

Determination of aflatoxin M₁ levels in goat milk consumed in Kilis province

Mehmet ÖZDEMİR

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200 Afyonkarahisar, Turkey.

Summary: Aflatoxin M₁ (AFM₁) may occur in milk and dairy products, resulting from the ingestion of feedstuffs contaminated with aflatoxin B₁ (AFB₁) by dairy goats. The aim of this study was to determine the levels of AFM₁ in goat milk commonly consumed in the city of Kilis. One hundred and ten samples of milk were collected randomly from individual farms in Kilis from March 2006 to April 2006, Turkey, samples were analysed for AFM₁ by ELISA. AFM₁ was not determined in 17 samples (15.46%), whereas 93 samples (84.54%) were found to contain AFM₁ at various levels. In 70 of the 110 samples, the presence of AFM₁ was detected in a concentration ranging between 5.16 and 116.78 ng/l. AFM₁ levels in 7 (6.36%) of 110 goat milk samples were found to be higher than the maximum tolerable limit (50 ng/l) accepted by the Turkish Food Codex. We concluded that AFM₁ was determined in 84.54% of the goat milk samples consumed by the people in the Kilis province. Moreover 6.36% of samples contained AFM₁ at hazardous levels for human health.

Key words: Aflatoxin M₁, ELISA, goat milk, Kilis.

Kilis bölgesinde tüketilen keçi sütlerinde aflatoksin M₁ düzeylerinin belirlenmesi

Özet: AFB₁ ile kontamine yemler süt keçileri tarafından tüketildiğinde, süt ve süt ürünlerinde AFM₁ meydana gelebilir. Bu çalışmanın amacı Kilis'te yaygın olarak tüketilen keçi sütlerindeki AFM₁ düzeylerini belirlemektir. 2006 yılı Mart-Nisan aylarında, Kilis'teki bireysel çiftliklerden elde edilen 110 keçi sütü örneklerinde ELISA yöntemiyle AFM₁ analizleri yapıldı. AFM₁ 17 (%15,46) örnekte belirlenemezken, 93 (%84,54) süt örneğinde farklı düzeylerde bulundu. AFM₁ 110 süt örneğinin 70'inde 5,16–116,78 ng/l düzeyleri arasındaki yoğunluklarda belirlendi. Örneklerin 7'sinde (%6,36) belirlenen AFM₁ düzeyleri Türk Gıda Kodeksi'nde belirtilen maksimum kabul edilebilir limitten (50 ng/l) daha yüksek düzeyde ölçüldü. Sonuç olarak, Kilis ilinde insanlar tarafından tüketilen keçi sütü örneklerinin %84,54'ünde AFM₁ belirlendi ancak örneklerin %6,36'sı insan sağlığı için risk oluşturacak düzeyde AFM₁ içermekteydi.

Anahtar sözcükler: Aflatoksin M₁, ELISA, keçi sütü, Kilis.

Introduction

Aflatoxins associated with both toxicity and carcinogenicity in human and animal populations are a group of naturally occurring toxins produced mainly by three species of *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*) which contaminate plants and their products (6, 18).

Aflatoxins are acute toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (18). When lactating mammals, such as cows, sheep and goats, are fed with feeds contaminated with AFB₁, this metabolite can be converted to hydroxylated form called AFM₁, which is cytotoxic and genotoxic substance (30). AFB₁ is biotransformed by hepatic microsomal cytochrome P450 to AFM₁ which possesses 10 times lower carcinogenic potential compared to the parent

molecule (8). AFM₁ was classified by the International Agency for Research on Cancer (IARC) as a Group 2B agent (possibly carcinogenic to humans) in 1993 (15).

AFM₁ could be detected in milk 12-24 h after the AFB₁ ingestion, reaching a high level after a few days. When the intake of AFB₁ is stopped, the AFM₁ concentration in the milk decreases to an undetectable level after 72 h (30). The ratio between AFB₁ ingested and AFM₁ excreted had been estimated to be 1-3% (5) but values as high as 6% had been reported by Pittet (25), however, the percentage varies from animal to animal, day to day and one milking to the other (21).

AFM₁ had a resistant to thermal inactivation used during food processing procedure such as pasteurization and autoclaving. Storage of various dairy products was not effective in the reduction of this toxin (18, 24, 27). Milk and its products are a major nutrient for human

especially children through the world. At the same time, these products may be contaminated with AFM₁ residues which extensively threaten the human health. For this reason, many countries have regulations to control the levels of AFB₁ in feeds and to propose maximum permissible levels of AFM₁ in milk to reduce this risk (2, 26). Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another (28, 30). The European Community and Codex Alimentarius prescribe that the maximum level of AFM₁ in liquid milk and dried or processed milk products should not exceed 50 ng/kg (7). However, according to US regulations the level of AFM₁ in milk should not be higher than 500 ng/kg (28). Turkish legal limits for AFM₁ in milk is 50 ng/l (29). In spite of the regulatory control measures taken by many countries, production of aflatoxin-free milk is not always achieved (12).

