Brief Communication

Aflatoxin M1 levels in the raw milk produced by a dairy factory and the milk distribution centers in Sanandaj, Iran (2015)

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ABSTRACT

Aflatoxins are fungal toxins with carcinogenic, mutagenic, and teratogenic properties. Aflatoxin M1 (AFM1) is resistant to autoclave, pasteurization temperature, and other food preservation procedures. The present study aimed to measure and compare the levels of AFM1 in 84 raw milk samples collected during six successive months, including 36 samples from a dairy factory and 48 samples from four major local dairy distribution centers. The day of sampling was selected randomly. AFM1 levels were measured using the ELISA assay. In distribution centers number one, two, three, and four, the levels of AFM1 were higher than the permissible limits in 33%, 25%, 8.3%, and 25% of the samples, respectively. Furthermore, the level of AFM1 in 19.44% of the factory samples was higher than the permissible limits in the United States. The mean AFM1 concentration in distribution centers number one, two, three, and four and the dairy factory was 0.0344, 0.243, 0.19, 0.301, and 0.175, respectively. The results of one-way analysis of variance (ANOVA) indicated no significant difference in the mean concentration of AFM1 with (P=0.279). According to the results, it is recommended that more preventive actions be taken in order to control the AFM1 content of livestock feeds and consumed milk in Kurdistan University, Iran.

Keywords: Aflatoxin M1, Raw milk, Dairy factory, Distribution centers

Introduction

Contamination of foodstuffs, especially livestock and agricultural products, with various types of molds is considered to be an important health issue in the food industry. According to the Food and Agriculture Organization (FAO), 25% of the global crop production is contaminated with molds and mycotoxins.[1] Improper harvesting and drying, excessive manipulation of the products, inappropriate packaging conditions, and improper storage and transfer of agricultural products increase the risk of fungal contamination in agricultural crops, as well as mycotoxin production.[2]

Aflatoxins are hazardous fungal products that threaten the health of consumers if they enter milk and other dairy products.[3,4] These harmful substances have cancerous, mutagenic, and mutagenic properties. Aflatoxins also cause acute and chronic toxicity in humans. Several studies have confirmed the adverse effects of these toxic compounds on the central nervous system, liver, kidneys, and brain.[1] If the human diet contains several mycotoxins, the symptoms of the poisoning become more severe depending on the age, gender, and health status of individuals.[5]

The permitted limit for aflatoxin M1 (AFM1) in dairy products is 0.5 µg/l.[6,7] According to the literature, consumption of foods containing aflatoxin leads to liver cancer in various regions in the world.[8] Furthermore, a survey on foods conducted in Cyprus indicated the significant contamination of foodstuffs by aflatoxin.[9] In a study in Taiwan, 13 samples of raw milk and formulas were assessed, none of which contained the AFM1 levels of above the standards in Taiwan[10]. In another research by Hazhir *et al.* performed in Kurdistan province (Iran), 65.91% of milk samples were

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contaminated by aflatoxin[11]. According to the findings of Gholampour Azizi *et al.* in Babol (Iran), 100% of milk samples were contaminated with aflatoxin, and the aflatoxin level was estimated at 230 ng/l, which was significantly higher than the permissible standards of Iran and other countries.[12]

Each year, several studies are carried out in Iran to measure the levels of AFM1 in milk. However, it seems that the responsible authorities do not use the obtained data to take proper action, and alarming data are published every year on the presence of this toxin in consumed milk. Due to the lack of proper action to control the entry of this toxin into milk in Iran, continuous measurements of AFM1 in milk and dairy products could be a practical measure to prevent the distribution and consumption of the products that contain unsafe amounts of AFM1.

Due to the lack of studies in this regard in Kurdistan province in recent years, the present study aimed to investigate the levels of AFM1 in the raw milk samples collected from a dairy factory and local milk distribution centers in Sanandaj, Iran in 2015.

Materials and Methods

This descriptive, cross-sectional study was conducted on 84 raw milk samples, which were collected in the summer and winter of 2015-2016 from a dairy factory and four major milk distribution centers in Sanandaj, including 36 samples from the dairy factory and 12 samples from each distribution center.

The samples were randomly selected, and 10 milliliters of each sample was separated for analysis. In the laboratory, the samples were centrifuged at 3000 rpm at the temperature of 10 ºC for 10 minutes. Following that, the supernatant was thoroughly discarded using a Pasteur pipette, and the underlying fluid was maintained to assess the levels of AFM1 at the temperature of -70 ºC.

After sample collection, a quantitative detection kit of AFM1 was used based on the ELISA method. ELISA is a competitive, immunogenic method based on antibody response. Microtiter wells were covered with an antibody against AFM1. Afterwards, the sample absorbance was measured at the wavelength of 450 nanometers using the ELISA reader, and AFM1 concentrations were calculated after drawing the curve.[13]

Results and Discussion

In order to protect the health of consumers, especially children, against the health risk of infected dairy products, many countries have set different rules to control the levels of AFM1 in raw milk.[7] Table 1 shows the findings regarding the AFM1 (µg/l) levels in the milk samples collected from the milk distribution centers in Sanandaj. According to the information in this table, the mean concentration of AFM1 in distribution centers number one, two, three, and four was 0.342, 0.243, 0.1964, and 0.3017 µg/l, respectively. In addition, the mean concentration of AFM1 in the samples obtained from the dairy factory was estimated at 0.175 µg/l.

