SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 132-140, DOI: 10.24323/akademik-gida.1350944

Research Paper / Araştırma Makalesi

Aflatoxin M1 Levels in Milk Samples Produced in the Northern Part of Cyprus

Cengiz Bereket[1,2], Gozde Girgin[2], Gonul Sahin[1]

[*1]Eastern Mediterranean University, Faculty of Pharmacy, İbn-i Sina Street, 99628, Famagusta, North Cyprus, via Mersin 10 Türkiye
[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100, Sıhhiye, Ankara, Türkiye

Received (Geliş Tarihi): 27.08.2022, Accepted (Kabul Tarihi): 07.06.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): cengiz.bereket@emu.edu.tr (C. Bereket)
+90 392 630 24 01 +90 392 630 2819

ABSTRACT

Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of aflatoxin B_1 (AFB_1), which is formed in the liver by cytochrome P450 enzymes and can be secreted into the urine, feces, and milk of mammals. AFM_1 is a carcinogenic, cytotoxic, teratogenic, mutagenic and genotoxic agent that poses a significant health risk to both humans and animals. This study was conducted to determine the presence of AFM_1 in both raw and ultra-high temperature (UHT) cow's milk samples produced in the northern part of Cyprus, and to determine whether it poses a risk to public health. In this survey, a total of 20 UHT cow's milk samples from 2 different milk brands produced in the northern part of Cyprus, and 22 raw cow's milk samples collected from the different dairies were analyzed for the presence of AFM_1 by high performance liquid chromatography (HPLC) with fluorescence detector after immunoaffinity cleanup. AFM_1 could not be detected in any of the analyzed raw and UHT cow milk samples. The LOD and LOQ values of the HPLC-FLD method were 1.038 µg/kg and 3.145 µg/kg, respectively. The mean recovery and repeatability values of the method were 95.6% and 4.9%, respectively. Although the presence of AFM_1 in milk samples produced in the northern part of Cyprus poses no major risk to public health, more milk samples and animal feed must be monitored on a regular basis to decrease potential consumer exposure.

Keywords: Mycotoxin, Aflatoxin M_1, Milk, High performance liquid chromatography (HPLC)

Kıbrıs'ın Kuzeyinde Üretilen Süt Örneklerinde Aflatoksin M_1 Düzeyleri

ÖZ

Aflatoksin M_1 (AFM_1), sitokrom P450 enzimleri tarafından karaciğerde oluşan ve memelilerin idrar, dışkı ve sütüne salgılanabilen, aflatoksin B_1 (AFB_1)'in hidroksillenmiş metabolitidir. AFM_1 karsinojenik, sitotoksik, teratojenik, mutajenik ve genotoksik bir ajan olduğundan hem insanlar hem de hayvanlar üzerinde önemli sağlık riskleri teşkil etmektedir. Bu çalışma, Kıbrıs'ın kuzeyinde üretilen çiğ ve ultra yüksek ısı (UHT) inek sütü örneklerinde AFM_1 varlığını tespit etmek ve halk sağlığı açısından risk oluşturup oluşturmadığını belirlemek amacıyla yapılmıştır. Çalışmada, Kıbrıs'ın kuzeyinde üretilen 2 süt markasından toplam 20 UHT inek sütü ve farklı mandıralardan toplanmış 22 adet çiğ inek sütü örneği, immünoaffinite kolon temizlemesinden sonra floresans dedektörlü yüksek performanslı sıvı kromatografisi (HPLC) ile AFM_1 varlığı yönünden analiz edilmiştir. Analiz edilen çiğ ve UHT inek sütü örneklerinin hiçbirinde AFM_1 tespit edilebilecek düzeyde bulunamamıştır. HPLC-FLD yönteminin LOD ve LOQ değerleri sırasıyla 1.038 µg/kg ve 3.145 µg/kg idi. Yöntemin ortalama geri kazanım ve tekrarlanabilirlik değerleri sırasıyla, %95.6 ve %4.9 olarak bulunmuştur. Kıbrıs'ın kuzeyinde üretilen süt örneklerindeki AFM_1 içeriği, halk sağlığı açısından önemli bir risk teşkil etmese de daha fazla sayıda süt örneğinin ve hayvan yeminin sürekli takibi, olası tüketici maruziyetini azaltmak için gereklidir.

Anahtar Kelimeler: Mikotoksin, Aflatoksin M_1, Süt, Yüksek performanslı sıvı kromatografisi (HPLC)

INTRODUCTION

Aflatoxins are secondary metabolites produced by diverse species of the fungal genus *Aspergillus* and are the most investigated group of mycotoxins [1, 2]. *A. parasiticus* and *A. flavus* are the most prevalent strains that produce aflatoxins under particular conditions (relative humidity above 70%, 24-35°C, 2.5-6.5 pH). *A. nomius*, *A. pseudotamarii*, *A. bombycis*, *A. ochraceoroseus*, and *A. australis* strains, on the other hand, are only seldom capable of producing them. Aflatoxins occur naturally in a wide variety of food commodities such as cereals (corn, sorghum, wheat, rice), oilseeds (soybean, peanut, sunflower, cottonseeds), spices (cayenne pepper, black pepper, coriander, turmeric, ginger), nuts, dried fruits, milk, and meat [3–6]. Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are the most prominent ones among the more than 20 types of aflatoxin molecules identified [7]. While acute exposure to high doses of aflatoxins in mammals usually results in hepatotoxicity, nephrotoxicity, and in some cases death; chronic exposure causes a variety of toxic effects, including immunosuppression, teratogenicity, carcinogenicity, mutagenicity, cytotoxicity, reproductive and estrogenic disorders [8, 9].

