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Original Article
Özgün Araştırma

Aflatoxin M_1 contamination of Anatolian Water Buffalo milk

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Abstract: Aflatoxin M_1 (AFM_1), a hepatotoxic metabolite, occurs due to the consumption of feeds contaminated with aflatoxin B_1 (AFB_1) by lactating animals. This study aims to specify the presence and levels of AFM_1 in water buffalo milk produced widely in our region.

Between the years 2019 - 2021, a total of 250 raw water buffalo milk samples were used as material. All samples were transported to the laboratory in the cold chain (4°C) and analyzed. Tests were done with ELISA (Enzyme-Linked Immunosorbent Assay) technique and AFM_1 specific test kits Ridascreen® Aflatoxin M_1, r-biopharm were used for detection of AFM_1. First, the samples were prepared as described in the kit manufacturer's instructions. Later, for the calculation of AFM_1 levels, RIDASOFT WIN.NET software was also used as recommended.

Two hundred and fifty raw water buffalo milk samples were analyzed in duplicate, and the average values of the results was taken into account. While AFM_1 was not detected in 174 samples (69.6%), 76 sample (30.4%) was contaminated with AFM_1. However, it was observed that the AFM_1 levels of these 76 samples did not exceed the levels specified in the Turkish Food Codex.

In conclusion, although water buffalo milk and dairy products pose a potential risk in AFM_1, this risk was found relatively low in samples belong to our region. However, this situation may vary depending on the feeding conditions of lactating animals and sampling season. Therefore, it is recommended that similar and further studies are needed to diversify the data in the future.

Keywords: Aflatoxin M_1, ELISA, milk, public health, water buffalo

Anadolu Manda sütünde Aflatoksin M_1 kontaminasyonu

Özet: Hepatotoksik bir metabolit olan aflatoksin M1 (AFM1), süt hayvanları tarafından aflatoksin B1 (AFB1) ile kontamine olmuş yemlerin tüketilmesi nedeniyle oluşur. Bu çalışma, bölgemizde yaygın olarak üretilen manda sütünde AFM1 varlığını ve düzeylerini belirlemeyi amaçlamaktadır.

Çalışmada, 2019 - 2021 yılları arasında toplam 250 adet çiğ manda sütü numunesi materyal olarak kullanılmıştır. Tüm numuneler soğuk zincirde (4°C) laboratuvara getirilmiş ve analiz edilmiştir. Testler ELISA (Enzyme-Linked Immunosorbent Assay) tekniği ile yapılmış olup ve AFM1'in tespiti için AFM1'e özgü test kitleri Ridascreen® Aflatoxin M1, r-biopharm kullanılmıştır. İlk olarak numuneler kit üreticisinin talimatlarında belirtildiği gibi hazırlandı. Daha sonra AFM1 seviyelerinin hesaplanması için de önerilen RIDASOFT WIN.NET yazılımı kullanılmıştır.

İki yüz elli çiğ manda sütü örneği iki tekrar halinde analiz edilmiş ve sonuçların ortalama değerleri dikkate alınmıştır. Yapılan analizler sonucunda, 174 örnekte (%69,6) AFM1 saptanmazken, 76 örnekte (%30,4) AFM1 tespit edilmiştir. Ancak bu 76 örneğin AFM1 seviyelerinin Türk Gıda Kodeksi'nde belirtilen seviyeleri aşmadığı gözlemlenmiştir.

Sonuç olarak, manda sütü ve süt ürünleri AFM1'de potansiyel risk oluştursa da bölgemize ait örneklerde bu risk nispeten düşük bulunmuştur. Ancak bu durum, emziren hayvanların beslenme koşullarına ve örnekleme mevsimine bağlı olarak değişebilir. Bu nedenle gelecekte verilerin çeşitlendirilmesi için benzer ve daha ileri çalışmalara ihtiyaç olduğu önerilmektedir.

