TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2011; 35(2): 87-91
© TÜBİTAK
doi:10.3906/vet-0810-5

Aflatoxin M1 contamination in cow's milk in Kayseri (central Turkey)

Hikmet Mavuş BULDU[1], A. Nedret KOÇ[1,*], Güven URAZ[2]

[1]Department of Microbiology, University of Erciyes, Kayseri - TURKEY

[2]Department of Microbiology, Faculty of Science and Arts, Gazi University, Ankara - TURKEY

Received: 07.10.2008

Abstract: The purpose of this study was to determine the levels of aflatoxin M1 (AFM1) in the milk of cows in the villages of Kayseri province, Turkey. Ninety milk samples were investigated for AFM1 contamination using the enzyme linked immunoassay (ELISA). The mean value of AFM1 levels was 59.9 ng/L, 95% confidence interval was 54.4-65.5 ng/L, and standard deviation was 26.6 ng/L. For 63 samples (70%) the values were significantly above the limit permitted by European Union criteria (EU) and the Turkish Food Codex (TFC) ($P < 0.05$). However, the differences in AFM1 levels between villages were not significant ($P > 0.05$). In conclusion, since the level of AFM1 in the milk of cows was higher in Kayseri province, this must be seen as a significant public health problem. Further research must be conducted on the levels of AFM1 in milk samples sterilized using ultra high temperature (UHT).

Key words: Aflatoxin M1, cow's milk, ELISA

Kayseri'deki inek sütlerinde aflatoxin M1 kontaminasyonu

Özet: Bu çalışmada, Türkiye'de Kayseri'nin köylerindeki inek sütlerindeki Aflatoksin M1 düzeyinin belirlenmesi amaçlanmıştır. Toplam 90 süt örneğinde AFM1 kontaminasyonu ELISA (Enzyme Linked Immunoassay) yöntemi kullanarak çalışıldı. AFM1 düzeyinin ortalama değeri 59,9 ng/L, % 95 konfidende intervali 54,4-65,5 ng/L, ve standart sapması 26,6 ng/L olarak belirlendi. Avrupa Birliği ve Türkiye gıda kodeks göre 63 (% 70)'ün limit sınırlarının üzerinde olması istatiksel olarak önemli bulunmuştur ($P < 0,05$). Bununla beraber köylere göre AFM1 düzeyindeki farklılık istatiksel olarak önemli değildir ($P > 0,05$). Bu bölgedeki inek sütlerinde AFM1 düzeyinin yüksek olması, önemli bir halk sağlığı sorunu olarak düşünülmelidir. Daha fazla araştırmalarla yüksek ısı altında steril edilen (UHT) süt örneklerinde Aflatoksin M1 düzeyi belirlenmelidir.

Anahtar sözcükler: Aflatoksin M1, inek sütü, ELISA

* E-mail: anedret@erciyes.edu.tr

Introduction

Aflatoxins, as known, are produced mainly by the *Aspergillus* species *A. flavus* and by most, if not all, strains of *A. parasiticus*, plus related species, *A. nomius* and *A. niger*. Moreover, studies revealed that there are 4 major aflatoxins: B1, B2, G1, and G2 plus 2 additional metabolic products, M1 and M2, which are of significance as direct contaminants of foods and feeds. The aflatoxins M1 and M2 were first isolated from milk of lactating animals fed aflatoxin preparations; hence the M designation. Aflatoxin M1(AFM1) is 4-hydroxy aflatoxin B1 and aflatoxin M2 is 4-dihydroxy aflatoxin B2 (1).

It was found that cows metabolize some of the aflatoxin B1 in their food to become AFM1, a compound that is closely related to aflatoxin B1. The aflatoxins, in particular B1, exhibit strong carcinogenic properties and thus have been classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC). As limited studies were carried out, the same qualitative conclusion is usually drawn, that AFM1 is also hepatoxic and carcinogenic.