AFB_1 is the most well-known human hepatocarcinogen and the most potent hepatotoxin among the aflatoxins [10]. According to research, AFB_1 is involved in 4.6-28.2% of hepatocellular carcinoma cases, which is the third leading cause of cancer death worldwide [6]. The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence for the carcinogenicity of AFB_1 in humans and has classified this mycotoxin as a “Group 1” carcinogen [9, 11].

AFM_1, the hydroxylated metabolite of AFB_1, is formed in the liver by microsomal enzymes and is excreted into urine, feces, and milk in mammals [12, 13]. AFM_1 has approximately 10% carcinogenicity and 10% mutagenicity of AFB_1, according to *in vivo* and *in vitro* studies, respectively [14]. Hence, AFM_1 has been identified as a potential risk to human health and has been classified as a “Group 1” carcinogen by the International Agency for Research on Cancer [3]. Humans are exposed to AFM_1 mostly through the consumption of contaminated milk and dairy products or through AFB_1 metabolism in the liver. AFM_1 may be present in milk and dairy products, since it is the major excretion product in the milk of lactating animals fed with AFB_1-contaminated diets. According to the data from several research, the carry-over rate of AFB_1 as AFM_1 into the milk of dairy cows varies between 0.3% and 6.2%. The extent of carry-over of AFB_1 from feed to milk in dairy cows is influenced by various factors including season, animal feeding regimen, milking mode, rate of ingestion, rate of digestion, hepatic biotransformation capacity, lactation stage, and actual milk production [14–16].

Since the presence of AFM_1 and its by-products in milk and dairy products causes global concern, many countries have established the maximum acceptable limits for AFM_1 in milk and dairy products, and legislative laws have been drafted accordingly. For example, the European Union (EU) has set the maximum acceptable AFM_1 limit for adults in raw milk, milk for manufacturing of milk-based products and heat-treated milk at 0.05 µg/kg (*ppb*). However, the more restrictive maximum acceptable limit for AFM_1 was set at 0.025 µg/kg for infant formulae and follow-on formulae, including infant milk and follow-on milk [17]. In accordance with European Union regulations, Turkey and the northern part of Cyprus have set the maximum AFM_1 level in milk and dairy products for adults and infants at 0.05 µg/kg and 0.025 µg/kg, respectively [18].

Due to their high stability, no significant reduction in the amount of AFM_1 in milk and milk-based products has been reported with pasteurization, ultra-high temperature, sterilization, cooking, ionizing radiation, addition of enzymes, and other conventional food processing methods [19, 20]. Therefore, the most effective strategy to prevent the occurrence of AFM_1 in the food chain is to inhibit mold growth in agricultural products and the subsequent production of AFB_1 in livestock feed [21].

The presence of AFM_1 in milk and dairy products is one of the most public health issues. The presence of this toxin in milk, even at low levels, poses a risk for consumers of large quantities of milk such as children and adults, especially in long-term exposure [22]. Although milk and dairy products are the primary sources of nutrition for infants, children, and adults, there is no literature data on the determination of AFM_1 levels in milk samples consumed in the northern part of Cyprus. To date, only a study on the determination of AFM_1 levels in Cypriot traditional cheese (Hellim), has been reported by Öztürk et al. [23].

This study aimed to measure the AFM_1 levels both in ultra-high temperature (UHT) cow's milk samples produced in the northern part of Cyprus and raw cow’s milk samples collected from several local dairy farms, and evaluate them in terms of current limits.

MATERIALS and METHODS

In this study, a total of 20 UHT cow's milk samples, 10 from each of the two dairy companies produced in the northern part of Cyprus, and 22 raw cow's milk samples obtained from different dairy farms were analyzed for the presence and concentration of AFM_1. On September 2020, 20 UHT cow's milk samples, all of which were manufactured on different dates, were collected from various markets in their original packaging (200 mL or 1 L) and transported to the Eastern Mediterranean University, Faculty of Pharmacy Laboratory within a cold chain. These samples were stored in the refrigerator at +4°C and analyzed as soon as possible. In September, 22 raw cow’s milk samples from 22 distinct cows were collected from dairy farms in three different villages in the Famagusta region by using 50 mL falcon tubes. Raw cow milk samples were transported to the Eastern Mediterranean University Faculty of Pharmacy

Laboratory within a cold chain and kept in the refrigerator at -20°C until analysis.