Although presence of aflatoxin in feed, milk and cheese was systematically controlled in USA, Europe and other developed countries, there were insufficient data on the contamination of milk and cheese with AFM₁ in Turkey. Milk and dairy products have been produced and consumed widely in Kilis province. There was no information about the occurrence of AFM₁ in milk and its products in this city. Therefore, the aim of this study was to investigate the presence of AFM₁ in goat milk samples consumed in Kilis by ELISA method.

Materials and Methods

One hundred and ten samples of milk obtained randomly from individual farms in Kilis from March 2006 to April 2006, Turkey, samples were investigated for AFM₁ by ELISA. The goat milk samples were transferred to the laboratory in ice boxes and they were stored in a cool place, protected from light. Analysis of samples was carried out in less than 24 h from the time of their arrival to the laboratory. The samples were analysed in Department of Pharmacology and Toxicology Faculty of Veterinary Medicine at Afyon Kocatepe University. AFM₁ concentrations were determined by competitive ELISA (Thermo LabSystems Multiskan Spektrum, 1500).

Determination of AFM₁ was based on an enzyme linked immunoassay using the Ridascreen test kit (Ridascreen[®], aflatoxin M₁ r-biofarm, Art. No.:R1101, Germany). According to the instructions for use of the Ridascreen kit, the recovery rate in milk (10–80 pg/ml) is 95%. This method is quick, reliable and cost effective for the estimation of AFM₁ and has been included in the official collection of test procedures by the German Federal Board of Health. The test shows cross-reaction to

AFB₁ (12.4%) but this is not relevant when analyzing AFM₁, considering that AFB₁ usually is not to be found in milk or milk products (17).

Preparation of samples: Preparation of samples was conducted according to the instructions of the RIDASCREEN kit. Briefly, milk samples (4 ml each) were chilled and then centrifuged for 10 min / 3500 g /10°C (Nüve NF 1000R). After centrifugation the upper cream layer was completely removed by aspirating through a pasteur pipette. An aliquot (100 µl per well) of the skimmed milk was used directly in the test.

Test procedure: ELISA test kit included AFM₁ standards solutions levels of 0, 5, 10, 20, 40 and 80 pg/ml. AFM₁ standards and the prepared sample solutions were added (100 µl) to microtiter wells. During incubation for 60 min at room temperature in the dark, the antibody binding sites were occupied proportionally to the AFM₁ concentration. The liquid was then removed completely from the wells, which were washed twice with distilled water. In the next step, any remaining free binding sites were occupied by the enzyme conjugate (enzyme labeled toxin), which was added (100 µl) and incubated for another 60 min at room temperature in the dark. Any unbound enzyme conjugate was then removed in a washing step. Enzyme substrate (urea peroxide, 50 µl) and chromogen (tetramethylbenzidine, 50 µl) were added to each well and incubated for 30 min at room temperature in the dark. Bound enzyme conjugate converts the colourless chromogen into a blue product. Then the addition of the reaction stop reagent (100 µl per well) led the colour change from blue to yellow. Change in colour was made photometrically at 450 nm. The mean values of the samples and the absorbances for standards were evaluated according to the Ridasoft Win (Version 1.44 R-Biopharm) computer program.

Statistical analyses: Means, percentages, minimum and maximum values of data were calculated as proposed by Zar (31).

Results

The present study, total of 110 milk samples obtained randomly from individual farms were analysed for AFM₁ with the ELISA method. Results are shown in Table 1. AFM₁ was not detected in 17 samples (15.46%), whereas 93 samples (84.54%) were found to contain AFM₁ at various levels. In 70 of the 110 samples, the presence of AFM₁ was detected in the concentration ranging between 5.16 and 116.78 ng/l. AFM₁ levels in 7 (6.36%) of 110 goat milk samples were found to be higher than maximum tolerable limit (50 ng/l) accepted by the Turkish Food Codex.

Table 1. Levels of AFM₁ in goat milk consumed in Kilis province.Tablo 1. Kilis bölgesinde tüketilen keçi sütlerinde AFM₁ düzeyleri.

