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Survey of the occurrence of aflatoxin M1 in cheeses produced by dairy ewe's milk in Urfa city, Turkey

Ayhan FİLAZİ[1], Sinan INCE[2], Fusun TEMAMOGULLARI[3]

[1] Department of Pharmacology and Toxicology, Ankara University, Faculty of Veterinary Medicine, Ankara/Turkey, [2]Department of Pharmacology and Toxicology, Afyon Kocatepe University, Faculty of Veterinary Medicine, Afyon/Turkey, [3]Department of Pharmacology and Toxicology, Harran University, Faculty of Veterinary Medicine, Urfa/Turkey.

Summary: The study was undertaken to determine the presence and levels of aflatoxin M1 (AFM1) in cheeses produced by dairy ewe's milk in Urfa city of Turkey. Fifty samples of cheese were analyzed for the occurrence of AFM1 using TLC. The cheese samples were purchased randomly from different markets. The presence of AFM1 was detected in concentrations between 20-2000 ng/kg in 14 of 50 samples (28%). Altogether, 5 cheese samples (10%) were found to have levels that exceed the legal limits of 250 ng/kg established by the Turkish Food Codex. It was therefore concluded that, widespread occurrence of AFM1 in ewe's milk cheese samples produced in Urfa city were considered to be possible hazards for human health. Continuous surveillance programme may be warranted to monitor regularly the occurrence of aflatoxins in foods and foodstuffs which consumed by human.

Key words: Aflatoxin M1; Cheese; TLC

Türkiye'nin Urfa İli'ndeki koyun sütlerinden üretilen peynirlerde aflatoksin M1 düzeylerinin belirlenmesi

Özet: Bu çalışma Türkiye'nin Urfa İlinde koyun sütlerinden üretilen peynirlerdeki Aflatoksin M1'in düzeyleri ve sıklığını belirlemek için yapıldı. Bunun için 50 adet peynir numunesi TLC kullanılarak Aflatoksin M1 yönünden analiz edildi. Peynir örnekleri farklı satış yerlerinden rastgele toplandı. Bunlardan 14'ünde (%28) aflatoksin M1 varlığı 20-2000 ng/kg arasında saptandı. peynir numunelerinin 5 tanesinin (%10), Türk Gıda Kodeksi tarafından bildirilen yasal tolerans limitlerinin üzerinde olduğu belirlendi. Böylece Urfa ilinde koyun sütünden üretilen peynirlerdeki aflatoksin M1'in yaygın olduğu ve insan sağlığına zararlı olabileceği sonucuna varıldı. Bunun için insanlar tarafından tüketilen ve mikotoksinler yönünden riskli olan gıdaların düzenli bir program dahilinde resmi otoriteler tarafından kontrol edilmesi gerekmektedir.

Anahtar sözcükler: Aflatoksin M1; peynir; TLC.

Aflatoxins produced by fungus such as *Aspergillus flavus, A. parasiticus* and *Penicillium puberulum* are genotoxic and cytotoxic carcinogens. When lactating mammals, such as cows, sheep and goats, are fed with feedstuffs containing aflatoxin B1 (AFB1) and B2 (AFB2), these metabolites can be converted to aflatoxin M1 (AFM1) and M2 (AFM2) (3).

About 1-2% of AFB1 in animal feed is transformed to AFM1 in milk with variatons from animal to animal, from day to day and from one milking to the next and when the intake of AFB1 is stopped, the AFM1 concentration in the milk decreases to an undetectable level after 72 h (14).

AFM1 had a resistant to thermal inactivation used during food processing procedures such as pasteurization and sterilization. Storage of various dairy products was not effective in the reduction of this toxin (2). Thus, if raw milk contains AFM1 cheese made from such milk will also contain AFM1.

To protect consumers, particularly children, from contaminated milk and dairy products, several countries have established legislation to regulate the levels of AFB1 in feeds and AFM1 in milk and cheese. The EU (European Union) and Turkish legal limits for AFM1 in milk and cheese are 50 ng/L and 250 ng/kg, respectively (13)

The purpose of this survey was to determine the natural occurrence and levels of AFM1 in samples of ewe's milk cheeses produced in Urfa city, Turkey. This paper reports the data of first survey on the presence of AFM1 in ewe's milk cheeses, in Urfa city of Turkey.

