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Aflatoxin M₁ contamination of cow's raw milk in different seasons from Qazvin province, Iran

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ABSTRACT

Aflatoxins are extremely teratogenic, mutagenic, toxic, and carcinogenic compounds. In the present study, 60 cow's raw milk samples were collected from Qazvin province, Iran during Dec 2015 till July 2016. Enzyme-linked immunoabsorbent assay (ELISA) was applied to determine Aflatoxin M₁ (AFM₁) in the milk samples. AFM₁ was detected in 34 raw milk samples ranging from 6.25×10^{-3} to 127.87×10^{-3} (part per billion). AFM₁ contents in all positive samples were far below the US legal limit (0.5 ppb), but AFM₁ in 30% of the raw milk samples exceeded the EU legal limit (0.05) and 5% of the samples exceeded the Iran legal limit (0.1 ppb). This study indicates a high occurrence of AFM₁ in cow's raw milk especially in winter (40.71×10^{-3} ppb) but the level of contamination were not significantly different in various seasons ($P < 0.05$). Since contamination of milk with AFM₁ is a potential risk for human health, in order to prevent the repetition, milk and milk products should be controlled periodically. The levels of AFM₁ contamination of milk in the present study showed that continuous examining of milk is necessary to improve public health and reduce consumer exposure to aflatoxins. Reducing the levels of AFB₁ in animal feedstuffs can be regarded as initial step to control the transfer of AFM₁ to the humans.

Key words: Milk, AFM₁, ELISA.

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1. INTRODUCTION

Mycotoxins are secondary metabolites produced by *filamentous fungi* (1). Almost 25% of food and food products are affected annually by mycotoxins (3). Among mycotoxins, AFs are extremely toxic compounds produced by three species of *Aspergillus* (*A. flavus*, *A. parasiticus*, and rare *A. nomius*) (1-4). *A. flavus* produces only AFB₁, while the others produce both B and G AFs (3, 4). Under favorable conditions of temperature and humidity, AFs can be produced during any stage of production (including harvesting, storage, transport and processing) (1). When cows in their lactation period consume AFB₁ contaminated feed, this toxin is metabolized to form the monohydroxy derivative, AFM₁; which is appeared in the cow's milk (3, 5, 6). Previous studies have shown that approximately 0.3-6.2% of AFB₁ ingested by livestock is metabolized into AFM₁ and excreted in their milk however, it mainly depends on the genetics of animals, seasonal variation, milking process

and the environmental conditions (7). The AFM₁ is the main hydroxylated metabolite of AFB₁ formed in liver by means of P450 cytochrome enzymes and may be found in milk products obtained from livestock that have ingested contaminated feed (3, 5, 6). The AFM₁ derivative can be detected in milk within 12-24 hr after the first intake of AFB₁, while its concentration decreases to an undetectable level 72 hr after the initial intake is stopped (6). AFs are associated with the incidence of certain types of cancers which provokes a global concern over food safety (8). AFM₁ carcinogenicity is approximately 2-10% that of AFB₁ (2). The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified AFM₁ as 2B group carcinogen (possibly carcinogenic to humans) (9) and this compound causes immunosuppressive, mutagenic, teratogenic and carcinogenic effects, which pose a health concern to humans (1, 10). Mycotoxin detoxification processes of human food are still not efficient in terms of food safety,

nutritional elements retention as well as cost (1). AFM₁ is a very stable AF which neither storage (4, 10) nor thermal processing i.e. pasteurization, autoclaving, ultra-high temperature (UHT) or other methods used in the production of fluid milk, significantly affected its toxin (7, 10).

Since milk is an important part of human diet, especially for children; it appears that milk is one of the most important exposure factors to AFM₁ (11). AFM₁ is the only mycotoxin that has a legal limit in milk all over the

world (12). The EU has implemented the maximum AFM₁ level in liquid milk and dried or processed milk products intended for adults i.e. 0.05 (ppb) and 0.025 (ppb) for milk intended for infants. However, the US Food and Drug Administration stated the maximum permissible level of 0.5 (ppb) in milk (7). Iran, have also conducted national surveys to assess the AFM₁ content in fluid milk in order to taking effective measures to ensure milk safety (Table 1) (6).

Table 1. Acceptable limit of AFM₁ level in liquid milk

| EU(ppb) | ISIRI(ppb) | FDA(ppb) |
|---------|------------|----------|
| 0.05 | 0.1 | 0.5 |

The purpose of the present study is to evaluate the seasonal AFM₁ contamination in raw cow milk distribution centers of Qazvin province for awareness AFM₁ in milk (as the basis for the preparation of other dairy products); for provide health decisions at managerial level.