Concentration of AFM ₁ (ng/l)	Sample, n	Incidence (%)	Average ^a	Range ^b Min-Max
ND	17	15.46	-	-
< 5	23	20.91	-	-
5-50	63	57.27	13.03	5.16-41.40
> 50	7	6.36*	75.05	55.07-116.78
Total	110	84.54	19.23	5.16-116.78

ND, not detected.

^a: Mean of positive samples.^b: Minimum – maximum.

*: Exceed Turkish legal limits.

Discussion and Conclusion

AFM₁ levels in milk and dairy products are important since many people use milk and dairy products in their diets frequently, especially babies and children. Milk has the greatest potential for introducing AFM₁ into the human diet. For this reason, AFM₁ in milk and dairy products should be controlled systematically.

Currently aflatoxin analysis are done by various methods including thin-layer chromatography (TLC), liquid chromatography (LC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) (2, 4). Although there are several techniques for detecting AFM₁ in milk and dairy products, ELISA is the most useful technique due to its quickness, sensitivity, ease of application and cheapness. Therefore, ELISA technique was used for detecting AFM₁ in goat milk samples in present survey.

Evidence of hazardous human exposure to AFM₁ through milk and dairy products had been shown by several investigators (4, 12, 19, 21). AFM₁ level in the milk was significantly affected by the geographical region, country, and seasons. It was demonstrated that milk produced in summer was less contaminated than that produced in the winter season (12).

In a study conducted by Alborzi et al. (1), AFM₁ was found in 100% of examined milk samples. 390 samples (62.5%) had contamination less than 45 ng/l AFM₁, 123 samples (19.7%) contained 45-50 ng/l, 94 samples (15.1%) contained 50-80 ng/l and 2.7% of samples contained more than 80 ng/l of AFM₁ in Shiraz (south of Iran). Alborzi et al. (1) determined that 17.8% of samples had AFM₁ greater than the maximum tolerance limit (50 ng/l) accepted by European Union. In Campinas, Brazil (9), AFM₁ was only detected in four

samples of pasteurized milk in values between 73 and 370 ng/l, of a total of 204 samples of pasteurized milk, powdered milk, cheese and yoghurt. Galvano et al. (11) detected AFM₁ in 97 samples of dry milk for infant formula using HPLC; in 81 samples (84%) amounts ranged from <0.001 to 0.101 µg/l.

In Japan during the winter season, AFM₁ was detected in 207 (99.5%) of 208 milk samples at 1-29 ng/kg with a mean of 9 ng/kg (22). In a Korean study, the incidence of AFM₁ in liquid milk was 76%, with a mean concentration of 18 pg/g (19). The incidence of AFM₁ contamination in the raw milk analysed in Portugal was 80.6%, 17 samples (54.8%) contained low levels (5–10 ng/l), two samples (6.5%) had levels ranging from 11 to 20 ng/l, six samples (19.3%) had levels between 21 and 50 ng/l (21). During 1996, 161 samples of milk in Italy were checked for AFM₁. AFM₁ was detected in 125 (78%) of milk samples (ranging from <1 to 23.5 ng/l; mean level was 6.28 ng/l) (13). In a study in Argentina, a total of 77 various types of milk samples were analysed, only 18 samples (approximately 23%) were found to be contaminated with AFM₁ at levels of 0.01–0.03 µg/l. All concentrations were below the maximum tolerated levels of AFM₁ in liquid milk and powdered milk (0.05 µg/l) (20). In another study of 111 raw milk samples, 85 (76.6%) were found contaminated with AFM₁ at concentration between 0.15 and 0.28 µg/l (16). Elgerbi et al. (10) found AFM₁ in 71.4% of the milk samples (35 of the 49 samples) with contaminated levels between 0.03 and 3.13 ng/ml in North African. In Lucknow, India (26), a total of 87 infant milk products and liquid milk samples were analysed, 76 (87.3%) samples were found to be contaminated with AFM₁ at 28-1012 ng/kg with a mean of 299 ng/kg. Almost 99% of the contaminated samples exceeded the 50 ng/l limit.