In Turkey, the acceptable AFM1 level is listed as 0.00005 mg/kg = 50 ng/L = 50 ppt in the Regulations of the Turkish Food Codex (TFC), Official Gazette, 1997, and European Union criteria (2-4).

The aim of this study was to determine the AFM1 level in the milk of cows in villages (Germir, Cırgalan, and Salur) in Kayseri province, Turkey.

Materials and methods

A total of 90 milk samples were collected from cows in 3 central villages in Kayseri province in February 2002. Thirty-two samples were collected from village I (Germir), 26 from village II (Cırgalan), and 33 from village III (Salur).

These samples were taken into 10 mL sterile injectors and after labeling they were transported to the laboratories in an ultraviolet filtered special refrigerator. The milk samples underwent refrigerated centrifuging at 10 °C for 10 min at 3500 rpm for fat separation. After centrifuging, the upper cream layers were completely discarded and the lower phases were frozen for quantitative tests. An aliquot of the skimmed milk was used directly in the test.

The aflatoxin levels in the milk samples were analyzed using the ELISA test (R-biopharm, Germany) according to the procedure recommended by the company. This method is quick, reliable, and cost effective for the estimation of AFM1 levels and has been included in the official collection of test procedures by the German Federal Board of Health. The absorbance at 450 nm against an air blank was measured spectrophotometrically (Tecan-Columbus plus-Abbott, Austria). Calibration curves of AFM1 for the absorbance values were obtained for the standards and then the AFM1 levels for the milk samples were determined.

These results were assessed according to the Regulations of the Turkish Food Codex (TGK) and the European Union criteria (the upper limit of aflatoxin in milk is 0.00005 mg/L = 50 ng/L = 50 ppt) (2).

Chi-square test in a single example and one-way ANOVA were used for the statistical analysis of data. P values of less than 0.05 were considered statistically significant.

Results

AFM1 was found in 100% of the examined milk samples. The AFM1 levels ranged from 5 ng/L to 80 ng/L in the milk samples. Ten samples (11.1%) had a contamination of <5-9 ng/L of AFM1, 17 samples (14.4%) contained 10-49 ng/L, 15 samples (18.9%) contained 50-79 ng/L, and the remaining 16.6% of samples contained more than 80 ng/L of AFM1. AFM1 was found to be 70% higher than the TGK and EU criteria and this finding was statistically significant ($P < 0.05$, χ^2: 14.4). Table 1 shows the distribution of AFM1 levels according to villages.

The overall mean level of AFM1 was 59.9 ng/L, the 95% confidence interval was 54.4-65.5 ng/L, and the standard deviation was 26.6 ng/L. Individual sample values ranged from 5 ng/L to 80 ng/L. Village-by-village data and statistical test results are summarized in Table 2. According to these data, higher levels of AFM1 contamination were seen in village I (Germir), but the differences in the AFM1 levels between villages were not statistically significant ($P > 0.05$).

Table1. The distribution of aflatoxin M1 evels according to villages.

AFM1 level (ng/L)	village I	village II	village III	Total
<5-9	-	-	10	10
10-49	6	7	4	17
50-79	6	7	2	15
≥80	20	12	16	48
Total	32	26	32	90

$P > 0.05$, χ^2: 23

Table 2. Statistical summary of AFM1 determinations, based on regions.

Location	No. of samples	Statistically	Mean Difference (ng/L)	Std. Deviation (ng/L)	95% Confidence Interval of the Difference (ng/L)	
					Lower	Upper
Village I	32	0.890	68.3594	18.19528	61.7993	74.9195
Village II	26	0.242	61.8462	21.32171	53.2341	70.4582
Village III	32	0.370	49.9531	34.03896	37.6808	62.2255
Total	90	0.921	59.9333	26.62708	54.3564	65.5103

Discussion

After finding out the effects of the aflatoxins on human and animal health, many countries designated maximum limits for these mycotoxins that exist in foods and fodders. Since having levels under this limit does not mean that it is safe, the countries tended to decrease the limit (5).

