Aflatoxin M₁ 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 M₁ 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 M₁ was detected in concentrations ranging between 0.007 and 115.930 ng/l. AFM₁ 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). AFB₁ 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), AFB₁ and AFM₁ 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 AFM₁ in milk and AFB₁ in feed consumed by animals (Bakirci 2001, Dragacci et al 1995). When animals eat foodstuff containing AFB₁, it will be metabolized and excreted as AFM₁ 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 AFM₁. Occupational exposure to aflatoxins in
agricultural workers, people working in oil mills and granaries have been reported (Ramdell & Eaton 1990). The parameters affecting levels of AFM<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub>, 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<sub>1</sub> in milk produced in Sanandaj is presented for the first time in order to determine the presence and levels of AFM<sub>1</sub> 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<sub>1</sub> 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 10°C. 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](image-url) **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

**Table 1.** Aflatoxin M₁ concentration of raw and pasteurized milk samples.

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Samples</th>
<th>Negative</th>
<th>Positive</th>
<th>Mean Concentration (ng/l)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>240</td>
<td>14</td>
<td>226</td>
<td>12.65*</td>
<td>17.76</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>32</td>
<td>1</td>
<td>31</td>
<td>12.43*</td>
<td>17.53</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>15</td>
<td>257</td>
<td>12.63</td>
<td>17.70</td>
</tr>
</tbody>
</table>

**Table 2.** Distribution of Aflatoxin M₁ concentration by season.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>No. of Samples</th>
<th>Mean Concentration (ng/l)</th>
<th>SD</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>49</td>
<td>9.40a</td>
<td>13.09</td>
<td>52.24</td>
<td>0.000</td>
</tr>
<tr>
<td>Summer</td>
<td>65</td>
<td>5.14a</td>
<td>5.14</td>
<td>33.50</td>
<td>0.000</td>
</tr>
<tr>
<td>Autumn</td>
<td>79</td>
<td>11.01a</td>
<td>12.39</td>
<td>60.31</td>
<td>0.000</td>
</tr>
<tr>
<td>Winter</td>
<td>79</td>
<td>22.35a</td>
<td>25.59</td>
<td>115.93</td>
<td>0.410</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>12.60</td>
<td>17.67</td>
<td>115.93</td>
<td>0.000</td>
</tr>
</tbody>
</table>

According to Table 1 Aflatoxin M₁ was found in
257 (94.49%) of the examined samples with no
significant differences between raw and pasteurized
milk samples. The levels of aflatoxin M₁ 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
AFM₁ levels in milk and milk products in Iran
(Tajik et al 2007, Kamkar 2005, Alborzi et al 2006,
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 AFM₁
above 50ng/l (Karim et al 1998). The incidence of
the AFM₁ level in 328 milk and infant milk products
in Tehran were 96.3%. The presence of AFM₁ 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.
AFM₁ 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 AFM₁ (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, AFM₁ 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 AFM₁ 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 M₁ contamination in raw milk samples

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

The incidence of AFM₁ 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 AFM₁; of which 27 samples (36%) were contaminated with concentrations <50 ng/l and 48 samples (64%) exceeded the legal level of Aflatoxin M₁ 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 AFM₁ in liquid milk was 76% with a mean concentration of 18 pg/g (Kim et al 2000). In this study the level of AFM₁ 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 (Oveisí 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 AFM₁ level derived from pasteurized samples were lower compared with other studies conducted in Shiraz (Alborzi et al 2006), Tehran (Oveisí 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 M₁ contamination in pasteurized milk samples.

<table>
<thead>
<tr>
<th>Countries</th>
<th>No. of Pasteurized Samples</th>
<th>Percent of Samples&gt;50 ng/l</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanandaj</td>
<td>32</td>
<td>6.25</td>
<td>Tajik et al. (2007)</td>
</tr>
<tr>
<td>Urmia</td>
<td>72</td>
<td>0.0</td>
<td>Alborzi et al. (2006)</td>
</tr>
<tr>
<td>Shiraz</td>
<td>624</td>
<td>17.8</td>
<td>Oveisí et al. (2006)</td>
</tr>
<tr>
<td>Tehran</td>
<td>128</td>
<td>78</td>
<td>Movassagh Ghazani (2009)</td>
</tr>
<tr>
<td>Tabriz</td>
<td>50</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Mashhad</td>
<td>110</td>
<td>5.4</td>
<td>Markaki et al. (1997)</td>
</tr>
<tr>
<td>Turkey(Anatoli)</td>
<td>129</td>
<td>47</td>
<td>Unusan (2006)</td>
</tr>
<tr>
<td>USA</td>
<td>85</td>
<td>64</td>
<td>Cathey et al. (1994)</td>
</tr>
<tr>
<td>India</td>
<td>12</td>
<td>33</td>
<td>Rastogi et al. (2004)</td>
</tr>
<tr>
<td>Turkey(Ankara)</td>
<td>27</td>
<td>3.7</td>
<td>Gurbay et al. (2006)</td>
</tr>
<tr>
<td>Argentina</td>
<td>16</td>
<td>0</td>
<td>Lopez et al. (2003)</td>
</tr>
<tr>
<td>Brazil</td>
<td>57</td>
<td>7.4</td>
<td>Oliveira et al. (2007)</td>
</tr>
<tr>
<td>Italy</td>
<td>316</td>
<td>0.6</td>
<td>Nachtmann et al. (2007)</td>
</tr>
</tbody>
</table>

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.

References


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