

## Levels of Aflatoxin M1 in Different Types of Milk Collected in Jahrom, Iran, Winter-Spring 2013

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**Abstract:** Aflatoxins as a group of mycotoxins, are produced by *Aspergillus* species under environments that favor the growth of molds. Aflatoxin M1 (AFM1) has been considered as a class 2Bcarcinogen. As milk is a major nutrient, extensive attention must be taken on the presence of AFM1 in commercial milk and dairy foods. The aim of this study was to evaluate AFM1 contamination in raw and pasteurized milk consumed in Jahrom city located in southern of Iran. The level of AFM1 in 120 milk samples collected from winter to spring 2013 from Jahrom, Iran market or domestically produced was determined using ELISA method. There was significant difference between the mean concentration AFM1 in milk collected during winter ( $7.53 \pm 12.86$  ng/L) and spring ( $21.56 \pm 28.84$  ng/L),  $p \leq 0.001$ . Inspection of milk and dairy products is recommended for AFM1 contamination specially during winter.

**Keywords:** Aflatoxin M1, Pasteurized Milk, Raw Milk, Iran

### Introduction

Aflatoxins as a group of mycotoxins, are produced by *Aspergillus* species under environments that favor the growth of molds (Van Egmond, 1991).

Animals are exposed to mycotoxins such as the aflatoxins (AFs) by consumption of feeds contaminated by mycotoxin-producing molds during growth, harvest and/or storage. When lactating cows consume aflatoxin B1 (AFB1) contaminated feed, AFB1 is metabolized to form the monohydroxy derivative, aflatoxin M (AFM1), which is produced in the cow's milk. The AFM1 is the main hydroxylated derivative of AFB1 formed in liver by means of P450 cytochrome enzymes and secreted into milk through the mammary gland of dairy cows (Van Egmond, 1991) and defecated as AFM1 in urine and also in milk (Vagef and Mahmoudi, 2013). AFM1 has been considered by the International Agency for Research on Cancer as a class 2Bcarcinogen and its carcinogenicity was valued to be one-tenth of that of AFB1 by the Joint Expert Committee on Contamination and Food Additives in 2001 (FAO/WHO, 1999).

As milk is considered to be a perfect natural food for consumers of all age groups due to its high nutritional value, extensive attention must be taken on the presence of AFM1 in commercial milk and dairy foods (Oveisi *et al.*, 2007). The European Commission Regulation 1881/2006 sets a maximum limit of 0.05 mg/kg for AFM1 in raw milk, heat-treated milk and milk for the manufacture of milk based products (CAC, 2001).

AFM1 is moderately stable during pasteurization, sterilization, preparation and storing of different dairy products (Fallah, 2010).

Infants in Iran usually consume pasteurized and sterilized milk after breast stopping and up to 3 years of age as the basic source of food, so the problem is most important in this age group (Oveisi *et al.*, 2007).

Therefore, it is vital to evaluate AFM1 level in milk and dairy foods in order to inform customers about its potential hazard. There is little information on the basic diet, including milk and milk products as well as AFM1 concentrations in milk, in some cities of Iran (Tajkarimi *et al.*, 2007).

The aim of this survey was to determine AFM1 concentrations in raw and pasteurized milk marketed

in Jahrom city located in southern of Iran during winter and spring 2013.

## Materials and Methods

A total 120 milk samples were randomly collected from supermarkets in Jahrom (a southern city in Iran), during winter and spring 2013. Of these, 90s samples were raw milk and 30 samples were pasteurized milk. All of the samples were transported at 2-4°C in the cold box to the laboratory and centrifuged at 3500 g for 10 min at 10°C. The upper creamy layer was removed by Pasteur pipette and lower liquid was evaluated for AFM1 concentration. The quantitative analysis of AFM1 in samples was performed by competitive ELISA using commercial AFM1 test kit (R-Bio pharm AG, Darmstadt, Germany).

According to Iranian national standard and accentuated by European Union (EU) and codex Alimentarius Commission, (European Commission (EC), the presence of AFM1 level more than 50 ng/l in milk, considered as positive and less than 50 ng/l considered as negative for AFM1 (CAC, 2001).

The results analysis was done by one-way Analysis Of Variance (ANOVA) and considered statistically difference at 95% confidence levels.

## Results

The result of analyses of AFM1 level showed that 10 milk samples (8.33%) including 6 raw milk samples (6.7%) of and 4 pasteurized milk samples (13.3%) of had positive AFM1 contamination, i.e., higher than standard limitation (50 ng/l).

