Journal of Animal and Veterinary Advances 7 (5): 643-645, 2008
ISSN: 1680-5593

Aflatoxin M1 Levels in Milk Powder Consumed in Turkey

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Abstract: Aflatoxins are secondary metabolites produced by certain strains of *Aspergillus* species. They have immunsupresive, genotoxic, hepatoxic and carcinogenic (particularl liver cancer) effects which are the common problems worldwide for people of all age groups. This study was aimed to investigate the presence and levels of AFM1 in the milk powder sold in retail stores of Kars, Erzurum, Mersin, Konya and Ankara vicinities in Turkey. AFM1 was determined in 62.5% of all samples analyzed. Amount of the AFM1 in samples was higher in 45% of the samples than the maximum allowed level according to the Turkish Food Codex (TFC) criteria (500 ng kg^{-1}). Consequently, milk powders sold in retail stores in Turkey pose a great risk for public health. Serious programmes controlling the occurence of the aflatoxin will surely help on dealing with the risk factors.

Key words: AFM_1, aflatoxin, milk powder, milk product

INTRODUCTION

Natural contaminats in foods may come from either chemical or biological origins. Mycotoxins are biological in origin. Despite efforts to control mould contamination, toxigenic mould are ubiquitous in nature and occur regularly in worldwide food supplies due to mould infestation of susceptible animal feeds. A great number of mycotoxins exist in the environment, but only a few present significant food safety challenges (Murphy *et al*., 2006). Among these, aflatoxins are immunsupresive, genotoxic, hepatotoxic and carcinogenic (potent liver carcinogen) mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Casttegnaro and Mcgregor, 1998; Concon, 1988; Egmond, 1989).

Aflatoxins include four main components (B1, B2, G1, G2). Following the ingestion, Aflatoxin B1 and B2 are metabolized into M1 and M2 derivatives in the liver, respectively (Concon, 1988; Egmond, 1989). Aflatoxin M1 and M2 are then excreted from the body through the milk of lactating animals (IARC, 1993a). However, AFM1 is the primary mycotoxin in terms of monitoring dairy products for aflotoxins in milk industry.

International Agency for Research on Cancer (1993b) reported that AFB1 is more toxic than AFM1. However, AFB1 and AFM1 share first and second place in toxicity classification, respectively. Toxic effects of AFs can be manifested by various ways including disruption of DNA-RNA and protein synthesis, reduction of glycose metabolism and interference with the lipid metabolism (Murphy *et al*., 2006).

Since, milk is a basic source of protein, it is widely consumed by all age groups of people particularly by children. Therefore, milk is always a potential risk factor for babies and developing children in terms of AFM1 (Kim *et al*., 2000). In addition to direct consumption, milk is also indirectly consumed as cheese, ice-cream and milk powder. Cheese and ice-cream consumption in Turkey have been increasing, eventhough drinking milk as a regular habit in adults is uncommon. Milk powder has been a frequently marketed product in retail stores during the recent years. It is used in chocolate, cake, ice-cream, yoghurt, baby food and ready-to-eat foods. According to Turkish Food Codex, the maximum allowed AFM1 level in milk powder is 500 ng kg^{-1} (TFC, 2002).

Reports on the levels of AFM1 in milk and milk products (cheese, butter and yoghurt) have been accumulating in Turkey (Aycicek *et al*., 2002; Aycicek *et al*., 2005; Baskaya *et al*., 2006; Gurbuz *et al*., 1999; Oruc and Sonal, 2001) as well as around the

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world (Barrios *et al*., 1997; Galvano *et al*., 1996; Karaioannoglu *et al*., 1989; Martins and Martins, 2004; Saitanu, 1987; Stoloff and Wood, 1981). However, there are very few studies documenting the levels of AFM1 in milk powder, contrary to the increasing use of milk powder (Kim *et al*., 2000; Deveci and Sezgin, 2005; Galvano *et al*., 1998; Srivastava *et al*., 2001).

This study, therefore, was aimed to report the presence and level of the AFM1 in the milk powder sold in retail stores of Kars, Erzurum, Mersin, Konya and Ankara cities in Turkey. In addition, this study will underline the potential risc factors for public health with respect to AFM1.

