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Aflatoxin M_1 contamination of raw and pasteurized milk produced in Sanandaj, Iran

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ABSTRACT

This study was conducted to evaluate and compare the levels of aflatoxin M_1 in raw and pasteurized milk samples during different seasons by Enzyme- Linked Immuno Sorbent Assay in Sanandaj, Iran. In 257 (94.49%) out of 272 milk samples the presence of aflatoxin M_1 was detected in concentrations ranging between 0.007 and 115.930 ng/l. AFM_1 level in 12 (4.4%) of positive samples were higher than the maximum tolerance limit (50 ng/l) accepted by Iran and European Union countries. Statistical evaluations showed that the differences between raw and pasteurized samples were not significant ($p<0.05$). There was no significant difference between spring and summer but the differences between other seasons were statistically significant. Winter samples with 22.35 ng/l and summer samples with 5.14 ng/l had the highest and lowest concentration, respectively ($p<0.05$). Since contamination of milk with aflatoxin is a potential risk for human health, milk and milk products should be controlled periodically for Aflatoxin contamination.

Keywords: Milk, Aflatoxin, measurement, ELISA

INTRODUCTION

Aflatoxins are toxic by-products produced by the mold fungus varieties *Aspergillus flavus* and *Aspergillus parasiticus* (Harper 2003). AFB_1 is the most potent of the group and has been shown to be a potent carcinogen. According to the International Agency Research on Cancer (IARC), AFB_1 and AFM_1 are categorized as class 1A and class 2B carcinogens, respectively (International Agency for Research on Cancer 1993, Maurice 2002). Many researchers have reported that there was a linear relationship between the amount of AFM_1 in milk and AFB_1 in feed consumed by animals (Bakirci 2001, Dragacci *et al* 1995). When animals eat foodstuff containing AFB_1, it will be metabolized and excreted as AFM_1 in the urine and also in milk (Bakirci 2001, Gurbay *et al* 2006, Lopez *et al* 2003). Apart from this, exposure to aflatoxin can be through ingestion of contaminated milk containing AFM_1.Occupational exposure to aflatoxins in

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agricultural workers, people working in oil mills and granaries have been reported (Ramdell & Eaton 1990). The parameters affecting levels of AFM_1 contamination in milk are the sources of animal feeds, ecologic and economic factors on the farm, and also farm management. It seems that the kind of animal feed and the harvesting time and temperature could be effective parameters in this regard (Kuiper 1999). According to the European Union and Codex Alimentarius the maximum level of AFM_1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg (Codex Alimentarius Commissions 2001) but based on the US regulations it should not be higher than 500 ng/kg (Stoloff *et al* 1991, Tajik *et al* 2007). Susceptibility to aflatoxin is greatest in the young, and there are very significant differences between species, individuals, and gender.The toxicity of aflatoxin also varies according to many nutritional factors and recovery from protein malnutrition is delayed by exposure to aflatoxin.With increasing levels of aflatoxin in the diet, reduction in feed intake and growth of rate become severe. If aflatoxin levels are high enough, liver damage can occur (Williams *et al* 2004). The results of the numerous studies on the effect of milk processing on the concentration of AFM_1 are variable. It is resistant to thermal inactivation, pasteurization, autoclaving (Pittet 1998, Deshpande 2002, Creppy 2002, Park 2002, Soha *et al* 2006, Galvano *et al* 1996) and production of yoghurt, cheese, cream, milk powder, or butter does not lead to loss of AFM_1, although it is redistributed differentially into the products resulting from these processes (Kamkar 2005, Food and Agriculture Organization/World Health Organization). In this work, data on the natural occurrence of AFM_1 in milk produced in Sanandaj is presented for the first time in order to determine the presence and levels of AFM_1 in milk during different seasons and to compare the results with maximum tolerable limits in milk that may be accepted by the European Union.

MATERIALS AND METHODS

Sampling. During the years 2006-2007, 240 raw and 32 pasteurized milk samples were obtained from Sanandaj Dairy and Milk Company. Samples of raw milk were taken with raw milk jar samplers from raw milk tankers, arriving directly from traditional dairy farm and after pasteurization process, samples of pasteurized milk were taken from pasteurized milk tankers. All of milk samples were collected based on Iranian National Standards (INS) milk sampling method No.419 (Institute of Standard and Industrial Research of I.R. 1965). The samples were prepared for analysis of AFM_1 with the competitive enzyme-linked immunosorbent assay (ELISA) method described by R-Biopharm AG, Darmstadt, Germany (Aflatoxin M130/15 Kit) by ELISA Reader ELX800, USA.