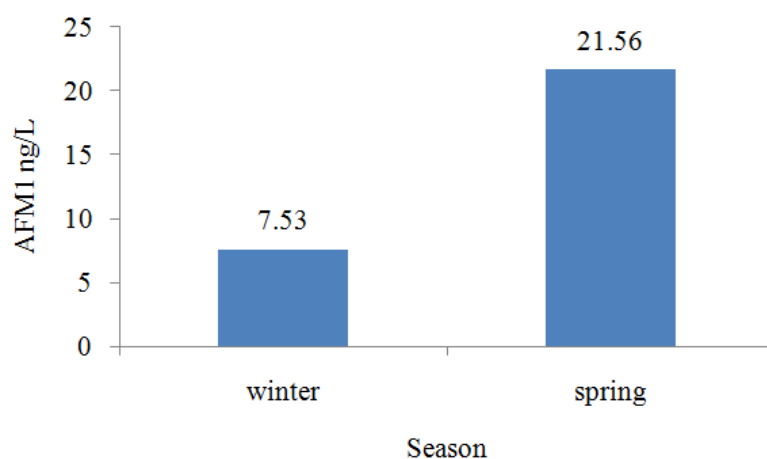


Fig. 1. AFM1 mean contamination in milk samples collected in different seasons

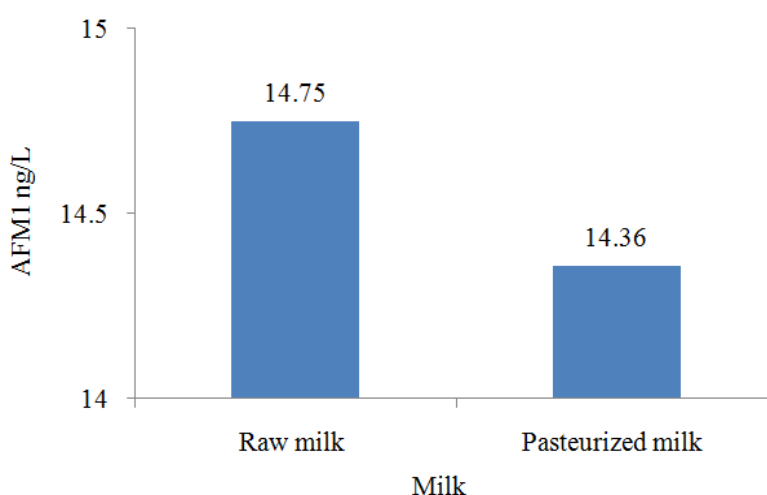


Fig. 2. AFM1 mean contamination percentage in milk samples

There was not found significant difference between the level of AFM1 in raw and pasteurized milk samples ( $p > 0.05$ ).

There was significant difference between the mean concentration AFM1 in milk collected during winter ( $7.53 \pm 12.86$  ng/L) and spring ( $21.56 \pm 28.84$  ng/L), ( $p \leq 0.001$ ), (Fig. 1).

There was not significant difference between the mean concentration AFM1 in raw milk ( $14.75 \pm 21.76$  ng/L) and pasteurized milk ( $14.36 \pm 28.31$  ng/L), ( $p = 0.164$ ), (Fig. 2).

## Discussion

The results of this study showed that 8.33% consuming milk in Jahrom as a city in southern of Iran had AFM1 contamination more than 50 ng/l.

In present study the level of AFM1 in raw and pasteurized milk samples showed no significant difference ( $P = 0.164$ ).

There are few literature data on the occurrence of AFM1 levels in milk and milk products in Iran (Oveisi *et al.*, 2007; Tajkarimi *et al.*, 2007; Tajik *et al.*, 2007; Kamkar, 2005; Alborzi *et al.*, 2006).

The AFM1 mean contamination found in present study is lower than reported in other cities of Iran (44-76 ng/l) (Tajkarimi *et al.*, 2007; Tajik *et al.*, 2007; Kamkar, 2005; Alborzi *et al.*, 2006).

Our results about the prevalence positive AFM1 contamination of pasteurized milk and raw milk are in agreement with Shiraz (southern city of Iran) 17.8% (Alborzi *et al.*, 2006) and with the result of study in Urmia (north-west city of Iran) 6.25% (Tajik *et al.*, 2007).

AFM1 levels in milk produced in Argentina were found to be very low and in no case did the levels exceed the recommended limits for milk products (50 ng/L) (Lopez *et al.*, 2003). The incidence of positive AFM1 milk contamination rate were reported 80.6% and 64% in Portugal (Martin and Martin, 2000) and Turkey (Çelik *et al.*, 2005), respectively.

AFM1 contamination of milk is the result of cows feeding by AFB1 having material. The concentration of this mycotoxin in animal feedstuffs is influenced by the type, the time and system of harvesting and temperature and relative dampness of storage facilities, all of each factors are changed in various cities of Iran (Tajkarimi *et al.*, 2007).

In present study, there was significant difference between the mean concentration AFM1 in milk collected during winter and spring, ( $p \leq 0.001$ ) which is in agreement with the results of other researches. For instance, Tajkarimi reported that AFM1 level in milk samples were significantly higher in winter than in summer (Tajkarimi *et al.*, 2008).

Milk AFM1 contamination found in our study are similar to those reported in other countries, especially those in Asian and Africa, although higher than those in many European countries (Wood and Trucksess, 1998).

To reduce the level of AFM1, it is essential to conduct training programs for producers about the toxicity potential of aflatoxins, decrease AFB1 concentration in animal feed by appropriate manufacturing and storage, integrating it with Hazard Analysis and Critical Control Points (HACCP) based safety program and performance regular monitoring and evaluation of milk and milk products for AFM1 level (Dairy HACCP-Evaluation, 2003).

## Conclusion

The results indicated that the milk AFM1 contamination in such level could be a hazardous for public health. For this reason, milk and dairy products should be inspected and controlled unceasingly for AFM1 contamination. Where concentrations are unacceptably high, careful investigation of feedstuffs for AFB1 contamination are suggested.

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## Authors' Contributions

All authors had read and approved the final manuscript:

**Reyhaneh Rouhi and Akbar Kazemi:** Participated in Designed the study, data collection and contributed to the revision of the manuscript.

**Abdolreza Sotoodeh Jahromi, Hasdsan Zabetian, Alireza Yusefi, Hossien Hakimelahi and Abdolhossien Madani:** Carried out data collection, analysis and writing the manuscript.

## Ethics

All dairy companies whose milk were evaluated in this research, will be kept anonymous.

## Competing Interests

The authors declare that they have no competing interests.

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