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## Presence of Aflatoxin M<sub>1</sub> in Raw Milk at Cattle Farms in Babol, Iran

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**Abstract:** In this study, raw cow milk samples were collected from milk churns at 40 traditional and semi-industrial cattle farms located in Babol (Northern Iran) in winter 2006. In total, 120 raw milk samples were tested for Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) contamination by competitive ELISA. In 68 out of 120 samples (56.7%) the presence of AFM<sub>1</sub> was detected by concentration ranging from 50 to 352.3 ng L<sup>-1</sup>. Fifty two samples (43.3%) contained AFM<sub>1</sub> at levels of 4-50 ng L<sup>-1</sup> (The AFM<sub>1</sub> contamination levels were between 4-352.3 ng L<sup>-1</sup> by the average of 102.73 ng L<sup>-1</sup>). In general, 56.7% of samples were beyond the limit of European community regulations (50 ng L<sup>-1</sup>). In other words, the AFM<sub>1</sub> contamination levels in raw milk were more than twice over standard levels. The AFM<sub>1</sub> contamination levels, (>50 ng L<sup>-1</sup>), in January, February and March were 40, 65 and 65%, respectively. Estimation of contamination of AFB<sub>1</sub> using AFM<sub>1</sub> in feed stuff showed that it was about 0.25 to 22 µg kg<sup>-1</sup> holding the average of 46.7%, being higher than European community limit (5 µg kg<sup>-1</sup>).

**Key words:** Aflatoxin M<sub>1</sub>, ELISA, raw milk, Iran

### INTRODUCTION

Aflatoxins are structurally related to a group of toxic compounds, found in most plant products such as peanuts, copra, soya, maize, rice and wheat. They are generally produced by special strains of *Aspergillus flavus* and *Aspergillus parasiticus*. These toxigenic fungi contaminate food products in different phases of production and processing especially in suitable heat and moist conditions. Aflatoxicosis depends on both environmental, social and economical conditions and the climate (Aycicek *et al.*, 2005). AFM<sub>1</sub> and AFM<sub>2</sub> are oxidative metabolic AFB<sub>1</sub> and AFB<sub>2</sub> which are made by live mycosomal enzymes functions. AFM<sub>1</sub> and AFM<sub>2</sub> are discharged via milk, urinary and feces of cattle and some species of mammals that consume feed stuff contaminated to Aflatoxin (Creppy, 2002; Aycicek *et al.*, 2005).

Their main target organ for toxicity and carcinogenicity is liver. Although mutagenic and carcinogenic level of AFM<sub>1</sub> is lower than AFB<sub>1</sub>, its genotoxic activity is high. Aflatoxin in some human diseases, particularly, liver primary cancer is involved by engaging DNA, mutagen P<sub>53</sub> gene (Kocabas and Sekerel, 2003). European community and Codex Alimentary determined the maximum level of AFM<sub>1</sub> 50 ng kg<sup>-1</sup> in raw milk, liquid, powder, heat-treated milk and processed dairy products and it must not exceed the amount.

The permitted contamination limit to AFB<sub>1</sub> in feed stuff is 5 µg kg<sup>-1</sup>. AFM<sub>1</sub> appears in milk by cattle's consuming AFB<sub>1</sub> after 12-24 h. Milk and dairy products consumption is high in human particularly among children; therefore, they are exposed to AFM<sub>1</sub>. On the other hand, milk is consumed not only as liquid but as children formulae, yoghurt, cheese and milk-based sweets such as chocolate and donuts. Therefore, AFM<sub>1</sub> recognition in milk and dairy products is crucial. As a result, consumers of various ages are protected from its potential risks (Bakirci, 2001).

Many countries have conducted inspection program and controlling on mycotoxins for several years to promote public health. Some studies have been conducted in some provinces in Iran. No research is observed to be done in the Northern provinces. The aim of the study is to survey the occurrence of AFM<sub>1</sub> in raw milk at cattle farms in Babol, Iran. There has been no detection in area.

### MATERIALS AND METHODS

**Samples:** A total of 120 raw cow milk samples randomly collected from milk churns at 40 traditional and semi-industrial cattle farms located in Babol (Northern Iran) in winter 2006. In total, 120 raw milk samples were tested for Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) contamination by competitive

Enzyme Linked Immunosorbent Assay (Tecna, AFLA M<sub>1</sub>-code MA418). Solid phase in plastic micro wells was coated with anti-Aflatoxin M<sub>1</sub> antibodies.

