The study of aflatoxin $M_1$ in UHT milk samples by ELISA

Kamkar, A.*

Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran.
(Received 16 September 2005, Accepted 22 April 2007)

Abstract: Aflatoxin $M_1$ (AFM$_1$) levels in 52 samples of UHT milk that were produced by different plants in province of Tehran were analyzed by competitive ELISA. AFM$_1$ was found in 100 percent of 52 of the UHT milk samples that were analyzed in this study. The range of contamination levels varied in two seasons of summer and autumn. AFM$_1$ in summer and autumn samples ranged from 22.40 to 84.80 and 19.40 to 93.60 ng/kg respectively, while the mean values were 69.22 and 65.50 ng/kg respectively. Statistical evaluation showed that there were not significant differences (p<0.05) between the concentrations of AFM$_1$ of UHT milk samples produced in summer and autumn. In other words, AFM$_1$ contents of UHT milk samples produced in summer were not lower than UHT milk samples produced in autumn. Almost 79.92% of the contaminated samples exceeded the maximum acceptable levels (50 ng/kg) that accepted by some of the European countries. It was, therefore, concluded that, high occurrence of AFM$_1$ in UHT milk samples was considered possible hazard for human health.

Key words: aflatoxin $M_1$, UHT milk.

Introduction

Aflatoxins are actually toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds to animals and humans, and contamination of feed and food is a current problem (Piva et al., 1995; Kotsonis, Burdock, and Flamm, 1996; Peraica et al., 1999; Kocabas and Sekerel, 2003).

International Agency for Research on Cancer (IARC) of WHO included AFB$_1$ (Aflatoxin B$_1$) as primary and AFM$_1$ (Aflatoxin M$_1$) as secondary groups of carcinogenic compounds (Anonymous, 1993; Cathey et al., 1994; Dragacci et al., 1995).

Aflatoxins can be produced by three species of Aspergillus. A. flavus, A. parasiticus and the rare A. nomius. A. flavus only produces B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxins M$_1$ (AFM$_1$) and M$_2$ (AFM$_2$) are hydroxylated metabolites of Aflatoxins B$_1$ (AFB$_1$) and B$_2$ (AFB$_2$), respectively, and may be found in milk products obtained from livestock that have ingested contaminated feed (Van Egmond, 1991; Wood, 1991; Cathey et al., 1994; Creppy, 2002). Many researchers reported that there was a linear relationship between the amount of AFM$_1$ in milk and AFB$_1$ in feed consumed by animals (Wood, 1991; Dragacci et al., 1995; Bakirci, 2001).

According to Stoloff (1980) milk has the greatest demonstrated potential for introducing AF residues from edible animal tissues into the human diet, and taking into account that pasteurization processes (even those using UHT, Ultra High Temperature, techniques) do not affect AFM$_1$ concentration because of its heat stability (Gelosa, and Buzzetti, 1994; Galvano, Galofaro, and Galvano, 1996), moreover, as milk is the main nutrient for growing young, whose vulnerability is noteworthy and potentially more sensitive than that of adults, the occurrence of AFM$_1$ in human breast milk, commercially available milk, and milk products is one of the most serious problems of food hygiene. For this reason, many countries have regulations to control the levels of Aflatoxins B$_1$ in feeds and to purpose maximum permissible levels of AFM$_1$ in milk to reduce this risk (Rastogi et al., 2004;
Sarimehmetoglu, Kuplulu, and Celik, 2004).

Regulatory limits throughout the world are highly variable, depending on the degree of development and economic involvement of countries, and may vary from one country to another (Van Egmond, 1989; Stahr et al., 1990; Stoloff, Van Egmond, and Parks, 1991; Chen and Gao, 1993). The European Community and Codex Alimentarius prescribe that the maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg (Codex Alimentarius Commission, 2001). However, according to US regulations the level of AFM1 in milk should not be higher than 500 ng/kg (Stoloff et al., 1991). In Austria and Switzerland, the maximum level is further reduced to 10 ng/kg for infant food commodities (FAO, 1997). There are thus differences in maximum permissible limit of AFM1 in various countries (Van Egmond, 1989), and many including Iran, have no legal limit for AFM1 in milk and dairy products.

