Asian Journal of Chemistry Vol. 21, No. 3 (2009), 1966-1970

Occurrence of Aflatoxin M_1 in Raw Ewe's Milk Produced in Sanliurfa, Turkey

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The presence and concentration range of aflatoxin M_1 (AFM_1) were investigated by Enzyme Linked Immunosorbent Assay (ELISA) technique in 90 samples of raw ewe's milk obtained from 2 dairies in Sanliurfa, Turkey. Analytical results showed that 88.9 % of the samples were contaminated with AFM_1. A total of 61.1 % of the samples tested exceeded 50 ng/L set by the Turkish and European regulations for AFM_1 in raw milk. AFM_1 levels ranged from 17 to 232 ng/L and mean value was 89.89 ng/L. The incidence of AFM_1 in milk from these dairies was 77.8 and 100 %, respectively. As a result, high incidence and levels of AFM_1 in raw milk samples produced in Sanliurfa were determined.

Key Words: Aflatoxin M_1, Raw milk, Ewe's milk, Occurrence, ELISA.

INTRODUCTION

Aflatoxins are a group of extremely toxic metabolites produced by *A. flavus*, *A. parasiticus* and the rare *A. nomius*, during the growth on foods and feeds. *Aspergillus flavus* produces only B aflatoxin, while the other two species produce both B and G aflatoxins[1,2]. These fungi are capable of growing on a great variety of food commodities and animal feed materials when the conditions of temperature, relative humidity and product moisture are favourable[3,4]. Aflatoxins are a significant threat to both human and animal health because they present carcinogenic, teratogenic and mutagenic potential. Among aflatoxins, aflatoxin B_1 (AFB_1) is considered to be the most potent naturally occurring hepatocarcinogen known, the risk assessment of which is well established[1]. The International Agency for Research on Cancer (IARC) has classified as a Group 1 human carcinogen[5].

Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1 excreted in milk when lactating animals are fed with AFB_1 contaminated feeds[6]. The formation of AFM_1 occurs in the liver and it is secreted into the milk. There is a linear relationship between the amount of AFM_1 in milk and AFB_1 in feed consumed by the animals. It has been reported that 0.3-6.2 % of AFB_1 in animal feed is transformed to AFM_1 in milk[2], but this transmission rate varies from animal to animal, from day-to-day and from one milking to the next[7]. AFM_1 could be detected in milk 12-24 h after the first AFB_1 ingestion, reaching peak levels after a few days. When the

intake of AFB_1 has finished, AFM_1 concentration in the milk decreases to an undetectable level[8] after 72 h. Studies have clearly demonstrated that AFM_1 causes toxic and carcinogenic effects[6,9]. Therefore, AFM_1 is classified as a Group 2B human carcinogen by IARC document[5].

The possible presence of AFM_1 in milk and dairy products is a worldwide concern since these products are largely consumed by children, including infants who are considered more susceptible to the adverse effects of mycotoxins. Milk is not only consumed as liquid milk, but also utilized for the preparation of infant formulas, yoghurt, cheese, and milk-based confectioneries including chocolate and pastry. Therefore, it is important to determine AFM_1 levels in milk and dairy products in order to protect consumers in various age groups, from its potential hazards[10]. The Commission of the European Communities has set at 50 ng/L for AFM_1 in raw milk, heat-treated milk and milk for the manufacture of milk-based products. However, the maximum level of AFM_1 in infant formulae[11] has been set at 25 ng/L. The maximum limit has been fixed[12] by Turkish government in milk at 50 ng/L.

Taking all this information into account, the purpose of this survey was to investigate the occurrence and levels of AFM_1 in samples of raw ewe's milk used cheese and yoghurt manufacturing in Sanliurfa, Turkey.

EXPERIMENTAL

Sampling: Ninety samples of raw ewe's milk were analyzed for the presence of AFM_1. Samples were obtained from two dairies (A and B) located in the province of Sanliurfa. At each dairy, a total of 9 sampling (5 samples from each unit) were performed on a weekly basis from March to April 2006. The samples were taken in 500 mL quantities and transported within an insulated container at about 4 ºC for analysis. Samples were protected against light until the day of analysis. Analysis of samples was carried out in less than 24 h from the time of their arrival in the laboratory.

Method: The AFM_1 concentrations of the samples were determined by competitive ELISA method (RIDASCREEN AFM1 Art no: R1101; R-Biopharm GmbH, Darmstadt, Germany) according to the procedure described by R-Biopharm GmbH[13]. This method is the test procedure employed by the German Federal Board of Health.

Preparation of samples: Ten mL aliquots of milk samples were chilled to 10 °C and then centrifuged for degreasing 10 min at 3500 g. After centrifugation, the upper cream layer was completely removed by aspirating with a Pasteur pipette and skimmed milk was used directly in the test.

ELISA test procedure: A sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples. 100 µL standard solutions and prepared samples were added in separate wells and incubated for 1 h at room temperature (20 ºC) in the dark. The liquid was removed from the wells and the microwell holder was tapped upside down vigorously (3 times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Then the wells were washed twice with 250 µL of distilled water. 100 µL of the diluted

enzyme conjugate (peroxidase conjugated AFM_1) was added and incubated for 1 h at room temperature in the dark. The wells were again washed with 250 µL of distilled water as described above. In the next stage, 50 µL of substrate (urea peroxidase) and 50 µL of chromogen (tetramethylbenzidine) were added to each well and mixed thoroughly and incubated for 0.5 h at room temperature in the dark. Then 100 µL of the stop reagent (1 N H_2SO_4) was added to each well and mixed and the absorbance was measured at 450 nm in ELISA reader (ELX-800, Bio-Tek Instruments, Winooski, VT, USA).

