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# Seasonal Survey in Content of Aflatoxin M1 and Somatic Cell Count in Collected Raw Milk Samples from Qazvin Province, Iran

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## ABSTRACT

The presence of aflatoxin M1 (AFM1) and somatic cell (Sc) in milk and milk products is a public health concern; also, it is an important index for evaluating the quality and safety of milk. Therefore, monitoring their level in milk has high health importance. The aim of this study was to determine the content AFM1 and somatic cell count (Scc) in raw cow milk samples in Qazvin province during various seasons (warm and cold). In this cross sectional study, 92 raw cow milk samples (produced by six semi industrial farms) was randomly collected from milk collection centers in Qazvin province (Iran) during the warm and the cold seasons in 2016 (23samples for each season). All samples were examined by Enzyme-linked immunosorbent assay (ELISA) and somatic cell counter; the archived data were analyzed using ANOVA and Chi-square test. The results showed that all collected samples were contaminated with AFM1 and AFM1 in raw cow milk samples that were above the maximum residue limits (MRL) (Iran legal limit = 100 ng $L^{-1}$) in %36.95 samples; in addition, the SCC was above the MRL (Iran legal limit = 500000 cell $ml^{-1}$) in 45.65 % of milk samples. AFM1 Contamination in warm seasons (%52.17) was significantly higher than in cold seasons (%21.73) ($P < 0.05$), but there was not any significant difference between the Scc and season of sampling ($P > 0.05$). According to the results of this research, comprehensive and careful supervising of the production and supply of milk and evaluation of AFM1 and Scc are necessary.

**Key words: Aflatoxin M1, Somatic cell count, Cow milk, Elisa kit, Qazvin.**


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## 1. INTRODUCTION

Aflatoxin has a wide spreading occurrence in different kind of matrices, such as fruits, vegetables, spices, meat, milk, oils, cereals, etc. Peoples can be encountered to aflatoxins by the periodic usage of contaminated food, participating to an incensement in immunosuppression, hepatocellular carcinoma and nutritional deficiencies (1). Aflatoxins are prevalent crop contaminants, that maybe occurred during storage or harvest of milk and milk products. The principal aflatoxins are including aflatoxin G1, G2, B1 (AFB1) and B2. Due to their low molecular weight, once ingested these compounds, they are rapidly adsorbed in the gastro-intestinal tract by a passive transition mechanism (2) and quickly emerge as a metabolite in milk as soon as 12 h post-feeding (3) and in blood after just 15 min (4). Aflatoxins (AFs) produced primarily by two kinds of *Aspergillusfungus* which are usually found in regions with warm and humid climates. Aflatoxins (AFs) produced primarily by three kinds of Aspergillus (*A. flavus, A. parasiticus,* and rare *A. nomius*) which are usually found in regions with warm and humid climates. A.flavus produces only AFB, while the others produce both B and G AFs. (5). Ingested AFB1 is metabolized by the hepatic microsomal cytochrome P450 enzyme family to aflatoxin M1 (AFM1) which can be excreted in the milk of lactating animals and because their products are used by peoples (especially children), the presence of AFM1 in dairy products and milk is very important (6). The level carcinogenic $AFM_1$ is almost 0.1 AFB1and its acute toxicity is similar or slightly lighter than AFB1 (7). The EU, Regulation 2174/2003 (European Union, 2003) set a limit of 50 ng $kg^{-1}$ for AFM1 in milk destined, raw milk for the produce of milk-based

products and heat-treated milk, whereas the Codex Alimentarius Commission (2001) established a limit of 500 ng $kg^{-1}$ (8). Thus, in order to prevent of toxicity, the values of aflatoxins and other similar toxic compounds in foodstuffs should be evaluated carefully and protected with exact control continuously. Otherwise, associated health effects such as chronic and acute intoxications, and even deaths, will be a challenge (9). Dairy cattle are sensitive to many diseases especially mastitis; in this regard, somatic cell count (SCC) is used as a basic indicator for milk quality (10) and it reflects the risk of nonphysiological variations in the milk composition and the health status of the mammarygland. High SCC milk is associated with reduced milk yield (11, 12), as well as increased expenses correlated with culling, treatment, and alterations in milk quality. Total SCC includes leucocytes (such as neutrophils, lymphocytes and monocytes) and epithelial cells; during milk processing, high SCC in milk has adversely effects on cheese production, as a result of reduced curd consistence, increased fat and less casein in whey, decreased milk yield and hazard susceptible quality (13, 14). Moreover, High SCC milk reduces the shelf life of pasteurized liquid milk and effects the production of milk protein concentrate (15, 16). Somatic cell count limits are also a basic component of national and international rules for milk quality (16). The European Union (EU) regulatory SCC limit has been accepted as the international export standard in various countries (16, 17). The United States has a national penalty limit of 750,000 cells $mL^{-1}$ for local utilization (US Food and Drug Administration, 2011), but recently recommended a European Union Health validation Program, determining an SCC threshold of 400,000 cells $mL^{-1}$ for companies exporting productions into the EU (18). Although numerous studies have focused on somatic cell count (SCC) all over the world, the results cannot be generalized to all regions in the world, which can be attributed to differences in race, region, climate, animal nutrition and other environmental factors that affect the collected data (19). In our country, this disease is undoubtedly one of the most important problems in milk industry, and very few studies have focused on the effect of increasing of SCC on the quality of raw milk particularly milk products. Iranian National Standard for SCC is 500000 (20). Considering the importance of milk quality control and also the status of this valuable product, the present study was conducted in order to examine and assess the AFM1 level and somatic cell count in raw cow milk produced in Qazvin province.