A number of survey and monitoring programs have been carried out in several countries attempting to obtain general pattern of extent of food contamination (Galvano et al., 1996; Abdulkadar, Abdulla, and Jasim, 2000; Oruc and Sonal, 2001; Aycicek et al., 2002; Gunseven and Buyukyörük, 2002; Rastogi et al., 2004).

There is little information about the occurrence of AFM1 in milk and milk products. In Tehran, of 73 samples taken in 1998, 60 (82.2%) samples contained AFM1, and all contaminated samples had a level of aflatoxin M1 above the European countries standard (Karim et al., 1998). In another study from 52 liquid milk samples that were analyzed, 48 (92.3%) were contaminated with aflatoxin M1 at concentration between 23 and 3000 µg/l (Karim et al., 1982).

The purpose of this survey was to determine natural occurrence and levels of AFM1 in UHT milk which were produced and consumed in Tehran province of Iran, and to compare the obtained results with maximum AFM1 tolerance limits (50 ng/kg) accepted by some European countries.

Materials and Methods

Fifty-two samples of UHT milk were taken randomly from markets located in the various districts of Tehran, and examined for the presence and levels of AFM1. All samples were collected between the periods of May 2004 and November 2004, and aflatoxin M1 concentration was determined before their expiration dates exceeded. Randomized block experiment was used to evaluate the differences between AFM1 occurrence levels of the milk samples. Furthermore, Independent-samples t-test was used to obtain significant differences between sampling periods of all tests.

The milk samples were centrifuged 10min/3500 rpm/10°C. After centrifugation, the upper cream layer was completely removed by aspirating through a Pasteur pipette. The skimmed milk was used directly in the test (100 µl per well). A sufficient number of micro titer wells were inserted into the micro well holder for all standards and prepared samples. One hundred µl of standard solutions and prepared samples were added in separate wells and incubated for 60 min at room temperature in the dark. The liquid was poured off the wells and the micro well holder was tapped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with 250 µl of washing buffer and emptied as described earlier. The washing procedure was repeated twice. One hundred µl of the enzyme conjugate was added and incubated for 60 min at room temperature in the dark. The washing sequence was repeated three times. Fifty µl of substrate and 50 µl of chromogen were added to each well and mixed thoroughly and incubated for 30 min at room temperature in dark. Then 100 µl of the stop reagent was added to each well, mixed and absorbance measured at wave length of 450 nm against air.

Results

In this study, 52 UHT milk samples were analyzed for AFM1 with the competitive ELISA. The presence of AFM1 in UHT milk samples is shown in tables 1 and 2.

The prevalence rate of AFM1 contamination in UHT milk samples was 100 percent. In the other words, of 52 UHT milk, 52 samples (100%) were
Table 1: Occurrence of aflatoxin M₁ in UHT milk samples and concentration (ng/kg). Values in parentheses indicate percentages of positive samples.

<table>
<thead>
<tr>
<th>Samples Tested (n)</th>
<th>Level of positive samples (%)</th>
<th>Number and percent of samples with AFM₁ in ng/kg ranges</th>
<th>Exceeding EC/Codex regulation (50 ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>ND</td>
<td>&lt;20, 20-40, 40-60, 60-80, 80-100</td>
<td>Number, Range (ng/kg)</td>
</tr>
<tr>
<td></td>
<td>52(100)</td>
<td>0 (0), 1(1.92), 8(15.4), 7(13.5), 21(40.4), 15(28.8)</td>
<td>40(76.92), 52.7 - 93.66</td>
</tr>
</tbody>
</table>