Samples Preparation. Milk samples were centrifuged for degreasing at 3500 G for 10 min at 10ºC. The upper cream layer was removed by aspirating through a Pasteur pipette. The skimmed milk was used directly in the test (100 µl per well).

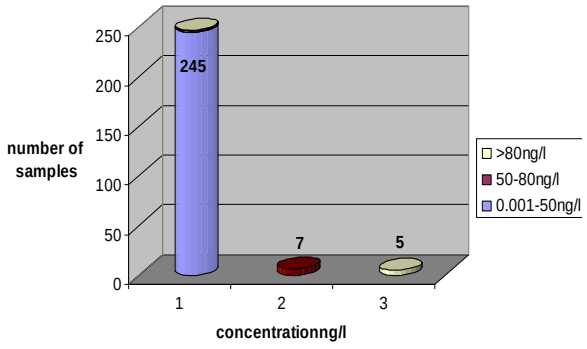

Figure 1. Level of Aflatoxin M1 in analyzed milk samples.

ELISA test procedure: 100 µl standard solutions and prepared samples in separate wells were added and incubated for 30 min at room temperature in the dark. After the washing steps, 100 µl of the enzyme conjugate was added to each well and incubated for 15 min at room temperature in the dark. Again the washing steps were done, then 100 µl of substrate/ chromogen were added to each well and incubated

for 15 min at room temperature in the dark. 100 µl of the stop solution was added to each well, mixed gently and the absorbance measured at 450 nm against an air blank within 15 min after addition of stop solution.

Statistical methods. sequential differences among means were calculated at the level of $p<0.05$, using SPSS 12 software.

RESULTS

In general 12 (4.67%) of positive samples, 10 (4.17%) of raw milk and 2 (6.25%) of pasteurized milk samples, was exceeded the legal level of AFM_1 accepted by Iran and European countries (Codex Alimentarius Commissions 2001, Institute of Standard and Industrial Research of I.R.2002).

Table 1. Aflatoxin M_1 concentration of raw and pasteurized milk samples.

Type	No.of Samples	Negative	Positive	Mean Concentration (ng/l	SD
Raw	240	14	226	12.65[a]	17.76
Pasteurized	32	1	31	12.43[a]	17.53
Total	272	15	257	12.63	17.70

Table 2. Distrbution of Aflatoxin M_1 concentration by season.

Seasons	No.of Samples	Mean Concentration (ng/l)	SD	Maximum	Minimum
Spring	49	9.40[a]	13.09	52.24	0.000
Summer	65	5.14[ba]	5.14	33.50	0.000
Autumn	79	11.01[c]	12.39	60.31	0.000
Winter	79	22.35[d]	25.59	115.93	0.410
Total	272	12.60	17.67	115.93	0.000

According to Table 1 Aflatoxin M_1 was found in 257 (94.49%) of the examined samples with no significant differences between raw and pasteurized milk samples. The levels of aflatoxin M_1 were different between seasons. The overall contamination levels in winter were higher than other seasons.

DISCUSSION

There are few literature data on the occurrence of AFM_1 levels in milk and milk products in Iran (Tajik *et al* 2007, Kamkar 2005, Alborzi *et a*l 2006, Tajkarimi *et al* 2007, Karim *et al* 1998, Oveisi *et al* 2007, Sefidgar *et al* 2008). In one study in 1998, out of 73 milk samples delivered to Tehran milk pasteurization plants, 60(82.2%) were contaminated. All contaminated samples had a level of AFM_1 above 50ng/l (Karim *et al* 1998). The incidence of the AFM_1 level in 328 milk and infant milk products in Tehran were 96.3%. The presence of AFM_1 in pasteurized liquid milk (n=128), infant formula (n=120) and milk-based cereal weaning food was 72.2±23.5, 7.3±3.9 and 16.8±12.5 ng/kg, respectively (Oveisi *et al* 2007). In Shiraz (Southern Iran) 624 pasteurized milk samples were analyzed. AFM_1 was found in 100% of the examined samples, 390 of them with contamination less than 45 ng/kg, 123 samples (19.7%) contained 45-50 ng/kg, 94 samples(15.1%) had 50-80 ng/kg and 17 (2.7%) of samples contained more than 80 ng/kg (Alborzi *et al* 2006). Tajik et al. (2007) found a high incidence rate of AFM_1 (100%) in 144 raw and pasteurized milk samples in Urmia, Iran of which the level in 6.25% of samples was higher than 50 ng/l (Tajik *et al* 2007). In 85 of 111 raw milk samples (76.6%) examined in Sarab city of Iran, AFM_1 was detected in concentrations ranging between 0.015 and 0.28 µg/l and in 40% of positive samples, its level was higher than 50 ng/l (Kamkar 2005). Lopez et al. (2003) suggested that levels of AFM_1 in samples of milk produced in Argentina were found to be very

low and in no case did the levels exceed the recommended limits for milk products (0.05µg/l).

