

A Study on Contamination of Aflatoxin M₁ in Milk and Infant Milk Products in Iran

Ebrahim Rahimi

Member of Young Research Club, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Abstract: Aflatoxin M₁, a mutagenic and carcinogenic metabolite aflatoxin B₁, occur in milk from animals fed on food contaminated with some species of *Aspergillus*. In this survey the incidence of contamination of aflatoxin M₁ (AFM₁) in milk and infant milk products collected from the Iran market was investigated by using the contitive enzyme linked immunosorbent assay (ELISA) technique. During February 2007 to December 2009 a total of 167 samples composed of liquid milk (47 samples), infant formula (40), infant milk food (35), and milk based weaning food (45) showed that 81.4% of tested samples were contaminated with various levels of AFM₁ raying from 7 to 476 ng/l. The concentration of AFM₁ in all of the liquid milk samples were lower than Iranian national standard and US tolerance limit (500 ng/l), but in 44.7 % of liquid milk and 10 % infant milk formula, 17.1 % infant milk food and 15.6 % of milk based weaning food samples were higher that maximum tolerance limit accepted by (European union/ codex Alimentarius has prescribed a limit of 50 ng/l for AFM₁ in milk and 25 ng/l in infant milk products). This study suggests a regular monitoring of AFM₁ in milk and milk products in the country.

Key words: Aflatoxin M₁ • Raw milk • Infant milk products

INTRODUCTION

Mycotoxins are secondary metabolites of molds and have adverse effects on humans, animals, and crops that result in illnesses and economic losses [1]. One of the mycotoxins, aflatoxin M₁ (AFM₁) is the hydroxylated metabolite of aflatoxin B₁ (AFB₁) and can be found in milk or milk products obtained from live stock that have ingested contaminated feed [2-4]. About 1-2% of AFB₁ in animal feed is transformed to AFM₁ in milk; it may vary from animal to animal, from day to day and from milking to the next. 12-24 h after the first AFB₁ ingestion, the toxin can be detected in the milk. When the intake of AFB₁ is stopped, the AFM₁ cocentration in the milk decreases to an undetectable level after 72 hours [2].

There is sufficient evidence that AFM₁ is a genotoxic carcinogen and that it is less toxic than AFB₁ [5]. Exposure of children, including infants, to AFM₁ is worrisome, because they are considered more susceptible to its adverse effects, and their capacity for biotransformation of carcinogens is generally shower than in adults [6-7].

Many countries have carried out studies about the incidence of AFM₁ in milk. In most of them, samples have been found whist exceed the limit imposed by many

countries of 50 ng/l. Unfortunately, in our country, in spite of the fact that the dairy industry has evolved a lot in the last years, as regards production levels and technology, there are very few data about AFM₁ incidence on fluid milk, powder milk, infant milk products and other milky derivatives. The purpose of this survey was to determine the status of AFM₁ contamination in infant milk products and liquid milk in Iran.

MATERIALS AND METHODS

Sampling: During February 2007 to December 2009 a total 167 samples were collected from the central cities of Iran, during. These samples were composed of raw cow milk 47 samples, infant formula 40 samples, milk based cereal weaning food 45 Samples and infant milk food 35 Samples.

Sample Preparation: Powder based samples (10g) were suspended in 100 ml of warm deionised water. Subsequently, these samples as well as liquid milk samples were centrifuged at 3500g for 10 min at 4°C.

Method of analysis: The quantitative analysis of AFM₁ was performed using enzyme immunoassay: Ridascreen®

aflatoxin M1 kit (R-Bipharm AG, Germany). The test is based on the antigen-antibody reaction. The wells in the microtitre strips are coated with specific antibodies to AFM1. By adding AFM1 standards or the sample solutions, the antibody binding sites are occupied proportionally to the AFM1 concentration. Any remaining free binding sites are occupied in the next stage by enzyme labeled toxin (enzyme conjugate). Unbound enzyme conjugate is then removed in a washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethyl benzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow which is measured photometrically at 450 nm. Light absorption is inversely proportional to the AFM1 concentration in the sample. Milk samples were centrifuged for 10 min at 3500 g and 10°C. After centrifugation, the upper cream layer was removed completely by aspiration with a Pasteur pipette and the skimmed milk used directly in the test (100 µl per well). The mean lower detection limit of the RIDASCREEN® AFM1 test was 5 ng /l.

