DOI: 10.29050/harranziraat.1247936
Harran Tarım ve Gıda Bilimleri Derg. 2023, 27(3): 435-443

Research Article/Araştırma Makalesi

Evaluation of aflatoxin M1 content in milk and dairy products by high-performance liquid chromatography in Tehran, Iran

Tahran, İran'da yüksek performanslı sıvı kromatografi ile süt ve süt ürünlerinde aflatoksin M1 içeriğinin değerlendirilmesi

Nazanin SHABANSALMANI[1], MohammadHosein MOVASSAGHGHAZANI[2*]

[1]Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran
[2]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

[1]https://orcid.org/0000-0002-1371-5190; [2]https://orcid.org/0000-0003-2201-4529

To cite this article:
Shabansalmani, N. & Movassaghghazani, M. (2023). Evaluation of aflatoxin M1 content in milk and dairy products by high-performance liquid chromatography in Tehran, Iran. Harran Tarım ve Gıda Bilimleri Dergisi, 27(3): 435-443.
DOI: 10.29050/harranziraat.1247936

Address for Correspondence:
MohammadHosein MOVASSAGHGHAZANI
e-mail:
drmhmg@gmail.com

Received Date:
05.02.2023
Accepted Date:
23.06.2023

ABSTRACT

Aflatoxin M1 (AFM1) is the most important aflatoxin in milk and dairy products, which is carcinogenic and hepatotoxic. This study aimed to evaluate the AFM1 content in the milk and distributed dairy products in Tehran. 75 samples, including 15 samples of raw milk, 15 samples of pasteurized milk, 15 samples of ultra-high temperature milk, 15 samples of pasteurized yogurt, and 15 samples of pasteurized cheese, were collected from October to December 2020 in Tehran by simple random sampling. The dietary exposure or estimated dietary intake (EDI) and hazard index (HI) were calculated for milk and dairy product consumers. The AFM1 content in the samples was determined by using high-performance liquid chromatography (HPLC) along with a fluorescence detector. AFM1 was observed in all samples. The values of AFM1 in all samples were higher than the acceptable range determined by the European Union. 100% of milk and yogurt samples and 82% of cheese samples exceeded the Iranian maximum limit (100 ng kg^{-1} in milk and yogurt, and 250 ng Kg^{-1} in cheese). Mean AFM1 content in raw milk samples, pasteurized milk samples, UHT milk samples, pasteurized yogurt samples, and pasteurized cheese samples were 337±17.7, 306±15.5, 305±17.4, 320±17.6, and 309±18.5 ng Kg^{-1}, respectively. The highest value of HI was observed in children of Tehran, Iran. Based on the results, the aflatoxin content in milk and distributed dairy products in Tehran in the autumn is inconvenient. It is recommended that the aflatoxin levels should be measured at different times of the year, especially in raw milk, and feed monitoring is intensified for contamination with toxin-producing molds.

Key Words: Milk, Dairy products, Aflatoxin M1, HPLC, Tehran

ÖZ

Aflatoksin M1 (AFM1), süt ve süt ürünlerinde kanserojen ve hepatotoksik olan en önemli aflatoksindir. Bu çalışma, Tahran'da süt ve dağıtılan süt ürünlerindeki AFM1 içeriğini değerlendirmeyi amaçladı. Ekim-Aralık 2020 arasında Tahran'da basit rastgele yöntemle 15 çiğ süt, 15 pastörize süt, 15 ultra yüksek sıcaklıkta süt, 15 pastörize yoğurt ve 15 pastörize peynir olmak üzere 75 numune toplandı. örnekleme. Süt ve süt ürünleri tüketicileri için diyete maruz kalma veya tahmini diyet alımı (EDI) ve tehlike indeksi (HI) hesaplandı. Numunelerdeki AFM1 içeriği, bir floresan detektörü ile birlikte yüksek performanslı sıvı kromatografisi (HPLC) kullanılarak belirlendi. AFM1 tüm numunelerde tespit edildi. Tüm örneklerde AFM1 değerleri, Avrupa Birliği tarafından kabul edilen kabul edilebilir aralığın üzerindeydi. Süt ve yoğurt örneklerinin %100'ü ve peynir örneklerinin %82'si İran maksimum sınırını (süt ve yoğurtta 100 ng kg^{-1} ve peynirde 250 ng kg^{-1}) aştı. Çiğ süt, pastörize süt, UHT süt, pastörize yoğurt ve pastörize peynir örneklerinde ortalama

