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Assessment of Aflatoxin M1 Contamination in Raw Milk by ELISA in Urmia, Iran

[1]Pourdad Panahi, [2]Saeed Kasaee, [1]Alireza Mokhtari, [3]Amin Sharifi and [1]Amirhossein Jangjou

[1]Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Research and Development, Pegah Iran Dairy Industries Co. Urmia, Iran
[3]Department of Food Hygiene, Pegah Iran Dairy Industries Co. Urmia, Iran

Abstract: This study conducted to determined aflatoxin M_1 in raw milk. Aflatoxins are carcinogenic and toxic which is a secondary metabolic product of some *Aspergillus* spp. Aflatoxin M_1 has discovered in the milk of animals that have consumed feed contaminated with aflatoxins B_1. Afloatoxin M_1 is relatively stable during milk pasteurization and storage as well as during the preparation of various dairy products. During the spring, 100 samples of raw cow's milk were selected randomly from Urmia, Iran. The samples were analyzed with a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit. All samples (100%) were found to have levels that exceed the legal limits of 50 ng/l established by the EU/Codex. Aflatoxin high concentration in milk cause widespread negative impact on public health and demonstrate considerable economic losses for producers. Therefore, it is necessary to establish Strategies for reducing aflatoxin levels in animal feed.

Key words: Aflatoxin B · Mycotoxin · Food Hygiene · Dairy

INTRODUCTION

Although milk is the valuable source of nutrition but consumption of toxin contaminated milk adversely affect the health of people. Aflatoxin M_1 (AFM_1) is a toxin that found in milk. Four aflatoxins (B_1, B_2, G_1 and G_2) are produced by *Aspergilli* in crops. Among them, the aflatoxin B_1 (AFB_1) is the most widespread and toxic. AFM_1 is a hepatocarcinogen that is found in milk of animals have consumed feeds contaminated with AFB_1 [1].

The existence of AF in human foods may cause chronic health effects including immune-system suppression, impaired childhood development and cancer. Acute poisoning can led to death [2]. The aflatoxins produced by *A. flavus* and *A. parasiticus* fungi are assigned by the International Agency for Research on Cancer as first_class carcinogens[3]. They are potential carcinogens, teratogens, genotoxics, mutagens and they have severe hazards for animal and human health. Previous studies have demonstrated growth retardation on human child [4]. AFM_1 remain stable after pasteurization, sterilization, preparation and storage of various dairy products [5].

Milk also is a major nutrient for infants, children, convalescents and old people. Infants usually consume pasteurized and sterilized milk between weaning from the breast till 3 years old then they use it as the main source of food, so the problem is more critical in this group of consumer [6]. Beacuse milk also used for the preparation of infant formulas, yogurt and cheese, it is important to determine AFM_1 level in milk and dairy products in order to inform consumers from its potential hazard.

Since AFs are potential carcinogens, their quantity in food and feed is closely monitored and regulated in most countries [7]. maximum permitted level of AFM_1 concentration in milk is 100ng/l and 50ng/l in Iran and European countries, respectively [8]. Many studies have shown high incidence of AFM_1 In Iran [5, 9-11]. This study has conducted to determine the current situation of AFM_1 contamination in Urmia and see if there is any improvement in AFM_1 levels in milk.

MATERIALS AND METHODS

During the spring, one hundred samples of raw cow's milk were selected randomly from dairy farm of Urmia (North West of Iran). ELISA kit EuroProxima Aflatoxin M_1

Corresponding Author: Alireza Mokhtari, Department of Microbiology,
Science and Research Branch, Islamic Azad University, Tehran, Iran.
Hesarak, Ashrafi Esfahani Highway, Ponak sq. Tehran, Iran.
Tel: +98-65295125, Fax:+98-47912213.

Table 1: Frequency Distribution of AFM_1 in raw milk of Urmia (ng/l)

Sample	Number	Positive frequency	Positive percent	Mean ± se	S.D	Max	Min
Raw milk	100	100	100	76.5±1.43	2.28	1.3	68

Table 2: Prevalence Frequency Distribution of AFM_1 in raw milk of Urmia (ng/l)

AFM1Concentration	<50	51-60	61-70	71-80	81-90	90<
Sample Raw milk	0%	0%	0.3%	43%	40%	14%

EIA kit, (Euro Proxima, Netherlands), were used for the test. The samples prepared as manufacturer's instructions. All Samples defatted through cooling Centrifuge for 10 min, 2000xg at 4°C. The upper fat layer removed by spatula. For the ELISA test (Enzyme Linked Immunosorbent Assay) 100 µl of the defatted milk were used in each microplate well. According to the manufacturer's instructions, the following steps were performed:

Before starting the test, the reagents were brought up to room temperature. The AFM standards and test sample were added in duplicate to 96 microtiter coated plate then Incubated for 1 h in darkness at room temperature (24°C). The solutions have discarded from the microtitre plate and washed 3 times with rinsing buffer. Then 100 µl of conjugate (Aflatoxin M_1-HRPO) were added to all wells, except zero standard maximal wells. The microtitre plate had been shaken for 10 s on a microtitre plate shaker, then Incubated for 30 min in darkness at room temperature. The solution were discarded from the microtitre plate and washed 3 times with rinsing buffer. Volume 100 µl of substrate solution were added into each well and incubated 30 min at room temperature. Finally, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with microplate reader (ELX 800 UV, Bio-Tek Instruments, Inc.).

The mean optical density zero standard maximal wells subtracted from Individual O.D. of the wells containing the standards and the samples. The O.D. values of the standards and the samples (mean values of the Duplicates) were divided by the mean O.D. value of the zero standards then multiplied by 100. The absorbance percentage had been taken to calibration curve and performed with standard at different levels. Statistical analyses were performed using SPSS software version 12. Probability values less than 0.05 were considered significant.