Other studies have reported variable results in different countries. For instance, a study conducted in Brazil showed the level of AFM1 to be 0.016 µg/l,[14] while another research in Kenya reported this level to be 0.061 µg/l.[15] In the Middle East, the level of AFM1 was measured to be 0.063 in a meta-analysis.[16] Comparison of the results of the current research with the aforementioned studies shows a significant difference in this regard, which could raise awareness in the studied area of the present study. The standards in the United States have been set at the maximum AFM1 limits of 500 ng/l or 0.5 µg/l in dairy products.[7]

According to the results of the present study, the mean concentration of AFM1 in all the distribution centers and the dairy factory was below the permissible limits of the United States. However, in 33%, 25%, 8.3%, and 25%, of the milk samples that were collected from distribution centers number one, two, three, and four, respectively, the AFM1 levels were above the legislated limits. In addition, the concentration of AFM1 in 19.44% of the samples obtained from the dairy factory was higher than the permissible limits in the United States. Similar studies have also compared the measured AFM1 concentrations with these

limits. According to a research conducted in several cities in China, none of the samples had higher AFM1 contamination than the permissible limits,[17] while another study performed in five regions of Kosovo showed that 5.7% of the samples contained AFM1 level of higher than the legitimate standards.[18]

According to a Tanzania study, 83.8% of the samples had AFM1 contamination of above the permitted limits, which is substantially significant and higher than the results of the present study.[19] In another study in Brazil, the concentration of AFM1 in 13.95% of the samples was observed to be above the permissible limits, and the acceptable daily intake of AFM1 was also calculated in milk samples, indicating that the milk obtained from the studied region was safe for human consumption.[13]

In the current research, the results of one-sample t-test for the samples collected from the distribution centers number one and four indicated that AFM1 concentrations were significantly different between these samples (P=0.004 and P=0.028, respectively) (Table 2). However, the results of one-sample t-test for the samples collected from the distribution centers number two and three and dairy factory showed no significant difference between the samples in terms of AFM1 concentrations (P=0.09, P=0.141, and P=0.111, respectively) (Table 2). Moreover, the results of ANOVA demonstrated no significant difference in the mean concentration of AFM1 concentrations between the samples obtained from the studied regions (P=0.279) (Table 3).

Table 1. Aflatoxin M1 level in milk samples collected at different milk distribution centers and diary factory

	N	Mean	Std. Deviation	Min	Max
Distribution center 1	12	0.3442	0.23735	0.04	0.70
Distribution center 2	12	0.2433	0.26671	0.02	0.70
Distribution center 3	12	0.1900	0.19642	0.05	0.67
Distribution center 4	12	0.3017	0.27521	0.03	0.80
Diary factory	36	0.1750	0.27500	0.00	0.80
Total	84	0.2292	0.26102	0.00	0.80

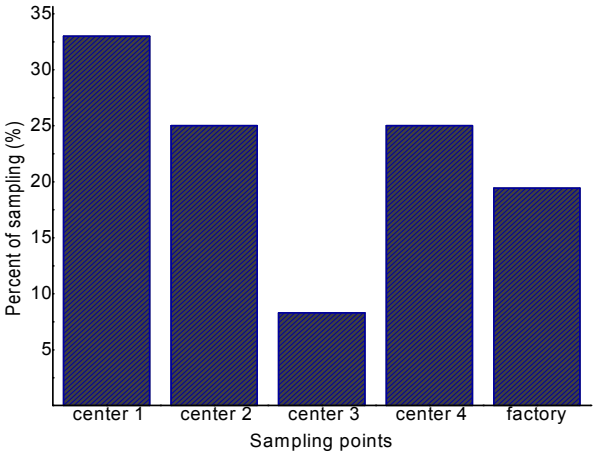

Fig. 1. Percentage of samples with higher AFM1 concentration than permissible limits in different sampling areas

Table 2. Results of One-sample T-test for samples from distribution centers and dairy factory

One sample t-Test	
	P-Value
Distribution center 1	0.004
Distribution center 2	0.09
Distribution center 3	0.141
Distribution center 4	0.028
Diary factory	0.111

Table 3. Comparison of different distribution centers in terms of AFM1 concentration using One-way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.348	4	.087	1.296	0.279
Within Groups	5.307	79	.067		
Total	5.655	83			

Conclusion

Aflatoxins are fungal toxins that could enter livestock milk through the consumption of the fungi-polluted fodder. These compounds have carcinogenic, mutagenic, and teratogenic properties. The pasteurized products that are produced in dairy factories, as well as the purchased raw milk from local dairy distribution centers, could become contaminated with AFM1 since it is resistant to pasteurization and sterilization processes. The present study aimed to measure and compare the AFM1 concentrations in the raw milk samples collected

from a dairy factory and milk distribution centers in Sanandaj, Iran. The raw milk samples were collected in winter and summer, and the results showed that in 33%, 25%, 8.3%, 25%, and 19.44% of the samples obtained from four major milk distribution centers and dairy factory, the AFM1 concentration was higher than the permissible limits in the United States (0.5 µ/l). In order to prevent the AFM1 contamination of dairy products, aflatoxin B1 should be prevented from entering the daily feeds of livestock. Due to the industrialization of animal husbandry in recent years, using concentrated fertilizers for animal fodder has become widespread. In case of the contamination of animal feeds with aflatoxin-producing molds, the level of milk contamination with this toxin has increased significantly. Therefore, it is recommended that the necessary interventions be considered and implemented in this regard.

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