MATERIALS AND METHODS

Eighty milk powder samples were obtained from the retail stores of Kars, Erzurum, Mersin, Konya and Ankara cities. The samples were analyzed for AFM1 in the Animal Research Center Laboratory of Kafkas University. The quantitative analysis of aflatoxin M1 (AFM1) in milk powder samples were performed by competetive enzyme immunoassay test procedure as indicated by R-Biopharm (2006) GmBH, Germany (Ridascreen® Aflatoxin M1 30/15, Art. No.: R1101).

Sample preparation: Preparation of samples and ELISA test procedure were done in accordance with the test booklet provided along with commercially available kit (Ridascreen®). The samples were diluted at 1/10 ratio and were stirred for 5 min. Then, they were centrifuged at 3500 g at 10°C for 10 min. Finally, the fatty layer on the top was removed and the rest was acquired by a pasteur pipet and used for analysis.

Test procedure: A hundred μL standart solutions and samples were parallely placed into the wells and then incubated in a dark room at room temperature for 30 min. The wells were reversed on Whatmann paper and the fluid was removed. The samples were washed with 250 μL of washing buffer. This process was performed two times. After washing, 100 μL diluted enzyme conjugate was added into the wells, slightly shaked and incubated in the dark at room temperature for 15 min. The samples were unloaded onto the Whatmann paper and washed with 250 μL washing buffer (this was repeated 3 times). After this procedure, 100 μL substrate chromogen was added into the each well, slightly shaked and incubated in the dark at room temperature for 15 min. Finally, 100 μL stop reagent was added into each well and assayed with ELISA (Spectra Max 384 Plus).

Table 1: Determined AFM1 levels in the milk powder samples analyzed

Number of samples	<1 ng kg^{-1}		1-500 ng kg^{-1}		501-600 ng kg^{-1}		>601 ng kg^{-1}	
	n	(%)	n	(%)	n	(%)	n	(%)
Total (80)	30	37.5	14	17.5	19	23.75	17	21.25

The data obtained from standards and samples were evaluated using a computer program (RIDA®SOFT Win, R-Biopharm, Germany) for windows.

RESULTS AND DISCUSSION

AFM1 levels in samples of milk powder were presented in Table 1. While AFM1 was detected in 62.5% of the samples, 45% of the samples was found to exceed maximum allowed level (500 ng kg^{-1}) indicated by TFC.

In related studies, Kim *et al*. (2000) reported that AFM1 was present in 18 (75%) and 17 (70.8%) milk powder samples out of 24 determined by ELISA and HPLC, respectively. Srivastava *et al*. (2001) found this level to be 0.01 μg L^{-1} in 60% of the milk powder samples. Similarly, Galvano *et al*. (1998) reported that AFM1 was present in 81 milk powder samples out of the 97 (83.5%) while the value was between in 10-50 ng in 47 samples analyzed. As compared with our findings, those of the Kim *et al*.(2000) and Galvano *et al*. (1998) are higher while the findings of the Srivastava *et al*. (2001) are in parallel. This varriation in the results may be due to the sample numbers, technique used and feeding conditions of the lactating animals.

Processes including sterilization, pasteurisation and fermentation in milk technology have been shown to have no effect on the levels of the AFM1 in the milk (Martins and Martins, 2004; Bakirci, 2001; Oruc *et al*., 2006). As the use of milk technology is increasing, milk powder is frequently used in chocolate, baby food and cake production. Determination of the AFM1 in milk powder is very essential since it is widely used in such productions. These food groups are commonly consumed by the children at developing ages.

CONCLUSION

Milk powder sold in Turkey pose a great risk for public health. Serious programmes should be applied on the aflatoxin occurence during the period beginning from animal feeding through the consumption of the milk and milk products. Particularly, eliminating every factors which may cause the AFB1 occurence in animal feeds is of vital importance. Detoxification of aflatoxins needs more effort and much more money. For this reason, storage conditions of the animal feeds should be improved and

contaminated feeds should be either destroyed or mixed with non-contaminated feeds to dilute the concentration of aflatoxin levels.

REFERENCES

Aycicek, H., E. Yarsan, B. Sarimehmetoglu and O. Cakmak, 2002. Aflatoxin M1 in white cheese and butter consumed in Istanbul, Turkey. Vet. Hum. Toxicol., 44: 295-296.

Aycicek, H., H. Aksoy and S. Saygi, 2005. Determination of aflatoxin levels in some dairy food products which consumed in Ankara, Turkey. Food Control, 16: 263-266.

Bakirci, I., 2001. A study on the occurence of the Aflatoxin M1 in milk and milk products produced in Van province of Turkey. Food Control, 12: 47-51.