2. MATERIALS AND METHODS

2.1. Sampling

This study was carried out from December 2015 to July 2016. A total of 60 cow's raw milk samples were collected in different seasons from raw cow milk distribution centers in Qazvin province (15 samples per season). Collected samples were transferred to the laboratory at 2-8 °C then frozen at -20 °C until examining for AFM₁ contamination (13).

2.2. Examining milk samples for AFM₁

The quantitative analysis of AFM₁ in the row cow milk samples was performed by competitive enzyme immunoassay using EuroProxima Aflatoxin M₁ Elisa kit.

Milk samples were chilled to 10°C and then centrifuged at 2000g for 5 min. The upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted supernatant) 200 µl was directly used per well in the test. ELISA test procedure was conducted according to the instructions of kit.

2.3. Statistical Analysis

The results of AFM₁ concentration were statistically analyzed and the data were presented as mean and range. The significant difference (p<0.05) between provinces was determined by one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc, IBM, NY, USA).

3. RESULTS AND DISCUSSION

In this study a total of 60 milk samples were examined for detection of AFM₁ contamination. Obtained results were summarized and shown in Table 2 (comparison based on ISIRI5925 Amendment No. 1).

Table 2. Occurrence of AFM₁ in cow's raw milk samples.

| season | Date (month/year) | Sample category | No | Range of concentration (ppb) Min-Max | Mean ^{NS} (ppb) | n> ISIRI limit ^a |
|--------|-------------------|-----------------|----|--------------------------------------|--------------------------|-----------------------------|
| autumn | Dec/2015 | Raw bulk milk | 15 | <0.00625-0.11929 | 0.03913 | 1(6.66%) |
| winter | Feb/2016 | Raw bulk milk | 15 | <0.00625-0.11730 | 0.04071 | 1(6.66%) |
| spring | June/2016 | Raw bulk milk | 15 | <0.00625-0.09381 | 0.03846 | 0 |
| summer | July/2016 | Raw bulk milk | 15 | <0.00625-0.12787 | 0.03417 | 1(6.66%) |

^{NS} Not Significant

Note: ^a Iran legal maximum level (0.1ppb).

Table 3. Comparative evaluation in term of AFM₁ contamination between countries

| Local of sampling | year | Category of samples | Evidence of AFM ₁ (%) | Contamination upper than EU (%) |
|-------------------|------|--------------------------|----------------------------------|---------------------------------|
| Iran | 2013 | raw and pasteurized milk | 47.91 | 0 |
| Serbia | 2014 | raw cow milk | 98.7 | 86 |
| Iran | 2014 | raw buffalo milk | 54.4 | 16.3 |
| Kosovo | 2016 | raw cow milk | 2.8 | 0 |
| Iran | 2016 | raw cow milk | - | 15.4 |

The linearity of the standard calibration curve was gaged by calculating the coefficient of the regression curve (R²), which stayed not under 0.999. 34 of samples from all

seasons were contaminated with AFM₁ in different levels. Analysis of 60 collected raw milk samples showed that the lowest percentage of samples contaminated with AFM₁

belonged to spring (100% Corresponded to ISIRI5925 Amendment No. 1) (Figure 1).

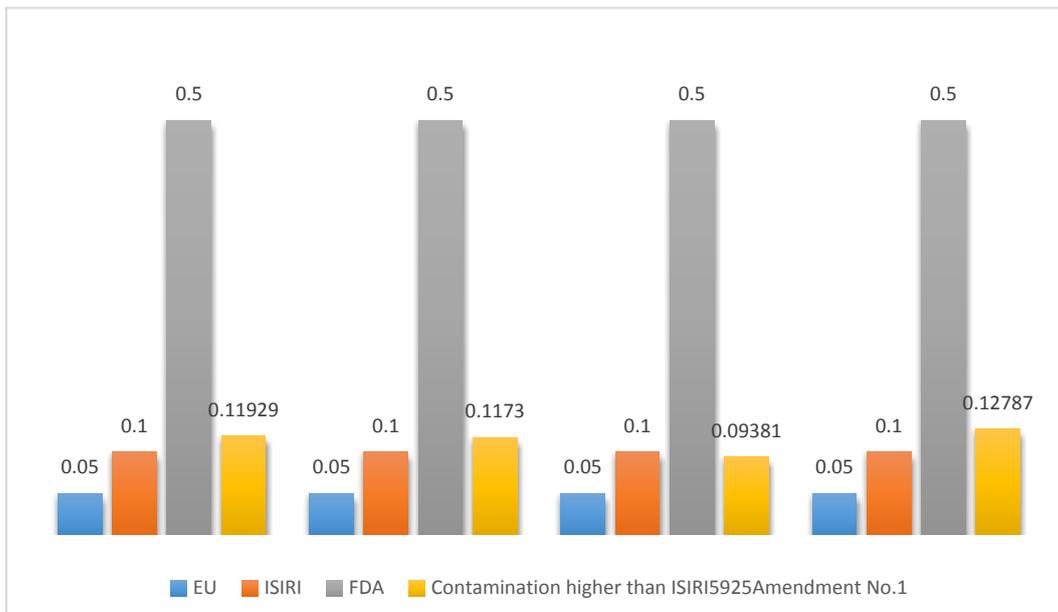


Figure 1. Comparison of the levels of AFM₁ contamination by existing standards (ppb)