The contamination ranges of milks with AFM1 were found to be low in many counties (2%-28%): in Czechoslovakia (3.3%), Brazil's Campinas City (2%), Spain (5.4%), Cyprus (12%), and Kuwait (28%), but high in some: United Arab Emirates (95%), Italy's 4 major cities (78%), Italy (86%), Portugal (80%), and Turkey (this study) (100%) (6-14).

European Union's National Reference Laboratories, established in 1992, is a committee commissioned in order to find out the aflatoxin ranges in milk. In the research this committee performed, natural controlled milk was taken from cows fed with aflatoxin B1. The aflatoxin found in the milk was within the range of 0.005-0.7 pg/L. It was declared that this result was similar to the EU's limit (0.05 pg/L) (3). In a study carried out in Italy from 159 milk samples collected in 1995, AFM1 was found in 136 (86%) of milks (in amounts ranging from <1 ng/L to 108.5 ng/L; mean level: 10.19 ng/L) by the HPLC test. However, only 2 samples exceeded the limits (14).

In another study, conducted in Kuwait, AFM1 range was measured by HPLC for 54 fresh cream, light milk, coffee cream, yogurt, and baby food samples. AFM1 was found as a contaminant in 28% of the samples and in 6% of the samples AFM1

was found to be over the limit. In coffee cream and in baby food no AFM1 contamination was seen. It was reported that Kuwaiti milk and milk products were not hazardous to human health. However, they also reported that the AFM1 range for animal fodders should be carefully observed (10).

Rodríguez Velasco et al. (8) conducted a study where the concentrations of AFM1 in milk were estimated by ELISA; in that study AFM1 was found in only 5.4% of the samples, and the concentrations in all these cases were lower than the maximum limit applicable to these products pursuant to EU legislation. In the same study, it was also reported that using a commercial ELISA kit to determine AFM1 concentrations is a very effective screening method when a large number of samples are involved.

In research carried out in Portugal, aflatoxin levels were determined using the ELISA test. In this study, in 31 uncooked milk samples the AFM1 levels were determined at a rate of 80.2%. In 70 cooked UHT milk samples, the rate was 84.2%.

The results of our study showed that the presence of AFM1 in UHT milk samples in central Anatolia, Turkey, had a high incidence rate (100%) and was well above (70%) the limit permitted by the EU. This contamination range was found to be higher than that in other studies on UHT milk samples done in Turkey (15-18). The higher incidence of AFM1 found in milk samples may be due to the fact that cow's milk was used compared to UHT milk in other studies.

In this area, in another study, in 86 samples taken from milk collecting tanks AFM1 was investigated using the ELISA test. In 20 of these samples 3.4-32.7 ng/L aflatoxin was found. Although AFM1 levels in Turkey are below the maximum limits, the investigators state that in Kayseri AFM1 is a potential contaminant (19). The reason for the current study's results being higher than that study's results may be that the milk was taken directly from its source and cooked without being mingled with other milks.

In our study, the overall mean level of AFM1 was 54.4-65.5 ng/L (95% confidence interval). The AFM1 level differences between villages (Germir, Cırgalan, and Salur) were not statistically significant ($P > 0.05$). In another study, in Iran, the overall mean level of AFM1 was 54.4-65.5 ng/L. The level of aflatoxin in milk from one region (Hanedan) was significantly lower ($P < 0.05$) than in those of the other regions (Gorgan, Rasht, Shiraz, and Tehran) (20). It seems that the kind of animal feeding and the harvesting time and temperature could be effective parameters in this regard.

In conclusion, the aflatoxin range was higher in the villages where the study was conducted. However, further research must be conducted on pasteurized milks and in different seasons.