AFM1 içeriği sırasıyla 337±17,7, 306±15,5, 305±17,4, 320±17,6 ve 309±18,5 ng Kg^{-1} olarak bulundu. En yüksek HI değeri İran'ın Tahran kentindeki çocuklarda gözlendi. Sonuçlara göre Tahran'da sonbaharda süt ve dağıtılan süt ürünlerindeki aflatoksin içeriği endişe vericidir. Özellikle çiğ sütte aflatoksin düzeylerinin yılın farklı zamanlarında ölçülmesi ve toksin üreten küflerle kontaminasyon için yem denetimlerinin yoğunlaştırılması önerilir.

Anahtar Kelimeler: Süt, Süt ürünleri, Aflatoksin M1, HPLC, Tahran

Introduction

Based on Food and Agriculture Organization, 25% of agricultural production is contaminated with aflatoxins, which annually causes a significant reduction in the volume of food and feedstuffs (Movassagh & Adinehvand, 2010). Milk and dairy products are important food stuffs because they supply many minerals to our bodies such as calcium and protein, so their contamination is a serious risk to society. Aflatoxins are one of the factors which cause the contamination of milk, followed by dairy products (Pardakhti & Maleki, 2019). Aflatoxins are of mycotoxins produced by Aspergillus species, especially Aspergillus flavus and Aspergillus parasiticus in the case of suitable conditions such as humidity, temperature and improper storage conditions which is a function of the climatic conditions of the region. Among the aflatoxins (B1, B2, G1, G2), B1 is the most dangerous, which is one of the first category of carcinogens based on the epidemiological studies and grouping of the International Agency for Research on Cancer (IARC), and these toxins can be found in food used in livestock (Fallah et al., 2016). Aflatoxins M1 is in group 1(IARC), the carcinogens are produced by the enzyme cytochrome P450 in the liver of lactating animals such as cattle (Kamkar et al., 2014).

Although the food crops cultivated and stored in the warmer regions of the world have the largest concentrations, the worldwide trade of these vital commodities assures that aflatoxins are an issue for both the producing and importing nations. When cows or other ruminants consume feed contaminated with these mycotoxins, aflatoxins M1 and M2 the hydroxylated metabolites of aflatoxins B1 and B2 are created. (Movassagh & Adinehvand, 2010).

World Health Organization (WHO) has declared the maximum permitted level of AFM1 in milk and dairy products to be 50 to 500 ng kg^{-1}; the European Codex Alimentarius Commission (CAC) has set the permitted level of this toxin in milk and processed dairy products at 50 ng kg^{-1}, this value is 500 ng kg^{-1} in the US, and also Iranian National Standards Institute has set the maximum permitted level for this toxin in raw milk at 100 ng kg^{-1} (Movassagh & Adinehvand, 2010). Considering that mycotoxin detoxification mechanisms known for human diets render the food inedible and that pasteurization processes (including those utilizing UHT techniques), do not impact AFM1 concentration due to its heat stability. As a result, monitoring programs are now the primary tactic for reducing exposure risk for both humans and animals (Lopez et al., 2003).

There are several analytical techniques for the determination of AFM1 are available in the literature. The Enzyme Linked Immunosorbent Assay (ELISA), High Performance Liquid Chromatography with Fluorescence Detector (HPLC-FLD), and Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) are the most frequently utilized techniques for this purpose, according to a number of recent research. HPLC has been used in recent years because of its ease of operation and better quantitation (Kos et al., 2016).

This study aimed to evaluate AFM1 level in milk and distributed dairy products in Tehran, Iran by HPLC method.

Materials and methods

Samples

75 samples including 15 samples of raw milk, 15 samples of pasteurized milk, 15 samples of ultra-high temperature (UHT) milk (pasteurized and UHT samples from three Iranian brands and

low-fat type), 15 samples of pasteurized yogurt, and 15 samples of pasteurized cheese from three Iranian brands from October to December 2020, milk and dairy products supply centers in Tehran were collected by simple random sampling.