Statistical analysis: Statistical analysis of results was performed with SPSS (version 16) software (SPSS Chicago, IL, USA). The mean AFM1 concentration in raw cow milk, infant formula milk based cereal weaning food and infant milk food Samples was compared by one way analysis of variance (ANOVA) and Tukey's (HSD) tests.

RESULTS AND DISCUSSION

The results of the analyses of aflatoxinM1 (AFM1) level (ng/l) in the milk liquid cow milk, infant formula, milk based cereal weaning food and infant milk food showed that the incidence of contamination with AFM1 is 81.4 %, the mean values of AFM1 in each group was 98.9 ng/l, 20.6 ng/l, 23.2 ng/l and 20.3 ng/l, respectively (Table 1). Significantly higher contamination rates of AFM1 ($P < 0.05$) were found in raw cow milk.

The US regulation and Iranian national standard has prescribed a limit of 500 ng/l for AFM1 in milk and dairy products. However, European Communities and Codex Alimentarius have fixed the limit to a maximum of 50 ng/l for AFM1 in milk and 25 ng/l in infant milk products [8-9]. Almost 29% of the contaminated samples exceeded the European Communities/Codex Alimentarius recommended limits; all of milk samples had AFM1 levels less than the prescribed limit of US regulations.

Table 1: Occurrence of aflatoxin M1 level in liquid milk and infant milk products

Type of milk sample	Samples tested (n)	Positive samples (n)	Mean±SD AFM1 concentration (ng/l)
Liquid milk	47	41	98.9±115.8
Infant formula	40	22	20.6 ±11.8
Infant milk food	35	35	20.3±9.8
Milk based weaning food	45	38	23.2±20.5

Limited internationally published data are available on the occurrence of AFM1 in milk products in Iran. In a study done by the present authors in 2008 with the ELISA method in 236 raw (n=140), pasteurized (n=48) and UHT (n=48) milk samples in Isfahan and shahrekord cities, Iran the grand mean of all samples was 12-218 ng/l (mean= 65 n/l); 117 samples had 0.01-50.0 ng/l, 93 samples had 51.0-100.0 ng/l and 26 samples had >10 ng/l [10]. In another study which was done in Tehran, Iran by the ELISA method, the amount of AFM1 in 100 (78%) of liquid milk samples and 24 (33%) of milk based weaning food was higher than the maximum tolerance limit accepted by European Union [11]. In Shiraz, 2006, the occurrence of AFM1 in 624 pasteurized milk samples was studied [12]. AFM1 was found in 100% samples and in 17.8% samples was greater than the maximum tolerance limit accepted by European Union.

Concentrations of AFM1 in milk found in the present study are similar to those reported in other countries, especially those in Asian and Africa like Pakistan, India, Turkey, Kuwait, Nigeria, Korea, Syria [13-19].

Due to high toxicity and carcinogenic properties of AFM1, its presence in milk is a concern. AFM1 is resistant to thermal inactivation, pasteurization, autoclaving and other varieties of food processing procedures [20]. So, to produce high quality milk, it is essential to keep feeds free from contamination by AFB1. The concentration of AFB1 in animal feed can be reduced by good manufacturing practice and good storage practices. If preventive measures fail, however, AFB1 can be reduced in feed by blending with feed that has lower concentrations or by chemical, physical or biological treatment [3].

In the recent past, it has been indicated that many countries of Europe showed relatively low levels of contamination of AFM1 in milk and milk products. The occurrence of AFM1 at such low levels in European countries may be a result of stringent regulation of AFB1 in complementary feedstuffs for dairy cattle.

