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Aflatoxin M1 contamination in pasteurised milk

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ABSTRACT

Aflatoxin M1 (AFM1) in milk and milk products is considered to pose certain hygienic risks for human health. These metabolites are not destroyed during the pasteurization and heating process. In this study, the contamination level of AFM1 in pasteurised milk that all age groups, including children, consume worldwide is defined. A total of 85 pasteurised milk samples were analysed for AFM1 with the ELISA technique. Seventy-five samples (88.23%) were found to be contaminated with AFM1, and 48 samples (64%) exceeded the legal level of AFM1 in milk according to the Turkish Food Codex and Codex Alimentarius limit (50 ng/kg-l). Serious risks for public health exist from milk consumption. Thus, milk and milk products have to be controlled periodically for AFM1 contamination. Also, dairy cow feeds should be stored in such a way that they do not become contaminated.

Key words: milk, aflatoxin M1, ELISA

Introduction

Mycotoxins are those secondary metabolites of fungi which are associated with certain disorders in animals and humans. The manifestation of toxicity in animals is as diverse as the fungal species which produce these compounds. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products (CASTEGNARO and MCGREGOR, 1998; D'MELLO and MACDONALD, 1997; EGMOND and PAULSCH, 1986).

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It has been defined that many kinds of moulds produce mycotoxins and that within these moulds mycotoxin species were presented in foods as natural pollutants, thereby causing acute and chronic toxications in both human and animals (PITTET, 1998; GALVANO et al., 2001b).

Aflatoxicosis is poisoning resulting from ingestion of aflatoxins in contaminated food or feed. Aflatoxins are a group of structurally-related toxic compounds produced by certain strains of the fungi Aspergillus flavus and A. parasiticus. Under favourable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins (ANON., 1979; CONCON, 1988; CREPPY, 2002; D'MELLO and MACDONALD, 1997; VELDMAN et al., 1992).

Aflatoxin contamination in milk and products is produced in two ways. Either toxins pass to milk with ingestion of feeds contaminated with aflatoxin, or it results as subsequent contamination of milk and milk products with fungi (APPLEBAUM et al., 1982; BLANCO et al., 1993; BARRIOS et al., 1997; SARIMEHMETOĞLU et al., 2003).

The major aflatoxins of concern are designated B1, B2, G1 and G2, also M1 and M2 as metabolic products of AFB. AFB1 to G2 belong to Group 1, and M1 belongs to Group 2B, according to IARC (CASTEGNARO and MCGREGOR, 1998). Additionally, the total reached 17 compounds, whether obtained from fungi culture or an animal's body (AFB2a, G2a, P, Q and aflatoxicol) (CONCON, 1988; PITTET, 1998).

It has been stated, in fact, that the contamination of milk and milk products with AFM1 displayed variations according to geography, country and season. The pollution level of AFM1 was differentiated further by hot and cold seasons, due to the fact that grass, pasture, weed, and rough feeds were found more commonly in spring and summer than in winter. At the end of summer, greens were consumed more than concentrated feed, causing a decreased level of AFM1 in milk (GALVANO et al., 1996; PITTET, 1998; SARIMEHMETOĞLU et al., 2003).

AFM1 has been reported to cause certain hygiene difficulties in milk and milk products used for food. During the obtaining of cream, AFM1 disperses heterogeneously in milk. AFM1 is not destroyed during the pasteurisation process or in yoghurt and cheese making (BARBIERI et al, 1994; CREPPY, 2002; GALVANO et al., 1996; PITTET, 1998). As aflatoxins pose more serious risks for public health, certain limits of aflatoxins in foods were determined. The limiting rates of AFM1 are shown in Table 1.

