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A survey on the occurrence of aflatoxin M1 in raw milk samples in Bafq and Bahabad, Iran

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ABSTRACT

Aflatoxins are a group of mycotoxins mostly produced by the fungi called *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus nomium*. Aflatoxin M1 (AFM1) is the major metabolite of aflatoxin B1 and is a hepatotoxic and carcinogenic toxin. The aim of this study was to determine the level of contamination of cow's milk with aflatoxin M1 in Bafq and Bahabad. For this study, samples of raw cow's milk were collected randomly from milk collection centers around the city of Bafq and Bahabad from March to April. The determination of aflatoxin M1 levels was based on the ELISA method. Contamination was observed in 100% of milk samples. According to the results of the study, the rate of contamination with aflatoxin M1 in 43.3% of milk samples was above the acceptable level (50 ng/L) in Iranian national standard. It is concluded that further monitoring of milk production should be carried out in the spring and winter seasons.

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1. Introduction

Nowadays, contamination of foodstuffs, including animal and agricultural products with various types of fungi, is one of the most important problems (1). According to estimates (1) by the World Food and Agriculture Organization, 25 percent of all agricultural products in the world are contaminated with mold and mycotoxins. Inappropriate harvesting of crops, improper drying, poor harvesting, poor cultivation conditions, storage and transport of agricultural products increase the fungal contamination of our crops and crop production. At least 18 types of aflatoxins have been identified in nature, of which B1, B2, G1, and G2 are the most important types (2). When animals are fed a diet contaminated with aflatoxin B1 (AFB1), this toxin is metabolized in the liver and is diagnosed as AFM1 in milk 12 to 24 h later. The proportion of AFB1 eaten to AFM1 excreted in milk is between 0.5% and 5% estimated in various sources (1, 3). AFM1 toxicity includes inhibition of RNA encoding and protein synthesis, mutagenesis, teratogenesis, carcinogenesis, liver, kidney, digestive, pulmonary and cerebral disorders as well as immune suppression. Studies have shown that AFM1 toxicity is about one-tenth of AFB1 (4). It is worth noting that AFM1 exhibits resistance to conventional heat treatment such as pasteurization, sterilization, and autoclave (5). Various methods such as TLC, LC, HPLC, and ELISA can be used for AFM1 assay, such as the advantage of ELISA, including reduced preparation time and easy test preparation. Sample extraction is of low cost and high sensitivity. To prevent the entry of aflatoxin M1 through milk into the human food chain, prior to any work, aflatoxin B1 should be avoided in dairy foods (6). However, in Iran there are annual studies of aflatoxin M1 toxin in milk, unfortunately, the authorities responsible do not seem to use the results adequately.

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This study aimed to survay the AFM1 in the milk consumed in Bafq and Bahabad cities, Iran.

2. Materials and Methods

Milk samples: A total of 60 milk samples were collected randomly from different areas of Bafq and Bahabad cities of Yazd province in Iran and the samples were transferred to the laboratory in accordance with health and laboratory principles.

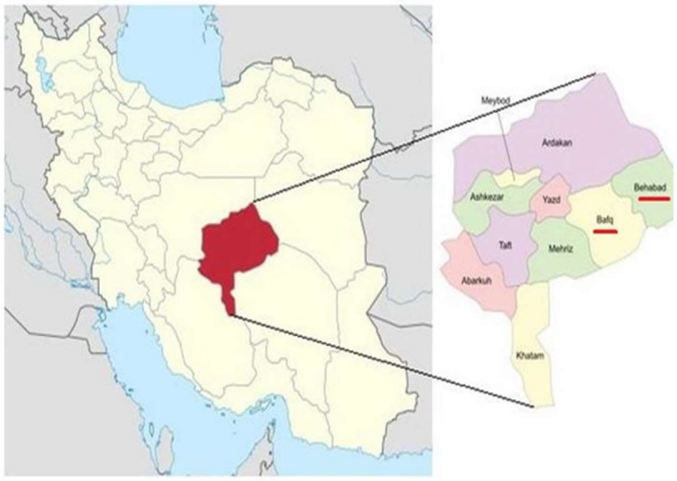

Figure. Yazd province map

Bafgh is one of the cities of Yazd province in Iran. The geographical coordinates of Bafgh are 55.3 to 19.56 east longitude and 195.19 to 32.3 north latitude. Bafgh city is adjacent to Bahabad city from the east and Kerman province from the south. Bahabad is a city in the easternmost part of Yazd province and at a distance of 200 km from Yazd city and 80 km from Bafgh city, on a satellite hill between two mountain ranges with geographical coordinates of 55 degrees and 36 min east longitude and 31 degrees and 33 min to 32 degrees and 29 min located north latitude.

Sample Preparation: All samples of cold milk were centrifuged in a refrigerated centrifuge at 3500 for 10 min and their fat layers were removed. Then, 100 µl of lean milk sample was removed for AFM1 assay.