Evaluation: The samples were evaluated according to the Rida Soft Win computer program (RIDAVIN.EXE) prepared by R-Biopharm. The lowest detection limit is 5 ng/L, the recovery rate 95 % and the average coefficient of variation 15 % for spiked milk.

RESULTS AND DISCUSSION

The data in Table-1 show that 88.9 % of the samples tested contaminated with AFM_1. The incidence of AFM_1 in milk from A and B dairies was 77.8 and 100 %, respectively. AFM_1 concentrations in the samples tested ranged from 17 to 232 ng/L. Eleven samples from dairy A and 44 samples from dairy B exceed the maximum level of 50 ng/L set by the Turkish and European regulations for AFM_1 in raw milk. A total of 61.1 % of the samples tested exceeded 50 ng/L. The highest contamination level of AFM1 (232 ng/L) was found in milk produced by dairy B. These findings indicate that animal feeds in Sanliurfa have probably been contaminated with high levels of AFB_1.

TABLE-1
OCCURRENCE AND DISTRIBUTION OF AFLATOXIN M_1 IN RAW MILK SAMPLES

Dairy	Samples						
	Analyzed (n)	Positive[a] n (%)	Distribution n (%)			Concentration[c] (ng/L)	
			< 5 ng/L	5-50 ng/L	> 50[b] ng/L	Mean ± SD	Range
A	45	35 (77.8)	10 (22.2)	24 (53.3)	11 (24.4)	50.71±35.64	17-130
B	45	45 (100)	0 (0)	1 (2.2)	44 (97.8)	120.36±69.04	50-232
Total	90	80 (88.9)	10 (11.1)	25 (27.8)	55 (61.1)	89.89±66.41	17-232

[a]≥ 5 ng/L; [b]Exceed Turkish legal limit; [c]Positive samples.

The consumption of ewe's milk is very common in Sanliurfa and other provinces in the southeast Anatolia region of Turkey. Since milk production is generally carried out in spring, the present survey was conducted from March to April. Zinedine *et al.*[14], Kamhar[15] and Ghiasian *et al.*[16] reported AFM_1 contamination in a large percentage of milk samples collected in winter, but Bakirci[17] obtained similar results from samples collected in March, April and May. Several studies have noted lower AFM_1 in milk samples collected during the summer months[14,17,18]. This phenomenon was attributed to the fact that lactating animals are receiving less contaminated feeds in

summer time when they are grazing. Thus, the overall incidence of AFM_1 observed in the present study might be attributed to the fact that during cold weather periods dairy ewes are usually fed higher amounts of concentrated feeds.

The relationship between AFB_1 content of feed and AFM_1 concentration in milk is well established[2,7,8]. The most important impact on the amount of AFB_1 in feeds is undoubtedly temperature and moisture, because some aflatoxin producing moulds can easily grow in feeds having moisture between 13 and 18 % and environmental moisture between 50 and 60 %, whereas they can also produce toxin under conditions of 25 °C, and 85 and 90 % relative humidities[17,19].

Since milk is a major commodity for introducing aflatoxins in the human diet, many studies have been done in Turkey and other countries. Other studies in Turkey have reported lower AFM_1 contamination rates (58.1 to 70.83 %) than the current study[10,20,21]. The concentrations of AFM_1 observed by Akdemir and Altintas[20] and Unusan[21] in raw and pasteurized milks (10 to 817 ng/L) were higher than values observed here, but Gurbay *et al.*[10] observed lower concentrations (10 to 50.5 ng/L) in ultra high temperature and pasteurized milks. The present results are in agreement with those obtained by Bakirci[17], who reported 87.8 % of raw milk samples contaminated and concentrations of AFM_1 ranging from 12.5 to 120 ng/L. Similarly, Celik *et al.*[22] found AFM_1 concentrations of 5.2 to 127.6 ng/L in 88.2 % of the pasteurized milk samples examined.

In comparison to studies performed in other countries, the AFM_1 contamination rates in raw ewe's milk samples reported here are higher than those reported for Argentina[23], Brazil[24-27], Greece[18,28], India[29], Iran[15], Italy[30] and Kuwait[31]. On the other hand, concentrations of AFM_1 observed by Sassahara *et al.*[25] in raw milk (258 to 1998 ng/L) were higher than values observed in the present study. The present results are in agreement with those obtained by Zinedine *et al.*[14], who observed AFM_1 concentrations of 1 to 117 ng/L in 88.8 % of the samples of pasteurized milk traded in Morocco and Kamhar[15], who found concentrations of 15 to 280 ng/L in Iranian raw milk. Conversely, Alborzi *et al.*[32] reported finding AFM_1 in 100 % of the raw milk samples traded in Iran with concentrations of 45 to > 80 ng/L.

In summary, 61.1 % of the ewe milk samples tested were found to contain concentrations of AFM_1 higher than maximum acceptable level of 50 ng/L established by Turkish Food Codex. This appears to be a serious problem for the public health, since all the age groups including infants and children consume milk and dairy products. With regard to AFM_1, milk and dairy products have to be inspected and controlled.

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(*Received*: 11 March 2008; *Accepted*: 10 November 2008) AJC-7013