Materials and methods

Sampling. A total of 85 pasteurised milk samples were obtained from markets in various districts of Ankara. These samples were brought to the laboratory in an ice box together with their original packaging. The samples were prepared for analysis of AFM1 with

the competitive ELISA method described by R-biopharm GmbH, Dermstadt, Germany. (ANON., 1999)

Table 1. Maximum limits for aflatoxin M1 in milk in various countries (ANON., 1997; CREPPY, 2002)

Mycotoxin	Country	Maximum limit (µg/kg or µg/l)	Food
Aflatoxin M1	Sweden	0.050	Liquid milk products
	Austria	0.050	Milk
	Germany	0.050	Milk
	Netherlands	0.020	Butter
		0.200	Cheese
	Switzerland	0.050	Milk and milk products
		0.250	Cheese
	Belgium	0.050	Milk
	USA	0.50	Milk
	Czech Republic	0.1	Children's milk
		0.5	Adult's milk
	France	0.03	Children's milk
		0.05	Adult's milk
	Turkey	0.05	Milk and products
		0.25	Cheese

ELISA test procedure. The quantitative analysis of AFM1 in pasteurised milk samples was performed by competitive ELISA, using AFM1 test kit (ANON., 1999).

Milk samples were centrifuged at 3.500 g. for 10 min. at 10 ºC. The upper, creamy layer was removed and aliquot of the lower phase (supernatant without fat) was carefully poured off with a pasteur pipette. The skimmed milk was used directly in the test (100 µl per well).

One hundred µl standard solutions and prepared samples in separate wells were added and incubated for 60 min. at room temperature in the dark. After the washing steps, 100 µl of the enzyme conjugate was added and incubated for 60 min. at room temperature in the dark. The washing step was repeated three times. Fifty µl of substrate and 50 µl of chromagen were added to each well and mixed thoroughly and incubated for 30 min in the dark. One hundred µl of the stop reagent was added to each well, mixed and measured at an absorbance of 450 nm against air.

Evaluation. The mean values of absorbances for the standards and the samples were evaluated according to the RIDAVIN.EXE for Windows (Version 1.2) software programme prepared by R-biopharm GmbH.

Results and discussion

A total of 85 pasteurised milk samples were analysed with the competitive ELISA. The occurrence of AFM1 is shown in Table 2. Of the 85 samples analysed, 75 samples (88.23%) were found to be contaminated with AFM1. Twenty-seven samples (36%) failed to reach the desired level of the Turkish Food Codex, defined as 50 ng/l. Despite this, 48 samples (64%) exceeded the legal level of AFM1 in milk (Table 3).

Table 2. Aflatoxin M1 distribution and percentage of pasteurized milk samples

AFM1 levels ng/l in positive samples						
	< 10	11 - 30	31 - 50	51 - 70	71 - 90	91>*
N[1]	11	3	13	29	12	7
%[2]	14.66	4	17.33	38.66	16	9.33

[1] Number of samples analysed
[2] Percentage of AFM1 positive samples
*127.6 ng/l in one samples, 5.2 ng/l in two samples

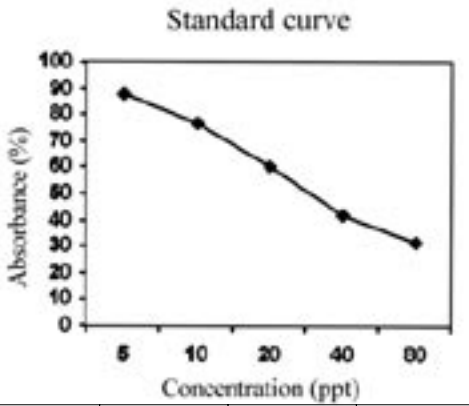
Table 3. Comparison of AFM1 levels with Turkish Food Codex

Sample		Under the Codex limit (0.05 µg/l)	Over the Codex limit (0.05 µg/l)	Percent value (%)
Positive	75	27	48	88.23
Negative	10	-	-	11.77
Total	85	27	48	
Percent value (%)		36	64	100

AFM1 was detected at 127.6 ng/l in the maximum level of only one sample, although the minimum level in 2 samples at 5.2 ng/l AFM1 was not determined in 10 samples. The results and calibration curve of test kit are shown in Fig. 1.

Although some studies showed no AFM1 detected in milk samples examined in various countries, such as Portugal, Japan and Turkey (BENTO et al., 1989; TABATA et al.,

1993; DEMIRER, 1973), many studies indicated that AFM1 contamination in milk was an important risk to human health (BAKIRCI, 1995; DOMAGALA et al., 1997; GALVANO et al., 1998; GALVANO et al., 2001a; MEERARANI et al., 1997; SAITANU, 1997).