Method of extracting AFM1 from milk and yogurt

5 mL of sample was mixed with 41 mL of distilled water and then centrifuged at 5500 rpm for 4 minutes at 4 °C. The fat was isolated, and the blue part was taken. It was passed through a 0.45 µm cellulose acetate filter (Millipore®-Merck-Germany). 20 mL of filtered liquid was placed on the immunoaffinity column with AFM1 (Waters-Vicam-USA) specific antibodies, and after the absorption was completed, it was the first rinse with distilled water and finally removed and concentrated with acetonitrile solvent. Then, 20 µL of sample was used for injection into HPLC (Unicam®-Crystal-200-England) (FDA, 2015).

Method of extracting AFM1 from Cheese

5 g of each cheese sample was carefully weighed and added to a balloon containing 40 mL of dichloromethane and stirred for 15 minutes. The resulting suspension was evaporated using filtered syringes, filter, and 10 mL of extract at 60 °C. Residue in a mixture contains half an mL of methanol, half a mL of phosphate buffer (0.55 g of disodium phosphate, 2.85 g of Na_2HP_4 $2H_2O$, and 9 g of sodium chloride which was made up to 100 mL with distilled water), (pH = 7.2) and one mL of heptane was dissolved. The obtained compounds were centrifuged for 15 minutes at a maximum temperature of 10 °C at 2700 rpm, and then the supernatant (heptane layer) was completely evacuated. Finally, 100 µL of sub-phase (methanol layer) was diluted with 400 µL of phosphate buffer. In the next step, 20 mL of PBS solution is completely passed via the AFM1 immunoaffinity column. Then, 20 mL of fat-free milk is passed via the column and the resulting solution is collected at a rate of 1 to 2 drops per second until the air leaves the column. Then, the top of the column was filled with water, and 10 mL of the solution was isolated and collected at a rate of one to two drops per second in a clean glass syringe. This operation was performed for the second time until the air came out of the syringe. Then, again (from this column) 1 mL of acetonitrile was passed at a rate of one drop every 2 to 3 seconds, and 1.5 mL of this solution was collected. This vial was dried under nitrogen vapor at 40 °C, and the dried material was reconstituted in 1 mL of AFM1mobile phase and from this solution, 20 µL of the last solution was injected into HPLC (Unicam®-Crystal-200-England) (Kamkar et al., 2008; Reuter & Hopkinton, 2016).

AFM1 measurement

HPLC method (Unicam®-Crystal-200-England) was used in this study. The AFM1 standard was prepared by Sigma-Aldrich (Germany). The column with a length of 25 cm and an inner diameter of 4.6 mm, with a particle diameter of 3 microns, was used at a temperature of 30 °C. The mobile phase is acetonitrile-methanol-water with a ratio of 17:23:60 and a rinse rate of 1.1 mL/min, and a pressure of 2900 psi. A fluorescence detector was used at the excitation wavelength of 362 nm and output wavelength of 435 nm. After injecting each sample, the toxin content was measured by measuring the area below the peak of its diagram at the time of inhibition and comparing it with the standard curve (ISIRI, 2013).

Risk assessment

The dietary exposure or estimated dietary intake (EDI) was calculated for milk and dairy products consumers according to the following equation (Serranio et al., 2019 ; Sootodeh et al., 2021; Ilievska, 2022) :

$$\text{Dietary exposure} = \frac{\text{Contamination level AFM1 mean} \times \text{daily milk (or dairy product) intake}}{\text{Average body weight}}$$

AFM1 mean: ng kg^{-1}

daily milk (or dairy product) intake: kg day^{-1}

Average body weight: kg

Hazard Index (HI)

According to the below-mentioned formula, the Hazard Index was obtained by dividing the EDI by TD50(threshold dose per body weight) of AFM1 (10.4 µg kg bw^{-1} day^{-1}), divided by an uncertainty factor of 50000. TD50 is a dose that induces tumors in half of the tested animals (Serranio et al., 2019 ; Sootodeh et al., 2021 ; Ilievska, 2022)

$$HI = \frac{\frac{EDI}{TD50}}{50000}$$

Statistical analyses

Analysis of variance (*ANOVA*) was used to evaluate the differences between AFM1 occurrence levels of the milk and dairy product samples by SPSS software (version 24). Duncan multiple comparison test was applied. A p-value less than 0.05 is considered statistically significant.

