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Occurence of Aflatoxin M1 Determined by ELISA in UHT (Sterilized) and Raw Milk Samples Produced in Turkey

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Aflatoxins are secondary metabolites of fungi produced by certain strains of *Aspergillus* spp. They are known to have potent carcinogenic, hepatotoxic, genotoxic and immunusuppressive effects leading to toxicities in human and animals. In this study, it was aimed to determine presence of AFM1 levels in raw and UHT milk produced in the Kars vicinity of Turkey which is commercialized in big retail markets and to evaluate the toxins levels in milk samples with respect to the levels allowed by Turkish Food Codex. Of the total 90 milk samples analyzed, while 87.8 % (79) of the milk samples were found to exceed the limits allowed by Turkish Food Codex, only 12.2 % (11) of the total number of milk samples were in accordance with Turkish Food Codex limits. The levels of AFM1 were above the allowed limits in 84.4 % (38 out of 45) of UHT milk and 91.1 % (41 out of 45) of raw milk samples. The presence of unusually high rate of AFM1 levels in both raw and UHT milk samples implies that children and people in all age groups are under risk of being toxicated by AFM1. Therefore, it is important to take preventive actions toward reducing contamination of milk with AFM1.

Key Words: Aflatoxin M1, Raw milk, Ultra high temperature milk, Enzyme-linked immunosorbent assay.

INTRODUCTION

Aflatoxins (AFs) are toxic metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* which are known to have hepatotoxic, genotoxic, immunosuppressive and carcinogenic effects on human and animal species[1-5]. AFB1 and AFB2 are metabolized and excreted in the milk of lactating cows as AFM1 and AFM2, respectively. According to International Agency for Research on Cancer[6], although AFB1 is more toxic than AFM1, AFM1 is classified as 2nd degree carcinogen agent.

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Milk and other dairy products constitute the major food source of people in every age group particularly in children and are considered to have a potential risk factor for aflatoxins. Therefore, monitoring the AFM1 level in milk and dairy products is of importance for public health safety. Aflatoxin levels in food could be determined through the advanced techniques such as HPLC and ELISA. The maximum allowed level of AFM1 set by FAO/WHO, Codex alimentarius[7] and Turkish Food Codex (TFC)[8] in milk and milk-based baby food is 50 ng/L, whereas the maximum limit for milk powder is 500 ng/kg.

Kars is one of the important cities of north eastern part of Turkey in terms of animal husbandry and the milk produced in this region is mainly utilized in cheese production as Kashar, White cheese, Cecil and Gravier. These products are sold in both local markets and metropolitan cities (Istanbul and Ankara) of western Turkey and consumed by different age groups including children in developing age.

Farmers in this part of Turkey perform hay-harvest in the spring and summer seasons and feed their cattle with this hay during harsh winter season. Fungi present in haystack can easily produce toxins in inappropriate storage conditions and pose a great risk associated with aflatoxin contamination.

In this study, it was aimed to determine presence of AFM1 levels in raw and Ultra High Temperature (UHT) milk produced in the Kars vicinity of Turkey which is commercialized in big retail markets and to evaluate the toxins levels in milk samples with respect to the levels allowed by Turkish Food Codex (TFC).

EXPERIMENTAL

Forty five packets of UHT milk from the retail markets and 45 raw milk samples from the local farms in the Kars vicinity were collected to analyze AFM1 levels. Following the collection of milk samples, the raw milk samples were taken to the laboratory under 4 ºC cold-chains and analyzed on the same day. The UHT milk samples were analyzed in between May and July 2006 in the Kafkas University Animal Research and Central Laboratory.

The quantitative analysis of aflatoxin M1 (AFM1) in milk samples were carried out by competitive enzyme immunoassay test procedure as described by R-Biopharm[9] GmBH, Germany (Ridascreen® Aflatoxin M1 30/15, Art. No.: R1101).

Preparation of samples for analysis: Preparation of samples and ELISA test procedure were carried out according to the test booklet obtained along with commercially available kit (Ridascreen®). Before the analysis, the temperature of milk samples were brought to 10 ºC and then the samples were centrifuged at 3500 × g at 10 ºC for 15 min. After the centrifugation, the upper oily layer was completely removed. An aliquot of lower layer was taken using a Pasteur pipette and used for AFM1 analysis.

A 100 µL of standard solution and the previously prepared samples were added to the microtiter wells and then incubated in a dark room at room temperature for 0.5 h. At the end of incubation, the liquid in the wells was poured and the microwell holder was tapped upside down on an absorbent paper to remove the liquid out of the wells. The wells were washed 2 times with 250 µL of washing buffer and then

the liquid in microtiter wells was poured out. A 100 µL of the diluted enzyme conjugate was added to the each well and the wells were gently shaken. The wells were incubated for 15 min at room temperature in a dark room. The microwell holder was tapped upside down on an absorbent paper again and the wells were washed 3 times with 250 µL of washing buffer. A 100 µL of the substrate chromogen was added to the each well and gently mixed. The samples were then incubated for 15 min at room temperature in a dark room. Following the addition of 100 µL of the stop reagent to the each well, absorbances were measured spectrophotometrically at 450 nm (Spectra Max 384 Plus) within 1 h. The data obtained from standards and samples were evaluated using a computer program (RIDA®SOFT Win, R-Biopharm, Germany) for windows.