## 2. MATERIAL AND METHODS

*2.1. Sampling*

A total of 92 samples of raw cow milk (produced by six semi industrial farms) was randomly collected from milk collection centers in Qazvin province (Iran) during the warm and the cold seasons in 2016 (23 samples for each season). All collected samples were transferred to the laboratory at 2-8 °C and then kept frozen at -20 °C until evaluating for AFM1 contamination.

*2.2. Determination of AFM1 by Competitive ELISA*

The quantitative analysis of AFM1 in the raw cow milk samples was applied by competitive enzyme immunoassay using Euro Proxima AFM1 Elisa kit, the Netherlands (detection limit 0.05 ng $L^{-1}$). Milk samples were chilled to 10°C and then centrifuged at 2000*g* for 5 min. The upper creamy layer was eliminated with aspirating through a Pasteur pipette and from the under phase 200 µl was aright used per well in the exam. ELISA test process was accomplished according to the ground rule of kit (21).

*2.3. Somatic Cell Count*

SCC analysis was performed electronically with flux cytometer (Fossomatic 90; England), and data of SCC was represented as $10^3$ mL.

*2.4. Statistical analysis*

All experiments were performed in triplicate, and Statistical analysis was conducted using Analysis of variance (ANOVA) and Chi-square test with SPSS 17.0 software. Significant level was considered $P<0.05$.

## 3. RESULTS AND DISCUSSION

The mean values of AFM1 and SCC in raw cow milk samples were determined as 97.24±53.3 ng $L^{-1}$ and 657.73±663.64 cells $mL\times10^3$, respectively (Table 1). The results showed that all collected samples were contaminated with AFM1 in ELISA kits. 58 samples (63.04%) were contaminated with less than 100 ng AFM1, Meanwhile 34 samples (36.95%) contained more than 100 ng AFM1 (ISIRI limit). The related results for distribution of AFM1 contamination in raw cow milk samples (ng $L^{-1}$) are presented in Table 2. In addition, the SCC in 50 samples (54.34 %) were less than 500000 cells $mL^{-1}$ whereas in 42 samples (45.65 %) were more than 500000 cells $mL^{1}$ (ISIRI limit). The results about status of somatic cell count (SCC) $ml^{-1}$ in raw cow milk samples presented in Table 3.

**Table 1. Mean values of AFM1 and SCC in raw cow milk samples**

| Season | Sample size | AFM1 concentration(ng $L^{-1}$) | | | | Somatic cell Count (1000 cells mL) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ±SD | Min-Max | n> ISIRI limit | %n> ISIRI limit | Mean ±SD | Min-Max | n> ISIRI limit | %n> ISIRI limit |
| Warm | 46 | 114.76±58.48 | 28-263 | 24 | 52.17[a] | 544.06±410.37×$10^3$ | 50-2383 | 22 | 47.82[c] |
| Cold | 46 | 79.72±41.21 | 17.88-214 | 10 | 21.73[b] | 771.41±850.02×$10^3$ | 108-3458 | 20 | 43.47[c] |
| Total | 92 | 97.24±53.3 | 17.88-263 | 34 | 36.95 | 657.73±663.64×$10^3$ | 50-3458 | 42 | 45.65 |

Data in the column with different letters are different significantly (P < 0.05).

