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Determination of Aflatoxin M1 in Buffalo Milk and Products

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A B S T R A C T

This study aimed to investigate the presence of Aflatoxin M1 (AFM1) in raw buffalo milk and buffalo milk products such as cheese, yogurt, cream, and ice cream by ELISA technique. In the study, 175 samples were investigated, and it was determined that 146 (83.43%) of the samples had AFM1 concentrations below the LOD (limit of detection) value. In 29 samples (16.57%), the AFM1 concentration exceeded the LOD value, and in 7 (4%) of these, the AFM1 concentration was above the legal limits (50 ng/L). While AFM1 was not detected in raw buffalo milk samples, the presence of AFM1 in cheese, yogurt, cream, and ice cream samples was found to be 2.85%, 2.85%, 62.85%, and 14.3%, respectively. 2.85% of cheese samples and 17.1% of cream samples were found contaminated with AFM1 above the allowed legal limits (50 ng/L). As a result, it was determined that the potential for exposure to AFM1 by consuming buffalo cream was higher than the consumption of buffalo milk and other buffalo milk products. However, the detection of AFM1 above legal limits in buffalo milk products poses a serious risk to public health. Therefore, it is extremely necessary for public health to develop effective monitoring programs to control aflatoxin contamination in milk and new strategies to maintain inspections.

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Introduction

Nutrition is one of the basic requirements for the continuity of life. Adequate and balanced nutrition is needed for a quality life, the metabolic processes of which are in a regular course.

The concept of "adequate and balanced nutrition" includes the total intake of the necessary energy for the organism to maintain its daily life healthily and the supply of this energy from all the required macro and micronutrients (Firmansyah et al., 2021). Milk and dairy products provided by nature and considered the main elements of a balanced diet are a source of proteins, carbohydrates, vitamins, and essential oils for people of all age groups, from infants to adults (Hussain et al., 2010). The consumption of milk and dairy products in the world is becoming more critical every day, and in this regard, milk production is also constantly increasing (FAO, 2021). Naturally, this increase in the world also affects the production in our country. As an example of this situation, we can show that milk production, which was 858,017 tons in Türkiye in 2019, increased by 6.1% and reached 10,434 tons in 2020 (TUIK, 2020). It has been observed that buffalo milk production in the world has increased by about 2.5% more than cow's milk's annual growth rate (FAO, 2021). Despite all these data, our country's 2019-2020 showed a yearly decrease in buffalo milk production (29.9%) (TUIK, 2020). Milk and dairy products are indispensable foods for people of all age groups. However, the presence of AFM1 in milk and dairy products poses severe risks to the consumer (Baysal, 2011).

Aflatoxin contamination is considered a ubiquitous health risk for humans and animals, and the FAO emphasizes that about 25% of plant-derived products worldwide are contaminated with aflatoxin (FAO, 2004). Among the significant aflatoxins, AFB1, AFM1, and AFG1 are classified as Group 1 carcinogens (IARC, 2002). Aflatoxin exposure causes liver failure, encephalopathy, Reye's syndrome; on the other hand; it negatively affects the growth process of fetuses and newborns (Bennett and Klich, 2003). While it causes different types of cancer, mainly resulting in liver metastasis, it also causes deterioration in liver and kidney functions. Also, high levels of aflatoxin consumption can cause death due to liver damage. The lethal dose is set at 20-120 µg/ kg body weight per day for 1-3 weeks (IARC, 2002).

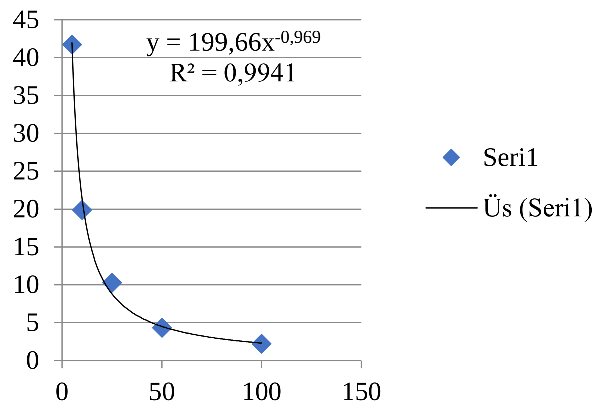

Figure 1. Calibration curve

Along with all this, aflatoxin contamination in the feed included in the nutrition of lactating animals is considered the greatest potential risk for transmission of AFM1 to humans. The study determined that the conversion of 5 mg of AFB1 taken by animals to the form of AFM1 in their milk took an average of 15 minutes. This clearly shows the rapid absorption and biotransformation of AFB1 to AFM1 (Moschini et al., 2006).

