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Occurrence of aflatoxin M1 levels in local yogurt samples in Gilan Province, Iran

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ABSTRACT

Aflatoxins are carcinogenic and toxic which is a secondary metabolic product of some Aspergillus spp. When animals consume contaminated feed stuff to aflatoxins, the toxin is metabolized in the liver and execrated as aflatoxin M1via milk. During the autumn of 2011, 60 samples of local yogurt were selected randomly from Gilan (Northern Iran). ELISA technique was used to determine the presence and the level of AFM1. In 59 of the 60 yogurt samples examined (98.33%), the presence of AFM1 was detected in concentrations between 6.2 - 87ng/l. The mean level of AFM1 of positive samples was 51.66 ng/l. AFM1 in 38 samples (63.33%) were higher than the maximum tolerance limit (50 ng/l) accepted by the European countries. Aflatoxin high concentration in milk and milk products cause widespread negative impact on public health. Therefore, it is necessary to establish strategies for reducing aflatoxin levels in animal feed and milk products.

Keywords: Aflatoxin M1, local yogurt, ELISA

INTRODUCTION

Mycotoxins are the secondary toxic metabolites of fungi synthesized during the end period of the logarithmic growth phase [1]. Aflatoxins are generally produced in animal feed by toxigenic fungi such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* [2, 3]. Aflatoxins have sub-acute and chronic effects such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis in humans, AFM1 is classified in Group 2 as a probable human carcinogen [1, 4]. 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 [5]. The consumption of milk and dairy products by human especially by infants and young children increases the risk of exposure to AFM1 [6]. AFM1 remain stable after pasteurization, sterilization, preparation and storage of various dairy products [4, 7]. Many researchers from different countries have carried out studies about the incidence of AFM1 in yogurt [5, 8, 9, 10, 11]. The contamination of milk and milk products with AFM1 display variations according to geography, country and season. The pollution level of AFM1 is differentiated further by hot and cold seasons, due to the fact that grass, pasture, weed and rough feeds are found more commonly in spring and summer than in winter [4, 8, 12]. The European Commission proposes a maximum permissible level of 50 ng/l AFM1 in milk and milk products [13]. Although, there are some studies about the contamination of aflatoxin M1 in milk and dairy products in different cities of Iran [12, 14, 15, 16, 17] but there is no published data regarding aflatoxin M1 in local yogurt samples in Gilan province (Northern Iran) .This study was carried out to evaluate the occurrence of aflatoxin M1 in local yogurt samples produced in this region by ELISA method.

MATERIALS AND METHODS

Preparation of samples: A total of 60 local yogurt samples was obtained randomly during autumn 2011 from Gilan province (Northern Iran). The samples were transported to the laboratory in insulated container at about 4°C. All yogurt samples were heated to 80°C for 3 min, in order to inactivate living yogurt bacteria and then pasteurized. Then, the samples were cooled down to a room temperature and diluted in 1:5 in PBS-buffer (pH 7.2) and 100 µl (per well) of this solution was used in the test.

ELISA test procedure: The samples were analyzed for the presence of AFM1 with the competitive ELISA using R-Biopharm AFM1 kit. Before starting the test, the reagents were brought up to room temperature. The AFM1 standards and test samples (100 µl per well) in duplicate were added to the wells of a micro-titer plate pre-coated with antibodies for AFM1 and incubated at room temperature in dark for 60 min. After the washing step, 100µl of peroxidase conjugate was added to the wells and plate was incubated again for 60 min at room temperature in dark. After the washing step, the unbound conjugate was removed during washing. Subsequently, 50 µl each substrate (urea peroxide) and chromogen (tetramethyl-benzidine) were added to the wells and incubated for 30 min in dark. Finally, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with ELISA plate reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations. Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

The distribution and evaluation of AFM1 levels in local yogurt samples are given in Tables 1. Of the 60 samples analyzed, 59 samples (98.33%) were found to be contaminated with AFM1. 38 samples (63.33%) failed to reach the desired level of the European Communities and Codex, defined as 50 ng/l. The aflatoxin M1 contamination levels were between 6.2 - 87ng/l with the mean of 51.66 ng/l.

Table 1: Occurrence of AFM1 in local yogurt samples from Northern Iran

AFM_1 levels ng/l	Sample No.	(%)	Mean	Range
Not detected	1	-	-	-
< 25	10	16.66	12.46	6.2-18.07
25-50	11	18.33	34.67	21.40-48.3
≥50	38	63.33	66.88	52.16-87
Total Sample	60	98.33	51.66	6.2-87

Several surveys were performed in order to determine the AFM1 levels in milk, yogurt and milk products. Galvano et al. reported [8] that 80% of all yogurt samples in Italy were contaminated with AFM1, renged between 1- 3.1 ng/kg. In Kuwait, 54 samples of dairy products were analyzed for aflatoxin M1, 28% were contaminated with AFM1 [10]. Gubry et al. [18] found AFM1in 22 yogurt samples out of 40, ranging 61.61-365.64 ng/kg. Sarimehmetoglu et al. [9] reported that most of the yogurt samples (62.88%) purchased at different markets in Ankara was free of AFM1, ranging from 50-800 ng/kg. Akkaya et al. [5] in Turkey,177 yogurt samples consisting of 104 samples of ordinary yogurt, 21 of fruit yogurt, and 52 of strained (Torba) yogurt were tested for AFM1 by ELISA method. The highest AFM1 concentration was 150 ng/kg in strained yogurt, 100 ng/kg in ordinary yogurt as well as in fruit yogurt. 11.53% of ordinary yogurt (12 samples), 9.52% of fruit yogurt (2 samples), and 21.15% of strained yogurt (11 samples) had higher AFM1 levels, than the acceptable levels (50 ng/kg for yogurt). Gurses et al. [19], analyzed 63 cheese samples and in 28(44.44%), AFM1 was detected in concentrations between 7-202 ng/l. Martins et al. [11] 96 local yogurt samples were tested by HPLC in Portugal and was found In 18 samples out of 78, ranging 19-98 ng/kg. In our study, Of the 60 samples analyzed, 59 samples (98.33%) were found to be contaminated with AFM1 and contamination levels were between 6.2 - 87ng/l. According to observations, the levels of contamination of local yogurt by AFM1 seem to vary in many studies. These variations may be related to different reasons such as yogurt manufacturing procedures, different milk contaminations, type of yogurt, conditions of yogurt ripening, geographical region, the country, the season and the analytical methods employed [3, 10, 17]. AFM1 is stable in unprocessed milk and processed milk products and is unaffected by pasteurization or processing of milk into cheese or yogurt. For this reason, milk and dairy products have to be inspected continuously for AFM1 contamination, at least twice a year.

CONCLUSION

According to results obtained, incidence and contamination levels of AFM1, seem to be a serious problem for public health. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1

contamination and animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control.

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