Occurrence of Aflatoxin M$_1$ in UHT Milk in Erzurum-Turkey

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Summary

In this study, 150 UHT milk samples were analyzed for aflatoxin M$_1$. They were obtained from supermarkets in Erzurum city. The occurrence and concentration range of AFM$_1$ in the samples were investigated by ELISA method. Fifty-nine percent of the UHT milk samples contained AFM$_1$. AFM$_1$ levels in 16 (10.7%) UHT milk samples exceeded the maximum tolerable limit of the European Community and the Turkish Food Codex. It was concluded that high AFM$_1$ level is an important problem threatening the public health in Turkey.

Keywords: Aflatoxin M$_1$, UHT milk, ELISA

INTRODUCTION

Aflatoxin is common contaminant of foods. This toxins is produced by fungal action during production, harvest, storage, and food processing. The toxin is produced as secondary metabolites by Aspergillus flavus and A. parasiticus and the rare A. nomius fungi. A. flavus produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxins M$_1$ and M$_1$ are the hydroxylated metabolites of aflatoxin B$_1$ and B$_1$. International Agency for Research and Cancer (IARC) of WHO included AFB$_1$ as primary and AFM$_1$ as secondary groups of carcinogenic compounds.

The residues of AFM$_1$ remain stable when milk is processed by heat or is fermented. There is no evidence that cold storage, freezing, heat-treating, fermenting, concentrating or drying of the contaminated milk changes the level of AFM$_1$. AFM$_1$ is mainly soluble in the aqueous phase of milk or adsorbed to casein particles; data of several studies show that a small proportion of AFM$_1$ in milk is carried-over to cream, and yet a smaller proportion to butter. The remainder of AFM$_1$ in milk, however, remains in skim milk and buttermilk.

Milk is a major nutrient for infants, children, convalescents and old people. However, milk and milk products are the most potent source of aflatoxin among foods. To protect consumers several countries have established legislation to regulate the levels of AFB$_1$ in
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feeds and AFM1 in milk and dairy products.\textsuperscript{10-12}

The European Community (EC) and the Turkish Food Codex (TFC) legal limit for AFM1 in milk is 50 ng/kg.\textsuperscript{13,14} Despite lots of studies on AFM1 in cheese varieties,\textsuperscript{11,15-18} only a few are concerned with UHT milk. The aim of this study was to investigate the occurrence of AFM1 in UHT milk and to compare the results with the maximum AFM1 tolerance limits which are accepted by the EC and TFC.

**MATERIAL and METHODS**

**Samples:** A total of 150 samples of UHT milk (whole milk) samples were obtained randomly from supermarkets between September 2006 and September 2007 in Erzurum city. The samples were transported to the laboratory in an insulated container at about 4°C and analyzed upon arrival. All samples were analyzed before their expiry date.

**Methods:** AFM1 concentrations of the samples were analyzed by competitive ELISA (RIDASCREEN Aflatoxin M1, R-Biopharm). The samples were evaluated according to the RIDAVIN computer program prepared by R-Biopharm.\textsuperscript{19} According to the instructions for use of the RIDASCREEN kit, the lower detection limit was 5 ng/kg.

Calculation of extrapolated values of AFB1 concentration in cattle feeding stuffs based on AFM1 in UHT milk samples

It has been suggested that only 1.6% of ingested AFB1 is converted to AFM1 by the dairy cattle.\textsuperscript{10,20-22} Hence, the values of AFB1 contamination in feeding stuffs were back calculated by using the formula:

$$AFB1 \, (\mu g/kg) = \frac{AFM1 \, (ng/kg) \times 100}{1.6 \times 1000}$$

**RESULTS**

In this study a total of 150 UHT milk (whole milk) samples were analyzed for AFM1 with the competitive ELISA. The occurrence and the distribution of AFM1 concentration in various ranges in UHT milk samples are presented in Table 1.

As shown in Table 1, AFM1 was found above the detectable level in 59% (89/150) in UHT milk samples. AFM1 levels in 10.7% (16/150) UHT milk samples were found to be higher than the maximum acceptable limits (milk; 50 ng/kg) of the EC\textsuperscript{13} and TFC\textsuperscript{14}. AFM1 content of positive samples were determined in UHT milk samples as minimum 5 ng/kg, maximum 185 ng/kg, and mean 36±38 ng/kg.

Earlier studies have shown that contamination of AFM1 in milk and dairy products is a result of exposure of AFB1 to dairy cattle through feedstuffs.\textsuperscript{23} Further, investigators have suggested that on an average 1.6% of AFB1 fed to the lactating cattle is excreted in milk as AFM1.\textsuperscript{20,21} Using this factor, the content of AFB1 in the dairy cattle feeding stuffs was extrapolated from AFM1 contamination in the UHT milk samples (Table 2).