Studies on the presence of AFM1 contamination in milk and dairy products are ongoing worldwide to provide data on aflatoxin exposure and analyze the health risks associated with chronically low doses of AFM1 intake. It was stated that although AFM11 is less toxic than AFB1, it is not affected by pasteurization, storage, and processing conditions. Therefore, it is crucial to determine the maximum legal limits on dairy products and develop measuring methodologies (Ketney et al., 2017).

This study is planned to determine the presence and contamination level of AFM1 in buffalo milk and buffalo milk products sold in Samsun, where 12% of the number of buffalo in Türkiye and 13% of the amount of buffalo milk are produced (TUIK, 2020). In Samsun, no studies related to AFM1 were found in different varieties of buffalo milk products.

Material and Method

Material

In this study, a total of 175 samples (raw buffalo milk (n:35) and cheese (n:35), yogurt (n:35), cream (n:35), and ice cream (n:35) from Samsun/Türkiye were analyzed. Samples were obtained from public markets, local businesses, animal breeders, and different outlets for six months between January and June 2021. While sterile bottles of at least 500 ml were used to collect raw milk samples, 500-gram sterile bags were used for dairy products.

Method

In our study, AFM1 levels were determined in raw buffalo milk and samples produced from buffalo milk by ELISA (Enzyme-Linked Immunosorbent Assay) method. AgraQuant® Aflatoxin M1 High Sensitivity COKAQ7300 (10002120_10002121) test kit was used in the analysis. According to the manufacturer's recommendations, samples were prepared and analyzed at an absorbance at 450 nm (reference wavelength 630 nm). The LOD value was determined as five ppt, and the upper limit specified in the Turkish Food Codex (TGK) Contaminants Regulation was 50 ppt. The calibration curve created with the standards and absorbance values used in the study is shown in Figure 1.

Results

A total of 175 samples were analyzed in this study. It was determined that 146 (83.43%) samples had AFM1 concentrations below the LOD (limit of detection) value. In 29 of the samples (16.57%), the AFM1 concentration exceeded the LOD value, and in 7 (4%) samples, the AFM1 concentration was found to be above the legal limits (50 ng/L, 50 ppt).

While AFM1 was not detected in raw buffalo milk samples, the presence of AFM1 in cheese, yogurt, cream, and ice cream samples was found to be 2.85%, 2.85%, 62.85%, and 14.3%, respectively. 2.85% of the cheese samples and 17.1% of the cream samples were contaminated with AFM1 above the allowed legal limits (50 ng/L).

An overview of AFM1 minimum and maximum values and standard deviations of all samples are given in Table 1.

Discussion

The data obtained from this study were discussed by comparing with the data of similar and different investigations in which AFM1 concentrations were determined in buffalo milk and dairy products in Türkiye and the world.

Kara and Ince (2014) used 126 buffalo and 124 cow's milk to detect AFM1 in Afyonkarahisar province. They also reported that none of the cow's milk samples exceeded the LOD (detection limit) value in AFM1. 36 (27%) buffalo milk samples were contaminated with AFM1, but no samples exceeding legal limits were found. The results of this study are similar to our values in terms of sample rates that exceed the legal limit.

In another study conducted in the Aksaray region, Kevenk (2021) investigated 250 buffalo milk obtained from breeders and reported that 76 (30.4%) samples were contaminated with AFM1. However, none of the samples have been reported to contain AFM1 above legal limits. These results are also similar to the values reached in our study when evaluated in terms of sample ratios that exceed legal limits.