AFM_1 levels in milk samples were detected by high performance liquid chromatography (HPLC) coupled with a fluorescence detector after immunoaffinity column (containing monoclonal AFM_1 antibodies immobilized to a solid support) purification. For sample preparation and immunoaffinity column (IAC) clean-up, the manufacturer's (R-Biopharm Rhone LTD.) protocols were followed [24].

Preparation of UHT Cow's Milk Samples

Prior to the analysis,100 mL of the milk samples were warmed to 37°C in an ultrasonic bath, then centrifuged at 4,000 rpm for 20 minutes. After centrifugation, the remaining fat on the surface was separated and discarded. In order to completely remove the fat layer, milk samples were filtered through whatman No:4 filter paper. 50 mL of the filtrate were passed through the immunoaffinity column (RP70N Easi-Extract Aflatoxin) at a flow rate of 2 mL per minute. Following the passage of the filtrate through the column, the column was washed with 20 mL of phosphate buffered saline (PBS) at a flow rate of approximately 5 mL per minute. Afterwards, the air was passed through the column to remove residual liquid. 1.25 mL of methanol: acetonitrile (40:60, v/v) solution was passed through the column at a flow rate of 1 drop per second to elute the AFM_1 from the column and collected in an amber glass vial. Backflushing was applied and repeated 2-3 times during this process. Following the elution, 1.25 mL of water was passed through the column and collected in the same vial to give a 2.5 mL total volume. The eluate collected in the amber vial was mixed by vortex and injected into the HPLC system in a volume of 100 µL.

Preparation of Raw Cow's Milk Samples

Minor changes were made in the preparation of raw cow's milk samples compared to UHT cow's milk samples. According to these changes, 100 mL of the milk samples were centrifuged at 4,000 rpm for 20 minutes. After centrifugation, the remaining fat on the surface was separated and discarded. To increase the filtration rate and prevent clogging of the immunoaffinity column, milk samples were warmed to 37°C in an ultrasonic bath. After warming, milk samples were filtered through whatman No:4 filter paper to remove the fat layer completely. 50 mL of the filtrate were passed through the immunoaffinity column (RP70N Easi-Extract Aflatoxin) at a flow rate of 2 mL per minute. Following the passage of the filtrate through the column, the column was washed with 20 mL of phosphate buffered saline (PBS) at a flow rate of approximately 5 mL per minute. Afterwards, the air was passed through the column to remove residual liquid. 1.25 mL of methanol: acetonitrile (40:60, v/v) solution was passed through the column at a flow rate of 1 drop per second to elute the AFM_1 from the column and collected in an amber glass vial. Backflushing was applied and repeated 2-3 times during this process. Following the elution, 1.25 mL of water was passed through the column and collected in the same vial to give a 2.5 mL total volume. After mixing the eluate by vortex, it was filtered through an RC 0.45 µm membrane filter into a new amber vial. Finally, 100 µL of the eluate collected in the amber vial was injected into the HPLC system.

High Performance Liquid Chromatography (HPLC) Analysis

Agilent Technologies 1260 Infinity HPLC system (SEM Lab Inc., Turkey) equipped with an autosampler and fluorescence detector (Agilent FLD cell 8 µL, Germany) was used for AFM_1 analysis. The excitation and emission wavelengths of the fluorescence detector were set to 365 and 435 nm, respectively. An Inertsil ODS-3V 4.6x150 mm column was used and the column temperature was set to 25°C. The isocratic mobile phase (acetonitrile/water, 25:75, v/v) with a flow rate of 1 mL/min was used. The retention time of AFM_1 was determined by injecting 100 µL of AFM_1 reference standard into the HPLC, which was prepared for analysis by passing the mobile phase for a certain period of time. Quantification was performed based on the calibration curve constructed using AFM_1 reference standard solutions for a total of six concentration points (2, 5, 10, 20, 50 and 100 µg/L).

Method Validation

The linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and recovery of the method were validated with an in-house model according to the decision of the European Union Commission 657/2002 [25].
Linearity was evaluated by injection of AFM_1 reference standard solutions at 6 concentration points (three replicates for each). LOD and LOQ values were calculated using Equation 1 and Equation 2, respectively:

$$LOD = 3.3 * S_a / b \quad (1)$$
$$LOQ = 10 * S_a / b \quad (2)$$

Where s_a is the standard deviation of the intercept; and b is the slope of the regression line obtained from the calibration curve [26].

Blank samples (milk samples which did not exhibit AFM_1 presence) spiked at three fortification levels (12.5, 25 and 37.5 µg/kg corresponding to 2.5, 5 and 7.5 µg/kg in eluate) were used to calculate repeatability and recovery. Analyzes were repeated 6 times for each concentration level.

Statistical Analysis

Data were analyzed by one-way ANOVA. The confidence level was established at 95% in all tests and probabilities less than 5% ($P<0.05$) were evaluated as significant. All statistical analyzes were made with Microsoft Excel statistical program.