There were many researches for AFM₁ analyses in milk and dairy products in our country. One of them, Bakirci (4) was found AFM₁ in 79 (87.77%) of 90 milk samples. He reported that 35 (44.3%) of the positive samples were found higher than the maximum limit accepted by Turkish regulation. Oruc and Sonal (23) examined AFM₁ levels in milk and cheese from Bursa. Their result showed that the contamination with AFM₁ were found in 89.5% of the cheese samples with range of 0–810 ng/kg and only one of milk sample (10%) in (10.8 ng/l) level. Aycicek et al. (3), studied the occurrence AFM₁ in 183 samples of white cheese and butter in Istanbul, in 2001. The incidence of AFM₁ in white cheese and butter samples were found as high as 65% and 81%, respectively. In another study, in Ankara, Gürbay et al. (14) detected AFM₁ in 16 (59.3%) of 27

milk samples using HPLC. However, they found that only one sample above maximum permissible limit accepted by Turkey for AFM₁. In another study, Aycicek et al. (2) found that AFM₁ in 90.58% of 202 dairy products samples. AFM₁ levels in 12 (12.76%) white cheese and 7 (13.2%) of Kashar cheese samples were found higher than the maximum acceptable levels (250 ng/kg). In this study, we detected in 93 (84.54%) of 110 goat milk samples AFM₁ contamination. AFM₁ levels in 7 (6.36%) of 110 milk samples were found to be higher than maximum tolerable limit (50 ng/l) accepted by the Turkish Food Codex.

Previous studies have reported different levels of AFM₁ in milk samples. The overall AFM₁ incidence of the milk samples analysed in this study were lower than the results reported by Alborzi et al. (1), Nakajima et al. (22), Rastogi et al. (26), Bakirci (4), and were higher than the results reported by De Sylos et al. (9), Kim et al. (19), Elgerbi et al. (10), Martins and Martins (21), Galvano (13), López (20), Kamkar (16), Oruç and Sonal (23), Gürbay et al. (14). The difference can be attributed to the geographic location, season of the year and sample size differences among studies. Those contradicting results might be mainly due to the different livestock management and dairy processing systems used in those countries.

Considering its risk to the human health, particularly liver cancer, this study indicated that the incidence of AFM₁ concentrations in the goat milk samples consumed in Kilis was high (84.54%). Furthermore, the levels of AFM₁ in milk samples were higher than 50 ng/l is 6.36% (7 samples) in the present survey. The results of this study about the factual contamination of milk and dairy products with AFM₁ imply that more emphasis should be given to the routine AFM₁ inspection in milk and dairy products. In addition, governmental agencies need to inform both farmers and dairy companies about the important of AFB₁ and AFM₁, and the consequences of AFM₁ presence in their products. In conclusion, these results show that the important of periodically monitoring the occurrence of AFM₁ in goat milk and dairy products in Kilis province.

References

1. **Alborzi S, Pourabbas B, Rashidi M, Astaneh B** (2006): *Aflatoxin M₁ contamination in pasteurized milk in Shiraz (south of Iran)*. Food Control, **17**, 582-584.
2. **Aycicek H, Aksoy A, Saygi S** (2005): *Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey*. Food Control, **16**, 263-266.
3. **Aycicek H, Yarsan E, Sarımehmetoglu B, Cakmak O** (2002): *Aflatoxin M₁ in white cheese and butter consumed in Istanbul, Turkey*. Vet Hum Toxicol, **44**, 295-296.
4. **Bakirci I** (2001): *A study on the occurrence of aflatoxin M₁ in milk and milk products produced in Van province of Turkey*. Food Control, **12**, 47-51.
5. **Barbieri G, Bergamini C, Ori E, Pesca P** (1994): *Aflatoxin M₁ in Parmesan cheese: HPLC determination*. J Food Sci, **59**, 1313-1331.
6. **Betina V** (1989): *Aflatoxins, sterigmatocystins and versicolorins*. 114-150. Mycotoxins: Chemical, Biological and Environmental Aspects. New York.
7. **Codex Alimentarius Commissions** (2001): *Comments submitted on the draft maximum level for Aflatoxin M₁ in milk*. Codex Committee on Food Additives and Contaminants 33rd sessions, Hauge, The Netherlands.
8. **Cullen JM, Ruebner BH, Hsieh LS, Hyde DM, Hsieh DPH** (1987): *Carcinogenicity of dietary Aflatoxin M₁ in male Fischer rats composed to Aflatoxin B₁*. Cancer Res, **47**, 1913-1917.
9. **De Sylos CM, Rodriguez-Amaya DB, Carvalho PRN** (1996): *Occurrence of aflatoxin M₁ in milk and dairy products commercialized in Campinas, Brazil*. Food Addit Contam, **13**, 169-172.
10. **Elgerbi AM, Aidoo KE, Candlish AAG, Tester RF** (2004): *Occurrence of aflatoxin M₁ in randomly selected North African milk and cheese samples*. Food Addit Contam, **21**, 592-597.
11. **Galvano F, Galofaro V, De Angelis A, Galvano M, Bognanno M, Galvano G** (1998): *Survey of the occurrence of aflatoxin M₁ in dairy products marketed in Italy*. J Food Prot, **61**, 738-741.
12. **Galvano F, Galofaro V, Galvano G** (1996): *Occurrence and stability of aflatoxin M₁ in milk and milk products: a worldwide review*. J Food Prot, **59**, 1079-1090.
13. **Galvano F, Galofaro V, Ritieni A, Bognanno M, De Angelis A, Galvano G** (2001): *Survey of the occurrence of aflatoxin M₁ in dairy products marketed in Italy: second year of observation*. Food Addit Contam, **18**, 644-646.
14. **Gürbay A, Aydın S, Girgin G, Engin AB, Şahin G** (2006): *Assessment of aflatoxin M₁ levels in milk in Ankara, Turkey*. Food Control, **17**, 1-4.
15. **IARC (International Agency for Research on Cancer)** (1993): *Aflatoxins. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic aromatic amines and Mycotoxins*. 245-395. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans.
16. **Kamkar A** (2005): *A study on the occurrence of aflatoxin M₁ in raw milk produced in Sarab city of Iran*. Food Control, **16**, 593-599.
17. **Kaniou-Grigoriadou I, Eleftheriadou A, Mouratidou T, Katikou P** (2005): *Determination of aflatoxin M₁ in ewe's milk samples and the produced curd and Feta cheese*. Food Control, **16**, 257-261.
18. **Kaya S** (2002): *Mikotoksinler*. 544-568. Veteriner Hekimliğinde Toksikoloji. İkinci Baskı. S Kaya, İ Pirinçci, A Bilgili (Ed), Medisan Yayınevi. Ankara.
19. **Kim EE, Shon DH, Ryu D, Park JW, Hwang HJ, Kim YB** (2000): *Occurrence of aflatoxin M₁ in Korean dairy products determined by ELISA and HPLC*. Food Addit Contam, **17**, 59-64.

