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AFLATOXIN M1 LEVEL IN RAW MILK SAMPLES OF MARAGHEH, BONAB AND MALEKAN CITIES, EAST AZERBAIJAN PROVINCE, IRAN

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ABSTRACT: Mycotoxins, especially aflatoxins are recognized as poisonous and potential carcinogenic compounds in food borne diseases. The aim of this study was to determine of aflatoxin M1 level in raw milk samples collected from traditional and semi-industrial dairy farms in the southern cities of East Azerbaijan province of IRAN. Present cross-sectional study was conducted on raw milk samples of three cities (Maragheh, Bonab and Malekan) in the southern of East Azerbaijan province, Iran. Aflatoxin M1 was determined in a total of 320 raw milk samples using a commercial competitive ELISA kit, according to the test kit instructions. Obtained results showed 83.75% and 16.25% of samples had aflatoxin M1 more and less than of 50 ng/L (standard limit of Iran and Europe Union), respectively. Continuous assessment of the aflatoxin M1 in produced milk of the understudy areas and confirmation with more accurate methods is necessary.

Keywords: Aflatoxin M1, ELISA, East Azerbaijan, Raw milk, Iran

INTRODUCTION:

Mycotoxins, particularly aflatoxins are recognized as poisonous and potential carcinogenic chemicals in food borne diseases (Celik et al., 2005). Three species of Aspergillus molds are involved in the production of aflatoxins included: A. flavous, A. parasiticus and A.numius (Chao-Zong et al., 2004; Chiavaro et al., 2001; Tajkarimi et al., 2008; Karim et al., 1998). Aflatoxin G1, G2, B1 and B2 are four types of the most important aflatoxins. Consumption of aflatoxin B1 (AFB1) contaminated food results hydroxylation of these toxins in liver and consequently producing aflatoxin M1 (AFM1) as a metabolite in liver, its entrance to blood and traceability in milk and urine of dairy animals. AFB1and AFM1 has been identified as the first and second group of carcinogenic compounds in human and animals, respectively (WHO, 1993). In addition to irreparable damages to the livestock industry, toxin entrance through milk to food industry can cause human's acute and chronic poisoning (EC, 2010). Several studies are demonstrated adverse effects of these toxins on the central nervous system, kidney, liver, brain and even death (Becker-Algeri et al, 2016).

The main effect of chronic intoxication with aflatoxins is liver cancer (Hazhir et al., 2009). There are a lot of reports of poisoning, illness and death in animals and human due to consumption of aflatoxins contaminated food (Deshpande, 2002; Jay, 2000). Because of the importance of mycotoxins and complications of the aflatoxins, limit for this toxin was determined in many countries in terms of conditions of country, including the development and economic problems (12 Nemati et al., 2010; Kamkar, 2008; Galvano et al., 1996). The allowable concentration of AFM1 has been determined 0.05-0.5µg/kg and 20µg/kg in milk and animal feed respectively, by credible organizations such as Food and Drug Administration of America (FDA) and Codex Alimentarius (CA) (Tajkarimi et al., 2008; Lopez et al., 2001; Mortazavi et al., 1996). Iran Standard National organization (ISIRI) has announced maximum allowable level (MAL) of AFM1 in 0.5µg/kg raw milk (ISIRI No. 7133, 2011). AFM1 content varies in raw milk due to geographical and seasonal conditions such as feeding of lactating animals, ambient temperature and relative humidity that can influence toxin production (Tajkarimi et al., 2008). Qualitative and quantitative methods such as ELISA (Enzyme-linked immunosorbent assay) as a quantitative screening method and chromatography methods such as HPLC and TLC are used for the detection and measurement of aflatoxins in milk and its products. Extensive studies were conducted in different countries including Iran using different analytical methods for determine AFM1 level in milk. Relatively, all studies have been reported milk contamination in various concentrations higher than standards limit (Table 1).

Since milk and dairy products are the main diet of human and due to the complications caused by the remnants of AFM1, continuous monitoring and control of AFM1 level in raw milk and preventing of distribution and consumption of contaminated milk in the community are necessary. Hence, the purpose of this study was to determine the amount of AFM1 in raw milk samples collected from traditional and semi-industrial dairy farms in the southern cities of East Azerbaijan province of IRAN.