RESULTS and DISCUSSION

Validation of HPLC Analysis

The HPLC method fulfilled the conditions outlined in Commission Regulation (EC) No. 401/2006 [27]. The calibration curve was constructed using the peak areas obtained from AFM_1 standard solutions at different concentrations. The calibration curve was found to be linear between 2 and 100 µg/L, with linear equation y=0.1463x+0.0652. The coefficient of determination (R^2) of the calibration curve was calculated as 0.9998 (Figure 1). The mean recovery and repeatability of the method were 95.6% and 4.9%, respectively as reported in Table 1. The chromatograms of the UHT cow's milk sample with and without the AFM_1 standard solution added, and the chromatogram of the AFM_1 standard solution at 5 µg/L concentration used for evaluating recovery are presented in Figures 2, 3 and 4, respectively.LOD and LOQ values were found to be 1.038 and 3.145 µg/kg, respectively.

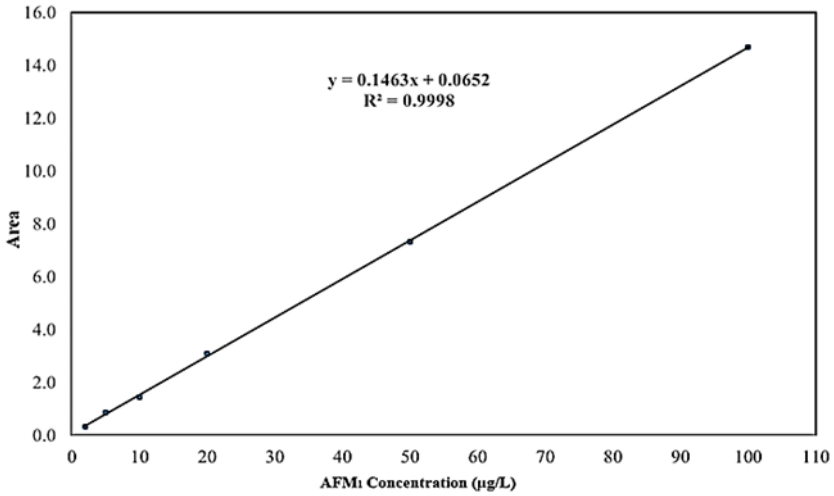

Figure 1. Calibration curve of AFM_1 standard solutions.

Table 1. Repeatability and recovery data.

Parameters	AFM_1 Spike Doses		
	12.5 µg/kg	25 µg/kg	32.5 µg/kg
Repeatability (coefficient of variation, % CV)	4.6	3.6	6.4
Recovery (% ± standard deviation, SD)	96.6±4.5	91.8±3.3	98.6±6.3

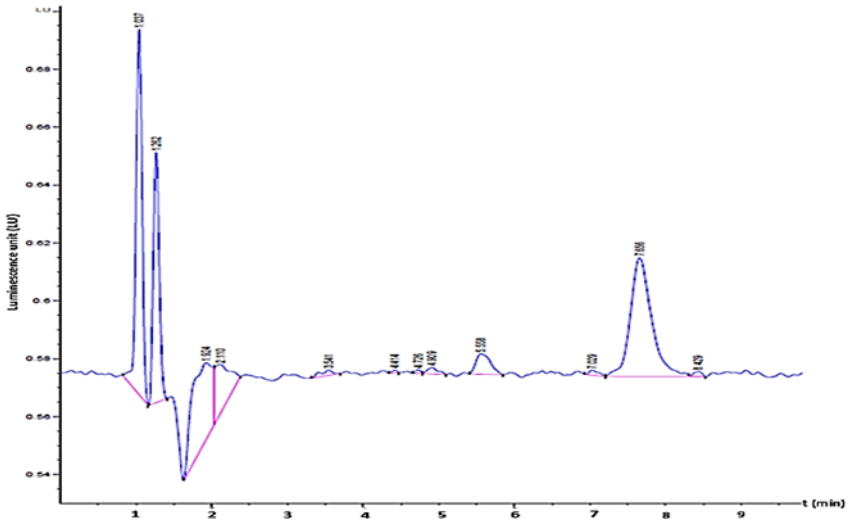

Figure 2. Chromatogram of UHT cow's milk sample containing 5 µg/kg AFM_1 standard solution.

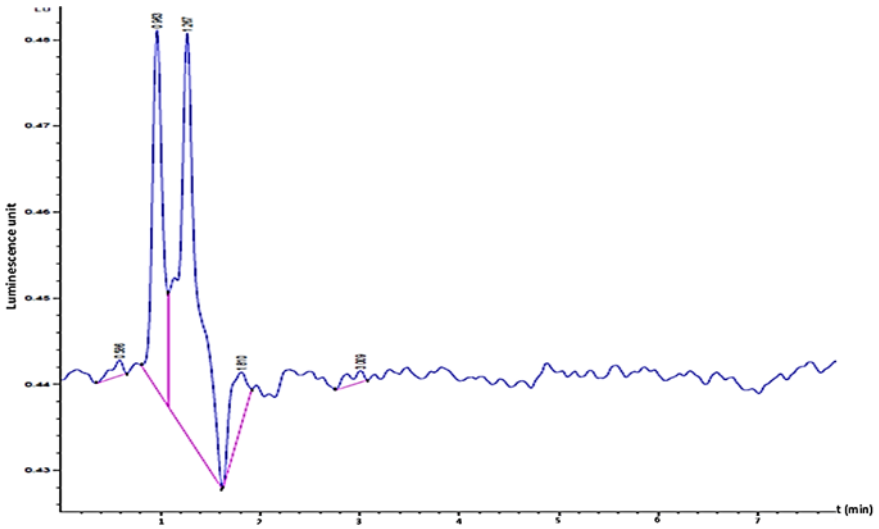

Figure 3. Chromatogram of UHT cow's milk sample without the addition of AFM_1 standard solution.