20. **López CE, Ramos, LL, Ramadán SS, Bulacio LC** (2003): *Presence of aflatoxin M₁ in milk for human consumption in Argentina*. Food Control, **14**, 31–34.
21. **Martins ML, Martins HM** (2000): *Aflatoxin M₁ in raw and ultra high temperature-treated milk commercialized in Portugal*. Food Addit Contam, **17**, 871-874.
22. **Nakajima M, Tabata S, Akiyama H, Itoh Y, Tanaka T, Sunagawa H, Tyonan T, Yoshizawa T, Kumagai S** (2004): *Occurrence of aflatoxin M₁ in domestic milk in Japan during the winter season*. Food Addit Contam, **21**, 472-478.
23. **Oruç HH, Sonal S** (2001): *Determination of aflatoxin M₁ levels in cheese and milk consumed in Bursa, Turkey*. Vet Hum Toxicol, **43**, 292–293.
24. **Park DI** (2002): *Effect of processing on aflatoxin*. Adv Exp Med Biol, **504**, 173-179.
25. **Pittet A** (1998): *Natural occurrence of mycotoxins in foods and feeds-an update review*. Rev Med Vet, **149**, 479-492.
26. **Rastogi S, Dwivedi PD, Khanna SK, Das M** (2004): *Detection of aflatoxin M₁ contamination in milk and infant milk products from Indian markets by ELISA*. Food Control, **15**, 287–290.
27. **Stoloff L** (1989): *Aflatoxin M₁ in perspective*. J Food Prot, **43**, 226-230.
28. **Stoloff L, Van Egmond HP, Parks DL** (1991): *Rationales for the establishment of limits and regulations for mycotoxins*. Food Addit Contam, **8**, 222–231.
29. **Türk Gıda Kodeksi Tebliğ** (2002): *Türk Gıda Kodeksi gıda maddelerinde belirli bulaşanların maksimum seviyelerinin belirlenmesi hakkında tebliğ (No:2002/63)*. Resmi Gazete, 23.09.2002, Sayı: 24885.
30. **Van Egmond HP** (1989): *Aflatoxin M₁: occurrence, toxicity, regulation*. 11-55. Mycotoxins in Dairy Products. In H.P. Van Egmond (Ed), Elsevier Applied Science, New York.
31. **Zar JH** (1984): *Biostatistical Analysis*. 2nd ed. Prentice-Hall International (UK) Limited. London.

Geliş tarihi: 07.04.2006 / Kabul tarihi: 21.09.2006

Address for correspondance

Yard.Doç.Dr. Mehmet Özdemir

Afyon Kocatepe Üniversitesi Veteriner Fakültesi

Farmakoloji ve Toksikoloji Anabilim Dalı

Ahmet Necdet Sezer Kampüsü, 03200, Afyonkarahisar

e-mail: mozdemir@aku.edu.tr