References

1. Doyle, M.P., Beuchat, L.R., Montuille, T.J.: Mycotoxigenic Molds, Food Microbiology, Asm Press, USA. 1997.
2. Anonymous.: European community comments for the codex committee on food additives and contaminants. CL 1999/13-GEN-CX 0016 FAC-Agenda item 16a. 2000.
3. Dragacci, S., Grosso, F., Pfouwathel-Marchan, N., Fremy, J.M., Venant, A.: Proficiency testing for the evaluation of the ability of European Union-National Reference laboratories to determine aflatoxin M1 in milk at levels corresponding to the new European Union legislation. Food. Addit. Contam., 2001;18: 405-415.
4. Goldblatt, L.A.: Aflatoxin and its control. Econ. Bot., 1968; 22: 51-62.
5. Taydaş, E., Aşkın, O.: Occurrence of aflatoxin in red pepper J. Food., 1995; 20: 3-8.
6. Fukal, I., Brezina, P. Determination of aflatoxin M_1 level in milk in the production of baby and children's food using immunoassay. Nahrung., 1991; 35: 745-748.
7. de Sylos, C.M., Rodriguez-Amaya, D.B., Carvalho, P.R.: Occurrence of aflatoxin M_1 in milk and dairy products commercialized in Campinas, Food. Addit. Contam., 1996; 13: 169-172.
8. Rodríguez Velasco, M.L., Calonge Delso, M.M., Ordónez Escudero, D.: ELISA and HPLC determination of the occurrence of aflatoxin M(1) in raw cow's milk. Food Addit. Contam., 2003; 20: 276-80.
9. Sun, Z., Lu, P., Gail, M.H. Pee, D., Zhang, Q., Ming, L., Wang, J., Wu, Y., Liu, G., Wu, Y., Zhu, Y.: Increased risk of hepatocellular carcinoma in male hepatitis B surface antigen carriers with chronic hepatitis who have detectable urinary oflatoxin metabolite M_1. Hepatology, 1999; 30: 379-383.

10. Srivasta, V.P., Bu-Abbas, A., Alaa-Basung, W., Al-Johar, W., Al-Mufti, S., Siddigui, M.K. Aflatoxin M_1 contamination in commercial samples of milk and dairy products in Kuwait, Food. Add. Con., 2001; 18: 993-997.

11. Saad, A.M., Abdelgadir, A.M., Moss, M.O.: Exposure of infants to aflatoxin M_1 from mother's breast milk in Abu Ahobi. Food. Addit. Contam., 1995; 12: 255-261.

12. Galvano, F., Galofaro, V., de Angelis, A., Galvano, M., Bognanno, M., Galvano, G.: Survey of the occurrence of aflatoxin M1 in dairy products marketed in Italy. J. Food. Prot., 1996; 1: 738-41.

13. Martins, H.M.: Aflatoxin M_1 in raw and ultra-high temperature-treated milk commercialized in Portugal. Food. Addit. Contam., 2000; 17: 871-874.

14. Galvano, F., Galofaro, V., Ritieni, A., Begnonno, M., De Angelis, A., Galvano, G.: Survey of the occurrence of aflatoxin M_1 in dairy products marketed in Italy, Food. Addit. Contam., 2001; 18: 644-646.

15. Bakırcı, I.: A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. Food. Control., 2001; 12, 47-51.

16. Oruç, H.H., Sonal, S.: Determination of aflatoxin M_1 levels in cheese and milk consumed in Bursa, Turkey., Vet. Hum. Toxicol., 2001; 43: 292-293.

17. Gürbay, A., Aydın, S., Girgin, G., Engin , A.B., Şahin G.: Assessment of aflotoxin M1 levels in milk in Ankara, Turkey. Food. Control., 2006; 17: 1-4.

18. Unusan, N.: Occurrence of aflatoxin M1 in UHT milk in Turkey. Food. Chem. Toxicol., 2006; 44: 1897-1900.

19. Liman, B.C., Şeybek, N.: Quantitative analysis of aflatoxin M_1 in raw milk contaminated at low levels. Toxicol. Lett., 2001; 123: 42.

20. Tajkarimi, M., Shojaee Aliabadi, F., Salah Nejad, M., Pursoltani, H., Motallebi, A.A., Mahdavi, H.: Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. Int. J. Food. Microbiol., 2007; 116: 346-9.