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Tab. 1
Widespread AFM1 occurrence and levels of milk in Iran and some countries

Milk sample	Country	Analytical method	Incidence rate (%)	Mean (ng/L)	% of Exceeding limit	Reference
Raw milk	Iran	ELISA	91.5	75.5	83.75	Present study
Raw milk	Iran-Ahwaz	ELISA	87.7	57.6±4.1	36	(Rahimi *et al.*, 2010)
UHT milk	Iran-Ardabil	ELISA	100	52.9±4.4	33	(Nemati *et al.*, 2010)
Pasteurized milk	Iran - Tabriz	ELISA	100	50.55±23.82	62	(Movassagh Ghazani, 2009)
UHT milk and pasteurized milk	Brazil	ELISA	27.5	23.24±24	5	(Duarte *et al.*, 2013)
Raw milk	Italy	HPLC-FD	2.2	72±48	0.5	(Bellio *et al.*, 2016)
Pasteurized milk	Serbia	UHPLC/HESI-MS/MS	80	0.3	76	(Skrbic *et al.*, 2014)
Pasteurized milk	Iran	TLC	54	0.06±0.14	10	(Tajkarimi *et al.*, 2008)
Pasteurized milk	Iran-Babol	ELISA	100	>200	100	(Sefidgar *et al.*, 2011)

MATERIALS AND METHODS:

Present cross-sectional study was conducted during January to June of 2016 with raw milk sampling in three cities (Maragheh, Bonab and Malekan) in the southern of East Azerbaijan province, Iran. A total of 320 samples were surveyed. Twice sampling was performed of traditional and semi-industrial dairy farms of these 3 cities in winter and spring seasons, randomly. In each season, 55, 50 and 55 raw milk samples were taken of Maragheh, Bonab and Malekan dairy farms, respectively. Milk samples were transferred to the laboratory immediately and were kept at -20 ºC until further tests.

AFM1 was determined in raw milk samples using a commercial competitive ELISA (RIDASCREEN, R-Biopharm, Germany), according to the test kit instructions. To prepare the samples, 10 ml of each milk samples were centrifuged 10 min at 3500 rpm and 10 ºC. The supernatant fat layer was removed completely using Pasteur pipette. Defatted residual milk was used for measuring AFM1 in accordance with the test kit instructions. All samples were tested in separated duplicated wells.

Statistical analysis of all samples was performed using SPSS software (Version 16; SPSS Inc., Chicago, IL) and the data were expressed as Mean ± Standard deviation (SD).

RESULTS AND DISCUSSION:

Results of this study showed that 293 samples of all milk samples had detectable levels of AFM1 and 27 samples were without aflatoxin. Twenty five samples of these 293 samples had AFM1 less than Iran and Europe standards limit (50 ng/L) and 268 samples had AFM1 more than Standard limit. Mean level of AFM1 in raw milk samples collected in the spring and winter was 48.8±20.77 ng/L and 56.04±16.34 ng/L, respectively (Table 2). AFM1 mean for two seasons was 52.42±19.03 ng/L which was more than of Iran and Europe standard limit.

Tab. 2
Mean concentration of AFM1 in raw milk samples of Bonab, Malekan and Maragheh cities in spring and winter seasons at 2016 (ng/L)

Cities	Spring	winter	*P value (seasons)*
Bonab	49.14±16.48	52.96±13.8	0.06
Malekan	45.32±20.82	51.90±16.8	0.07
Maragheh	51.98±23.53	62.98±15.72	0.1
Mean	48.80±20.77	56.04±16.34	
P value (cities)	0.07		

Based on the Results, 83.75% and 16.25% of samples had AFM1 more and less than of 50 ng/L (standard limit of Iran and Europe Union), respectively. The minimum and maximum level of AFM1 milk contamination in each city and seasons are presented in Table 3. Highest and lowest levels of AFM1 contamination were seen in the samples of Maragheh and Bonab cities with 75 ng/L and 4 ng/L, respectively, which was related to winter and spring seasons, respectively (Table 3). There is significant difference ($P \leq 0.1$) between the contamination level in milk samples of three cities and two seasons (Table 2).

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Milk contamination with AFM1 is an important and remarkable topic in Iran and many countries (Celik et al., 2005; Nurhan, 2006). Regard to the heat stability of the AF and its high shelf-life in milk and dairy products it can cause serious illnesses in milk and dairy products consumers (Dashti et al., 2009). Researchers use different quantitative methods (TLC, HPLC, …) and screening immunoassay such as ELISA for measuring AFM1 in milk and dairy products (Table 1). Investigations show that ELISA technique is a sensitive and suitable technique for measuring AFs even in low concentrations (Leszczynska et al., 2001).