Results and Discussion

100% of collected samples contained AFM1. The content values of AFM1 for five types of products (raw milk, pasteurized milk, UHT milk, yogurt, and cheese) have been shown in Table 1. There were no significant differences between means of AFM1 in different sample types ($p>0.05$). Determining AFM1 content in the chromatograms of samples was shown in Figures 1, 2, and 3. The EDI and HI calculated are shown in Table 2.

Table 1. Occurrence and level of AFM1 in milk and dairy products collected from Tehran

Sample	No. of sample	Mean±SD* (ng kg^{-1})	Number of contaminated samples (Exceeded the Iran limit- Milk & Yogurt <100 ng kg^{-1} Cheese <250 ng kg^{-1}) (%)	Range of AFM1 (ng kg^{-1})
Raw Milk	15	337±17.7	15 (100%)	230-461
Pasteurized Milk	15	306±15.5	15 (100%)	205-404
UHT Milk	15	305±17.4	15 (100%)	199-417
Yogurt	15	320±17.6	15 (100%)	224-446
Cheese	15	309±18.5	12 (80%)	211-430

*SD= Standard deviation

Table 2. EDI and HI from AFM1 from consumption of raw milk, pasteurized milk, UHT milk, yogurt, and cheese in Tehran, Iran

Dairy product	Raw Milk	Pasteurized Milk	UHT Milk	Yogurt	Cheese
AFM1 Mean concentration±SD* (ng kg^{-1})	337±17.7	306±15.5	305±17.4	320±17.6	309±18.5
Mean daily consumption (kg)	0.192	0.192	0.192	0.066	0.022
EDI mean (ng AFM1 kg^{-1} BW day^{-1})	0.92	0.83	0.83	0.30	0.097
HI values for adult (BW 70 kg)	4.44	4.03	4.02	1.45	0.46
HI values for children (BW 15 kg)	20.73	18.83	18.76	6.67	2.17

* SD= Standard deviation
HI–hazard index (<1 low risk, 1-10 medium risk, >10 high risk)
Adults (18-70 years)
Children (3-10 years)
BW: Body Weight

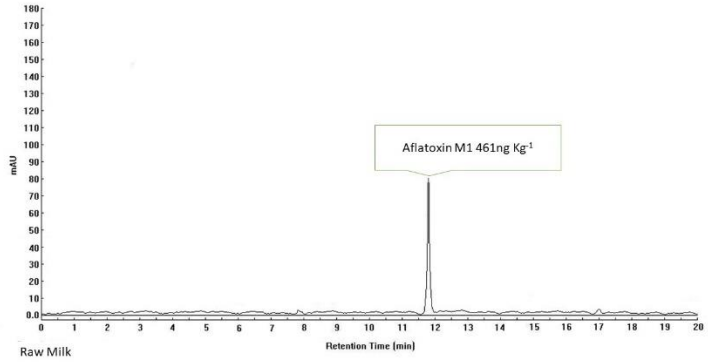

Figure 1. Chromatogram of the raw milk sample containing AFM1

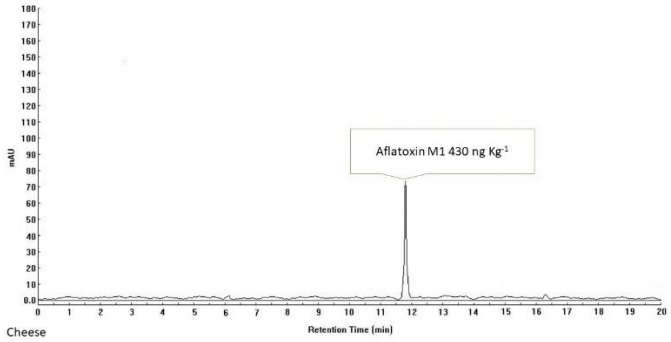

Figure 2. Chromatogram of the cheese sample containing AFM1

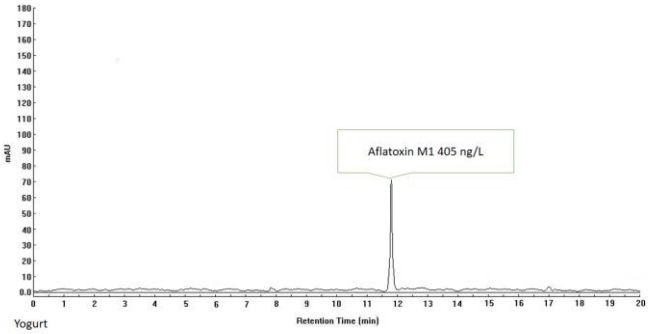

Figure 3. Chromatogram of the yogurt sample containing AFM1

In this study, the risk characterization from exposure to AFM1 was performed by calculating the HI value. Table 2 presents HI values for the mean exposure to AFM1 for consumers, the highest value is for milk in children in Tehran.