Table 3. The prevalence of aflatoxin M_1 contamination in raw milk samples

Countries		No. of Raw Samples	Percent of Samples> 50 ng/l	References
Iran	Sanandaj	240	4.17	
	Urmia	72	12.5	Tajik et al. (2007)
	Sarab	111	40	Kamkar (2005)
	Tehran	73	82.2	Oveisi et al. (2006)
	Babol	120	56.7	Sefidgar et al. (2008)
Greece		81	0	Markaki et al. (1997
Argentina		56	0	Lopez et al. (2003)
Italy		296	1.7	Nachtmann et al. (2007)

The incidence of AFM_1 contamination in raw milk analyzed in Portugal was 80.6%, 17 samples (54.8%) contained 0.005-0.010 µg/l, two samples (6.5%) had 0.011-0.02 µg/l, and six samples (19.3%) contained 0.021-0.050 µg/l (Martins *et al* 2000). Out of 85 milk samples in Ankara, Turkey, 75 samples (88.23%) were contaminated with AFM_1; of which 27 samples (36%) were contaminated with concentrations <50 ng/l and 48 samples (64%) exceeded the legal level of Aflatoxin M_1 in milk (Çelik *et al* 2005). During 2005, out of 45 raw milk samples analyzed in Italy, only one sample was positive to the ELISA test, but the confirmation test values (0.035 µg/l) were below the legal limit (Decastelli *et al* 2007). In Korea, the incidence of AFM_1 in liquid milk was 76% with a mean concentration of 18 pg/g (Kim *et al* 2000). In this study the level of AFM_1 in raw and pasteurized milk samples showed no significant differences ($p<0.05$). As compared to other studies the contamination level of raw samples of Sanandaj were lower than those of Urmia (Tajik *et al* 2007), Sarab (Kamkar 2005), Babol (Sefidgar *et al* 2008) and Tehran (Oveisi *et al* 2007) in Iran and it was higher than Argentina (Lopez 2003) and Greece (Markaki 1997) where in no case did the levels exceed the recommended limits. The results of the AFM_1 level derived from pasteurized samples were lower compared with other studies conducted in Shiraz (Alborzi *et al* 2006), Tehran (Oveisi *et al* 2007), Tabriz (Movassagh Ghazani 2009) India (Rastogi *et al* 2004), Brazil (Oliveira *et al* 2007), Turkey (Unusan 2006) and United State of America (Cathey *et al* 1994) and higher than samples examined in Urmia (Tajik *et al* 2007), Mashhad (Karimi *et al* 2007), Italy (Nachtmann *et al* 2007) and Argentina(Lopez *et al* 2003). The disagreement among the findings can be a result of differences in the level of contamination of consumed food stuffs. Seasonal evaluation of the data indicate meaningful differences in the contamination level ($p<0.05$).

Table 4. The prevalence of aflatoxin M_1 contamination in pasteurized milk samples.

Countries		No. of Pasteurized Samples	Percent of Samples> 50 ng/l	References
Iran	Sanandaj	32	6.25	Tajik et al. (2007)
	Urmia	72	0.0	Alborzi et al.(2006)
	Shiraz	624	17.8	Oveisi et al. (2006)
	Tehran	128	78	Movassagh Ghazani(2009)
	Tabriz	50	62	Karimi et al.(2007)
	Mashhad	110	5.4	
Turkey(Anatoli)		129	47	Unusan (2006)
USA		85	64	Cathey et al. (1994)
India		12	33	Rastogi et al. (2004)
Turkey(Ankara)		27	3.7	Gurbay et al. (2006)
Argentina		16	0	Lopez et al. (2003)
Brazil		57	7.4	Oliveira et al. (2007)
Italy		316	0.6	Nachtmann et al. (2007)

Some other studies also indicate a significant difference between winter and other seasons (Ghiasian *et al* 2007, Tajkarimi *et al* 2007, Kamkar 2005). This variation can be due to the fact that grass, pasture, weed, and rough feeds were found

more commonly in spring and summer than in winter. Therefore, obtained results were in agreement with prior studies. In order to achieve a low level of AFM_1, it is essential to conduct training programs for producers about the toxicity potential of aflatoxins, reduce the concentration of AFB_1 in animal feed by good manufacturing and storage and integrating it with Hazard Analysis and Critical Control Points (HACCP) based safety program and doing regular monitoring of milk and milk products.

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