Tab 3

Minimum and Maximum levels of AFM1 in raw milk samples of Bonab, Malekan and Maragheh cities in spring and winter seasons at 2016 (ng/L)

	winter		Spring	
City	Max	Min	Max	Min
Bonab	63.2	8.2	62.8	4.1
Malekan	62.7	9.5	61.4	5.3
Maragheh	75.6	11.4	68.6	6.7

ELISA technique as a simple and low-cost method is applicable to the screening tests. High obtained result with this technique is the limitation of this method and perhaps high prevalence in this study and other researches is because of it (Riazipour et al., 2010). Therefore, it is better along with the ELISA test, at least for a limited number of samples, higher precision techniques such as HPLC or TLC is used to confirmation the results. Several studies have reported incidence of AF contamination in milk and milk products in Iran and other countries. In similar studies conducted in Iran, a wide range of results can be seen out of the standard limit. For example Sadeghi et al. in a study on raw milk samples in Kermanshah at 2012, Tajik and coworkers on both pasteurized and raw milk samples in Urmia at 2006 and Nourian and coworkers on raw milk in Qazvin at 2015 have reported that 92.18%, 6.28% and 33.52% of the samples had AF contamination higher than standard allowed limit, respectively (Tajik et al., 2007; Norian et al., 2015; Sadeghi et al., 2012). While over limit AF contamination has been observed in 100% of milk samples in the studies carried out by Kamkar in 2008 on UHT milk samples in Tehran and Behfar et al. on pasteurized milk samples in Ahwaz (Behfar et al., 2012; Kamkar, 2008). Similar studies in other countries also have reported high AFM1 contamination in milk samples and dairy products. For example, contamination rate of pasteurized and sterilized milk samples were 64% and 47% higher than allowed limit in Turkey, respectively at 2005-2006, or only 4% of milk and dairy products samples were higher than standard level in India at 2004, (Celik et al., 2005; Skrbic et al., 2014; Shipra et al., 2004). There are many reports of the low contamination in milk samples, for example, Lopez et al. have been reported AFM1 content in milk samples in winter was lower than recommended limit for dairy products using ELISA method at 2003 (Lopez et al., 2003).

AFs are not produced in pasture and are rarely found in forages and their silage. It is believed, AFs persistence largely is due to feeding of animals with grain-based concentrates. Therefore a seasonal tendency in milk contamination is expected (Duarte et al., 2013). Feed type, cultivation, their preservation method and geographical conditions (local weather, humidity and warmth) can exacerbate contamination of milk, but rate and mode of action of these factors not clearly identified (Tajkarimi et al., 2008; CAC, 2001). Preventive measures by livestock related organizations,such as continuous sanitary quality control of livestock feeding for the presence of AFB1, farmers training to feed livestock with healthy and hygienic forage and silage are the most effective factors to reduce the AFM1 in milk and dairy products.

CONCLUSIONS:

This study shows that contamination of milk with aflatoxin M1 in some samples was significantly higher than the standard limit. Food safety is at risk with the growth of molds that capable to produce mycotoxins. Given the importance of milk in human nutrition, potential risks of aflatoxin in milk on the health of consumers especially children, it seems continuous assessment of the AFM1 in produced milk of the understudy areas and confirmation with more accurate methods is necessary.

REFERENCES:

Becker-Algeri TA, Castagnaro D, de Bortoli K, de Souza C, Drunkler DA, Badiale-Furlong E, Mycotoxins in Bovine Milk and Dairy Products. Journal Food Science 81(3), 544-552, 2016.

Behfar A, Nazari Khorasgani Z, Alemzadeh Z, Goudarzi M, Ebrahimi R, Tarhani N, Determination of Aflatoxin M1Levels in Produced Pasteurized Milk in Ahvaz City by

Using HPLC. Jundishapur Journal of Natural Pharmaceutical Products 7(2), 80-84, 2012.

Bellio A, Bianchi DM, Gramaglia M, Loria A, Nucera D, Gallina S, Gili M, Decastelli L, Aflatoxin M1 in Cow's Milk: Method Validation for Milk Sampled in Northern Italy. Toxins 8(3), 57, 2016.

Celik TH, Sarimehmetoglu B, Kuplulu O, Aflatoxin M1 contamination in pasteurized milk. Veterinary Archive 75 (1), 57-65, 2005.

Chao-Zong L, Guey-Yuh L, Gwo-Fang Y, Comparison of Aspergillus flavus and Aspergillus oryzae by amplified fragment length polymorphism. Botanical Bulletin of Academia Sinica, 45, 61-68, 2004.

Chiavaro E, Asta CD, Galaverna G, Biancardi A, Gambarelli E, Dossena A, Marchelli R, New reversed-phase liquid chromatographic method to detect aflatoxins in food and feed with cyclodextrins as fluorescence enhancers added to the eluent. Journal of Chromatography A 937(1-2), 31-40, 2001.

Codex Alimentarius Commission (CAC),Commission submitted on the draft maximum level for aflatoxin M1 in milk.

Dashti B, Al-Hamli S, Alomirah H, Al-Zenki S, Bu Abbas A, Sawaya W, Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. Food Control, 20: 686–690, 2009.

Deshpande SS, Handbook of Food Toxicology: Fungal Toxins. Marcel Decker, New York, 387-456. 2002.

Duarte SC, Almeida AM, Teixeira AS, Pereira AL, Falcão AC, Pena A, Lino CM, Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal exposure. Food Control, 30, 411-417, 2013.