Contaminated with AFM₁ ranging from 19.40 to 93.60 ng/kg (table 1). One sample (1.92%) contained level <20 ng/kg. Eight samples (15.38%) were contaminated with AFM₁ levels ranging from 20 to 40 ng/kg. Seven samples (13.46%) were contaminated with levels ranging from 40 to 60 ng/kg. Twenty-one samples (40.38%) showed contamination levels from 60 to 80 ng/kg and 15 samples (28.84%) presented high contaminations with levels from 80 to 100 ng/kg. Table 2 shows distribution by season of UHT milk samples and aflatoxin M₁ (AFM₁) concentration. The range of contamination levels varied in two seasons. AFM₁ in summer and autumn samples ranged from 22.40 to 84.80 and 19.40 to 93.60 ng/kg while the mean values were 65.50 and 69.22 ng/kg respectively. The highest mean concentration of aflatoxin M₁ (AFM₁) registered in autumn (93.60), while in summer samples highest concentration of aflatoxin M₁ (AFM₁) was 84.80 ng/kg. Statistical evaluation showed no significant differences (p>0.05) between the mean concentration of AFM₁ of milk samples produced in summer and autumn. Almost 79.92% of contaminated samples exceeded the European Communities/Codex Alimentarius recommended limits, while none of the samples exceeded the prescribed limits of US regulations.

Discussion

Since, milk is a major commodity for introducing aflatoxins in human diet, and several investigators (Galvano, Galofaro and Galvano, 1996; Stoloff, 1980) have showed evidence of hazardous human exposure to AFM₁ through dairy products, many countries have carried out studies about the incidence of AFM₁ in milk. In some of them, samples have been found which exceed the limit imposed by many countries (0.05 µg/l).

In Asia, high incidences and levels of aflatoxin M₁ contamination were found in In Thailand, of 310 liquid milk samples, more than 261 (>84%) were contaminated with aflatoxin M₁ concentrations > 0.05 µg/kg, and 58 samples (19%) contained >0.5 µg/kg, with a maximum of 6.6 µg/kg (Boriboon and suprasert, 1994; Saitanu, 1997).

In Philippine (Begino, 1998), indicated that 88% and 18% of 91 milk samples were contaminated with aflatoxin M₁ at > 0.05 µg/kg and >0.5 µg/kg, respectively. The data for Republic of Korea in 1995 and 1997 (Kim et al., 2000; Shon, Lim and Lee, 1996), indicated that of 134 liquid milk samples, 50 (37%) contained aflatoxin M₁ at a concentration > 0.05 µg/kg, with a maximum of 0.28 µg/kg. Of 504 samples taken in India in 1995, 89 (15.6%) samples contained AFM₁ at concentration of 100 - 3500 µg/l (Rajan, Ismail and Radhakrishnon, 1995).

In another study in India, 87.3% of 87 samples that were analyzed showed to be contaminated with AFM₁. The range of contamination of AFM₁ in liquid milk was 28- 164 µg/l. Almost 99 percentile of the contaminated samples exceeded the European Communities/Codex Alimentarius recommended limits (European Communities, 1992; Shipra et al., 2004). In a Korean study, the incidence of AFM₁ in liquid milk was 76%, with a mean concentration of 18 µg/g (Kim et al., 2000). In Kuwait, 54 samples of dairy products were analyzed for aflatoxin M₁ (AFM₁). Twenty-eight percent were contaminated.
Table 2: Distribution by season of UHT milk samples and aflatoxin M₁ concentration (ng/kg). Values in parentheses indicate percentages of negative and positive samples.

<table>
<thead>
<tr>
<th>Season</th>
<th>Tested</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>22</td>
<td>0(0)</td>
<td>22(100)</td>
<td>22.40</td>
<td>84.80</td>
<td>65.50</td>
<td>12.54</td>
</tr>
<tr>
<td>Autumn</td>
<td>30</td>
<td>0(0)</td>
<td>30(100)</td>
<td>19.40</td>
<td>93.60</td>
<td>69.22</td>
<td>20.14</td>
</tr>
</tbody>
</table>

with AFM₁ with 6% being above the maximum permissible limit of 0.2 µg/l, (Srivastava et al., 2001).

