

## Survey on Contaminated Raw Milks with Aflatoxin M1 in the Sarab Region, Iran

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**Abstract:** Mycotoxins are those groups of fungus metabolites which as the result of the consumption of contaminated food causes human or animal illness or death and the resulted illness from this is called mycotoxicosis. In this study, 100 samples from milk were analyzed for the presence of aflatoxin M1 by thin layer chromatography. This study showed that from 100 samples were analyzed a total of 84 samples (84%) were positive for aflatoxin M1 and 16 samples (16%) were negative. The range of aflatoxin M1 content was 30-630 ng L<sup>-1</sup>. Contamination rate of aflatoxin M1 in tanks milk in around (84%) with amounts ranging 30-630 ng L<sup>-1</sup>. All contaminated samples had a level of aflatoxin M1 above the European countries standard (50 ng L<sup>-1</sup>). Therefore, the following suggestions are made. Determined standard complications for aflatoxin limit value in feed and aflatoxin M1 in milk and dairy products use of effective methods for treatment of contaminated feed, milk and dairy products.

**Key words:** Raw milk, aflatoxin M1, mycotoxins, contaminated feed, Sarab, Iran

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### INTRODUCTION

According to the increasingly population growth need to diet also increased. In this mean, dairy products such as milk play an important role in relieving food needs specially from providing of proteins, minerals, vitamins and etc., aspect. Because of this reasons nurturing of dairy livestock industry have been expanded and daily requirement to dairy products has been increased. On the other hand, in unsafe order, milk as a risk agent can endanger the human life and child and neonates expose on a risk.

Understanding of toxicities subsequent fungi toxins has a long history so that disease which called aspergyllosis yields to wasting of 100,000 turkeys. More studies about exploring of the causative agent of death yields to this finding that toxins were produced by two groups of fungus which are called aspergillus flavus and aspergillus parasiticus (Van Egmond, 1989; Egmond, 1989). After this time, more studies were done on aflatoxins and their carcinogenesis effect and their toxicity effects caused that countries legislated such laws that by this means can calculate this toxins in foods so that nowadays in 56 world countries, inspection of foods from existence of this toxins apply. Entrance of aflatoxins to milk products occurred by two way:

- Indirect contamination to wit consumption of contaminated products by animals and entrance of these toxins to milk that about 1-2% of ingested B<sub>1</sub>

aflatoxin expelled as M1 aflatoxin (Van Egmond, 1989; Egmond, 1989; Veldman *et al.*, 1992)

- Direct contamination to wit contamination of milk and its products by fungus and then production of mycotoxins (Van Egmond, 1989; Egmond, 1989)

According to this point that M1 aflatoxin is very similar with B<sub>1</sub> from terms of chemical structure and in fact is of 4-hydroxy aflatoxin B<sub>1</sub> derivatives and on the other hand because of carcinogenesis, mutation and teratogenic effects of aflatoxins (Lafont and Lander, 1989; Umeda, 1971; Vesely *et al.*, 1983) and resistance of several aflatoxins including M1 and M2 against common physical agents (Aman, 1995; Choudhary and Parker, 1998; Wiseman and Marth, 1983) there are many studies about detection of contaminated milks from one hand and routes to sanitation of contaminated milks from other hand. The objective of this study was to determination of the raw milks contamination to aflatoxin M1.

### MATERIALS AND METHODS

**Chemicals:** Fixed chloroform with 5% ethanol 96%, toluene, glacial acid acetic 99.6%, acetonitrile, diethyl ether without peroxide which its ethanol is <5%, N-hexane, acetone, methanol, dried sodium sulfate, NaCl, sodium dodecyl sulfate, silica gel 60 solely to column and standard M1 chromatography, filter paper, fiberglass. It must be remember that all material used were specialized to chromatography.