**Table 2. Distribution of AFM1 Contamination in raw cow milk samples (ng $L^{-1}$)**

| AFM1 concentration (ng $L^{-1}$) | Positive samples (n) | Percent |
|---|---|---|
| <50 | 18 | 19.56 |
| 50-100 | 40 | 43.47 |
| 100-200 | 27 | 29.34 |
| >200 | 7 | 7.6 |

**Table 3. Status of SCC $mL^{-1}$ in raw cow milk samples**

| SCC $mL^{-1}$ | Positive samples (n) | Percent |
|---|---|---|
| <100000 | 2 | 2.17 |
| Up to 200000 | 10 | 10.86 |
| 300000 | 15 | 16.30 |
| 400000 | 16 | 17.39 |
| 500000 | 7 | 7.60 |
| >500000 | 42 | 45.65 |

In order to comparison of percent contamination in various seasons (warm and cold) Chi-square test was used and the results indicated that AFM1 contamination in warm seasons (%52.17) was significantly higher than cold seasons (%21.73) (P < 0.05), but there was not any significant difference between the Scc and season of sampling (P > 0.05). In addition, the comparisons of mean values of AFM1 and SCC against existing standards are presented in Figure 1 and Figure 2, respectively.

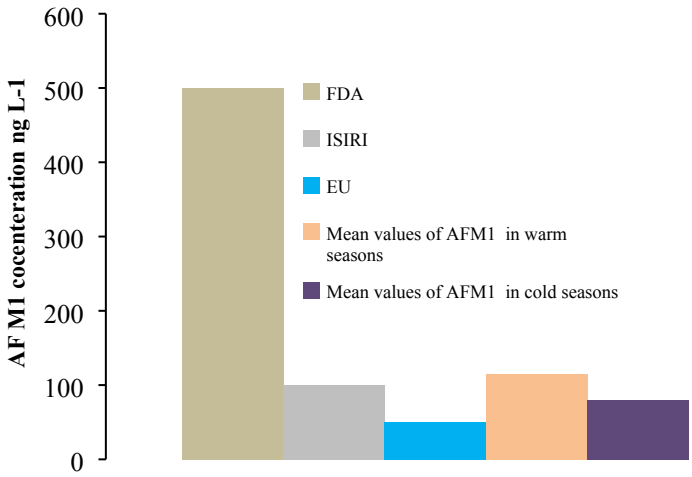


**Figure 1. Comparison the mean values of AFM1 against existing standards (ng $L^{-1}$)**

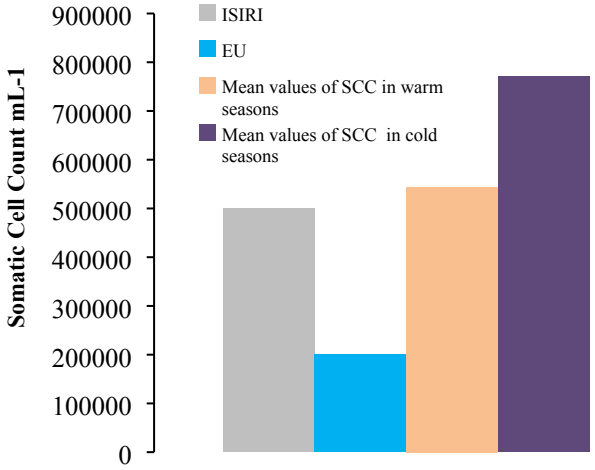


**Figure 2. Comparison the mean values of somatic cell count against existing standards (cells $mL^{-1}$)**

With increasing population growth, preparing adequate healthy food containing protein has become an important global issue. Among animal proteins, milk has special value because it is cheap and has numerous nutritional properties. Given the properties of milk, it is highly necessary for milk producers and industries of dairy products to control this valuable product and assess its components. Producing high quality milk has some challenges qua milk quality has a direct effect on milk processing and the quality of the milk products (22). Mycotoxins are biological materials that affect the production and health quality of foods after the growth of toxin-causing fungi in it. Therefore, it is vital to measure the level of different mycotoxins constantly and make necessary plans to reduce their level in food chain in order to ensure of the consumers' health. In the study carried out in Italy by Kerekes et al, Estimation of dietary exposure was performed based on the AFM1 in the milk samples also on Italian food consumption data. Hazard Indices (HI) and Estimated Daily Intakes (EDI) were calculated for various age groups of the population. The results showed that no harmful effects were expected for the adult population, but in the case of children under age three, the approximate HI values were significantly higher (23). The results of the present study showed that all of the prepared samples were more or less contaminated with AFM1. In general, the level of contamination with AFM1 in milk samples was increased significantly in warm (%52.17) seasons compared to cold seasons (%21.73) ($P < 0.05$) (Table 1). The average of contamination was determined as 97.24±53.3 ng $L^{-1}$; and %36.95 of the samples were contaminated higher than the defined standard level in Iran. There are various reports on the prevalence of contamination of milk samples with AFM1. Several studies that were previously carried out in Iran indicated a high level of contamination in most cases. Kamkar studied 111 raw milk samples produced in Sarab town and reported that the concentration of AFM1 in %40 of the positive samples was higher than the defined limit by the European Union (50 ng $kg^{-1}$) (24). Moreover, in a study that was performed by ELISA method in Shiraz, 100% of the collected pasteurized milk samples were contaminated