The AFM₁ maximum concentration (0.04071 ppb) observed in the winter. As moisture is one of the factors influencing fungal growth and AF production; the prevalence of AF in winter can be attributed to the high rainfall and high humidity in this season. Based on statistical analysis, AF levels of milk samples in four seasons were not significantly differed. Overall, three and 18 samples of whole samples were unsuitable for human consumption according to last Iranian Regulation and EU, respectively 117.30-127.87 ($\times 10^{-3}$ ppb) and 50.25-127.87 ($\times 10^{-3}$ ppb) (23,24). According to the study in Serbia, AFM₁ was detected in 98.7% of analyzed cow's milk samples with concentrations from 0.01 to 1.2 (ppb). In additional, AFM₁ level in 129 (86.0%) cow's milk samples was greater than maximum residue levels (MRL) of 0.05 (ppb) defined by European Union (EU) Regulation (14). The results of AFM₁ contamination in milk in Yazd, Iran showed that the 15.4% of cow milk, 11.5% of sheep milk, and 9.15% of goat milk samples had the AFM₁ level higher than of standard level, (0.05 ppb), while none of the camel milk samples exceeded this limit (15). Examining AFM₁ in raw milk throughout four seasons in Croatia showed the mean AFM₁ levels in the three regions over four seasons were in the ranges (ppb): eastern Croatia (7.25-26.6) $\times 10^{-3}$; western Croatia (5.91-9.26) $\times 10^{-3}$; other regions of Croatia (7.17-13.6) $\times 10^{-3}$. The highest AFM₁ levels were quantified in December (764.4 $\times 10^{-3}$ ppb) and January (383.3 $\times 10^{-3}$ ppb) (16). In an assessment of AFM₁ in milk consumed in Kosovo during 2009–2010, From 895 samples examined by ELISA method, 25 (2.8%) samples were contaminated with AFM₁, none of contaminated samples did not exceed the EU regulation limits (0.05 ppb) (17). The results of AFM₁ contamination of milk samples collected from individual farms in Qazvin province from March to February 2012, showed that the

AFM₁ contamination mean levels in milk in summer and winter were 0.08 and 0.18 ppb, respectively. The AFB₁ contamination level in winter feed (2.27 \pm 1.76) was higher than from summer (0.83 \pm 0.60) (P < 0.05) (18). In a seasonal pattern study on AFM₁ contamination in buffalo milk in the northwestern region of Iran, this mycotoxin was found in 54.4% of the samples by average concentration of 38.5 \pm 5.12 ($\times 10^{-3}$ ppb). The concentration of AFM₁ in all of the samples were lesser than Iranian national standard and FDA limit (0.5ppb), but in 16.3% of the milk samples the concentration of AFM₁ was higher than maximum tolerance limit established by European Union/Codex Alimentarius Commission (0.05ppb) (19). Examination of 144 milk samples (102 raw milk samples and 42 pasteurized milk samples) show that the AFM₁ was detected in 47.91% of the samples by average concentration of 39.45 \pm 18.40 ($\times 10^{-3}$ ppb). The highest mean concentration of AFM₁ (43.9 \pm 9.5 $\times 10^{-3}$ ppb) was recorded in traditional dairy farm samples (20) (Table 3). Traditional dairy farming is a common system in Iran, done mostly by farmers in a system of mixed farming, with animals in support of crop production. As a result, poor foodstuff quality might be related to the residual level of AFM₁ in the milk (21). However, the average AFM₁ contamination of the milk samples in the present study was in agreement with the results of the previously mentioned studies, but there is no significant difference between samples from various seasons (Table 2).

4. CONCLUSION

The results of this study didn't reveal a relatively significant occurrence of AFM₁ contamination in cow's raw milk. Contamination of milk samples with AFM₁ could be regarded as a potential public health problem.

Codex as an international organization is responsible for food-related regulations to facilitate traditional exchange and establish AFM₁ standard in milk about 0.05 (ppb). There is a seasonal trend of AFM₁ contamination in the milk of cow, sheep, and goat, with higher occurrence and levels of the toxin during cold seasons. Appropriate measures for decontamination of animal are feed in order to prevent the producing of fungal toxins in feedstuffs are essential. The government should have more supervision on traditional dairy farms, and these dairies should be gradually replaced by industrial ones.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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