The prevalence rate of aflatoxin M₁ (AFM₁) contamination in the raw milk analyzed in Portugal was 80.6%, 17 samples (54.8%) contained low levels (0.005 - 0.010 µg/l), two samples (6.5%) had levels ranging from 0.011 to 0.02 µg/l and six samples (19.3%) had levels between 0.021 and 0.050 µg/l (Martins and Martins, 2000). During 1996, 161 samples of milk in Italy were checked for aflatoxin M₁ (AFM₁). AFM₁ was detected in 125 (78%) of milk samples (ranging from <1 ng/l to 23.5 ng/l; mean level: 6.28 µg/l) (Galvano et al., 2001).

In Van province of Turkeys, AFM₁ found in 79 (87.77%) of 90 milk samples examined. AFM₁ concentration in 35 (44.30%) of the positive samples were higher than the maximum tolerance limit (0.05 µg/l) accepted by Turkey and some other countries (Bakirici, 2001).

There is little information about the occurrence of AFM₁ in milk products in Iran. In a study in Tehran, (Karim et al. 1998), analyzed 73 milk samples delivered to Tehran milk Pasteurization plants for the presence of aflatoxin M₁. AFM₁ was detected in 60 samples (82.2%). All contaminated samples had a level of aflatoxin M₁ above the European countries standard that is 50 µg/l. In another study, of 52 liquid milk samples, 48 (92.3%) were contaminated with aflatoxin M₁ at concentration between 23 to 3000 µg/l (Karim et al., 1982).

According to results obtained in Iran and other countries, incidence and contamination levels of AFM₁ in these countries, (especially Asian countries) seem to be a serious problem for the public health. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM₁ contamination. The most effective way of controlling aflatoxin M₁ in the food supply is to reduce contamination of raw material and supplementary feedstuffs for dairy cattle with aflatoxin B₁. Specific regulation exist in many countries (FAO, 1997), and practical programmes are being developed; e.g. the Codex Committee on Food Additives and Contaminants has developed, a code of practice for reducing aflatoxin B₁ in raw materials (Van Egmond et al., 1997). Reduction can be achieved by good manufacturing practice and good storage practices. If preventive measures fail to reduce fungal growth and aflatoxin B₁ formation in agricultural commodities intended for use as animal feeds, the last means for avoiding or reducing the occurrence of aflatoxins in feed is to eliminate (part of) the toxins. Feeds that have higher concentrations of aflatoxin B₁ may be acceptable for feeding to dairy animals if they are blended with feeds that have lower concentrations, to make sure that the resultant aflatoxin M₁ concentration in milk does not exceed levels considered being safe.

References


مطالعه آفلاکسین M۱ در شیرهای استریلیزه شده با روشنایی

ابراهیم کامکار
گروه میکروبا و واکنش‌های ویروسی، دانشکده احیایی، دانشگاه تهران، تهران، ایران

در این مطالعه میزان آفلاکسین M۱ در تعداد ۳۴ نمونه شیر استریلیزه عرضه شده در شهر تهران که توسط کارخانه مختلف تولید و ورود به بازار مصرف شده، بررسی شد. نتایج حاصله نشان داد که مقدار میزان آفلاکسین M۱ در نمونه‌های مورد بررسی بالا قرار گرفت. تاکنون فاقد داستانی در مورد نکاتی که خاصیت چنین مشابهی را در بافت‌هایی که این مقدار را دارند ندارد. 

کلیدواژه‌ها: آفلاکسین M۱، شیرهای استریلیزه، بافت‌های دارویی، بافت‌های عصبی، بافت‌های انتهایی.