Standard	Std1	Std2	Std3	Std4	Std5	Std6
Conct. (ng/L)	0.0	5.0	10.0	20.0	40.0	80.0
$B/B_{0\,(\%)}$	100.0	87.4	77.0	60.1	41.5	31.7

Fig. 1. Calibration curve of a RIDASCREEN® AFM1 kit and reference values

In our study, 75 of the examined samples (88.23 %) as AFM1-positive were found to have results similar to those of Ahmad, Bakırcı and Rastogi. These studies showed that AFM1 levels of various milks were found to range from 0.03 to 0.98 µg/kg., 27.7 - 53.4 ng/kg., and 28 to 164 ng/kg., respectively (AHMAD et al., 1996; BAKIRCI, 1995; RASTOGI et al., 2004). Also, KIM et al. (2000) determined the incidence of AFM1 in pasteurized milk as 76% in Korea, with a mean concentration of 18 pg/g, when determined by ELISA.

LÓPEZ et al. (2003), suggested that levels of AFM1 in samples of milk produced in Argentina were found to be very low and in no case did the levels exceed the recommended limits for milk products (0.05 µg l^{-1}). The authors reported that the AFM1 levels ranged from 0.013 to 0.017 µg l^{-1} in 8 positive pasteurised fluid milk samples. The lowest level of AFM1 was probably caused by the treatment of detoxification in feedstuffs, using lesser amounts of compound feeds, and the lesser amounts of samples analysed.

ROUSSI et al. (2002) examined raw and market milk samples for AFM1 contamination, over two periods. In the first sampling, the incidence rates of AFM1 contamination in pasteurised milk were 85.4%. In the second sampling, incidence rates were 79.6%. They

found that none of the pasteurised milk samples exceeded the limit of 50 ng/l. Their finding was that the current regulatory status in Greece is effective. In our study, the contamination level of AFM1 was similar, but the level of excess was determined at 64% of the samples examined.

European Communities and Codex Alimentarius have fixed the limit to a maximum of 50 ng AFM1/kg (ANON., 2001). In our study, 48 samples (64%) exceeded the regulatory limits, ranging from 50 to 127.6 ng/kg. RASTOGI et al. (2003) reported that 75% of liquid milk samples exceeded ECI Codex Regulations. Also, some studies indicated that contamination by AFM1 was relatively much higher, ranging from 28 to 1012 ng/kg in some European countries (GALVANO et al., 1998; MARKAKI and MELISSARI, 1997; MARTIN and MARTIN, 2000).

In the Turkish Food Codex (ANON., 1997), AFM1 levels in milk were limited to 50 ng/kg, similar to that of EC/Codex Regulations. Thus, the pasteurised milk samples analysed in this study exceeded the regulatory limit at a range of 50 to 127.6 ng/kg in 48 samples (64%).

In conclusion, this study has shown the serious risk for public health since all age groups, including infants and children, consume milk worldwide. For this reason, milk and milk products have to be controlled continuously for presence of AFM1 contamination. It is also extremely important to maintain low levels of AFM1 in the feeds of dairy animals. In order to achieve this, dairy cow feds should be kept away from contamination as much as possible. Therefore, animal feeds should be checked regularly for aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled.

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SAŽETAK

Aflatoksin M1 (AFM1) u mlijeku i mliječnim proizvodima predstavlja rizik za ljudsko zdravlje. Takvi metaboliti ne mogu se uništiti pasterizacijom ni zagrijavanjem. U ovom istraživanju određivana je kontaminacijska razina AFM1 u pasteriziranom mlijeku kojeg uzimaju sve dobne skupine diljem svijeta, uključujući i djecu. Ukupno je pretraženo 85 uzoraka pasteriziranog mlijeka na AFM1 imunoenzimnim testom. Ustanovljeno je da je 75 uzoraka (88,23%) bilo kontaminirano M1 aflatoksinom, a 48 uzoraka (64%) premašilo je dozvoljenu razinu AFM1 u mlijeku po Turskom kodeksu o hrani i Kodeksu alimentarius-u (59 ng/kg^{-1}). Uzimanjem kontaminiranog mlijeka javlja se ozbiljan rizik za javno zdravstvo. Stoga mlijeko i mliječne proizvode treba povremeno kontrolirati na prisutnost AFM1. Također treba voditi računa da se ne kontaminira hrana za mliječne krave.

Ključne riječi: mlijeko, aflatoksin M1, imunoenzimni test