**Laboratory equipment:** TLC-scanner modeled camac Swiss made, condenser evaporator Swiss made, chromatography glass plates 20×20 cm modeled Whatman USA made, Syringe 10 and 100 µL Hamilton Co. made, glass column for columnar chromatography longed 30 and 1 cm in diameter and 15 mL volume that lower terminal of the column were striped and makes 4 mm in diameter a small circle that equipped to an appropriate plug, mixer, regulatory ultraviolet lamp on 360 nm wavelength, appropriate equipments to evaporation of the chloroform in conical tubes in azotes gas flow and at 40-50°C temperature, regulatory drier at 70-105°C, micro-pipette 100 and 1000 µL modeled Eppendorf, regulatory bain marie at 0-100°C made by Swiss gerber Co., shaker mixer and other common laboratory materials. In this study of 246 Sarab milk collection centers by chance about 25 samples of 100 centers were collected in each season in different process and same intervals and after transportation to lab, samples were evaluated by International Dairy Federation (IDF) method to existence probability of M1 aflatoxin.

In this study, prior 50 mL milk transferred to a 250 mL separator funnel and 10 mL chilled to 4°C saturated NaCl were added to it and then 10 mL sodium dodecyl sulfate 5% in room temperature were added to it. Then 125 mL chilled to 4°C chloroform were shaded on it and were shaking slowly for 1 min so that two blue and chloroform phases were make. Chloroform layer were transferred to a conical Erlenmeyer and 5 g sulfate sodium were added to it and were fixed for 15 min and sometimes was shook. Finally by a filter paper noted solution were filtered and 75 mL of it were taken.

Achieved extract were transferred to chromatography column and logged solution were washed with 25 mL toluene+glacial acid acetic (9+1) to the extent that solution receive to upper level of sodium sulfate. Then washing solution was out and column was washed with 25 mL n-hexane and then was washed with 25 mL mixed acetonitrile, diethyl ether and n-hexane (10+30+60). Then remnant aflatoxin M1 was washed with 60 mL mixed chloroform and acetone and washing result were collected in bottom round balloons and evaporated by rotary evaporator.

It must be remember that all washing processes must be continue without interruption and in all stages chromatography column not be dried. Solution was evaporated at 50°C under neutralized gas including nitrogen. After chilling of the tube by a Hamilton syringe 100 µL chloroform was added to it and mixed for 1 min. After extraction, spots related to standard and analysis samples were located on a TLC plates. Then plates were transferred rapidly to chromatography tanks with 100 mL mixed isopropanole, acetone and chloroform (5+10+85)

and finally after preparation of the extraction, plates of chromatography were transferred to TLC scanner and based on under curve area and below formula, concentration of aflatoxin M1 were calculated by ng. Aflatoxin M1 concentration (ppb) = (Sample under curve area/standard under curve area)×standard concentration×(10<sub>0</sub>/recycling%).

## RESULTS AND DISCUSSION

According to the obtained results, among 100 understudying raw milk samples numbered 84 samples had aflatoxin M1 in range of 30-630 ng L<sup>-1</sup> and 16 cases in terms of existence of aflatoxin were detected negative. On the other hand, based on data in Table 1, maximum contamination rate is belonged to autumn and winter with 388 and 390 ng L<sup>-1</sup>, respectively. Whereas, contamination rate in spring and summer was 372 and 320 ng L<sup>-1</sup>, respectively. It has been revealed that contamination rate in summer was lower than other seasons (Fig. 1). In fact, ANOVA test consider this amounts as significant. However, aflatoxin M1 in Sarab area milks is higher than accepted permissible ranges in other countries such as European Union countries that in countries permissible range is approximately between 0.05-0.5 µg L<sup>-1</sup>. Based on contamination of milk and its products to aflatoxin M1 is worldwide (Karim and Kordy, 1982; Karim *et al.*, 1999; Aman, 1995; Bluethgen and Heesch, 1995; Cerutti and Elder, 1997; Galvano *et al.*, 1998; Ioannou-Kakouri *et al.*, 1999; Kim *et al.*, 2000; Saitanu, 1997; Markaki and