In the current study, the EDI of AFM1 in milk was 0.92 ng kg BW^{-1} day^{-1}. This value was higher than those reported by Mozafari Nejad et al.(2019), in the west of Iran, Leblanc et al.(2005) in France (0.01 ng kg BW^{-1} day^{-1}), Shundo et al.(2009) in Brazil (0.08 ng kg BW^{-1} day^{-1}) and Duarte et al.(2013) in Portugal (0.08 ng kg BW^{-1} day^{-1}).

Mozafari Nejad et al.(2019),In west of Iran, showed that the EDI of AFM1 in milk for an adult with a BW of 70 kg, was 0.107 ng kg BW^{-1} day^{-1}. Whereas this indicated a high incidence of AFM1 in milk samples, HI was 0.53, and it showed milk intake in the west of Iran did not have any potential risk for liver cancer in public (Mozafari Nejad et al., 2019). In north Macedonia, HI values for milk, yogurt, ice cream, and cheese were less than 1 which is controversial of our study's result (Ilievska, 2022). The carcinogenic risk assessment of AFM1 in milk in Kerman was estimated, indicating that adult consumers are not at considerable cancer risk (HI<1) and that for children was a medium risk (Sootodeh, 2021).

Based on the results, AFM1 content in three types of raw, pasteurized and, UHT milk, as well

as pasteurized yogurt and cheese in Tehran, was high and, this can be dangerous for the consumers of these products because this toxin is carcinogenic. There were few studies conducted in Tehran during the last ten years; however, their results show that the highest content of AFM1 in pasteurized milk (Riazipour et al., 2010), and the lowest level was 7.8% in raw milk samples (Khaneghahi Abyaneh et al., 2019). Compared to other studies, the AFM1 content in all samples of milk and dairy products in Tehran (current study) is higher than the Iranian standard limit (Table 3). The most important reason for the high level of AFM1 in milk and dairy products is the decrease in the supply of animal feed at the time of sampling and the increase in the consumption of moldy animal feed.

Table 3. The incidence AFM1 in milk and dairy products in Iran and other countries

Province	Sample	Number of samples	Mean±SD* (ng kg^{-1})	Percent of contaminated Samples (Exceeded the European Union's limit -<50 ng kg^{-1})	Reference
Tabriz, Iran	Raw Milk	90	148±19.2	47.77	(Movassagh & Adinehvand, 2010)
Mashhad, Iran	Raw Milk	60	61±8	40	(Hajmohammadi et al., 2017)
Shiraz, Iran	Raw Milk	22	36.1	36.36	(Abdali et al., 2020)
Yemen	Raw Milk	38	183	36.84	(Murshed, 2020)
Bangladesh	Raw Milk	50	699	97	(Tarannum et al., 2020)
China	Raw Milk	136	37.4±18.7	5.9	(Guo et al., 2019)
Khuzestan	Raw Milk	90	32.18±4.07	16.54	(Ghasemian, 2019)
Turkey	Raw Milk	35	25.4±3.38	14.28	(Turkoglu & Keyvan, 2019)
Tehran, Iran	Raw Milk	257	31±8.7	7.8	(Khaneghahi Abyaneh et al., 2019)
Brazil	Raw Milk	40	16.66	0	(Venancio *et al.*, 2019)
Kashan, Iran	Raw Milk	48	27±3.95	20.83	(Movassaghghazani & Ghorbiani, 2017)
East Azerbaijan, Iran	Raw Milk	320	52.4±19	16.25	(Koutamehr et al., 2017)
India	Raw Milk	50	30.2±1.8	22	(Nile et al., 2016)
Tehran, Iran	Raw Milk	15	337±17.7	100	Current study
Bangladesh	Pasteurized Milk	25	99.77	46	(Tarannum et al., 2020)
Turkey	Pasteurized Milk	35	12.8±1.05	0	(Turkoglu & Keyvan, 2019)
Hamedan, Iran	Pasteurized Milk	63	40	33.3	(Mozafari Nejad et al., 2019)
Tabriz, Iran	Pasteurized Milk	50	50.5±23.8	62	(Movassagh & Adinehvand, 2010)
Tehran, Iran	Pasteurized Milk	15	306±15.5	100	Current study
China	UHT Milk	26	22.4±10.9	0	(Guo et al., 2019)
Turkey	UHT Milk	13	52.59	53.84	(Yesil et al., 2019)
Turkey	UHT Milk	35	20.2±2.77	8.57	(Turkoglu & Keyvan, 2019)
Hamedan, Iran	UHT Milk	25	37	28	(Mozafari Nejad et al., 2019)
Bangladesh	UHT Milk	25	35.46	0	(Tarannum et al., 2020)
Tehran, Iran	UHT Milk	15	305±17.4	100	Current study
Yemen	Cheese	90	1198±114	42.2	(Murshed, 2020)
China	Cheese	17	43.1±12.3	23.52	(Guo et al., 2019)
Malaysia	Cheese	2	4.6±2.7	0	(Nadira et al., 2017)
Tehran, Iran	Cheese	15	309±18.5	80	Current study
Yemen	Yogurt	62	399	83.8	(Murshed, 2020)
China	Yogurt	27	17.2±9.5	0	(Guo et al., 2019)
Malaysia	Yogurt	5	25.7±7.2	0	(Nadira et al., 2017)
Tehran, Iran	Yogurt	15	220±17.6	100	Current study

