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Occurrence of Aflatoxin M_1 in Raw Milk in Trakya Region, Turkey

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The occurrence of aflatoxin M_1 (AFM_1) was investigated in raw cow's milk samples using an ELISA method (Ridascreen). The lower detection limit of AFM_1 test was 0.005 μg/L. The presence of AFM_1 in 90 samples of raw milk obtained at March and June 2005 from a dairy plant and 45 samples collected randomly at June 2005 in Trakya region of Turkey were analyzed. In all of the samples obtained in March the presence of aflatoxin M_1 was detected in concentrations ranging between 0.0077 and 0.0683 μg/L. Aflatoxin M_1 level in only one of positive samples was higher than the maximum tolerance limit (0.05 μg/L) accepted by Turkey and European Union countries. Incidence level of AFM_1 in month of March was higher than the month of June samples. Statistical evaluations showed that mean contamination level of AFM_1 in March samples were significantly higher ($p < 0.01$) than June. In addition the results showed that there were no statistical differences between AFM_1 contents of March and June samples.

Key Words: Milk, Trakya Region, Aflatoxin M_1.

INTRODUCTION

Aflatoxins are carcinogenic, highly toxic metabolites of the mold fungus varieties such as *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M_1 (AFM_1) is produced as a metabolite of aflatoxin B_1. It is secreted with the milk of lactating cows that are given aflatoxin B_1 containing feed. As AFM_1 is relatively stable towards the pasteurizing process, comprehensive routine checks have to be performed not only raw materials to be processed is required, but also of the final products. On the first January 1999 EU has been limited it as 0.05 μg/L (50 ppt).

Aflatoxin M_1 is a derivative of the AFB_1, which is produced in cow liver and cause damage to liver. The International Agency for Research on Cancer (IARC) reported AFB_1 as primary and AFM_1 as secondary groups of carcinogenic compounds[1]. Many countries have carried out various control and inspection programs on this subject fairly concerning about public health for many years. According to the results, obtained maximum aflatoxin levels were determined for food and feed by considering each country's conditions and finally regulations were established[2-4]. Many countries have carried out studies about the incidence of AFM_1 in milk. In most of them, samples have been found to exceed 0.05 μg/L, which is imposed as the limit by many countries[1,3,5]. In Turkey, a limited amount of research was carried out on determination of AFM_1 in foods. However, a legal limit (0.05 μg/L form milk and milk products) has been stated in 2002, by Ministry of Agriculture[6].

There have been a number of studies on the presence of AFM_1 in milk and milk products in the world (Table-1). In France, Blanc and Karleskind[7] reported that 40 % of 1046 milk and milk product samples were found to be contaminated (AFM_1 > 0.05 µg/L). On the other hand in USA, none of 182 milk and milk products was determined AFM_1 by Wood[8]. In Germany[9], 418 milk samples were analyzed and found AFM_1 contents for these samples between 0.0033-0.333 µg/L. In Italy, Quintavalla and Casalari[10] reported that AFM_1 contents were found between 0.18 and 0.75 µg/L in 70 milk samples. Tutelyan *et al.*[11] determined that AFM_1 contents to be below 3 µg/L in 6.9 % of 115 milk samples. Heeschen *et al.*[12] found AFM_1 above 0.05 µg/L in 19 of 473 milk samples. In India, Rastogi *et al.*[13] reported that 87.3 % of 87 samples of milk and milk products were found to be contaminated with AFM_1. In Turkey, AFM_1 was found in 79 of the 90 examined milk samples[14]. Ozkaya *et al.*[15] found AFM_1 in 159 out of 360 milk samples and the maximum AFM_1 content reported as 1.4 µg/L in 48 milk samples. Kamkar[16] analyzed the presence of AFM_1 in 111 samples of raw milk obtained from dairy plants of Sarab city of Iran. In 85 of the 111 samples (76.6 %) the presence of AFM_1 was detected in concentration ranging between 0.015 and 0.28 µg/L and AFM_1 level in 40 % of positive samples were found higher than the maximum tolerance limit (0.05 µg/L) accepted by some European Countries. Kamkar[16] informed incidence levels of AFM_1 on January, February, April and December, were higher than the other months'. Lopez *et al.*[17] collected 77 milk samples in a period of March-September for determining the contamination degree of milk in their region during winter. In result of this research, approximately 23 % of total 77 various types of milk samples were found to be contaminated with AFM_1 at 0.01-0.03 µg/L.

TABLE-1
OCCURRENCE OF AFM_1 IN RAW COW MILK REPORTED IN PREVIOUS STUDIES

Commodities	Number of samples	Percentage of positive samples	Average concentration of AFM_1 (µg/L)	Reference
Milk and milk products	1046	40	>0.05	7
Milk and milk products	182	-	-	8
Milk and milk products	418	-	0.0381	9
Milk and milk products	70	30 (43 %)	0.18-0.75	10
Milk	115	6.9	< 0.05	11
Milk	473	19 (4 %)	>0.05	12
Milk and milk products	87	87.3	-	13
Milk	90	79 (88.77%)	0.0125-0.1236	14
Milk	360	44.2	1.4 max	15
Milk	360	13.3	>0.05	15
Milk	111	85 (76.6%)	0.015-0.28	16
Milk	77	(23 %)	0.01-0.03	17

The present investigation was carried out to gain some data on the aflatoxin M_1 levels of raw cow's milk samples produced in Trakya region of Turkey and for the evaluation of the effect of AFM_1 on human health.

EXPERIMENTAL

Total 135 milk samples were evaluated for the occurrence of AFM_1. The milk samples were: 45 of them were collected from the same cows in the farm in March-June period. The rest of the milk samples randomly obtained 45 different milk producers who were selling their milk to the dairy plants. The samples were stored in a cool place and protected from light.