In another study conducted in Afyonkarahisar Kara and İnce (2018), 90 cream samples were analyzed, and 23/90 (32.2%) samples were reported to be contaminated with AFM1. However, it has been reported that none of the samples exceeded the legal limits. The results of this study were found to be lower than the results obtained in our study.

Bahraman et al. (2020) investigated the presence of AFM1 in raw, pasteurized, and UHT milk samples from buffalo, cow, sheep, goat, and camel in Iran. According to the results of their study, 82% of their samples were contaminated with AFM1. These determined values were found to be higher than our study.

Table1. Distribution of Samples by AFM1 Amount

	<5	5- 10	10– 25	25- 50	> 50	Min	Max	SS
Cheese	34	*	*	*	1	1.88	162.94	27.08
Yogurt	34	1	*	*	*	2.08	8.24	1.09
Cream	13	5	5	6	6	2.23	101.01	27.04
Ice-cream	30	5	*	*	*	0.89	7.19	1.32
Milk	35	*	*	*	*	1.96	2.75	0.18

Ghariby (2017) reported that all 60 raw buffalo milk samples were contaminated with AFM1 in Iran. On the other hand, they also reported that 54 (90%) samples exceeded the legal limits in their study. It was observed that the reported analysis results were higher than the results obtained in our study.

Nile et al. (2016) found AFM1 contamination of 38.6%, 45.3%, 36.6%, and 33.3%, respectively, in a total of 600 milk samples obtained from 150 buffalo, 150 cows, 150 sheep, and 150 goats in India. It was determined that the AFM1 concentrations of the samples were 16%, 44%, 12%, and 10%, respectively, above the limit value. The prevalence of contamination exceeding the legal limit they found for buffalo milk is higher than the values determined in our study.

Mahmoudi and Zare (2014) reported that in their study in Iran, all 30 raw buffalo milk samples were contaminated with AFM1. In addition, 12 samples (40%) of AFM1 levels were found above the legal limits. These data are higher than the analysis results obtained in our study.

Kamkar et al. (2014) reported that in a study conducted in Iran with 60 samples of buffalo milk, 69% of the samples had AFM1 contamination above the detectable limit. The sample rate exceeding the legal limit was found to be 28%. These results were found to be higher than our study.

Hussain et al. (2010) investigated the prevalence of AFM1 in Pakistan with 55 buffalo, 40 cows, 30 goats, 24 sheep, and 20 camel milk samples. In 19 (34.5%) buffalo milk samples, it was reported that the level of AFM1 was above the detectable limit. These values were higher than the values reached by our study.

Rahimi et al. (2007) reported that 29/75 (38.6%) buffalo milk samples were contaminated with AFM1 in their AFM1 screening study. Also, it was reported that the AFM1 level in 6 (8%) samples was above the legal limits. The analysis results determined in this study were higher than the results we reached.

Different AFM1 formations and levels in milk and dairy products obtained from animals of the same species depend on many external factors and variables. In samples collected from different geographies, Aflatoxin exposure varies depending on climatic conditions and feed quality. Similarly, studies conducted in nearby geographies are associated with the sampling season. However, agricultural policies also differ from state to state, and the production, transfer, and storage conditions of feeds used in animal feeding also vary. Considering all these variables, it was concluded that the milk of buffaloes consuming these feeds might contain different amounts of AFM1(Oruç et al., 2006; İşleyici et al., 2012; Alatalı, 2017).

Conclusion

The first step in assessing human exposure to chemicals and toxins is to determine the likelihood of the chemical or toxin occurring in the relevant environment. Detoxification technologies applied against toxins in milk and dairy products are not yet satisfactory regarding food safety, preservation of food composition, and economic values. Therefore, further studies are needed to minimize the health risks associated with these toxins. Today, the most critical strategy for reducing the risk of exposure to aflatoxins in both humans and animals is the effectiveness of monitoring programs.

The presence of the toxin in foods indicates the need to monitor AFB1 in animal feeds. The presence of AFM1 in milk and dairy products poses a significant risk, especially for children and those who consume these products heavily in their daily diets. Regarding the findings, further studies are recommended to determine the effect of AFM1 detection methods on milk formation and contamination levels.

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