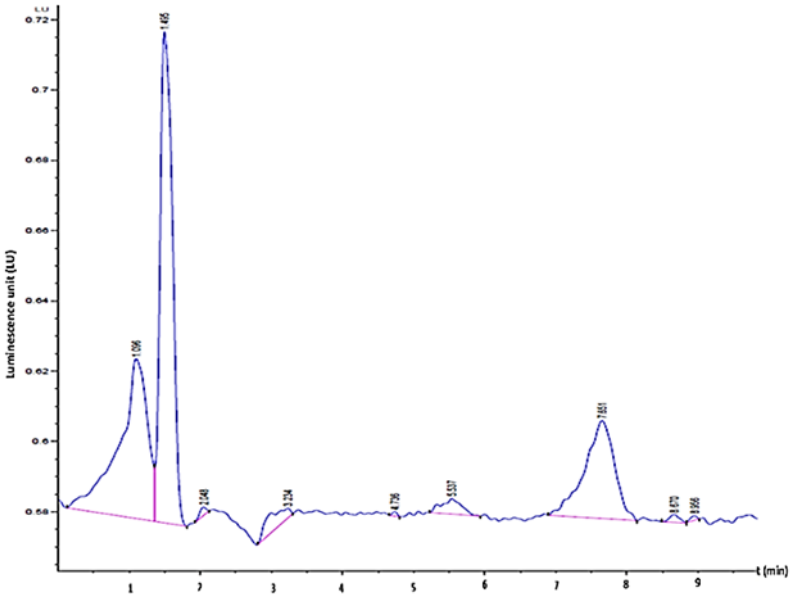

Figure 4. Chromatogram of AFM_1 standard solution at 5 µg/L concentration.

Sample Analysis

20 µg/L of AFM_1 standard solution was injected into HPLC and the retention time of AFM_1 was found to be 7.637 minutes. It was aimed to measure the AFM_1 levels in milk samples by comparing the chromatograms obtained from milk samples with the chromatograms obtained from AFM_1 standard solutions. AFM_1 was not detected in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed. In the analyzed UHT cow's milk and raw cow's milk samples, some examples of the chromatograms of AFM_1 levels that could not be detected are shown in Figures 5 and 6, respectively.

In this study, the presence of AFM_1 was determined in ultra-high temperature (UHT) cow's milk samples manufactured in the northern part of Cyprus and raw cow's milk samples collected from several local dairy farms. AFM_1 was not detectable in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed. According to the Meteorology Department in the northern part of Cyprus, the average temperature and the standardized precipitation index (SPI) in September 2020 (when the milk samples were collected) was 28°C, and between 0.50 and - 0.50 ('near normal', which is between slightly humid and slightly arid), respectively [42, 43]. It is well known that the optimum water activity, temperature and relative humidity required for the aflatoxin formation are 0.99, 33°C and >70%, respectively [6]. Therefore, the lack of ideal conditions for aflatoxin formation could explain the undetectable AFM_1 levels in milk samples.

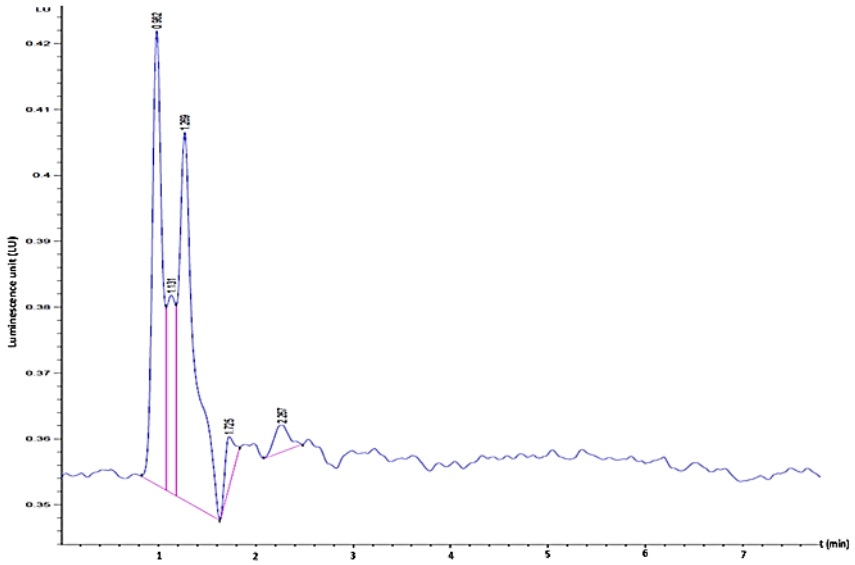

Figure 5. Chromatogram of UHT cow's milk sample which AFM_1 level could not be detected.

