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Occurrence of aflatoxin M_1 in raw cow milk in the Afyonkarahisar province of Turkey

Vorkommen von Aflatoxin M_1 in roher Kuhmilch in der Provinz Afyonkarahisar in der Türkei

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Summary

Aflatoxins (AF) are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M_1 (AFM_1) is transferred to animal milk via biotransformation by consumption of feeds that contain aflatoxin B_1 (AFB_1). The aim of this study was to determine the levels of AFM_1 in raw cow milk samples in Afyonkarahisar city (Turkey). In this study, totally 120 raw milk samples were randomly obtained from five different cow farms located in the area of Afyonkarahisar during the year 2006. The analysis was carried out by using immunoaffinity columns and a fluorometer. The results showed that 22.5 % of the samples were above the maximum level of 0.05 ppb. Of the milk samples, 77.5 % were in the range of 0–0.05 ppb and 4.17 % were in the range of 0.11–0.15 ppb, while 0.15 ppb was the highest value measured.

Keywords: raw cow milk, aflatoxin M1, fluorometer, immunoaffinity columns

Zusammenfassung

Aflatoxine werden hauptsächlich von *Aspergillus flavus* und *Aspergillus parasiticus* produziert. Aflatoxin M_1 (AFM_1) wird nach Biotransformation von Aflatoxin B_1 (AFB_1) von Tieren mit der Milch ausgeschieden, wenn diese AFB_1 befallenes Futter fressen. Das Ziel dieser Studie war die Ermittlung der Werte von AFM_1 in roher Kuhmilch in der Provinz Afyonkarahisar (Türkei). In dieser Studie wurden insgesamt 120 aus fünf verschiedenen Rinderhaltungsbetrieben in der Umgebung von Afyonkarahisar im Laufe des Jahres 2006 genommene Rohmilchproben untersucht. Die Analyse wurde mittels Immunoaffinitätssäulen und Fluorometer durchgeführt. Die Ergebnisse zeigten, dass 22,5 % der Proben über dem Höchstwert von 0,05 ppb lagen, d. h. 77,5 % waren im Bereich von 0–0,05 ppb und 4,17 % im Bereich von 0,11–0,15 ppb, wobei 0,15 ppb der höchste ermittelte Wert war.

Schlüsselwörter: rohe Kuhmilch, Aflatoxin M1, Fluorometer, Immunoaffinitätssäulen

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Introduction

Aflatoxins (AF) are extremely toxic mycotoxins basically produced by the common fungi *Aspergillus (A.) flavus, A. parasiticus* and *A. nominus*. These mycotoxins are found in many plant products such as peanuts, copra, soya, maize, rice and wheat (Alborzi et al., 2006). AFs are highly dangerous and carcinogenic for animals and humans, can lead to acute and chronic diseases such as liver damage, liver cirrhosis and tumor induction and are also teratogenic and hepatotoxic. Aflatoxin M_1 (AFM_1) can commonly be seen in the milk of many animals such as cows, goats and sheep which ingest feed contaminated with Aflatoxin B_1 (AFB_1) (Alborzi et al., 2006; Kamkar, 2005; Hansen, 1990).

AFM_1 is a strong and resistant toxin which is not inactivated by pasteurization, autoclaving and other food processing procedures. Since milk and milk products are largely consumed by infants and children, which are considered more susceptible to the adverse effects of mycotoxins, the presence and determination of AFM_1 in milk and milk products is very important.

Therefore, many studies have been carried out in many countries and inspection programs on this subject have been established for many years in order to protect public health. AF limits vary from country to country, for example the maximum level is 0.05 ppb in the EU (Codex Alimentarius Commission, 2001), 0.5 ppb in US regulations (FAO, 2003) and 0.05 ppb in Turkey (Turkish Food Codex, 2002). Worldwide limits for AFM1 in milk are seen in Figure 1 (FAO, 2003).