*SD= Standard deviation

Regarding Table 3, it is found that the content value of AFM1 in Tehran is higher compared to countries such as India, China, Turkey, Malaysia, and Brazil. Hence, these differences may also be due to differences in the method of testing and identifying aflatoxins in studies. High-performance liquid chromatography is a more

accurate method than other aflatoxin identification methods such as ELISA and TLC, and it is currently the gold standard for aflatoxin identification. For example, in the study conducted by Hajmohammadi et al. (2017) in Mashhad and also in the study by Ghasemian (2019), unlike the current study, the ELISA method was used to evaluate the AFM1 content. The study by Movasaghghazani and Ghorbiani (2017) in Kashan, although conducted in the same season as the current study (autumn), has achieved different results due to using the ELISA method. Furthermore, the study season is one of the factors affecting the differences in aflatoxin levels in different studies. The study by Tarannum et al. (2020) was conducted in Bangladesh in the spring, and the content value of AFM1 in the samples of this study is less than our study. The climate of the region, storage of forage in unfavorable humidity, and using dry bread to feed livestock due to the lack of awareness of farmers are also the underlying factors for the growth of fungi in animal feed (Barami et al., 2012). In Iran, due to the industrialization of animal husbandry in recent years, using concentrated animal feed has become common, which if contaminated with aflatoxin-producing molds, subsequently increases the contamination of milk with this toxin. According to the obtained statistics, Iran does not have a good position among dairy-producing countries and is not ranked high, which itself can be a factor for insufficient attention to the production and storage of animal feed also dairy products. While, more developed provinces, such as Tehran, East Azerbaijan, Alborz and Chaharmahal and Bakhtiari, have the highest per capita milk consumption (Movassagh & Adinehvand, 2010).

Conclusion

It seems that the reason for the high level of AFM1 in the collected samples was due to the contamination of animal feed, and monitoring organizations should continuously control the aflatoxin B1 content in animal feed. However, the method of identifying this toxin in this study (HPLC) is more accurate than other methods. Due to the carcinogenicity of AFM1, the reduction of toxins in milk and dairy products should be considered. Furthermore, the carcinogenic risk assessment of AFM1 in cheese in Tehran was low for adult consumers, but for other samples was at considerable cancer risk. It is possible to reduce the AFM1 content in milk and dairy products by increasing the level of awareness of farmers and teaching methods to reduce the content value of aflatoxin in animal feed and also using toxin binders in animal feed.