**Methods:** The samples were stored in a refrigerator (-20°C) and were centrifuged at 2-8°C for 10 min at 3000 g, then fat was completely separated from skimmed milk by pipette/pastur with micropipette. We added 100 µL skimmed milk and Aflatoxin M<sub>1</sub> standard solutions in each well. In each micro-plate, we appointed 7 wells for standards and then incubated the plate at 20-25°C for 45 min. Each well was washed four times by washing buffer 20X concentration. Then 100 µL conjugated solution (100X) was added to each well. After that, the plate was incubated at 20-25°C for 15 min. Next, the wells were washed. Substrate then was added in the wells. Then we incubated the plate at 20-25°C in a dark place for 15 min. The reaction was stopped by the stop solution. At most after one hour, light absorption was read at 450 nm by ELISA reader.

It is suggested that only 1.6% of AFB<sub>1</sub> fed by cattle convert to AFM<sub>1</sub>; therefore, AFB<sub>1</sub> level found in foods is calculated by the following equation (Rastogi *et al.*, 2004):

$$\text{AFBI}(\mu\text{g kg}^{-1}) = \frac{\text{AFMI}(\text{ng kg}^{-1}) \times 100}{1.6 \times 1000}$$

## RESULTS AND DISCUSSION

In the study, in total, 120 raw milk samples were tested for aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) contamination by competitive ELISA. In 68 out of 120 samples (56.7%) the presence of AFM<sub>1</sub> was detected by concentration ranging from 50 to 352.3 ng L<sup>-1</sup> and 52 samples (43.3%) contained AFM<sub>1</sub> at levels of 4-50 ng L<sup>-1</sup> (Table 1). The AFM<sub>1</sub> contamination levels were between 4-352.3 ng L<sup>-1</sup> by the average of 102.73 ng L<sup>-1</sup>. The AFM<sub>1</sub> contamination levels (>50 ng L<sup>-1</sup>) in January, February and March were 40, 65 and 65%, respectively (Table 2). In general, 56.7% of

samples were beyond the limit of European community regulations (50 ng L<sup>-1</sup>). In other words, the AFM<sub>1</sub> contamination levels in raw milk were more than twice over standard levels. (Table 3). In 40 cattle farms of the 26 were traditional and 14 were semi-industrial. The AFM<sub>1</sub> contamination levels (>50 ng L<sup>-1</sup>) in traditional and semi-industrial cattle farms were 58.97 and 52.38%, respectively (Table 3). The AFM<sub>1</sub> contamination levels (>50 ng L<sup>-1</sup>) in traditional cattle farms in January, February and March were 23.46, 69.38 and 69.38%, respectively, but in semi-industrial cattle farms this levels were 42.86, 57.14 and 57.14% (Table 3). Estimation of contamination of AFB<sub>1</sub> using AFM<sub>1</sub> in feed stuff shows that it is about 0.25-22 µg kg<sup>-1</sup> which bears the average of 46.7% (Table 4), being higher than European community limit (5 µg kg<sup>-1</sup>). The lowest contamination was observed in January. The highest contamination was observed in February and March (Table 5). Statistical evaluations show that there is not a significant relationship between AFM<sub>1</sub> contamination and different months of winter, the type of the cattle farm and the kind of feed stuff.

To decrease AFM<sub>1</sub> in milk to the lowest point, feed stuff ration should be checked regularly and it should be kept away from fungal contamination. AFM<sub>1</sub> seasonal contamination with low level in summer and high level in winter is considerable, since cattle feed concentrated materials in winter and in summer they graze in pastures. To increase milk quality, it is necessary for feed stuff to be without AFB<sub>1</sub> contamination (Kim *et al.*, 2000). In northern Iran due to favorable aflatoxigenic moulds growth especially in cold seasons, some measures should be taken in production, processing and storage of feed stuff.