RESULTS AND DISCUSSION

Aflatoxin M1 levels in 45 UHT and 45 raw milk samples are presented in Table-1. Of the total 90 milk samples (UHT and raw milk) analyzed, while 87.8 % (79) of the milk samples were found to exceed the limits (50 ng/L) allowed by Turkish Food Codex, only 12.2 % (11) of the total number of milk samples were in accordance with Turkish Food Codex limits. The levels of AFM1 were above the allowed limits in 84.4 % (38 out of 45) of UHT milk and 91.1 % (41 out of 45) of raw milk samples.

TABLE-1
AFM1 LEVELS IN ULTRA HIGH TEMPERATURE (UHT) AND RAW MILK SAMPLES PRODUCED IN TURKEY

Number of samples	< 50 ng/L		51-75 ng/L		> 75 ng/L	
	n	%	n	%	n	%
UHT milk (45)	7	15.55	33	73.33	5	11.11
Raw milk (45)	4	8.88	30	66.66	11	24.44
Total (90)	11	12.22	63	70.0	16	17.77

Studies monitoring the AFM1 levels in milk samples demonstrate that occurrence of AFM1 in milk samples are varied in both Turkey and other parts of the world. Celik *et al.*[10] reported that 88.2 % of the pasteurized milk samples (75/85) produced in different parts of Turkey was contaminated with AFM1 and 64 % of all the analyzed samples (48/85) were above the limits allowed by Turkish Food Codex. The number of heat-processed milk samples contaminated with AFM1 in the report of Celik *et al.*[10] was found to be lower than in the present study.

Martins and Martins[11] studied the incidence of AFM1 in milk samples produced in Portugal. According to this study, AFM1 were detected in 80.6 % of 31 raw milk samples and in 84.2 % of 70 heat-processed milk samples. In another study performed in Thailand, Saitanu[12] reported that AFM1 levels were found to be greater than 0.05 ppb in 57 raw milk samples (out of 68), in 63 pasteurized milk samples (out of 63) and 53 UHT milk samples (out of 60). Furthermore, Meerarani *et al.*[13] reported that AFM1 was detected in 36 of the 325 milk samples produced in India and the levels

of AFM1 in contaminated milk samples were found to be between 0.1-1 µg/L. Lopez *et al.*[14] showed that in Argentina, 10.71 % of farm milk samples (6/56) and 50 % of pasteurized milk samples (8/16) were contaminated with AFM1. The levels of AFM1 in farm milk, pasteurized milk and concentrated milk samples were at 0.016, 0.013 and 0.0125 µg/L, respectively. In another study monitoring the market milks for the presence of AFM1, Kim *et al.*[15] reported that AFM1 were detected in 53 milk samples out of 70 using ELISA method, but the detected number of milks contaminated with AFM1 was 39 out of 70 using HPLC. The levels of AFM1 in contaminated milk samples were between 18-31 ppm/mL. In another study conducted in Italy, of the 159 milk samples analyzed, 86 % of the samples (136) contained average of 10.2 ng/L AFM1, whereas 1 % of the samples had AFM1 level less than 50 ng/L[16]. In a similar study performed on 14 raw milk samples, Srivastava *et al.*[17] reported that AFM1 was detected in 70 % of milk samples with the average contamination level of 0.2 µg/L.

A great variation in the occurrence of AFM1 in milk samples produced in different geographical parts of the world could depend on the differences in climate, geographical conditions, feed hygiene and the methods used in the detection of the toxins. Comparing to the previous reports, milk samples contaminated with AFM1 in the present study were found to be higher than that of previous reports by Martins and Martins[11], Meerarani *et al.*[13], Lopez *et al.*[14], Srivastava *et al.*[17] and Roussi *et al.*[18], whereas results of Saitanu[12] were similar to the results obtained in the present study. With respect to the heat-processed milk (UHT and Pasteurized milk), the results of Martins and Martins[11], Saitanu[12] and Galvano *et al.*[16] were found to be similar to the results of this study. The incidence of AFM1 occurrence in pasteurized and UHT milk samples reported by Roussi *et al.*[18] was similar to our results. On the other hand, Lopez *et al.*[14] reported a lower rate of AFM1 occurrence compared to the present study.

Studies conducted in both Turkey[19-24] and other countries of the world[4,25,26] showed that milk and other dairy products including cheese, milk powder and yogurt contain AFM1 levels with great diversity depending on the year, country and climate. In spite of different type of processing techniques in milk technology, presence of AFM1 in milk and other dairy products found in different levels is of continuous problem for public safety.

AFB1 is metabolized by the mixed function oxidase system in the liver to produce less toxic metabolites of AFM1. However, AFM1 has still a great toxic potential to the human health[1,2,4-6]. Therefore, it is important to eliminate the risk factors affecting toxin production such as providing appropriate storage conditions for animal feed and avoiding inappropriate conditions favouring toxin productions. For this purpose, several management practices have been employed for the last years. For example, feedstuff contaminated with the mycotoxins can be mixed with uncontaminated feed and addition of propionate and gentian violet to the feed as the mycotoxin binding agent can be employed toward reducing mycotoxins levels in contaminated feeds[27]. However, protection through these methods should be considered to be still limited.

In conclusion, contamination of feed and edible animal products with aflatoxins is often unavoidable because of ubiquitous nature of mycotoxins. The results of this study indicate that AFM1 in raw and ultra high temperature milk samples produced in Turkey poses a great risk to human health particularly to the children suggesting that it is important to take preventive actions in every stage of food chain in addition to effective detoxification methods.

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