Acknowledgments

The present paper is an excerpt from the thesis of Doctor of Veterinary Medicine. The authors of article thank Dr Ali Dini and Dr. Alireza Ahmadzadeh for their help in risk assessment and conducting statistical analyzes, respectively.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mohammadhosein Movassaghghazani and Nazanin Shabansalmani. The first draft of the manuscript was written by Mohammahosein Movassaghghazani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

Abdali, F., Zare, M., Abbasi, A., & Berizi, E. (2020). Aflatoxin M1 Occurrence in Local Dairy Products in Shiraz, Southern Iran. International Journal of Nutrition Sciences, 5(3), 146-151.

Barami, A. R., Pour elmi, M. R., Irani, M.(2012). Contamination levels of aflatoxin M1 in bulk raw milk of Chaloos and Ramsar. Journal of Food Hygiene. 1(4) : 53-60.

Duarte S.C., Almeida A.M., Teixeira A.S., Periera, L.A., Falcao, L.C., Pena, A. & Lino, C.M. (2013). Aflatoxin M1 in marketed milk in Portugal: Assessment of

human and animal exposure. Food Control. 30(2):411–7.

Fallah, A. A., Fazlollahi, R., & Emami, A. (2016). Seasonal study of aflatoxin M1 contamination in milk of four dairy species in Yazd, Iran. Food Control, 68, 77-82.

FDA. (2015). CPG Sec 527.400 Whole Milk, Lowfat Milk, Skim Milk - Aflatoxin M1. Retrieved from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-527400-whole-milk-lowfat-milk-skim-milk-aflatoxin-m1

Ghasemian, S. O. (2019). Aflatoxin M1 Contamination in Raw Cow Milk of Dairy Farms in Behbahan area, Khuzestan province, Iran. The Journal of Tolooebehdasht. 18(2):46-56.

Guo, L., Wang, Y., Fei, P., Liu, J., & Ren, D. (2019). A survey on the aflatoxin M1 occurrence in raw milk and dairy products from water buffalo in South China. Food Control, 105, 159-163.

Hajmohammadi, M., Valizadeh, R., Naserian, A., & Nourozi, M. (2017). Composition and occurrence of a fl atoxin M 1 in cow ' s milk samples from Razavi Khorasan Province , Iran. International Journal of Dairy Technology, 70, 1-6.

Ilievska, G., Stojanovska-Dimzoska, B., Koceva, D., Stojkovic, G., Angeleska, A., Dimitriska-Stojkovic, E.(2022). Exposure and Health Risk Assessment of Aflatoxin M1 in Dairy Products Consumed by Population of North Macedonia. Journal of Food Quality and Hazards Control. 9 (1) ,14-22.

ISIRI. (2013). Milk and milk prouducts . Determination of aflatoxin M1 by HPLC method and immunoaffinity column clean up-Test method. Iran National Standards Organization(1), 1-5.

Kamkar, A., Fallah, A., & Mozaffari nejad, A. (2014). The review of aflatoxin M1 contamination in milk and dairy products produced The review of aflatoxin M 1 contamination in milk and dairy products produced in Iran. Toxin Reviews, 33(4), 160-168.

Kamkar, A., Karim, G., Aliabadi, F., & Khaksar, R. (2008). Fate of aflatoxin M 1 in Iranian white cheese processing. Food and Chemical Toxicology, 46(6), 2236-2238.

Khaneghahi Abyaneh H, A, B., Noori, N., Yazdanpanah, H., & Shojaee AliAbadi, M. H. (2019). Exposure to Aflatoxin M1 through Milk Consumption in Tehran Population, Iran. Iranian Journal of Pharmaceutical Research, 18(3), 1332-1340.

Kos, J., Hajnal, E.J., Jajic, I., Krstovic, S., Mastilovic, J., Saric, B. & Jovanov, P. (2016). Comparison of ELISA, HPLC-FLD and HPLC-MS/MS methods for determination of aflatoxin M1. in natural contaminated milk samples. Acta Chimica Slovenica, 63:747-756.

Koutamehr, M. E., Akbari, H., Akbari, S., & Hassanzadazar, H. (2017). Aflatoxin M1 Level in Raw Milk Samples of Maragheh , Bonab and Malekan Cities , East Azerbaijan Province , Iran. Studia Universitatis Vasile Goldis, 27(2), 85-89.

Leblanc J.C., Tard A., Volatier J.L., & Verger, P.(2005). Estimated dietary exposure to principal food mycotoxins from the first French Total Diet Study. Food Additives and Contamtaminants, 22(7):652–72.