**Table 1.** Occurrence and distribution of AFM1 in UHT milk samples

<table>
<thead>
<tr>
<th>Kind of Samples</th>
<th>Samples Tested (n)</th>
<th>Proportion of Positive Samples n (%)</th>
<th>Distribution of Samples * n (%)</th>
<th>Proportion of Samples Exceeding the EC and TFC Legal Limit &gt;50 ng/kg</th>
<th>Quantity of AFM1 (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHT Milk</td>
<td>150</td>
<td>89/150 (59)</td>
<td>61/150 (40.0)</td>
<td>42/150 (28)</td>
<td>36±38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31/150 (20.7)</td>
<td>10/150 (6.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6/150 (4)</td>
<td>16/150 (10.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-185</td>
</tr>
</tbody>
</table>

\* distribution of negative samples, a: ng/kg, EC: European Community, TFC: Turkish Food Codex, (): indicates percent, x±Sx: mean±standard deviation

**Table 2.** Extrapolated AFB1 concentration in cattle feedstuffs based on AFM1 contamination in UHT milk samples

<table>
<thead>
<tr>
<th>Kind of Samples</th>
<th>Samples Tested (n)</th>
<th>Level of Positive Samples n (%)</th>
<th>Range (μg/kg)</th>
<th>Exceeding EC and TFC (5 μg/kg)</th>
<th>Positive Samples (μ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHT Milk</td>
<td>150</td>
<td>89/150 (59)</td>
<td>0-11.6</td>
<td>6/150 (4)</td>
<td>2.3±2.4</td>
</tr>
</tbody>
</table>

x±Sx: mean±standard deviation, EC: European Community, TFC: Turkish Food Codex, (): indicates percent x±Sx: ortalama±standart sapma, EC: Avrupa Birliği, TFC: Türk Gıda Kodeksi, (): yüzde ifadesi
It can be seen from the results that the contamination of feed with AFB1 in cattle feed may range of 0-11.6, with an average of 2.3±2.4 μg/kg. Moreover, 4% (6/150) of the samples exceeded the limits recommended by EC and TFC regulations.

In some studies made on UHT milk, presence and level of AFM1 were showed in Table 3.

Table 3. AFM1 contents of UHT milk reported in previous studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>Country</th>
<th>No. of Samples Positive</th>
<th>Range of Samples Positive (ng/kg)</th>
<th>Exceed Legal Limit *</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHT Milk</td>
<td>Turkey</td>
<td>75/129 (58.1)</td>
<td>10-543.6</td>
<td>61/129 (47)</td>
<td>Unusan 27</td>
</tr>
<tr>
<td></td>
<td>Portugal</td>
<td>60/70 (85.7)</td>
<td>5-61</td>
<td>20/70 (2.9)</td>
<td>Martin and Martin 27</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>14/24 (58.3)</td>
<td>10-50.5</td>
<td>1/24 (4.2)</td>
<td>Gurbay et al. 26</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>67/100 (67)</td>
<td>10-630</td>
<td>31/100 (31)</td>
<td>Tekinsen and Eken 25</td>
</tr>
</tbody>
</table>

* Turkish Food Codex limits in UHT milk is 50 ng/kg. (%) indicates percent

DISCUSSION

In our study, AFM1 was determined in 59% of the UHT milk samples. These results are in parallel with the findings of some previous reports 22,24,25 which pointed out the presence of AFM1 in all or most of the UHT milk samples in Turkey (Table 3).

These reports and the present findings suggest that the milk which is processed in to dairy products may contain a high concentration of AFM1 and/or be contaminated with Aspergillus spp. The results confirm the findings of Bakirci 26 who reported high concentrations of the AFM1 in raw milk during spring. The AFM1 levels (as incidence) also were lower than the reported results by Martin and Martin 27, Oliveira and Ferraz 28. In addition, the AFM1 level in the milk was significantly affected by the geographical region and the country 26,29. Moreover, differences in the hygiene and storage conditions at the dairies and retail points are other key factors on the variations of the results 30-31.

Some of the AFM1 amounts in the UHT milk samples were at the risk level for human health because the AFM1 in 16 UHT milk samples (10.7%) exceeded the EC and TFC legal limits of 50 ng/kg. In this study AFM1 incidence of exceeding legal limit in UHT milk samples was lower than the reported results by Unusan 22, Tekinsen and Eken 25, Oliveira and Ferraz 28 and were higher than the results reported by Gurbay et al. 26, Martin and Martin 27. Very high AFM1 levels (51 - >100 ng/kg) in 10.7% of the UHT milk samples are of great importance. Also it should be kept in mind that, total daily aflatoxin intake from other foods could be an important risk factor for people as well. The weighted mean concentration of AFM1 in milk is 0.023 μg/kg in the European-type diet, 0.022 μg/kg in the Latin American diet, 0.36 μg/kg in the Far Eastern diet, 0.005 μg/kg in the Middle Eastern diet and 0.002 μg/kg in the African diet. These mean concentrations are based on a large number of milk samples analyzed. The intake of AFM1 from milk is calculated to be 6.8 ng/person per day for the European diet, 3.5 ng/person per day for the Latin American diet, 12 ng/person per day for the Far Eastern diet, 0.7 ng/person per day for the Middle Eastern diet and 0.1 ng/person per day for the African diet 22,22.

The content of AFB1 in the dairy cattle feeding stuffs was extrapolated from AFM1 contamination in the UHT milk samples (Table 2). In a previous study, contamination of AFB1 in Turkey cattle feedstuffs was found to be in the range of 0-33.98 μg/kg. 32. At the present study, AFB1 incidence of exceeding legal limit in UHT milk samples was lower than the reported results by Unusan 22.

In conclusion, AFM1 is common contaminant UHT milk can be considered to be a main concern for public health. So the public health authorities should take necessary measures and the producers should be informed. Moreover the prevention of aflatoxin formation in feeds is very important. Because the consumption of contaminated feeds by dairy animals causes AFM1 formation in milk. So the easiest and shortest way of reducing AFM1 amount forming in milk to minimum focuses on the prevention of AFB1 formation in feeds. For this, it is necessary to control well the feeds given to dairy animals and to reduce AFB1 amount permitted to take place in feeds to lower levels. In addition, it is considered that food substances should be produced and kept in convenient conditions to prevent aflatoxin formation.

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