with AFM1, and contamination level in 17.8% of them was over 50 ng $kg^{-1}$ (25). In the study carried out in 14 regions of Punjab, Pakistan by Imtiaz and Jamil, %99.4 of the milk samples had concentration of AFM1 over the limit permitted by the European Union (26). Tajkarimi et al studied 98 raw milk samples obtained from milk factories in Golestan, Gilan, Fars, Tehran and Hamedan provinces. They reported the average concentration of AFM1between 41-65 ng $kg^{-1}$, and in all cases it was lower than the standard 500 ng $kg^{-1}$ (United States of America standard and Iran's old standard). The factories were 400 Km away from each other and had different ecological and nutritional conditions for dairy cattle (27). Compared to our results, Sifuentes dos Santos et al and Ghajarbeygi et al observed that there was no significant difference in AFM1 content during warm seasons compared to cold seasons (28, 29). Sifuentes dos Santos et al studied 84 milk samples produced in Brazilian, 43 from organic (15 pasteurized, 28 raw milk) and 41 from conventional (15 pasteurized, 26 raw milk) production systems and reported that the concentration of AFM1 in 63 (75 %) of the positive samples was higher than the defined limit of detection. Also, there was no significant difference in the AFM1 levels in milk samples taken in various seasons (28). Ghajarbeygi et al investigated 60 raw milk samples produced in Qazvin province, Iran during Dec 2015 - July 2016 by ELISA method. AFM1 was detected in 34 raw milk samples ranging from $6.25\times10^{-3}$ to $127.87\times10^{-3}$ (ppb) and contamination level in all positive samples were lower the US legal limit (0.5 ppb), but AFM1 in 30% of the raw milk samples was higher than the defined EU legal limit (0.05) and 5% of the samples exceeded the Iran legal limit (0.1 ppb). This survey represented a high occurrence of AFM1 in raw milk samples especially in winter (40.71×10- 3ppb), but there was no significant difference in the AFM1 levels in milk samples taken in different seasons (29). The results of a study carried out on two hundred and twenty seven. One hundred and ninety two raw and thirty five processed milk samples were produced in Bomet County, Kenya. The found results demonstrated that the overall occurrence of AFM1 contamination higher than the threshold limit of 0.05 ppb defined by Food and Agriculture Organization (FAO) and World Health Organization (WHO) was 43.8% (81/185) (30). In general, effective factors in the emergence of AFM1 in milk and different reasons for the difference among the results of various studies are not completely known. The level of AFM1 is more dependent on AFB1 intake rather than the amount of lactation. Among other factors that affect the presence level of AFM1 in milk, the metabolism of aflatoxin in liver and its excretion rate through other ways such as urine and feces should be considered. Factors that cause difference among the results of the studies that carried out on the amount of toxin excretion through milk are probably related to the difference among mastitis, differences in the amounts and purity of the used aflatoxin, and differences in study methods. In addition, the AFM1 concentration and somatic cell count (SCC) are two important factors in assessment of quality and health of milk. Increasing in SCC as an index of appearance of clinical and subclinical mastitis is associated with decreasing the quality of raw milk and milk products; the related economic loss is estimated about 35 billion dollars per year (31, 32). According to the results of the recent study, it was concluded that SCC was higher than Iranian standard (500000 cell $mL^{-1}$) in %45.65 of the samples, and the mean of SCC in samples was determined as $657.73\pm663.64\times10^{3}$ cell mL. In Iran, numerous studies have focused on SCC. For example, a study was carried out in order to examine the quality of raw milk in animal husbandry tanks of Garmsar (10 traditional husbandries, 10 semi-industrial husbandries, and 10 industrial animal husbandries). In this study, the mean of SCC in the traditional, semi-industrial, and industrial animal husbandries was determined as $5.25\times10^{5}$, $5.13\times10^{5}$, and $4.325\times10^{5}$, respectively (33). The results of a study carried out on 11,000 heads of cattle in Iran indicated that SCC varied from 100000 to 5000000 cell $mL^{-1}$ with a mean ~ 2200000 cell $mL^{-1}$ (34). Furthermore, in a study conducted in Dezful, the total mean of SCC was determined as 342059±18174 (35).

## 4. CONCLUSION

Since some of the raw milk samples were over contaminated with AFM1 and SCC, it is recommended that all relevant organizations especially the authorities of agricultural and veterinary organizations should apply effective actions such as:

- Controlling animal feed
- Feed storage conditions (particularly in warm seasons)
- Raising the dairy farmers' awareness
- Preventing and treating mastitis
- Decreasing somatic cell count by observing health standards and using optimal management methods
- Constantly supervising the performance of dairy farmers, milk collectors and processors.

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## AUTHORS CONTRIBUTION

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## 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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