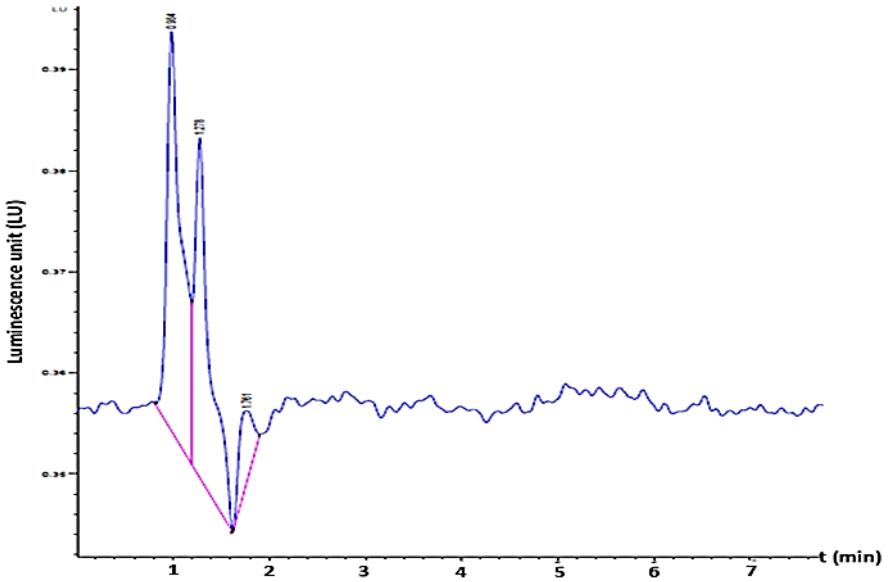

Figure 6. Chromatogram of raw cow's milk sample which AFM_1 level could not be detected.

In the study conducted by Kutlubay and Sökmen at Giresun University, AFM_1 was not detected in any of the 30 cow milk samples analyzed [28]. Bellio et al. [29] reported that only eight (0.5%) of a total of 1668 cow milk samples collected and evaluated in Italy, did not meet the EU regulation limit for the presence of AFM_1. Piva et al. [30] examined 276 milk samples for the presence of AFM_1 and reported that only 7 had AFM_1 levels of more than 50 ng/L. In any of the 100 pasteurized milk samples produced in Iran, AFM_1 levels did not exceed 50 ng/L, which is the maximum limit for milk, according to Behfar et al. [31]. In a study conducted by Cammilleri and his colleagues, AFM_1 levels in 170 cow's milk samples collected in Southern Italy were found to be below the European Commission regulation 1881/2006 limit [26], [27]. In a study of AFM_1 formation in 100 raw milk samples in South Korea, none of the samples exceeded the Korean legal limit of 0.5 µg/kg for AFM_1 [32]. The fact that the AFM_1 levels in the milk samples analyzed in the above-mentioned studies did not exceed the legal limits, or only exceeded the legal limits in a few samples, is consistent with our findings. The level of AFM_1 in milk increases in proportion to the amount of AFB_1 in the feeds [33]. In comparison to the winter months, when animals are fed more concentrated feed, AFM_1 contamination is lower in the spring and summer, when fresh grass and roughage are more abundant and pasture feeding is the common practice [34]. Therefore, various factors such as geographical, territorial and seasonal differences, feeding regimes of animals, lactation period from which milk samples were taken, and different analysis methods used are among the reasons for the different findings regarding the prevelance of AFM_1 in milk [35].

The HPLC-FLD method's LOD and LOQ values were found to be 1.038 and 3.145 µg/kg, respectively, which were much higher than the values determined by other

researchers [29, 36–40]. On the other hand, the linearity, repeatability and recovery values of the HPLC method were compatible with the criteria specified in the commission regulation [41], proving the reliability and validity of the method.

CONCLUSION

The AFM_1 levels in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed in this study were not detectable, and the AFM_1 level in the milk samples did not exceed the Turkish Food Codex (TGK) and European Union (EU) limit values [18, 44].

It is a pleasing situation for both dairy consumers and producers that the AFM_1 levels detected in the analyzed milk samples did not exceed the regulatory limits. The findings of the study can be explained by drying the animal feeds properly after harvest, storing them in appropriate conditions, and avoiding exposure to high temperatures and humidity. Although the AFM_1 content in milk samples produced in the northern part of Cyprus poses no major risk to public health, the need for larger research with more milk samples is becoming apparent. Moreover, an ongoing surveillance program is required to monitor the risk of aflatoxin contamination throughout the animal feed supply chain. Apart from all these considerations, the likelihood of total aflatoxin or mycotoxin exposure based on consumer dietary habits, also poses a concern. When all factors are considered, it has been concluded that good agricultural practices, good preservation practices, good hygiene practices, and application of hazard analysis and critical control points (HACCP) based food safety systems will be beneficial in preventing aflatoxin exposure in the food chain.