Samples: During the period of July-August 2005, total of 50 samples of commercial ewe's milk cheeses were randomly purchase from different markets in Urfa,

Turkey and transferred to the laboratory in plastic bags under cold chain and stored at 2-4 °C until being analyzed.

Reagents: All reagents were analytical quality and purchased from Merck.

AFM1 standard: Stock solution for AFM1 (obtained from Sigma Chem.) was prepared in chloroform in 1 µg/ml concentration and is kept frozen -20 °C. It was diluted in chloroform at proper concentrations (final concentration became 0.05 µg/ml) before using. This solution was well sealed and stored in a dark place at room temperature and was not used longer than 14 days.

Apparatus: Thin layer plates; Cut plates measuring 10x10 cm from the thin layer plates, Silica gel 60 (Merck, dimensions 20x20 cm^2, thickness 0.2 mm) was used in this study.

Ultraviolet lamp; An ultraviolet (UV) lamp with 254 and 364 nm wavelengths was obtained from CAMAG.

Methods: Each sample was analyzed by thin layer chromatography (TLC) for AFM1 levels by the technique described by Stubblefield (1979) and Kaya (1982). This method is a semi-quantitative method. AFM1 was extracted from the samples with acetone and diatomaceous earth. The extract was purified using column chromatography; finally the extract was evaporated to a known volume, and subjected to a two-dimensional TLC. AFM1 levels in cheeses were expressed as microgram per kilogram (ppb). The lowest detection limit of the method used is 0.02 µg/kg (ppb) and recovery value is 85.6%. The amount of aflatoxin was estimated visually by comparing the fluorescence intensity of AFM1 from the sample with that of one or more amounts of AFM1 standard. The identity of AFM1 was confirmed by the formation on the plate of a derivative using trifluoroacetic acid and comparison of the thin layer chromatographic properties of the derivatives of the sample spot and the standard.

Results are shown in Table 1.

Table 1. Levels of AFM1 in ewe's milk cheeses.
Tablo 1. Koyun sütünden yapılmış peynirlerdeki AFM1 miktarı.

Samples tested (n)	Levels of positive samples	Distribution of samples†		
		<20‡	21-250	251-2000
50	28% (14)	72% (36)	18% (9)	10% (5)

†*: ng/kg*

‡*: distribution of negative samples*

While no AFM1 was found in 36 samples (72%), 14 samples (28%) were found to contain AFM1 at variable levels. The AFM1 levels were higher than the limits of Turkish Food Codex (250 ng/kg for cheese) in 5 positive (10%) cheese samples.

AFM1 in cheeses may be hazardous to human, particularly children. For this reason, there are many studies concerning the presence of AFM1 in dairy products. Seyrek (2001) showed that of a total of 110 cheese samples, 9 did not contain AFM1, while 101 samples were found to be contaminated with AFM1 in the range of 10-2000 ng/kg. It was reported that 5.5% of the samples had greater levels of AFM1 than the permissible levels. Sarimehmetoglu et al. (2004) detected in 327 (81.75%) of 400 cheese samples AFM1 contamination. The number of cheese samples which exceeded the legal limits of 250 ng/kg was 110 (27.5%). Oruc and Sonal (2001) examined AFM1 levels in milk and cheese from Bursa, Turkey and found in 89.5% of cheese samples with range of 0-810 ng/kg. Dagoglu et al. (1995) analysed 75 white cheese samples and AFM1 contamination was 42%.

Gurses et al. (2004) examined AFM1 levels in some cheese types (23 white cheeses, 14 Kashar cheeses, 11 Tulum cheeses, 9 civil cheeses and 6 Lor cheeses) from Erzurum, Turkey and found in 44.44% of cheese samples with range of 2-202 ng/kg. This situation is positive for the microbial quality of some cheese types produced in Turkey.