Anahtar kelimeler: Aflatoksin M_1, ELISA, halk sağlığı, manda, süt

Introduction

Aflatoxins are known as toxic and heterocyclic compounds synthesized by strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are divided into four groups, such as aflatoxin B_1, B_2, G_1, and G_2. Aflatoxin M_1 (AFM_1) is the monohydroxylated hepatic metabolite of aflatoxin B_1 (AFB_1) that can be found in the milk and milk products of livestock due to feeding with AFB_1 contaminated feed (Nguyen et al. 2020). The International Agency Research on Cancer (IARC) has classified Aflatoxin M_1 in group

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1 human carcinogen (Guo et al. 2019; Hussain et al. 2010; Kara and Ince 2014).

It has been reported that Aflatoxin M_1 is an exceptionally durable compound under milk processing conditions such as pasteurization or ultra-high temperature (De Roma et al., 2017; Oruc et al. 2006). Furthermore, milk and dairy products are essential food substances for humans because of containing valuable animal protein, vitamins, and essential fatty acids. At the same time, aflatoxin contamination is overly critical in terms of public health since milk and dairy products occupy an important role in infant nutrition (Galvano et al. 1998). Because children are more sensitive to aflatoxins than adults. It has been determined that the long-term effects of consumption of aflatoxin M_1 contaminated milk and dairy products may cause DNA damage, chromosomal abnormalities, and genotoxic effects (Galvano et al. 1996; Prandini et al. 2009).

As reported and standardized by the Turkish Food Codex, the maximum limit of contaminants for aflatoxins has to be less than 50 ng/l in milk and dairy products (TFC, 2011).

Anatolian Water Buffalo is a registered species originating from the Mediterranean Water Buffalo and adapted to the geography of Turkey, where it has been breeding for approximately 1500 years in Anatolia (Şahin, 2016).

According to the Turkish Statistical Institute (TSI), there are around 183500 water buffaloes in Turkey, and 20% of this population is in the Central Anatolian Region. Aksaray province is located in the Central Anatolia Region, where water buffalo breeding is quite common. Likewise, according to TSI data, in 2018, 66300 and 2019, 68000 tone water buffalo milk was produced in Turkey (TSI, 2020).

This study was planned due to the lack of up-to-date and detailed data about Anatolian water buffalo milk, widespread consumption of water buffalo milk and dairy products in our region, and the fact that aflatoxin contamination is a biological threat that should not be ignored in terms of public health.

Material and Methods

Sampling

A total of 250 raw water buffalo milk samples were collected from the breeders in Aksaray region of Turkey between September 2019 – June 2021. The samples were procured from seven different breeders. All samples (approx. 50 mL) were taken into sterile centrifuge tubes and brought to the laboratory in an icebox at 2–4°C and stored at –20°C until analysis.

Methods

The ELISA screening method was applied for the analysis of water buffalo milk samples. For this purpose, AFM_1 specific test kits Ridascreen® Aflatoxin M_1 (r-biopharm R1121) were used (Biopharm, 2021).

Preparation of Samples

The raw water buffalo milk samples were prepared according to the kit manufacturer's instructions. First, samples were centrifuged at 3500 g for 10 min (Nüve, NF800R) to obtain skimmed milk. After defatted supernatant was isolated, 100 µl of it was used for analysis.

Test Procedure

According to the manufacturer's instructions, firstly, all reagents were brought to room temperature before usage. Furthermore, all samples in this study were analyzed twice to get more precise results. After inserting enough wells into the microwell holder, 100 µl antibodies were filled into wells. Then, plates were mixed gently and incubated at room temperature (20-25°C) for 15 minutes. After completion of incubation, the liquid in wells were poured out by micropipette, and wells were tapped forcefully on absorbent paper three times to ensure complete removal of liquid. Next, all wells were washed by Phosphate Buffered Saline (PBS, Sigma-Aldrich P4417). In the following step, 100 µl of standards or samples were added into wells, then plates were mixed gently and incubated at room temperature (20-25°C) for 30 minutes in the dark. Then, the washing procedure was repeated twice. Before the next washing procedure, 100 µl conjugate was pipetted into wells and mixed gently by shaking the plate manually. Then, they were incubated for 15 minutes at room temperature in the dark. Later, 100 µl of substrate/chromogen was added to each well, and the incubation step was repeated as written above. In the last phase, 100 µl of stop solution was added to each well, and the absorbance of samples was measured at 450 nm with an ELISA reader (ELX800, Bio-Tek Inst Inc USA). Specific software, the RIDASOFT® Win.NET, was used for the evaluation of data.