Table 1: Contamination rate of raw milks in Sarab area to aflatoxin M1 during 2009-2010

| Seasons                                       | No. of samples | Average | Minimum | Maximum |
|-----------------------------------------------|----------------|---------|---------|---------|
| -----Contamination (ng L <sup>-1</sup> )----- |                |         |         |         |
| Spring                                        | 25             | 372     | 30      | 341     |
| Summer                                        | 25             | 320     | 69      | 390     |
| Autumn                                        | 25             | 388     | 50      | 592     |
| Winter                                        | 25             | 390     | 91      | 630     |

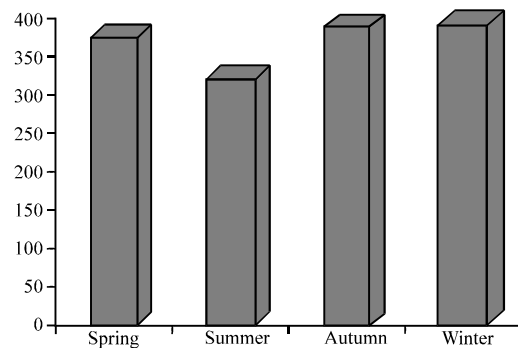


Fig. 1: Comparative columnar diagram of contamination rate of raw milks at different season to aflatoxin M1

Melissari, 1997; De Sylos *et al.*, 1996; Van Egmond, 1989; Vesely *et al.*, 1983; Weber, 1989; Fu, 1996) our country also is not exception so that done studies in our country about aflatoxin M1 in contaminated milks indicates this fact that not only produced milks about contamination to aflatoxin M1 are in high frequency but contamination rate in these milks is higher than standard levels exist in European countries. It must be noted that until there is not any complied standards in Iran thus European countries standards considered. In other study by Karim on 61 milk samples (52 samples was belonged to Tehran around cow keepers and 9 samples was belonged to pasteurized milks) revealed that 92.31% of raw milks and all of the pasteurized milks were contaminated to aflatoxin M1. In that study, maximum contamination rate were determined 25 and 20.1  $\mu\text{g L}^{-1}$  in raw and pasteurized milks, respectively (Karim and Kordy, 1982). In other study that carried out by Karim by Eliza method demonstrated that 82.2% of samples were contaminated to aflatoxin M1 and contamination rate in referred milks to Tehran milk factory was 259.5  $\text{ng L}^{-1}$  (Karim *et al.*, 1999). In the research of 100 raw milk samples numbered 84 samples were contaminated to aflatoxin M1 and contamination rate was 30-630  $\text{ng L}^{-1}$ . According to this and previous researches results revealed that contamination rate of aflatoxin M1 in Iran produced milks is higher than other countries and standard limits. Also demonstrated that contamination rate in cold seasons is higher than hot seasons so, this findings are consistent with other researches results (Karim *et al.*, 1999; Pikal, 1991) whereas this relationship is not referred in some others. Because of its worldwide until there is not any sufficient assume to minimizing the aflatoxin M1 from milks. In sum, appropriate management procedures in safe feeding and without contamination or minimizing the animals feed contamination to aflatoxin M1 is one of the most important way to minimizing the milk contamination.