European communities (EC), Regulation (EC) No 401/2006, Laying down regulation, the sampling methods of analysis for the official control of the levels for certain contaminants in food staffs. Official Journal of the European Union, 1-30, 2010.

Galvano F, Pietri A, Bertuzzi T, Fusconi G, Galvano M, Reduction of carry over of aflatoxin from cow feed to milk by addition of activated carbon. Journal of Food Protection, 59, 551-554, 1996.

Hague, The Netherlands: Codex Committee on Food Additives and Contamination. 33rd Session, 2001.

Hazhir MS., Tahaee N., Rashidi K, Sheikhi, H, Study of aflatoxin M1 in row milk delivered to pasturized milk company of Sanandaj. Journal of Unversity ofi Medical Sciences of Kurdestan, 13(3), 44-50, 2009.

Institute of Standards and Industrial Research of Iran (ISIRI), Milk and milk products, Determination of Aflatoxin M1 by HPLC Method and Immunoaffinity Column Clean up-Test Method. National Standard No. 7133, 2011.

Jay JM. Modern food microbiology. An Aspen publication. 6th ed.: 595-600, 2000.

Kamkar A, The study of aflatoxin M1 in UHT milk samples by ELISA. Journal of Veterinary Research, 63 (2), 7-12, 2008.

Karim G, Bekaeei S, Khorasani A, Contamination of raw milk with aflatoxin M1 in Tehran using ELISA. Pajoohesh Journal, 40(3), 163-165, 1998.

Leszczynska J, Maslowaska J, Owczarek A, Determination of aflatoxins in products by ELIZA methods, Czech Journal of Food Sciences, 19(1), 8-12, 2001.

Lopez C, Ramos L, Ramadan S, Bulacio L, Perez J, Distribution of aflatoxin M1 in cheese obtained from milk artificially contaminated. International Journal of Food Microbiology, 64, 211-215, 2001.

Lopez CE, Ramos LL, Romadan SS, Bulacio LC, Presence of aflatoxin M1 in milk for human consumption in Argentina. Food Control, 14(1), 31-34, 2003.

Mortazavi A, Ghods-Roohani M, Joyande H, Technology of milk and dairy products. 2nd ed. Mashhad: Ferdowsi University press, 169, 1996.

Movassagh Ghazani MH, Aflatoxin M1 contamination in pasteurized milk in Tabriz (northwest of Iran). Food and Chemical Toxicology, 47, 1624–1625, 2009.

Nemati M, Mesgari Abbasi M, Parsa Khankandi H, Ansarin M, Survey on the occurrence of aflatoxin M1 in milk samples in Ardabil, Iran. Food Control, 21,1022–1024, 2010.

Norian R, Mahmoudi R, Porfarzaneh A, Mashatian F, Kaboudari A, Pir Mahalleh SFR, Katiraee F, Determination of aflatoxin M1 levels in raw milk samples using ELISA and high-performance liquid chromatography in Qazvin, Iran. Journal of Mycology Research, 2(1), 41-48, 2015.

Nurhan U, Occurrence of aflatoxin M1 in UHT milk in Turkey, Food and Chemical Toxicology 44, 1897-1900, 2006.

Rahimi E, Bonyadian M, Rafei M, Kazemeini HR, Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. Food and Chemical Toxicology, 48(1), 129-131, 2010.

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

Sadeghi E, Almasi A, Bohloli-Oskoii S, Mohamadi M, The evaluation of aflatoxin M1 level in collected raw milk for pasteurized dairy factories of Kermanshah in 2010-2011. Zahedan Journal of Research in Medical Sciences, 13(9), 1-7, 2012.

Sefidgar SAA, Mirzae M, Assmar M, Naddaf SR, Aflatoxin M1 in Pasteurized Milk in Babol city, Mazandaran Province, Iran. Iranian Journal of Public Health, 40(1), 115-118, 2011.

Shipra R, Premendra D, Subhash K, Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Control, 15(4), 287-290, 2004.

Skrbic, B., Zivanceva, J., Antica, I. and Godula, M, Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. Food Control, 40:113-119, 2014.

Tajik, H., Rohani, S.M. and Moradi, M, Detection of aflatoxin M1 in raw and commercial pasteurized milk in Urmia Iran. Pakistan Journal of Biological Sciences 10(22), 4103-4107, 2007.

Tajkarimi M, Shojaee Aliabadi F, Salah Nejad M, Pursoltani H, Motallebi AA, Mahdavi H, Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. International Journal of Food Microbiology, 116(3), 346-349, 2008.

World Health Organization (WHO), IARC Monographs on the evaluation of carcinogenic risks to humans – some naturally occurring substances. Food items and constituents heterocyclic aromatic amines and mycotoxins, 65, 445–466, 1993.