In Greece, AFM<sub>1</sub> was measured by ELISA and HPLC methods in pasteurized milk, found at supermarkets. Of 81 milk samples, 32 had 2-2.5 ng L<sup>-1</sup>, 9 cases above 5 ng L<sup>-1</sup>, 31 cases 0.5-1 ng L<sup>-1</sup> and 9 cases had no AFM<sub>1</sub>. In India AFM<sub>1</sub> was measured in milk and children dairy products at supermarkets by applying competitive ELISA method. Out of 87 samples 87.3% were contaminated. Children

Table 1: The AFM<sub>1</sub> level distribution in raw milk at Babol's cattle-farms in different months

Months	Samples tested	Positive sample*		Negative samples		Mean±SE	SD	Max	Min	Total	
		sample*	%		%					N	%
Jan	40	16	40.0	24	60.0	104.96±18.4	114.09	3523.0	6.5	40	33.3
Feb	40	26	65.0	14	35.0	103.56±15.71	99.39	299.0	4.0	40	33.3
Mar	40	26	65.0	14	35.0	99.65±11.59	73.29	299.0	8.2	40	33.3
Total	120	68	56.7	52	43.3	102.73±8.79	96.27	352.3	4.0	120	100.0

\*: >50 ng L<sup>-1</sup>, SD = Standard Deviation, SE = Standard Error of Mean, N = Number

Table 2: The AFM<sub>1</sub> level distribution in raw milk in Babol, Iran

Frequency distribution of sample (ng L<sup>-1</sup>)

>251		201-250		151-200		101-150		51-100		26-50		5-25		<5	
%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
12.5	15	7.5	9	10	12	5	6	21.7	26	12.5	15	29.2	35	1.7	2

Table 3: The level of AFM<sub>1</sub> contamination and the type of cattle farms

Months	Samples tested	Traditional				Semi-industrial				Total			
		Positive sample*		Negative samples		Positive sample*		Negative samples		Positive sample*		Negative samples	
		N	%	N	%	N	%	N	%	N	%	N	%
Jan	26	10	38.46	16	61.54	14	6	42.86	8	57.14	40	16	40.0
Feb	26	18	69.23	8	30.77	14	8	57.14	6	42.86	40	26	65.0
Mar	26	18	69.23	8	30.77	14	8	57.14	6	42.86	40	26	65.0
Total	78	46	58.97	32	41.20	42	22	52.38	20	47.62	120	68	56.7

\*: >50 ng L<sup>-1</sup>, SD = Standard Deviation, SE = Standard Error of Mean, N = Number

Table 4: The AFB<sub>1</sub> level distribution in ration in Babol, Iran

Frequency distribution of sample (µg kg<sup>-1</sup>)

>20		16-20		11-15		5-10		<5	
%	N	%	N	%	N	%	N	%	N
1.7	2	10.8	13	9.2	11	25	30	53.3	64

Table 5: The AFB<sub>1</sub> level distribution in ration at Babol's cattle-farms in different months

Months	Samples tested	Positive sample*	%	Negative samples	%	Mean±SE	SD	Max	Min	Total	
										N	%
Jan	40	16	40.0	24	60.0	6.55±1.13	7.13	22.00	0.40	40	33.3
Feb	40	20	50.0	20	50.0	6.47±0.98	6.21	18.70	0.25	40	33.3
Mar	40	20	50.0	20	50.0	6.23±0.72	4.58	14.34	0.51	40	33.3
Total	120	56	46.7	64	53.3	6.42±0.55	6.01	22.00	0.25	120	100.0

\*: >50 ng L<sup>-1</sup>

dairy products (65-1012 ng L<sup>-1</sup>) were more contaminated than the liquid milk (28-164 ng L<sup>-1</sup>). Almost permitted by European community Alimentary, whereas 9% of samples had higher level allowed by US standard (500 ng L<sup>-1</sup>) (Rastogi *et al.*, 2004). In 2001 winter, AFM<sub>1</sub> was detected in 207 (99.5%) samples out of 208 (0.001-0.24 µg kg<sup>-1</sup>) which was found no significant difference in AFM<sub>1</sub> contamination in various areas in Japan (Nakajima *et al.*, 2004).

The results of the study show that AFM<sub>1</sub> contamination level in milk is high. It is a serious public health problem, because all age groups including babies and children extensively consume the product. As a result, milk and dairy products should be controlled regularly at least twice a year. Beside this, keeping low AFB<sub>1</sub> level in feed stuff is of importance. To reach the goal, feed stuff should be kept away from probable contamination.

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