AFM1 assay by ELISA method: In this experiment, AFM1 ELISA kit (Germany, Biopharm-R) was used to measure AFM1 in milk. The test was performed according to the kit manufacturer's instructions. In summary, 50 µl of each of the standards (0, 250, 500, 1000, and 2000 ng/L was added to 50 µl of lean milk sample in the well. Then 50 µl of conjugate and 50 µl of antibody were added to each well, respectively. The kit was manually moved several times in different directions to blend all the contents of each well perfectly. The kit was kept at room temperature for 10 min. After 10 min all contents of the kit were removed and the kit was washed three times with distilled water. The kit is flipped upside down to drain all the distilled water in the wells and dry completely. Then 100 µl of the chromogen solution was added to each well and the kit was manually moved several times in different directions to mix all the well contents. The kit was then placed in the dark for 5 min. After 5 min, 100 µl of stop solution was added to each well. The kit was shaken several times and then measured with a 450 nm wavelength ELISA reader (Germany, Tech-Bio) and AFM1 concentration calculated.

3. Results

In this study, 100% of milk samples collected from both cities were contaminated with AFM1. However, 13 samples had aflatoxin levels above the Iranian standard (50 ng/L).

Table. Comparison of aflatoxin contamination in Bafq and Bahabad samples

City	Total number of samples	Samples above the Iranian	Aflatoxin M1 (mean ± SD) ng/L		Percentage %
			Winter	Spring	
Bahabad	30	13	33.2±1.6	27. 1±1.1	43.3
Bafq	30	13	41.7±1.9	25.6±0.7	43.3

4. Discussion

Nowadays, it has been shown that aflatoxin M1 in milk is the result of animal feed contaminated with aflatoxin B1 toxin-producing molds (7). Also, the observable differences in the studies can be due to the regional, climatic and species differences of livestock breeding systems in different countries (8). Due to industrialization of livestock systems in Iran in recent years, the use of concentrate and animal feed has become common, and in case of contamination of feed with aflatoxin-producing molds, the rate of milk contamination with this toxin may increase. Although, it is somewhat difficult to prevent aflatoxin formation before harvesting in the field due to high humidity and heat, proper storage of these products can significantly reduce aflatoxin levels (9). Of course, given the country's approach to the development of animal husbandry and milk production, the use of management practices in livestock breeding and the regular control of livestock feed contamination and feed utilization systems could reduce the AFM1 occurance in obtained milk (10).

Various studies have been conducted on the contamination of raw milk with AFM1 in other countries. In a study in China, 280 (52.8%) of the 530 raw milk samples were infected with AFM1, and 28.9% of them had concentrations exceeding the standard limit of 50 ng/L (11). The incidence of AFM1 contamination in raw milk collected in Turkey was 30.1% which 17% of the samples having concentrations greater than 50 ng/L (12). Also in a study in Tabriz, which was conducted on the growth of infants who were exclusively breastfed in both urban and rural areas, AFM1 was observed in the breast milk of 20 out of 91 mothers (22%) in rural areas. AFM1 contamination was not present in urban samples. The presence of AFM1 was significantly associated with local milk consumption and stunted growth in children(13).

In another study in Shiraz, Iran, which collected 624 samples of pasteurized milk over six months, (17.8%) of the samples had aflatoxin more than the maximum tolerance accepted by the European Union (14). In another study in Tabriz, Iran, 50 samples of pasteurized milk were examined for 6 months which 62% of the samples had aflatoxin more than the maximum tolerance accepted by the European Union. It can be concluded that currently AFM1 level in samples purchased in Tabriz is a serious public health problem (15). In another study in Mashhad, Iran, 110 samples of pasteurized milk were collected from supermarkets in the city during three months in the spring season. AFM1 was found in (100%) milk samples. About (5.4%) of the samples contained AFM1 higher than the maximum limit accepted by the European Union. There was no significant difference in the mean amount of AFM1 in three months (16). Due to the toxic and carcinogenic effects of aflatoxins, investigating and inhibiting them in food is important. By carefully controlling, the formation of aflatoxins can be largely prevented.

Due to the structure of aflatoxins, a number of processes used to degrade aflatoxins affect the double bond in the lactone ring and cause a gap in the structure. To remove aflatoxins, methods such as physical methods including heating, microwave, gamma rays and UV, chemical methods such as the use of chlorine, hydrogen peroxide, sodium bisulfate, ammonia, ozone, bases, bioavailable agents and mechanical methods can reduce and eliminate aflatoxins (17).

5. Conclusion

Milk producing units with toxin levels above the permitted level can produce unhealthy dairy products. It is recommended that aflatoxin residual level standards to be updated continuously. Contaminated animal feed and milk must be avoided and continious surveillance programs for monitoring are demanded.

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Conflict of interest

Authors declare to have no conflict of interest.

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