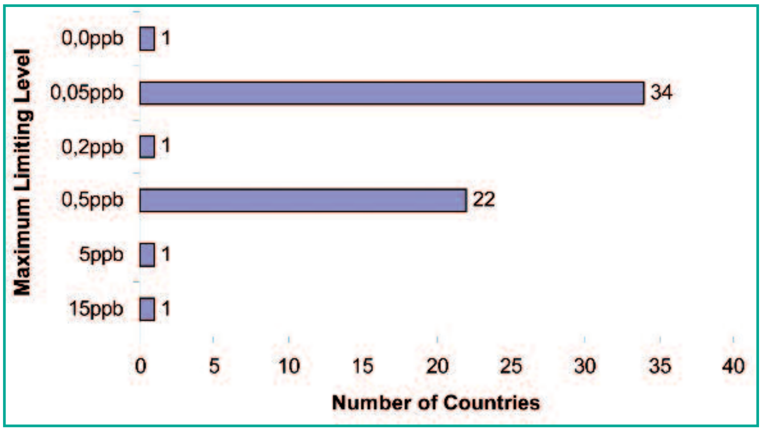

FIGURE 1: *Worldwide limits for AFM_1 in milk.*

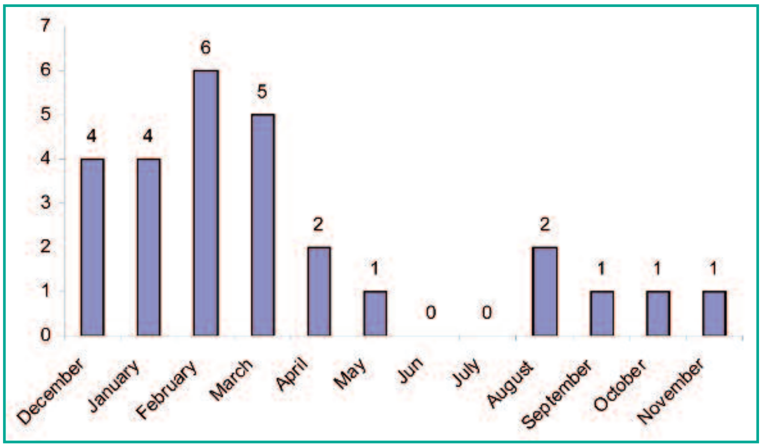

FIGURE 2: *Distribution of AFM_1 positive samples during the year.*

Oliveria and Ferraz (2007) used pasteurized UHT milk and milk powder from goats in their study. They observed that, although 69.4 % of the samples were positive for AFM_1, the levels (0.011–0.161 ppb) were lower than the tolerance limit of Brazilian regulations (0.5 ppb). Alborzi et al. (2006) studied 624 pasteurized milk samples from different supermarkets in Shiraz city (South of Iran) during six months in 2003. In this study, 17.8 % of the samples had AFM_1 levels above the maximum limit accepted by the EU (0.05 ppb). Günşen and Büyükyörük (2003) used an ELISA method in their study and determined AFM_1 levels of 0.20623 ppb ± 0.01588 ppb in cheese in Turkey.

The Afyonkarahisar province and the neighboring areas are important for milk production in Turkey. The purpose of our study was to determine AFM_1 levels in the Afyonkarahisar area. To achieve this goal a direct fluorescence measurement technique was used and the results were compared to previous studies.

Materials and methods

Sampling

In our study, we analyzed a total of 120 samples of raw milk randomly obtained from cow farms located in the center, the south, the north, the west and the east of Afyonkarahisar province. Samples were collected on the 7th and 22nd day of each month during the year 2006.

Analytic methodology

Since the direct fluorescence measurement method is the most up-to-date technique for the extraction and purification of AFs and combines specified AF binding with the sensitivity and accuracy of fluorescence measurements, this method was used for AFM_1 determination (Hansen, 1990).

All milk samples were analyzed for AFM_1 in a fluorometer (Vicam series-4 Fluorometer, Model Vicam V 2.0, Vicam, Milford, MA, USA) at a detection limit of 0.05 ppb, based on the method of Hansen (1989) with some adaptations proposed by the manufacturer of the immunoaffinity columns (Vicam, 1999).

For analysis, the samples were brought to room temperature. A 50 ml portion was mixed with 1 g NaCl (Merck, Darmstadt, Germany) and centrifuged at 2000 x g for 10 min. The fat layer was removed and the skim portion was filtered (11 cm microfiber filters) before analysis. Next, 25 ml was passed (flow: 1 ml in 20 s) through the immunoaffinity column, which was connected to a glass syringe (10 ml) and a pump system (airpump), followed by washing with two 10 ml portions of 10 % methanol (Merck, gradient grade for liquid chromatography) in pure water (Nüve, Ankara, Turkey). A glass test tray was placed under the column and AFM_1 was eluted with 1 ml 80 % methanol. After the elution solution passed through the column, the complete solution was collected in the glass test tray. Following this, 1.0 ml AflaTest developer was added, mixed (vortex)

TABLE 1: *AFM_1 levels in positive samples during the year.*

AFM₁ levels [ppb]	<0.05	0.05–0.075	0.076–0.1	0.11–0.15	***
N*	93	15	7	5	120
%**	77.50	12.50	5.83	4.17	

*Number of samples; **Percentage of AFM_1 positive samples; ***Total number of samples analyzed

TABLE 2: *AFM_1 levels in positive samples during winter.*

AFM₁ levels [ppb]	<0.05	0.05–0.075	0.076–0.1	0.11–0.15	**
N	16	8	4	2	30
%*	53.33	26.67	13.33	6.67	
%***	13.33	6.67	3.33	1.67	

N: Number of samples; *Percentage of AFM_1 positive samples; **Total number of samples analyzed; ***Percentage in the year

TABLE 3: *AFM_1 levels in positive samples during spring.*

AFM₁ levels [ppb]	<0.05	0.05–0.075	0.076–0.1	0.11–0.15	**
N	22	3	2	3	30
%*	73.33	10.00	6.67	10.00	
%***	18.33	2.50	1.67	2.50	

N: Number of samples; *Percentage of AFM_1 positive samples; **Total number of samples analyzed; ***Percentage in the year

and after 1 min the AF concentration was read in the fluorometer as ppb and was printed.