RESULT

All 100 tested samples were AF positive. The average of AFM_1 concentration in March, April and May, were 84.23, 72.71 and 83.34 ng/1 respectively. No statistically significant correlation between month and AFM_1 concentration ($p<0.05$) were observed in percent study. The Result and Statistics analysis are shown in Table 1 and 2.

DISCUSSION

AFM_1 is the hydroxylated metabolite of AFB_1 which formed in liver by means of cytochrome P450 associated enzymes [12]. It has been shown to be excreting in milk; following exposure to AFB_1 contaminated food and transferred to dairy products, which represents an important risk factor for consumers. AFM_1 is cytotoxic, as demonstrated in human hepatocytes *in vitro* and its acute toxicity in several species is similar to that of AFB_1 [13, 14]. In ducklings and rats, the acute and short-term toxicity of AFM_1 was similar to or slightly less than that of AFB_1. AFM_1 can also cause DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalians cells *in vitro*, in insects, lower eukaryotes and bacteria [14].

According to present study, occurrence of AFM_1 milk is highly prevalent and all samples have unacceptable aflatoxin concentration according to EU\codex. By comparing the result of present study to the same study, which conducted in 2005 by Tajik *et al.* in this geographical area, milk contamination by AFM_1 have increased.

Some Pervious studies show the differences observed in different seasons were not statistically significant [9, 10]. In present study also there was not statically significant different between samples collected in different months.

AFM_1 appears in milk or dairy products as a direct result of the ingestion of food contaminated with aflatoxin and contaminated feed not only creates risks of residues in milk, but it also reduces animal performance and overall health. Furthermore, aflatoxin associated with reduced feed consumption and overall retarded growth and development in dairy cattle [15]. When cows feed with an aflatoxin free diet, milk production increase over 25% [16].

There is no procedure for eliminating AF after producing in food. Controlling mold growth and mycotoxin production is important to the feed manufacturer and livestock producer. Regular analysis of animal feed, feed ingredients and employment of proper mycotoxin deactivation strategy will help to reduce the economic losses largely. Testing for AF concentrations should be the first step in proper feeding management.

For the future course of action, it is necessary to legislating new rule and encouragement policies to elimination the AF levels in milk. Awareness creation and education of farmers is an important factor to reduce AF in animal feed.

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REFERENCES

1. Henry, S.H., F.X. Bosch, T.C. Troxell and P.M. Bolger, 1999. Reducing liver cancer--global control of aflatoxin. Sci., 286: 2453.
2. Williams, J.H., T.D. Phillips, P.E. Jolly, J.K. Stiles, C.M. Jolly and D. Aggarwal, 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. The American J. Clinical Nutrition, 80: 1106.
3. IARC, 1987. Overall Evaluations of Carcinogenicity. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Supplement 7. Lyon, France: International Agency for Research on Cancer. pp: 440.
4. Khlangwiset, P., G.S. Shephard and F. Wu, 2011. Aflatoxins and growth impairment: A review. Critical Reviews in Toxicol., 41: 1-16.
5. Fallah, A.A., T. Jafari, A. Fallah and M. Rahnama, 2009. Determination of aflatoxin M1 levels in Iranian white and cream cheese. Food and Chemical Toxicol., 47: 1872-1875.
6. Oveisi, M.R., B. Jannat, N. Sadeghi, M. Hajimahmoodi and A. Nikzad, 2007. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. Food Control, 18: 1216-1218.
7. Yin, Y., L. Yan, J. Jiang and Z. Ma, 2008. Biological control of aflatoxin contamination of crops. J. Zhejiang University Sci. B., 9: 787-792.
8. National standard of Institute of Standard and Industrial Research of the Islamic Republic of Iran (ISIRI), 2009. Food & Feed - Mycotoxins- Maximum Tolerated level Amendment No.1,Vol a-5925.
9. Tajkarimi, M., F. Shojaee Aliabadi, M. Salah Nejad, H. Pursoltani, A. Motallebi and H. Mahdavi, 2007. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. International J. Food Microbiol., 116: 346-349.
10. Riazipour, M., H. Tavokoli, M. Razzaghi-Abyaneh, H. Rafati and S.M. MT, 2010. Measuring the amount of M1 Aflatoxin in pasteurized milks. Kowsar Medical J., 15: 89-93.
11. Tajik, H.S., M.R. Rohani and M. Moradi, 2007. Detection of Anatoxin Ml in Raw and Commercial Pasteurized Milk in Urmia, Iran. Pakistan J. Biological Sci., 10: 4103-4107.
12. Gallagher, E.P., K.L. Kunze, P.L. Stapleton and D.L. Eaton, 1996. The kinetics of aflatoxin B1 oxidation by human cDNA-expressed and human liver microsomal cytochromes P450 1A2 and 3A4. Toxicology and Applied Pharmacology,141:595-606.
13. Massey, T.E., R.K. Stewart, J.M. Daniels and L. Liu, 1995. Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B1 carcinogenicity. Experimental Biology and Medicine, 208: 213-227.
14. Prandini, A., G. Tansini, S. Sigolo, L. Filippi, M. Laporta and G. Piva, 2009. On the occurrence of aflatoxin M1 in milk and dairy products. Food and Chemical Toxicol., 47: 984-991.
15. Akande, K., M. Abubakar, T. Adegbola and S. Bogoro, 2006. Nutritional and health implications of mycotoxins in animal feeds: A Review. Pakistan J. Nutrition, 5: 398-403.
16. Guthrie, L. and D. Bedell, 1979. Effects of aflatoxin in corn on production and reproduction in dairy cattle. Proceedings of the 83rd Annual Meeting of the United States Animal Health Association. pp: 202-204.