## REFERENCES

- Aman, I., 1995. Stability of aflatoxin M1 milk samples. *Chem. Technol. Food Microbiol.*, 17: 161-163.
- Bluethgen, A. and W. Heeschen, 1995. Aflatoxin M1 and B1 contamination of tank milk and animal feeds in North Germany in early 1994. *DMZ-Labansmittelindustrie-und-Milckwirtschaft*, 116: 4-13.
- Cerutti, G. and M.E. Elder, 1997. Aflatoxins in foods. *Latte*, 22: 145-147.
- Choudhary, P.L. and F. Parker, 1998. Effect of chilling and heating on aflatoxin M1 content of contaminated Indian cow s milk. *Egypt. J. Dairy Sci.*, 26: 223-329.
- De Sylos, C.M., D.B. Rodriguez-Amaya and P.R. Carvalho, 1996. Occurrence of aflatoxin M1 in milk and dairy products commercialized in Campinas Brazil. *Food Additives Contam.*, 13: 169-172.
- Egmond, H.P., 1989. *Mycotoxins in Dairy Products*. Elsevier Applied Science, London, ISBN: 9781851663699, pp: 15.
- Fu, Y.M., 1996. Determination of aflatoxin M1 in milk and milk powder using immuno-affinity column and fluorescence measurement. *J. Food Drug Anal.*, 4: 175-183.
- Galvano, F., V. Galofaro, A. de Angelis, M. Galvano, M. Bognanno and G. Galvano, 1998. Survey of the occurrence of aflatoxins M1 in dairy products marketed in Italy. *J. Food Prot.*, 61: 738-741.
- Ioannou-Kakouri, E., M. Aletrari, E. Christou, A. Hadjioannou-Ralli, A. Koliou and D. Akkelidou, 1999. Surveillance and control of aflatoxins B1, B2, G1, G2 and M1 in foodstuffs in the Republic of Cyprus: 1992-1996. *J. AOAC Int.*, 82: 883-892.
- Karim, G. and J. Kordy, 1982. Survey on pasteurized milk aflatoxin contamination in Tehran region. *Iran. Public Health J.*, 11: 78-83.
- Karim, G., S. Bokaei and A. Khorasani, 1999. Survey on contamination rate of Tehran milk to aflatoxin M1 by using ELISA technique. *J. Pajohesh Va Szandeghi*, 40: 163-165.
- Kim, E.K., D.H. Shon, D. Ryu, J.W. Park, H.J. Hwang and Y.B. Kim, 2000. Occurrence of aflatoxin M1 in Korean dairy products determined by ELISA and HPLC. *Food Additives Contam.*, 17: 59-64.
- Lafont, P. and D.E. Lander, 1989. Genotoxicity of hydroxyl-aflatoxins M1 and M4. *Microb. Alim. Nutr.*, 7: 1-8.
- Markaki, P. and E. Melissari, 1997. Occurrence of aflatoxin M1 in commercial pasteurized milk determined with ELISA and HPLC. *Food Addit. Contam.*, 14: 451-456.
- Pikal, I., 1991. Immunoassay determination of aflatoxin M1 in milk. *Nahrung*, 37: 742-748.
- Saitanu, K., 1997. Incidence of Aflatoxin M1 in Thai milk products. *J. Food Prot.*, 60: 1010-1012.
- Umeda, M., 1971. Cytomorphological changes of cultured cells from rat liver, kidney and lung induced by several mycotoxins. *Jpn. J. Exp. Med.*, 41: 195-207.
- Van Egmond, H., 1989. Carry-Over of Aflatoxin in ToafM1 in Milk. *Mycotoxin in Dairy Production*. Elsevir, London, UK., pp: 15.
- Veldman, A., J.A.C. Meijs, G. J. Borggreve and J.J. Heeres-van der Tol, 1992. Carry-over of aflatoxin from cows food to milk. *Anim. Prod.*, 55: 163-168.
- Vesely, D., D. Vesela and R. Jelinek, 1983. Comparative assessment of the aflatoxin B1, B2, G1, G2 and M1 embryotoxicity in the chick embryo. *Toxicol. Lett.*, 15: 297-300.
- Weber, R., 1989. TLC determination of aflatoxin M1 in milk and milk products. *Fed. Health Gazette*, 32: 95-100.
- Wiseman, D.W. and E.H. Marth, 1983. Heat and acid stability of aflatoxin M1 in naturally and artificially contaminated milk. *Milchwissenschaft*, 38: 464-466.