Barrios, M.J., L.M. Medina, M.G. Cordoba and R. Jordano, 1997. Aflatoxin-producing starins of *Aspergillus flavus* isolated from cheese. J. Food Prot., 60: 192-194.

Baskaya, R., A. Aydin, A. Yildiz and K. Bostan, 2006. Aflatoxin M1 levels of some cheese varieties in Turkey. Med. Wetery., 62: 778-780.

Biopharm, R., 2006. Enzyme immunoassay for the quantitative analysis of aflatoxin M1. GmBH, Germany (Ridascreen® Aflatoxin M1 30/15, Art. No.: R1101).

Casttegnaro, M. and D. Mcgregor, 1998. Carcinogenic risk assesement of mycotoxins. Rev. Vet. Med., 149: 671-678.

Concon, J.M., 1988. Contaminants and Additives. Food Toxicology, Part B, Marcel Dekker, Inc., New York, pp: 667-743.

Deveci, O. and E. Sezgin, 2005. Aflatoxin M1 lebels of skim milk powders produced in Turkey. J. Food and Drug Analy., 13: 139-142.

Galvano, F.B., V. Galofaro, A. Angelis, M. Galvano, M. Bognanno and G. Halvano, 1998. Survey of the Occurence of Aflatoxin M1 in Dairy Products marketed in Italy. J. Food Prot., 61: 738-741.

Galvano, F., V. Galofaro and G. Galvano, 1996. Occurrence and stability of Aflatoxin M1 in milk and milk products: A Worldwide Review. J. Food Prot., 59: 1079-1090.

Gurbuz, U., M. Nizamlioglu, F. Nizamlioglu, I. Dinc and Y. Dogruer, 1999. The investigation of Aflatoxin B1 and M1 in some meat, dairy products and spices. Veterinarium (in Turkish), 10: 34-41.

International Agency For Research On Cancer (IARC), 1993a. Aflatoxins: Naturally occuring aflatoxins (Group 1), aflatoxins M1 (Group 2B). Int. Agency Res. Cancer, 56: 245.

International Agency For Research On CancER (IARC), 1993b. web: http://monographs.iarc.fr/ENG/Classification/crthall.php.

Karaioannoglu, P., A. Mantis, D. Koufidis, P. Koidis and J. Triantafillou, 1989. Occurence of aflatoxin M1 in raw and pasteurized milk and Feta and Teleme cheese samples. Milchwissench, 44: 746-747.

Kim, E.K., D.H. Shon, D. Ryu, J.W. Park, H.J. Hwang and Y.B. Kim, 2000. Occurence of Aflatoxin M1 Korean dairy products determined by ELISA and HPLC. Food Add. Contam., 17: 59-64.

Martins, M.L. and H.M. Martins, 2004. Aflatoxin M1 in yoghurt in Portugal. Int. J. Food Microbiol., 91: 315-317.

Murphy, P.A., S. Hendrich, C. Landgren and C. Bryant, 2006. Food Mycotoxins: An update. J. Food Sci., 71: 51-65.

Oruc, H.H., R. Cibik, E. Yılmaz and O. Kalkanli, 2006. Distribution and stability of Aflatoxin M1 during processing and ripening of traditional white pickled cheese. Food Add. Contam., 23: 190-195.

Oruc, H.H. and S. Sonal, 2001. Determination of aflatoxin M1 levels in cheese and milk consumed in Bursa. Vet. Hum. Toxicol., 43: 292-293.

Saitanu, K., 1997. Incidence of Aflatoxin M1 in Thai milk products. J. Food Prot., 60: 1010-1012.

Srivastava, V.P., A. Bu-Abbas, A. Ala-Basuny, W. Al-Johar, S. Al-Mufti and M.K.J. Siddiqui, 2001. Aflatoxin M1 contamination in commercial samples of milk and dairy products in Kuwait. Food Add. Contam., 18: 993-997.

Stoloff, L. and G. Wood, 1981. Aflatoxin M1 in manufactured dairy products producedin the United States in 1979. J. Dairy Sci., 64: 2426-2430.

Turkish Food Codex (TFC), 2002. Gida Maddelerinde Belirli Bulasanlarin Maksimum Seviyelerinin Belirlenmesi Hakkinde Teblig. 16.10.2002 tarihli 24908 sayili resmi gazete. Ankara, Turkey (in Turkish).

Van Egmond, H.P., 1989. Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Add. Contam., 6: 139-188.