The detection limit of the Ridascreen® Aflatoxin M_1 kit was 5 ng/l, and the specificity was 100% to aflatoxin M_1.

Results

In this research, 250 raw water buffalo milk samples were investigated for Aflatoxin M_1 presence by ELISA technique, and all analyzes were repeated twice. Aflatoxin M_1 was detected in 76 (30.4%) samples; however, the result of 174 (69.9%) samples was found below the detection limit. Moreover, the AFM_1 values of samples was detected between 5.12 – 36.7 ng/l. In Aflatoxin M_1 positive samples, the contamination levels 5-10, 11-20, 21-30, and 31-50 ng/l, 60.5% 25%, 7.9% and 6.6% was determined, respectively.

In this study, the resulting level of Aflatoxin M_1 in raw water buffalo milk samples did not exceed the levels specified in the Turkish Food Codex (50 ng/l).

The results are shown in Table 1.

Table 1. Occurrence of Aflatoxin M1 in Raw Anatolian Water Buffalo Milk Samples

	Negative Samples <5 ng/l	Positive Samples			
		76/250 (30.4%)			
Raw Water Buffalo Milk Samples	174/250 (69.6%)	5-10 ng/l	11-20 ng/l	21-30 ng/l	31-50 ng/l
		46/76 (60.5%)	19/76 (25%)	6/76 (7.9%)	5/76 (6.6%)

Discussion and Conclusion

In related investigations, Kamkar et al. (2014) reported that 46 (79%) of their samples were contained Aflatoxin M_1. Moreover, %52 of positive water buffalo milk samples contained higher levels than the maximum limit of the European Union and Codex Alimentarius (50 ng/l). These results show that our region harbors relatively lower risk in terms of AFM_1 contamination. In another study, De Roma et al. (2017) determined that 28 (7,2%) of water buffalo milk samples were observed to contain AFM_1. The AFM_1 contamination level in water buffalo milk samples were found between 4-31 ng/l in the mentioned study, and similar to our outcomes, they do not exceed the levels specified in the Turkish Food Codex (50 ng/l) as well. In Pakistan, Hussain et al. (2010) reported that 34.5% of their water buffalo milk samples contained AFM_1. In line with our findings, it was detected that 84,2% of contaminated samples were below the EU action level of 50 ng/l for AFM_1. Guo et al. (2019) revealed that 62,5% (85) of their samples contained AFM_1 in South China. In addition, similar to our results, 90,5% of their samples contained less than 50 ng/l AFM_1. Rahimi et al., (2010) investigated 75 water buffalo milk samples in their study and in 66/75 samples AFM_1 was found below the Turkish Food Codex (50 ng/l) levels consistent with our results.

There is very limited research in Turkey about AFM_1 contamination in raw Anatolian water buffalo milk. Kara and Ince (2014) reported that the presence of AFM_1 in raw water buffalo milk samples in Afyonkarahisar, Turkey was 27% (34/126), and paralel to our results, none of the AFM_1 levels were above the Turkish Food Codex.

It is known that several factors such as feed quality, environmental contamination, and seasonal factors affect the composition of milk. It has been reported that the risk of Aflatoxin M_1 arises as a result of feeding farm animals with aflatoxin B_1 contaminated feed or silage. These specific and local circumstances can be cited as the main reason for different contamination rates between the present study and others.

The results of this study indicated that the AFM_1 contamination in raw Anatolian water buffalo milk samples were not higher than the limit specified in the Turkish Food Codex. However, these results should be taken seriously in terms of public health because people from all age groups can consume milk and dairy products in different amounts. Furthermore, in the strategy of "from farm to fork" it is vital to struggle with AFB_1 contamination in animal feeds by improving process and storage conditions, so the AFM_1 risk does not exist in milk and dairy products. Finally, milk, dairy products, and animal feed should be monitored regularly to keep the AFM_1 hazard under control.

Ethical Statement: This study does not present any ethical concerns.

Conflict of Interest: The author has no conflicts of interest to declare.

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