livestock food, especially in spring. As livestock is fed by musty forage and bread, there is aflatoxin in the milk transported to dairy factories. In addition, due to the inappropriate storing of the forage crops, the mold of them is frequently seen in Iran. Moreover, other research is underway which study the amount of the different kinds of aflatoxin in livestock food and other dairy products. With respect to high amounts of aflatoxin in raw milk of Kermanshah province factories, it is highly important for the health authorities to have utmost control over this toxic and hazardous substance by managing the livestock food in a correct way and guarantee the public health through the accurate supervision of livestock feed and controlling the contents of this toxin in dairy products. In addition, with respect to the considerable difference in quality and contamination of aflatoxin, it is recommended to factories to use the industrial milk instead of the traditional milk for production.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The author declare no conflict of interest.

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References

1. Karim G, Kamkar A. [Study on the effect of Lactoperoxidase system (LPS) and LPS plus riboflavin on the aflatoxin M1 in milk] Persian. J Veterinary Med 2001; 55(4): 5-7.
2. Afzal M, Cheems RA, Chudhary RA. Incidence of aflatoxin and aflatoxin producing fungi in animal feed stuffs. Mycopathologia 1979; 69(3): 149-51.
3. Shotwell OL, Hesseltine CW, Goulden ML. Incidence of aflatoxin in southern comcereal. Sci Today 1973; 18(4): 192-95.
4. Van Egmond HP. Significance of Mycotoxin in dairy production. Neth Milk dairy J 1989; 15(3): 166-69.
5. Van Egmond HP, Paulsch WE. Mycotoxin in milk and milk products. Neth Milk Dairy J 1986; 40(3): 175-86.
6. Mortazavi A, Ghods-Roohani M, Joyande H. Technology of milk and dairy products. 2nd ed. Mashhad: Ferdowsi University press; 1996: 169.
7. Rastogi S, Dwivedi DP, Khanna KS and Das M. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Cont 2004; 15(2): 287-90.
8. Hazir MS, Tahaee N, Rashidi K and Sheikhi H. [Study of aflatoxin M1 in row milk delivered to pasturized milk company of Sanandaj] Persian. J Med Sci Univ Kordestan 2009; 13(3): 44-50.
9. Trucksess MW, Pohland AE. Mycotoxin Protocols, series methods in molecular biology. 2nd ed. Boston Humana Press; 2000: 15.
10. Nyikal J, Misore A, Nzioka C. Outbreak of aflatoxin poisoning eastern and central provinces, Kenya. MMWR 2004; 53(34): 790-3.
11. Tajkarimi M, Shojaee-Aliabadi F, Salah-Nejad M, et al. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. J Food Microbiol 2008; 116(3): 346-9.
12. Salunkhe DK, Adsule RN, Padule DN. Aflatoxins in foods and feeds. BV Gupa. Managing Diverter Metropolitan 1987; 510.
13. Ismair Y, Ruston S. Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. Food Chem 1996; 59(1): 57-64.
14. Tajkarimi M, Ghaemmaghami SS, Motalebi A, et al. [Seasonal survey in content M1 aflatoxin in raw milk taken from 15dairy factory] Persian. Pajouhesh va Sazandegi 2007; 75(2): 2- 9.
15. Nakajima M, Tabatas-Akiyma H. Occurrence of aflatoxin M1 in domestic milk in Japan during the winter season. Food Addit Contam 2004; 21(5): 472-81.
16. Gholampoor A, zizi E, Khosravi A and Hashemi J. [Comparison of Aflatoxin M1 level in animal row milk on zemestan and tabestan season in babol city milk] Persian. J Food sci 2008; 5(3): 332-342.
17. Barjaste MH, Gholampoor-Azizi E, Nooshfar E. [Measure Aflatoxin M1 in local and pasteurized yougart in mazandaran province by ELISA method] Persian. Global Veterinary 2010; 4(5): 459-96.
18. Khaksar R, Karim G, Kamkar A. [Methods validation of aflatoxin M1 in Iranian white cheese by high performance liquid cheromatogeraphy and immunoaffinity Colums] Persian. J Food Technol Nutr sci 2007; 4(1): 73-9.
19. Panariti E. Seasonal variations of aflatoxin M1 in the farm milk in Albania. Arh Hig Rada Toksikol 2001; 52(1): 37-41.
20. Kamkar A, karim G, Shojaee A and Khaksar R. Fate of aflatoxin M1 in Iranian white cheese processing. Food Chem Toxicol 2008; 46(6): 2236- 8.
21. Ioannou-Kakouri E, Aletrari M, Christou E, et al. Surveillance and control of aflatoxins B1, B2, G1, G2, and M1 in food stuffs in the Republic of Cyprus: 1992-1996. J AOAC Int 1999; 82(4): 883-92.
22. Lopez C, Ramos L, Ramadan S, et al. Distribution of aflatoxin M1 in cheese obtained from milk artificially contaminated. Int J Food Microbiol 2001; 64(1-2): 211-5.
23. Mokhtarian-Dalooee H, Mohsenzadeh M. [Evaluation of aflatoxin M1 in pasteurized milk in Gonabad city] Persian. Ofogh-e- Danesh 2005; 11(3): 3-9.
24. Shipra R, Premendra D, Subhash K. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Cont 2004; 15(4): 287-90.
25. Carvajal M, Bolanos A, Rojo F and Mendez I. aflatoxin M1 in pasteurized and ultra pasteurized milk with different fat content in Mexico. J Food Prot 2003; 66(10): 1885-92.

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