As is seen, the levels of contamination of cheese by AFM1 seem to vary in many studies. These variations may be related to different reasons such as cheese manufacturing procedures, different milk contaminations, type of cheese, conditions of cheese ripening and the analytical methods employed. In addition, AFM1 level in the milk is significantly affected by the geographical region, the country and the season. It is demonstrated that summer milk was less contaminated than milk produced in the winter season (5). Thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA) are the most common techniques for detecting AFM1 in milk and dairy products. Therefore, differences between these techniques may affect the results of different studies by different investigators (1).

These differences may be related, not only to the use different analytical methods for AFM1 determination but also to different recoveries of AFM1 related to the chemical composition of the cheeses. Another aspect to be taken into account is the use different processing methods for cheese manufacture. The presence of AFM1 in cheese may be due to the fact that on one hand this toxin binds to casein and, on the other hand, a part of the whey remains trapped in curd (8). Additionally, the method used this study is a semi quantitative and screening test and therefore results must be confirmed by HPLC.

In conclusion that the level of contamination of the cheese samples with AFM1 observed in this study was high. For this reason, animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control. At the same time surveillance programs must be continuous and widespread for feedstuff and milk across the country.

References

1. **Amra HA** (1998): *Survey of aflatoxin M1 in Egyptian raw milk by enzyme-linked immunosorbent assay*. Rév Med Vet, **149***,* 695.
2. **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.
3. **Cole RJ, Cox RH** (1981): *The aflatoxins*. 1-66. In: **RJ Cole, RH Cox (Eds),** Handbook of Toxic Fungal Metabolites, Academic Press, LDT, London.
4. **Dagoglu G, Keles O, Yıldırım M** (1995): *Peynirlerde aflatoksin düzeylerinin ELISA testi ile araştırılması.* İstanbul Univ Vet Fak Derg, **21,** 313-317.
5. **Galvano F, Galofaro V, Galvano, G.** (1996): *Occurrence and stability of aflatoxin M1 in milk and milk products: A worldwide review.* J Food Protect, **59,** 1079-1090.
6. **Gurses, M., Erdogan, A. & Cetin, B.** (2004): *Occurrence of aflatoxin M1 in some cheese types sold in Erzurum, Turkey.* Turk J Vet Anim Sci, **28,** 527-530.
7. **Kaya S** (1982): *Süt yemi ve çiğ sütte aflatoksin kalıntılarının kromatografik yöntem ile araştırılması.* Ankara Univ Vet Fak. Derg, **29,** 443-457.
8. **Lopez C, Ramos L, Ramadan S, Bulacio L, Perez J** (2001): *Distribution of aflatoxin M1 in cheese obtained from milk artificially contaminated.* Int J Food Microbiol, **64,** 211-215.
9. **Oruc HH, Sonal S** (2001): *Determination of aflatoxin M1 levels in cheese and milk consumed in Bursa, Turkey.* Vet Hum Toxicol, **43,** 292-293.
10. **Sarimehmetoglu B, Kuplulu O, Celik TH** (2004): *Detection of aflatoxin M1 in cheese samples by ELISA.* Food Control, **15,** 45-49.
11. **Seyrek K** (2001): *Türk Silahlı Kuvvetlerine bağlı birliklerde tüketilen beyaz peynirlerdeki aflatoksin M1 seviyesinin ELISA metodu ile saptanması*. Vet Hek Dern Derg, **72**, 55-58.
12. **Stubblefield RD** (1979): *The rapid determination of aflatoxin M1 in dairy products*. J Am Oil Chem Soc, **56**, 800-802.
13. **Türk Gıda Kodeksi Tebligi** (2002). Resmi Gazete 23 Eylül 2002, 24885 sayı, Başbakanlık Basımevi, Ankara.
14. **Van Egmond HP** (1989): *Aflatoxin M1: occurrence, toxicity, regulation.* 11-49. In: H.P. Van Egmond (Ed), Mycotoxins in Dairy Products, Elsevier Applied Science, London.

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Yazışma Adresi:
Prof. Dr. Ayhan Filazi
Ankara Üniversitesi Veteriner Fakültesi
Farmakoloji ve Toksikoloji Anabilim Dalı
06110-Dışkapı/ANKARA
E-mail: filazi@veterinary.ankara.edu.tr