

Presence of Aflatoxin M1 Ewe's Milk in the Northwest Region of Iran

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Abstract

Aflatoxin M1 is the hydroxylated metabolite of aflatoxin B1 and may be found in milk or milk products obtained from livestock that have ingested contaminated feed. Iranian white cheese is made from ewe's milk. The aim of this study was to evaluate Aflatoxin M1 contamination in ewe's milk samples in Tabriz city (Iran) by ELISA (Enzyme Linked Immunosorbent Assay). Ten ewe milk samples from different cheese maker centers in Tabriz city were collected during 2 months (May to June 2009). AFM1 was found in 30% of the analyzed samples. Results show that in 3 samples (30%) the AFM1 concentrations were less than 5 ng/l. It can be concluded that AFM1 levels in the samples purchased in Tabriz city, appear to be safe at the moment.

Key words: Aflatoxin M1; Ewe milk; ELISA, Tabriz

INTRODUCTION

Milk is a good source of many nutrients. However it could be a source of toxic substances like Aflatoxin M1 (AFM1). Contaminated milk with AFM1 could be a threat for children are consumed milk and dairy products. Aflatoxin may be produced by three species of *Aspergillus-A.flavus*, *A.parasiticus*, and rare *A.nomius* – that contaminate plants and its products. *A.flavus* produces only B aflatoxins, while the others produce both B and G aflatoxins. Aflatoxins M1 and M2 are the hydroxylated metabolites of aflatoxin B1 and B2 and may be found in milk products obtained from live stock that have ingested contaminated feed [1].

In 1987 the WHO classified the aflatoxins as Group 1 carcinogens. Aflatoxins are potent liver carcinogens and DNA-damaging agents from natural source[2].

It has been stated, in fact, that the contamination of milk and milk products with AFM1 displayed variations according to geography, country and season[3,4, and 5]. AFM1 is resistant to thermal inactivation; pasteurization, autoclaving and other varieties of food processing procedures are not effective in the reduction of this toxin [6,7].

In Iran, the cheese was produced from ewe milk traditionally, and it is important to determine the levels of Aflatoxin M1 in ewe milk. There is no information on ewe milk contamination by AFM1 in Iran. For the first time, this study was carried out to evaluate the prevalence of ewe milk contamination with AFM1 in Tabriz city (northwest region of Iran). This is the first report, as far as we are aware, of AFM1 contamination of ewe's milk in the northwest region of Iran.

MATERIALS AND METHODS

A total of 10 ewe milk samples from different cheese maker centers in Tabriz city were collected randomly during 2 months (May to June 2009).

The milk samples were centrifuged in 10 °C for 10 min with 3500 × g. After centrifugation, upper cream layers were completely discarded and the lower phases were freezed for the quantitative test. The quantity

of AFM1 was determined by I⁷ screen aflatoxin M1 test (Tecna, Italy) which is a competitive enzyme immunoassay based on antigen-antibody reaction. Sample solutions of 100 µl were added to the wells to occupy the binding sites proportionately then mixed gently and incubated for 45 min at room temperature (20-25 °C). The liquid was poured out of the wells and the wells filled with 250 µl washing buffer and poured out the liquid again. This washing step repeated four times. In the next stage 100 µl of enzyme conjugate were added to occupy the remaining free binding sites and incubated for 15 min at room temperature and repeated washing step. Then 100 µl of developing solution was added to each well and incubated for 15 min at room temperature. By using a multichannel pipette, 50 µl of stop solution was added to each well. The measurement of AFM1 was done photometrically at 450 nm against air blank within 60 min in ELISA reader (Sunrise, USA). Then data were analyzed by chi square test [1].

RESULTS

The standard curve for AFM1 detection by competitive ELISA is given in fig 1. As can be seen from the figure, the calibration curve was found virtually linear in the 5-250 ng/l range. The detection limit was found to be 5 ng/l. AFM1 was found in 30% of the analyzed samples. Results show that in 3 samples (30%) the AFM1 concentrations were less than 5 ng/l.

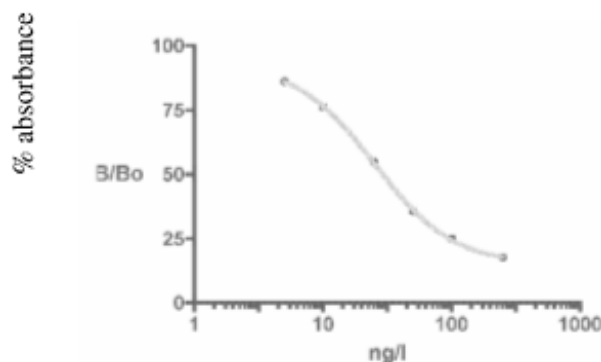


Fig. 1) Calibration curve of AFM1.

DISCUSSION

In the Iranian food standard (Anonymous, 2002), AFM1 levels in raw milk were limited to 50 ng/l, similar to EC regulations. Aflatoxin M1 was found in 30% of examined ewe milk samples [1].

According to the results obtained by Bognanno and et al in Italy, Aflatoxin M1 was found in 81% of examined ewe milk samples and 1.25% of all samples were over the legal limits (50 ng/l) [8]. In Greece, ewe's milk and the produced curd and feta cheese samples were examined

for presence of AFM1 and levels of AFM1 in milk were found far below the tolerance level (highest value 18.2 ng/l) which was parallel to our results [9]. Montagna and et al (2008) reported AFM1 contamination in 12.9% of cheese (made by ewe's milk) samples in Italy [10].

We are not aware of other surveys on the AFM1 content of ewe milk, so that comparison of results is not possible. As compared to other studies on cow's milk, which is shown in Table 1, AFM1 contamination in ewe's milk is lower than cow's milk in Iran.

Table-1 the prevalence of cow's milk contamination in other studies

Location	Milk samples (number)	Number of contaminated milk samples > 50 ng/l	Percent	Reference
Babol (Iran)	78	78	100	11
Japan	208	207	99.5	12
Tehran (Iran)	73	60	82.2	13
Libya	49	35	71.4	1
Korea	180	143	76.6	14
Tabriz (Iran)	50	31	62	1
Turkey	90	35	44.3	1
Sarab (Iran)	111	44	40	13
Brazil	139	29	20.9	1
Shiraz (Iran)	624	101	17.8	15
Mashhad (Iran)	110	6	5.4	13
Pakistan	168	1	0.6	16
Germany	379	2	0.5	1
Argentina	77	0	0	1
Italy	161	0	0	1

It is known that contamination of AFM1 in milk is a result of exposure of AFB1 to ewes through feed-stuffs [17]. The wide variations in AFM1 levels among studies could be related to geographic and climatic differences but also to differences in feeding systems, and farm management practices. This study showed that contamination with AFM1 in Tabriz is lower than standard levels. In Iran, especially in Tabriz, ewe milk is used for cheese making. It is concluded that consuming of ewe milk is safe for people in Tabriz, and the level of AFM1 in cheese should be lower than standard levels.

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