ACKNOWLEDGEMENTS

We thank Prof. Dr. Muberra Kosar for her support and assistance in the usage of the HPLC-system.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

Raw data were generated at Eastern Mediterranean University. Derived data supporting the findings of this study are available from the corresponding author, Bereket, C. on request.

REFERENCES

[1] Hussein, H., Brasel, J. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, 167(2), 101-134.

[2] Mehenktaş, C. (2019). Süt ve süt ürünlerinde aflatoksin M1. *Akademik Gıda*, 17, 439-443.

[3] International Agency for Research on Cancer. (2002). International agency for research on cancer ıarc monographs on the evaluation of carcinogenic risks to humans. *Iarc Monographs on the Evaluation of Carcinogenic Risks to Humansarc Monographs on the Evaluation of Carcinogenic Risks to Humans*, 82, 1-556.

[4] Sweeney, M., Dobson, A. (1998). Mycotoxin production by aspergillus, fusarium and penicillium species. *International Journal of Food Microbiology*, 43(3), 141-158.

[5] Yentür, G., Er, B. (2012). The evaluation of the aflatoxin presence in foods. *Turk Hijyen ve Deneysel Biyoloji Dergisi*, 69(1), 41-52.

[6] Neme, K., Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies - A review. *Food Control*, 78, 412-425.

[7] Ismail, A., Gonçalves, B.L., de Neeff, D.V., Ponzilacqua, B., Coppa, C.F.S.C., Hintzsche, H., Sajid, M., Cruz, A.G., Corassin, C.H., Oliveira, C.A.F. (2018). Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Research International*, 113, 74-85.

[8] Luo, Y., Liu, X., Li, J. (2018). Updating techniques on controlling mycotoxins - A review. *Food Control*, 89, 123-132.

[9] Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: Mechanisms of action. *International Journal of Environmental Research and Public Health*, 17(2), 1-28.

[10] Soni, K.B., Lahiri, M., Chackradeo, P., Bhide, S.V., Kuttan, R. (1997). Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. *Cancer Letters*, 115(2), 129-133.

[11] Rawal, S., Kim, J.E., Coulombe, R. (2010). Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. *Research in Veterinary Science*, 89(3), 325-331.

[12] Bognanno, M., Fauci, L.L., Ritieni, A., Tafuri, A., De Lorenzo, A., Micari, P., Renzo, L. Di, Ciappellano, S., Sarullo, V., Galvano, F. (2006). Survey of the occurrence of aflatoxin m1 in ovine milk by HPLC and its confirmation by MS. *Molecular Nutrition and Food Research*, 50(3), 300-305.

[13] Santini, A., Ritieni, A. (2013). Aflatoxins: Risk, exposure and remediation. *Aflatoxins - Recent Advances and Future Prospects*. INTECH Open Access Publisher,343p.

[14] Iqbal, S., Jinap, S., Pirouz, A., Faizal, A. (2015). Aflatoxin m1 in milk and dairy products, occurrence and recent challenges: A review. *Trends in Food Science & Technology*, 46(1), 110-119.

[15] Duarte, S.C., Almeida, A.M., Teixeira, A.S., Pereira, A.L., Falcão, A.C., Pena, A., Lino, C.M. (2013). Aflatoxin m1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control*, 30(2), 411-417.

[16] Giovati, L., Magliani, W., Ciociola, T., Santinoli, C.,

Conti, S., Polonelli, L. (2015). AFM1 in milk: Physical, biological, and prophylactic methods to mitigate contamination. *Toxins*, 7(10), 4330-4349.

[17] The Commission of the European Communities. (2010). Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*, 50(2009), 8-12.

[18] Türk Gıda Kodeksi. (2011). Türk gıda kodeksi bulaşanlar yönetmeliği mikotoksinler (Ek1). *Resmi Gazete Tarih: 29*, *12.*

[19] Santini, A., Raiola, A., Ferrantelli, V., Giangrosso, G., Macaluso, A., Bognanno, M., Galvano, F., Ritieni, A. (2013). Aflatoxin m1 in raw, UHT milk and dairy products in Sicily (Italy). *Food Additives and Contaminants:* Part B, 6(3), 181-186.

[20] Matabaro, E., Ishimwe, N., Uwimbabazi, E., Lee, B. (2017). Current immunoassay methods for the rapid detection of aflatoxin in milk and dairy products. *Comprehensive Reviews in Food Science and Food Safety*, 16(5), 808-820.

[21] Choudhary, A.K., Kumari, P. (2010). Management of mycotoxin contamination in preharvest and post harvest crops: Present status and future prospects. *Journal of Phytology*, 2(7), 37-52.

[22] Rodríguez Velasco, M., Calonge Delso, M., Ordónez Escudero, D. (2003). ELISA and HPLC determination of the occurrence of aflatoxin m1 in raw cow's milk. *Food Additives and Contaminants*, 20(3), 276-280.

[23] Öztürk, B., Çelik, F., Çelik, Y., Kabaran, S., Ziver, T. (2014). To determine the occurence of aflatoxin M1 (AFM1) in samples of cyprus traditional cheese (halloumi): A cross-sectional study. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 20(5), 773-778.

