Detection of Aflatoxin M1 in Milk from Qom
(Aried and Semiared) Province of Iran

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ABSTRACT

Background: Aflatoxins are secondary metabolites fungal When animals consume contaminated feed stuff to Aflatoxin B1 (AFB1), the toxin hydrolyzed and changed to the aflatoxin M1 and transmit to the milk consumers.

Methods: seventy milk samples from different milk producers who were selling their milk to the dairy plant during two season in winter and summer and investigated by Enzyme Linked Immuno Sorbent Assay (ELISA).

Results: AFM1 was found in 100% of the milk samples in winter. About 15% of the samples had AFM1 greater than the maximum tolerance limit (50ng/l) accepted by European Union. Mean concentrations of AFM1 in Winter, Summer were 122, 53 ng/L, respectively. Mean concentrations of AFM1 in winter and Summer samples were significantly higher (P<0.05).

Conclusion: these results show that the important of periodically monitoring the occurrence of AFM in raw milk and dairy products in Qom province.

Keywords: Aflatoxin, Food contamination, Milk, Iran

INTRODUCTION

That some of the moulds produce various toxic metabolites under appropriate temperature and moisture conditions is known. These metabolites, which may be hazardous for human health, are called mycotoxins (1-2). When lactating mammals, such as cows, sheep and goats, are fed with feeds contaminated with Aflatoxin B1 (AFB1), this metabolite can be converted to hydroxylases form called Aflatoxin M1 (AFM1), which is cytotoxic and Geno toxic substance (3).it is bioactived by liver metabolism and by hepatic microsomal cytochrome P450 to AFM1 which possesses 10 times lower carcinogenic potential compared to the parent molecule(4).Aflatoxins are acute toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive compounds. The main target organ for toxicity and carcinogenicity is the liver (5).The occurrence of AFM1 in milk and milk products is a public health concern because the International Agency for Research on Cancer (IARC) in the previous classification from 1993 year put AFM1 in second group of carcinogens (6) as a potent carcinogen, but in next classification from 2002 year this toxin was moved to the first group, as a proved carcinogen (7) ,a probable human carcinogen with a high risk of hepatotoxicity and mutagenicity (8) and high Geno toxic activity (9-10).AFM1 is relatively stable during pasteurization, sterilization, preparation, and storage of various dairy products (11). Aflatoxin M1 (AFM1) may
be found in the milk of animals that are fed with aflatoxin B1 (AFB1) containing feed (1). The forming of AFM1, metabolite of AFB1, occurs in liver and it is secreted in to milk in the mammary gland of dairy cows. As a result of the studies conducted in many countries, the availability of AFM1 in commercial milk and milk products is an evidence of that. Between the amounts of AFB1 in feed consumed by the animals AFM1 in milk was a linear relationship (12). The goal of this study was to determine the contents of AFM1 in milk and consumed in Qom province (has a different climatic condition as its precipitation is between 70-350mm and elevation is from 800 to 3250 meter) in center of Iran to investigate the effects of season on the AFM1 content of milk and finally to compare the obtained results with maximum AFM1 tolerance limits accepted by European Union Food Codex.

MATERIALS AND METHODS
A total of seventy milk samples taken from various districts of Qom province, and examined for the presence and levels of AFM1. The milk samples were transferred to the laboratory in ice boxes at temperatures 4°C. All samples were collected between the period of winter (Jun, FEB, March, 2007) and summer (July, Aug, Sep 2008). The samples were analyzed for AFM1 using the competitive ELISA procedure as described by R-Biopharm GmbH (13-14). A sufficient number of microliter wells were inserted into the micro well holder for all standards and samples. 100 µl standard solutions and prepared samples in separate wells were added 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 250µl of distilled water and emptied as described earlier. The washing procedure was repeated once. 100µl of the enzyme conjugate was added and incubated for 60 min at room temperature in the dark. The washing sequence was repeated three times. 50 µl of substrate and 50µl of chromogenic were added to each well and mixed thoroughly and incubated for 30 min at room temperature in the dark. 100 µl of the stop reagent was added to each well and mixed and measured at an absorbance of 450 nm against an air. Randomized samples were used to evaluate the differences between AFM1 occurrence levels of the milk samples. Furthermore, Duncan multiple comparison test was applied to obtain significance level between sampling periods. The results are expressed as mean SD. Data were analyzed by ANOVA. Sequential differences among means were calculated at the level of p<0.05.

RESULTS
The present study, total of 70 milk samples obtained randomly from individual farms were analyzed for AFM1 with the ELISA method. Results are shown in Table 1. AFM1 was not detected in 5 samples (3.50%), whereas 65 samples (96.50%) were found to contain AFM1 at various levels. There are differences in maximum permissible limit of AFM1 in various countries, and many including Iran have no legal limit for AFM1 in milk. Mean concentrations of AFM1 in winter, summer were 122, 53 ng/l, respectively. Mean concentrations of AFM1 in summer and winter samples were significantly higher (P<0.05). The lowest mean of AFM1 concentration was found as 11ng/l on summer and the highest AFM1 as 296ng/l on winter.