REFERENCES

1. Hussain, I. and J. Anwar, 2008. A study on contamination of aflatoxin M1 in raw milk in the Punjab province of Pakistan. *Food Control*, 19(4): 393-395.
2. Van Egmond, H.P. 1989. Introduction. In: van Egmond, H.P. (Ed.), *Mycotoxins in dairy products*. Elsevier Applied Science. London and New York, pp. 1-10.
3. Galvano, F., V. Galofaro, A. Ritieni, M. Bognanno, A. De Angelis and G. Galvano, 2001. Survey of the occurrence of aflatoxin M1 in dairy products marketed in Italy: second year of observation. *Food Addit. Contamin.*, 18: 644-646.
4. Tajkarimi, M., F. Shojaee Alibadi, M. Salah Nejad, H. Pursoltani, A.A. Motallebi and H. Mahdavi, 2007. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. *Int. J. Food Microbiol.*, 116: 346-349.
5. Creppy, E.E. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Letters*, 127: 19-28.
6. Lopez, C.E., L.L. Ramos, S.S. Ramadan and L.C. Bulacio, 2003. Presence of aflatoxin M1 in milk for human consumption Argentina. *Food Control*, 14: 31-34.
7. International Agency for Research on Cancer, 2002. Monograph on the evaluation of carcinogenic risk to humans. Vol. 82: 171 (Lyon: IARC).
8. Codex Alimentarius Commission., 2001. Comment submitted on the draft maximum level for aflatoxin M1 in milk. In Codex committee on food additives and contaminants 33rd session. Hauge, The Netherlands.
9. European Commission Regulation, 2001. No 466/2001/EC of 8 March 2001, setting maximum levels for certain contaminants in food stuffs. *Official Journal of the European Commission*, L077: 1-13.
10. Rahimi, E., A. Shakerian, M. Jafariyan, M. Ebrahimi and M. Riahi, 2009. Occurrence of aflatoxin M1 in raw, pasteurized and UHT milk commercialized in Esfahan and Shahr-e Kord, Iran. *Food Security*, 1: 317-320.
11. Oveisi, M.R., B. Jannat, N. Sadeghi, M. Hajiohamoodi and A. Nikzad, 2007. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. *Food Control*, 18:1216-1218.
12. Alborzi, S., B. Pourabbas, M. Rashidi and B. Astaneh, 2006. Aflatoxin M1 contamination in pasteurized milk in Shiraz (south of Iran). *Food Control*, 17: 582-584.
13. Srivastava, V.P., A. Bu-Abbas, D. Alaa-Basuny, W. Al-Johar, S. Al-Mufti and M.K.J. Siddiqui, 2001. Aflatoxin M1 contamination in commercial samples of milk and dairy products in Kuwait. *Food Addit. Contamin.*, 18: 993-997.
14. Rastogi, S., D.P. Dwivedi, S.K. Khanna and M. Das, 2004. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. *Food Control*, 15: 287-290.
15. Kamkar, A., 2005. A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab city of Iran. *Food Control*, 16 (70): 593-599.
16. Atanda, O., A. Oguntubo, O. Adejumo, J. Ikeorah and I. Akpan, 2007. Aflatoxin M1 contamination of milk and ice cream in Abeokuta and Odeda local governments of Ogun State, Nigeria. *Chemosphere*, 68:1455-1458.
17. Ghanem, I. and M. Orfi, 2008. Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market. *Food Control*, 20: 603-605.
18. Viridis, S., G. Corgiolu, C. Scarano, A.L. Pilo and E.P.L. Desanti, 2008. Occurrence of aflatoxin M1 in tank bulk goat milk and ripened goat cheese. *Food Control*, 19(1): 44-49.
19. 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 Chemic. Toxicol.*, 47: 984-991.
20. Stoloff, L., H.P. Van Egmond and D.L. Parks, 1991. Rationales for the establishment of limits and regulations for mycotoxins. *Food Addit. Contamin.*, 8: 222-231.