[24] R-Biopharm Rhone. (2017). *Rp70n easi extract aflatoxin milk and milk powder V11-2017-09*.

[25] European Commission (EC). (2002). Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, 221, 8-36.

[26] Cammilleri, G., Graci, S., Collura, R., Buscemi, M., Vella, A., Macaluso, A., Giaccone, V., Giangrosso, G., Cicero, Antonello., Maria Lo Dico, G., Pulvirenti, A., Cicero, N., Ferrantelli, V. (2019). Aflatoxin m1 in cow, sheep, and donkey milk produced in Sicily, Southern Italy. *Mycotoxin Research*, 35(1), 47-53.

[27] European Commission (EC). (2006). Commission Regulation (EC) No 118/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union,* (364), 5-24.

[28] Kutlubay, Z., Sökmen, B. (2016). Investigation of aflatoxin M1 in milks in the Giresun region. *Giresun Üniversitesi Fen Bilimleri Enstitüsü,* 1-67.

[29] Bellio, A., Bianchi, D.M., Gramaglia, M., Loria, A., Nucera, D., Gallina, S., Gili, M., Decastelli, L. (2016). Aflatoxin M1 in cow's milk: Method validation for milk sampled in northern Italy. *Toxins (Basel)*, 8(3), 57.

[30] Piva, G., Pietri, A., Galazzi, L., Curto, O. (1988). Aflatoxin M1 occurrence in dairy products marketed in Italy. *Food Additives & Contaminants*, 5(2), 133-139.

[31] Behfar, A., Khorasgani, Z.N., Alemzadeh, Z., Goudarzi, M., Ebrahimi, R., Tarhani, N. (2012). Determination of aflatoxin M1 levels in produced pasteurized milk in Ahvaz city by using HPLC. *Jundishapur Journal of Natural Pharmaceutical Products,* 7(2), 80-84.

[32] Kim, H.J., Lee, J.E., Kwak, B.M., Ahn, J.H., Jeong, S.H. (2010). Occurrence of aflatoxin M1 in raw milk from South Korea winter seasons using an immunoaffinity column and high performance liquid chromatography. *Journal of Food Safety,* 30(4), 804-813.

[33] Çiğdem, A., Arif, A. (2004). Ankara'da işlenen sütlerde aflatoksin M1 varlığının ve düzeylerinin HPLC ile araştırılması. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 51(3), 175-179.

[34] İşleyici, Ö., Morul, F., Sancak, Y.C. (2012). Van'da tüketime sunulan UHT sterilize inek sütlerinde aflatoksin M1 düzeyinin araştırılması. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 23(2), 65-69.

[35] Tunail, N. (2000). Mikrobiyal enfeksiyonlar ve intoksikasyonlar -Gıda mikrobiyolojisi ve uygulamaları. *Ankara Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü Yayını*, 82-88.

[36] Muscarella, M., Lo, S., Palermo, C., Centonze, D. (2007). Validation according to European Commission Decision 2002 / 657 / EC of a confirmatory method for aflatoxin m1 in milk based on immunoaffinity columns and high performance liquid chromatography with fluorescence detection. *Analytica Chimica Acta*, 594, 257-264.

[37] Kabak, B., Ozbey, F. (2012). Aflatoxin m1 in UHT milk consumed in Turkey and first assessment of its bioaccessibility using an in vitro digestion model. *Food Control*, 28(2), 338-344.

[38] de Oliveira, P.C., de Fátima Ferreira Soares, N., de Oliveira, T.V., Baffa Júnior, J.C., da Silva, W.A. (2013). Aflatoxin M1 occurrence in ultra high temperature (UHT) treated fluid milk from Minas Gerais/Brazil. *Food Control*, 30(1), 90-92.

[39] Iha, M.H., Barbosa, C.B., Okada, I.A., Trucksess, M.W. (2013). Aflatoxin M1 in milk and distribution and stability of aflatoxin M1 during production and storage of yoghurt and cheese. *Food Control*, 1(29), 1–6.

[40] Iqbal, S.Z., Asi, M.R., Selamat, J. (2014). Aflatoxin M1 in milk from urban and rural farmhouses of Punjab , Pakistan. *Food Additives & Contaminants: Part B*, 7(1), 17-20.

[41] Emea, Chmp, Ewp. (2011). Guideline on bioanalytical method validation. *European Medicines Agency Science Medicines Health*, 1-23.

[42] *Kuraklık Analizi - K.K.T.C. Meteoroloji Dairesi*. (2020). Retrieved November 20, 2020, from http://kktcmeteor.org/verianaliz/kuraklik-analizi

[43] *Sıcaklık Analizi - K.K.T.C. Meteoroloji Dairesi*.

(2020). Retrieved November 20, 2020, from http://kktcmeteor.org/verianaliz/Analyze-sic

[44] Alimentarius, C. (2001). Comments submitted on the draft maximum level for aflatoxin M1 in milk. *Codex Committee on Food Additives and Contaminants 33rd Session, Hague, The Netherlands Commission Regulation (EC),* 25, 1-6.