<table>
<thead>
<tr>
<th>Season*</th>
<th>Tested</th>
<th>Negative</th>
<th>Positive</th>
<th>Minimum (ng/l)</th>
<th>Maximum (ng/l)</th>
<th>Mean (ng/l)</th>
<th>SD (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>35</td>
<td>0</td>
<td>35</td>
<td>61</td>
<td>296</td>
<td>122</td>
<td>88.51</td>
</tr>
<tr>
<td>Summer</td>
<td>35</td>
<td>5</td>
<td>30</td>
<td>11</td>
<td>99</td>
<td>53</td>
<td>25.20</td>
</tr>
</tbody>
</table>

Table 1. Distribution by season of raw milk samples and aflatoxin M1 concentration (ng/l)
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Fig.1. Quantity of aflatoxin M1 in 70 milk sample
The results are shown in Fig.1. In general, regardless of the 23% of the samples that are in borderline limit (45–50 ng/l), the amount of FM1 in 15% of the samples was higher than the maximum tolerance limit (50 ng/l) accepted by European Union (14).

DISCUSSION
Currently aflatoxin analysis are done by various methods including thin-layer chromatography (TLC), liquid chromatography (LC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) (15,16) . Although there are several techniques for detecting AFM1 in milk and dairy products, ELISA is the most useful technique due to its quickness, sensitivity, ease of and cheapness. Therefore, ELISA technique was used for detecting AFM1 in Cow milk samples in present survey. Evidence of hazardous human exposure to AFM1 through milk and dairy products had been shown by several investigators (1, 17,18,19,16). AFM1 level in the milk was significantly affected by the geographical region, country, and seasons. In a study conducted by Alborzi et al (20), AFM1 was found in 100% of examined milk samples. 390 samples (62.5%) had contamination less than 45 ng/l AFM1, 123 samples (19.7%) contained 45-50 ng/l, 94 samples (15.1%) contained 50-80 ng/l and 2.7% of samples contained more than 80 ng/l of AFM1 in Shiraz (south of Iran). Alborzi et al. that 17.8% of samples had AFM1 greater than the maximum tolerance limit (50 ng/l) accepted by European Union. In Brazil (21), AFM1 was only detected in four samples of pasteurized milk in values between 73 and 370 ng/l, of a total of 204 samples of pasteurized milk, powdered milk, cheese and yoghurt. (22) Detected AFM1 in 97 samples of dry milk for infant formula using HPLC; in 81 samples (84%) amounts ranged from <0.001 to 0.101 µg/l.

In Japan during the winter season, AFM1 was detected in 207 (99.5%) of 208 milk samples at 1-29 ng/kg with a mean of 9 ng/kg (23). In a Korean study, the incidence of AFM1 in liquid milk was 76%, with a mean concentration of 18 pg/g (17). The incidence of AFM1 contamination in the raw milk analyzed in Portugal was 80.6%, 17 samples (54.8%) contained low levels (5_10ng/l), two samples (6.5%) had levels ranging from 11 to 20 ng/l, six samples (19.3%) had levels between 21 and 50 ng/l (19). During 1996, 161 samples of milk in Italy were checked for AFM1. AFM1 was detected in 125 (78%) of milk samples (ranging from <1 to 23.5 ng/l; mean level was 6.28 ng/l) (22). In a study in Argentina, a total of 77 various types of milk samples were analyzed, only 18 samples (approximately 23%) were found to be contaminated with AFM1 at levels of 0.01_0.03 µg/l. All concentrations were below the maximum tolerated levels of AFM1 in liquid milk and powdered milk (0.05 µg/l) (24). This phenomenon may be attributed to the fact that the cows were receiving higher
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concentrated feeds in the winter. A relationship between AFM1 occurrence level in milk and AFB1 content of feed was reported (1, 12). AFM1 contamination of milk is the result of cows feeding on material containing aflatoxin B1. The concentration of this mycotoxin in animal feedstuffs is influenced by the time, the method of harvesting and temperature and relative humidity of storage facilities, all of which vary among different regions of Iran (25, 27). AFM1 concentrations in milk samples were significantly higher in the colder seasons in agreement with the results of other authors. For example Tajkarimi et al. (2008) reported that AFM1 concentrations in milk samples in winter were significantly higher than summer (P<0.05), 30% of samples in winter were >50 ng/l; however, in summer 16% of samples were >50 ng/l. One reason for this is that milking animals are fed with compound feeds in winter that are prone to aflatoxin B1 contamination (22, 27, 26). The effective factor on low AFM1 level in summer was most likely out-pasturing of milking animals. his similar result was stated by some other researchers (29) and they found that low AFM1 level or no toxin production was obtained in summer season. Therefore, it is possible to say that the results obtained by us were parallel to the results of prior studies.

CONCLUSION

FM1 concentration of milk and milk products is potentially a serious public health problem as all age groups, including infants and children, consume these products world wide. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination. Where concentrations are unacceptably high, careful investigation of feedstuffs for contamination by AFB1 must be made, the reason for this established and the cause eliminated.

REFERENCES