Results

A total of 120 raw cow milk samples were analyzed for AFM_1. The number of positive (≥0.05 ppb) samples during the year is presented in Figure 2.

The AFM_1 levels of the positive samples are summarized in Table 1, while Tables 2 to 5 present the AFM_1 levels determined in the different seasons of the year.

Discussion

Of the total of 120 samples 22.5 % were above the maximum level of 0.05 ppb accepted by the EU and Turkey. Thus, 77.5 % of the samples were in the range of 0–0.05 ppb, while 4.17 % were in the range of 0.11–0.15 ppb. In total, the AFM_1 levels in the raw milk samples ranged from 0.05 ppb to 0.15 ppb.

An increase of positive samples in winter and a gradual decline in the spring months is striking. The increase in the winter months can be explained by high AFB_1 levels caused by bad storage conditions of the feed. The gradual decrease in spring is caused by open land feeding and usage of less feed remaining from the past years.

Tekinşen and Uçar (2008) analysed 92 butter and 100 cream cheese samples for AFM_1 and determined that the AFM_1 levels in these samples ranged from 0.01 ppb to 0.7 ppb. A study by Unusan (2006) examined 129 samples of bottled UHT milk (volume 250 ml) sold in supermarkets located in the provinces of Konya, Ankara, Sivas, Kayseri, and Niğde in the Central Anatolia Region of Turkey. It was found that 58 (45 %) samples had AFM_1 levels below 0.01 ppb, ten (7.9 %) samples were within the range of 0.01 to 0.049 ppb, 57 (43.9 %) samples were within the range 0.05–0.499 ppb, and four (3.2 %) samples had levels above 0.5 ppb. A study of milk samples conducted by Unusan (2006) found that eleven (40.7 %) samples had levels below 0.01 ppb, eight (29.6 %) samples were within the range 0.01–0.02 ppb, seven (25.9 %) samples were within the range 0.021–0.05 ppb, and one (3.7 %) sample exceeded the maximum limit of 0.05 ppb established in Turkey. Çelik et al. (2005) analyzed 85 pasteurized milk samples from supermarkets in different regions of the province of Ankara. It was found that eleven (14.66 %) samples had AFM1 levels below 0.01 ppb, three (4 %) samples were within the range 0.011–0.03 ppb, 13 (17.33 %) samples were within the range 0.031–0.05 ppb, 29 (38.66 %) samples ranged from 0.051 to 0.07 ppb, twelve (16 %) samples were within the range of 0.071–0.09 ppb, and seven (38.66 %) samples had levels in excess of 0.091 ppb.

A study by Akdemir and Altıntaş (2004) investigated the presence of AFM_1 in 48 raw milk samples from the provinces of Eskişehir, Burdur, Nevşehir, Bursa, Ankara, Lüleburgaz and Antalya, which were supplied to two different milk processing factories in Ankara. AFM_1 was found in 70.83 % of the samples and it was concluded that 33.3 % of the samples exceeded the maximum limit of 0.05. Liman and Şeybek (2002) examined 86 different raw milk samples from a milk collection center in the province of Kayseri and detected AFM_1 in 20 samples, with levels ranging from 0.034 to 0.327 ppb, while no evidence of AFM_1 was found in the remaining 66 raw milk samples. Bakırcı (2001) examined 90 raw milk samples collected from 15 different manufacturers. The presence of AFM_1 was determined in 79 (87.77 %) samples, 35 (44.3 %) of which had levels of AFM_1 in excess of the maximum limit of 0.05 ppb accepted in Turkey.

Consequently, AFM_1 in milk still presents a problem in the province of Afyonkarahisar and the neighboring area. However, this problem presents lower risks compared to other regions across Turkey which were studied by previous researchers. Furthermore, previous studies conducted across Turkey highlight the differences between regions. This may be attributed to the fact that Turkey covers a wide geographic area, with different climatic

TABLE 4: *AFM_1 levels in positive samples during summer.*

AFM₁ levels [ppb]	<0.05	0.05–0.075	0.076–0.1	0.11–0.15	**
N	28	2	0	0	30
%*	93.33	6.67	0.00	0.00	
%***	23.33	1.67	0.00	0.00	

N: Number of samples; *Percentage of AFM_1 positive samples; **Total number of samples analyzed; ***Percentage in the year

TABLE 5: *AFM_1 levels in positive samples during autumn.*

AFM₁ levels [ppb]	<0.05	0.05–0.075	0.076–0.1	0.11–0.15	**
N	27	2	1	0	30
%*	90.00	6.67	3.33	0.00	
%***	22.50	1.67	0.83	0.00	

N: Number of samples; *Percentage of AFM_1 positive samples; **Total number of samples analyzed; ***Percentage in the year

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conditions prevailing in the various regions. Another aspect may be the differing levels of awareness amongst farmers in the various regions. The current situation suggests that farmers and feed manufacturers should be better informed about factors other than the effects of climate conditions on the occurrence of AF in stored feed.

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