Lopez, C.E., Ramos, L.L., Ramadan, S.S. & Bulacio, L.C. (2003). Presence of aflatoxin M1 in milk for human consumption in Argentina. Food Control, 14(1): 31-34.

Movassagh, M. H., & Adinehvand, S. (2010). Study of aflatoxin M1 level in the collected raw cow milk from milk collection centers in Tabriz. Journal of Food Hygiene, 3(2), 63-70.

Movassaghghazani, M. H., & Ghorbiani, M. (2017). Incidence of Aflatoxin M 1 in Human and Cow Milk in Kashan , Iran. Journal of Food Quality and Hazards Control, 4, 99-102.

Mozafari Nejad, A. S., Heshmati, A., & Ghiasvand, T. (2019). The Occurrence and Risk Assessment of Exposure to Aflatoxin M1 in Ultra-High Temperature and Pasteurized Milk in Hamadan Province of Iran. Osong Public Health Res Perspect, 10(4), 228-233.

Murshed, S. (2020). Evaluation and Assessment of Aflatoxin M1 in Milk and Milk Products in Yemen Using High-Performance Liquid Chromatography. Journal of Food Quality, 1-8.

Nadira, A. F., Rosita, J., Norhaizan, M. E., & Redzwan, S. M. (2017). Screening of aflatoxin M1 occurrence in selected milk and dairy products in Terengganu , Malaysia. Food Control, 73, 209-214.

Nile, S. H., Park, S. W., & Khobragade, C. N. (2016). Occurrence and analysis of aflatoxin M1 in milk produced by Indian dairy species. Food and Agricultural Immunology, 27(3), 358-366.

Pardakhti, A., & Maleki, S. (2019). Risk Assessment of Aflatoxin M1 Contamination of Milk in Iran. International Journal of Environmental Research, 13, 265-271.

Reuter, W., & Hopkinton, M. (2016). Liquid Chromatography / Analysis of Aflatoxins in Milk by HPLC Using Kobra Cell and Fluorescence Detection. Retrieved from https://www.chromatographyonline.com/authors/wilhad-m-reuter

Riazipour, M., Tavakoli, H., Razzaghi Abyane, M., & Rafati, H. (2010). Measuring the amount of Aflatoxin M1 in pasteurized milks. Kowsar Medical Journal, 15(2), 89-93.

Serranio, A., Bonilauri, P., Kerekes, K., Farkas, Z., Giacometti, F., Canever, A., Zambrini, A., Ambrus, A.(2019). Occurrence of aflatoxin M1 in raw milk marketed in Italy: Exposure assessment and risk characterization, Frontiers in Microbiology, 10, 1-10.

Sootodeh, L., Dini, A., Rezaeian, M., Esmaeili, A., Asgarian, A. (2021). Evalution of Aflatoxin M1 contamination in pasteurized milk in Kerman and Rafsanjan cities in 2019. Journal of Rafsanjan University of Medical Science, 19(11), 1163-1178.

Shundo L., Navas S.A., Lamardo L.C.A., Ruvieri, V. & Sabino, M. (2009). Estimate of aflatoxin M1 exposure in milk and occurrence in Brazil. Food Control. 20(7):655–7.

Tarannum, N., Nipa, M. N., Das, S., & Parveen, S. (2020). Aflatoxin M1 detection by ELISA in raw and processed milk in Bangladesh. Toxicol Rep, 7, 1339-1343.

Turkoglu, C., & Keyvan, E. (2019). Determination of Aflatoxin M1 and Ochratoxin A in Raw, Pasteurized and UHT Milk in Turkey. Acta Scientiae Veterinariae, 47(1), 1-7.

Venancio, R.L., Ludovico, A., Walter de Santana, E.H., Amaral de Toledo, E., Cristine de Almeida Rego, F. &

Sifuentes dos Santos, J. (2018). Occurrence and seasonality of aflatoxin M1 in milk in two different climate zones. Journal of the Science of Food and Agriculture, 99: 3203–3206.

Yesil, O. F., HatipogLu, A., Yildiz, A., Vural, A., & Erkan, M. E. (2019). A Research on the Determination of Aflatoxin M1 Levels in Milk and Dairy Productsfor Sale in Diyarbakır by ELISA. Erzincan University Journal of Food Science and Technology, 12(1), 479-488.