Method: The quantitative analyses of AFM_1 levels were determined in the cow milk samples. The samples were analyzed using the AFM_1 test procedure (Art. no: R1201), which described by producer (r-biopharm)[18].

Preparation of samples: Preparation of samples was conducted according to the instructions of the Ridascreen kit. Briefly, milk samples (4 mL each) were centrifuged for 10 min at 3500 rpm, at 10 °C (Hettich Universal 32-R, Germany). An aliquot (100 µL per well) of the skimmed milk was used directly in the test.

Test procedure: Aflatoxin M_1 standards or the prepared sample solutions were added to microtiter wells in duplicate. During incubation for 1 h at room temperature in the dark, the antibody binding sites are occupied proportionally to the aflatoxin M_1 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 are occupied by the enzyme conjugate (enzyme labeled toxin), which was added (100 µL) and incubated for another 1 h 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 0.5 h at room temperature in the dark. Bound enzyme conjugate converts the colorless chromogen into a blue product. Then the addition of the stop reagent (100 µL per well) led to a colour change from blue to yellow. The measurement was made photometrically at 450 nm. All experiments were made in triplicate.

Evaluation of AFM_1: For the construction of the calibration curve, the mean of the absorbance values obtained for the standards was divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage maximum absorbance). The absorption is inversely proportional to the AFM_1 concentration in the sample. As can be seen from Fig. 1, the calibration curve was found to be virtually linear in the 10-80 ng/L range. The detection limit was found to be 5 pg/mL. In order to obtain AFM_1 actual sample concentration in ng/L, the concentration read from the calibration curve was further multiplied by a dilution factor 1.0 for milk.

RESULTS AND DISCUSSION

The standard curve for AFM_1 detection by competitive ELISA is depicted in Fig. 1. A concentration dependent decrease in per cent maximum absorbance at 450 nm was observed for AFM_1.

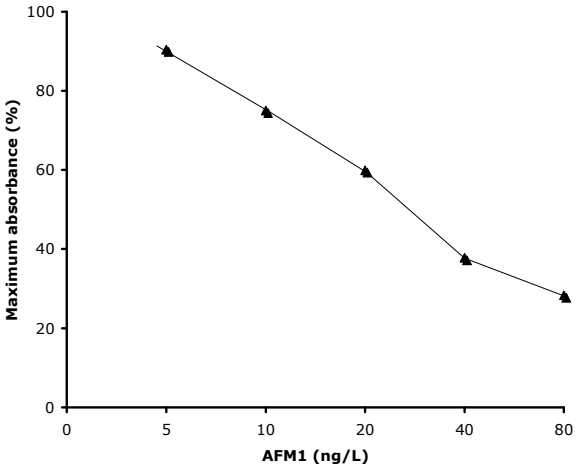

Fig. 1

In this study a total of 135 cow milk samples, which were 90 out of obtained from a dairy farm (45 in March, 45 in June 2005) and 45 collected randomly from Trakya region in June 2005, were analyzed for AFM_1 with the competitive ELISA. Aflatoxin M_1 ranged from non-detectable levels to 0.0683 µg/kg. The range of contamination levels varied relatively narrow categories. In all milk samples (totally 45), which were obtained a dairy farm in March 2005, were found AFM_1 between 0.0077 and 0.0683 µg/L (mean values of positive samples 0.0257 µg/L). In June 2005 period was determined AFM_1 between 0.005 and 0.020 µg/L (mean values of positive samples 0.0071 µg/L) only 8 out of 45 milk samples that obtained from the same dairy farm. In 7 out of 45 milk samples obtained randomly from Trakya region in June 2005 period, were found AFM_1 between 0.0050 and 0.0058 µg/L (mean values of positive samples 0.0051 µg/L).

The AFM_1 level in the milk was 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. The differences in the origin of the animal feed and different levels of contamination of raw milk could also affect these results[19]. Earlier studies have shown that contamination of AFM_1 in milk and dairy products is a result of exposure of AFB_1 to dairy cattle through feedstuffs[20].

The Turkish, European Communities and Codex Alimentarius regulations have prescribed a limit of 0.050 µg/L for AFM_1 in milk and dairy products[21,22]. Table-2 shows that the AFM_1 contamination in milk samples exceeding European Communities/ Codex Alimentarius and US regulations. Almost over 99 % of the contaminated samples not exceeded the Turkish, European Communities/Codex Alimentarius recommended limits. Only one sample exceeds the maximum level (0.0683 µg/L). It is known that AFM_1 in milk and dairy products may be a hazard for consumers. For this reason, there are many studies concerning the presence of AFM_1 in milk and dairy products.

TABLE-2
OCCURRENCE OF AFM_1 IN MILK SAMPLES COLLECTED FROM TRAKYA REGION OF TURKEY

Milk samples	Obtained at		Obtained (randomly) at	Total
Period	March	June	June	
Number of samples	45	45	45	135
Number of positive samples	45	8	7	60
Min (μg/L)	0.0077	0.0050	-	
Max (μg/L)	0.0683	0.0200	0.0058	
Mean value (μg/L)	0.0257*	0.0071*	0.0051*	

*Mean values of postive samples.

Conclusion

Result of this study indicated that the AFM_1 levels showed a seasonal variation. The presence of AFM_1 in raw milk can indicate that storage conditions were not suitable. A relationship between AFM_1 occurrence level in milk and AFB_1 content of feed was reported by Van Egmond[23] and Wood[24]. Since milk is one of the most important human foods and the main nutrient for growing young, be monitored for contaminants, including AFM_1. In addition, to have low levels of AFB_1, feed of dairy cows should be kept away from contamination as much as possible.

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