Use of Enzyme Linked Immunosorbent Assay for detection of aflatoxin M1 in milk powder

S. D. Al-Sawaf, O. A. Abdullah and O. H. Sheet

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

(Received May 2, 2011; Accepted January 9, 2012)

Abstract

Thirty five samples were collected from seven types of milk powder in Mosul city markets, and surveyed for the presence of aflatoxin M1 (AFM1), by using Enzyme Linked Immunosorbent Assay (ELISA) technique. Analytical results showed that 82.8% of the samples were contaminated with AFM1. The incidence of AFM1 in Al-mudhish, Angolac, Dielac, Lona, Nido, Melgro and Multi samples were 40, 50, 80, 100, 100, 100 and 100%, respectively. The occurrence of AFM1 in milk powder, 79.3% (23 out of 29) of positive samples were higher than the permissible limits, according to the European Commission (50 ng/kg), whereas 6.8% (2 out of 29) of positive samples were above the prescribed limit of US regulation (500 ng/kg). The level of AFM1 concentration in Melgro and Multi types was higher than Al-mudhish type and it had a low level of contamination, compared with other types of milk powder.

Keywords: Aflatoxin M1, ELISA, Milk powder.
Available online at http://www.vetmedmosul.org/ijvs

استخدام تقنية الاليزا للكشف عن سم الافلا M1 في الحليب المجفف

سناء داؤد الصواف، عمر احمد عبدالله و عمر هاشم شيت

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أظهرت نتائج الدراسة وجود سم الافلا M1 في سبعة أنواع من الحليب المجفف بعد جمعها من أسواق مدينة الموصل، تم تحليل ٣٥ عينة باستخدام تقنية الادمصاص المناعي المرتبط بألانزيم (الاليزا) إذ كانت نسبة تلوث الحليب المجفف بسم الافلا M1 ٨٢,٨%. نسبة حدوث التلوث بسم الافلا M1 للأنواع مدهش، انكولاك، ديالاك، لونا، نيدو، ميلغرو، المولتي ٤٠، ٥٠، ٨٠، ١٠٠، ١٠٠، ١٠٠ و ١٠٠ على التولي. وان ٧٩,٣ % من العينات الموجبة تجاوزت الحد الأعلى المسموح به طبقا للمواصفات القياسية للمفوضية الأوربية (٥٠ نانوغرام /كغم)، بينما كان ٦,٨ % من العينات الموجبة أعلى من الحد المسموح به طبقا للمواصفات المسموح بها في أمريكا (٥٠٠ نانوغرام /كغم). كشف البحث ان مستوى التلوث بسم الافلا M1 للنوعين ميلغرو والمولتي كان أعلى من النوع المدهش والذي كان اقل تلوثا مقارنة بباقي أنواع الحليب المجفف.

Introduction

Milk and it is products are regarded as a good nutritional source for infants and children who are considered to be more susceptible to adverse effect of mycotoxins (1). However, milk is a perishable food, so it may be contaminated after unsatisfactory processes through manufacturing of milk products or due to bad storage, leading to production food unfit for human consumption (2). Aflatoxins (AF) are a collective term for a group of toxic and carcinogenic secondary metabolites by some *Aspergillus* species during growth on feed and crops within optimum temperature (27 -38) ºC and relative humidity of 85% (3). Aflatoxin M1 (AFM1) is one of most hazardous metabolites of AF, causing deleterious health effects through consuming AFM1 contaminated milk or its

products (4). Aflatoxin B1 once ingested by mammals, is rapidly absorbed from gastrointestinal tract and appears as the metabolic AFM1 in the blood after as little as 15 minutes (5). AFM1 is relatively stable in raw, processed, heat treated, stored, freeze and dried milk or milk products (6,7). Initially AFM1 was classified by IARC as a group 2 as a human carcinogen, but recently as a group 1 for human carcinogen (8,9). AFM1 causes economic losses which values many millions of dollars throughout the world, because of the medical cost of human and animals, and destroyed the crops, in addition, AFM1 causes spreading of the Jaundice disease and increase the rate of the mortality in livestock (10). The legal regulations for AFM1 in milk and dairy products vary from one o another (11). United State Food and Drug Administration and Codex Alimentarius prescribed that the maximum level of AFM1 in milk should not exceeds 0.5 µg/L (12), whereas EU regulation speculate,the level to be not higher than 0.05 µg/L, and for infants to be not more than 25 ng/L (13). In Iraq, no regulations are present for the permissible limits of AFM1 in milk or its products, and few studies highlighted the current status of AFM1 levels in the local or the imported milk or its products. For this reason, we tried to evaluate this issue with special reference to the dried types of milk present in the local market of Mosul city.

Materials and methods

Sample preparation

A total of 35 samples from seven types of imported milk powder (Al-mudhish, Angolac, Lona, Dielac, Nido, Melgro and Multi) were collected from Mosul markets, during June –August 2010. Ten g of milk powder were taken in a flask and filled up to 100 ml with deionized water (dissolved by stirring for 5 minutes). Subsequently, milk powder samples were centrifuged for 10 minutes / 3500g/ 10 °C. The upper creamy layer was removed and the lower phase was used for the quantitative test.

Analysis of aflatoxin M1 by ELISA

Quantitative analysis of AFM1 in milk powder samples was conducted by RIDASCREEN®Fast Aflatoxin M1 test (R-Biopharm GmbH), which is an enzyme linked Immunosorbent Assay carried out using the procedure suggested in the AFM1 ELISA kit. Fifty µl of AFM1 standards or prepared milk powder samples were added to wells of a micro well plate. Fifty µl of enzyme conjugate and 50 µl of anti-aflatoxin M1 antibody solution were added to each wells then mixed gently and incubated for 10 minutes at room temperature. The liquid was poured out of the wells and the wells were filled with (250 µl per well) distilled water. After emptying the wells and removed all remaining liquid, washing step was repeated two more times. Subsequently, 100 µl of substrate (chromogen) added to each well, mixed gently and incubated for 5 minutes at room temperature in the dark. The reaction was stopped by adding 100 µl of stop solution to each wells, mixed gently by shaking the plate manually and measured the absorbance at 450 nm in micro plate reader. The intensity of absorbance was inversely proportional to the concentration of aflatoxin M1 in samples by using special software, the RIDA SOFT WIN, for evaluation of the RIDASCREEN®Fast Aflatoxin M1 ELISA kit results. The aflatoxin M1 concentration results from the ELISA assay were statistically analyzed by using the Analysis of Variance (14).

Results

The presence of AFM1 in seven types of milk powder is shown in (Table 1). Of the 35 samples analyzed, 82.8% were found to be contaminated with AFM1. The range of contamination levels varied among different types of milk powder. AFM1 in Al-mudhish, Angolac, Lona, Dielac, Nido, Melgro and Multi samples ranged from 32 to 44, 80 to 160, 10 to 270, 30 to 310, 50 to 280, 135 to 534, 200 to 640 ng/kg while the mean values were 15.2, 60, 106, 109.3, 157.1, 297.4, 314.4 ng/kg, respectively.

Table 1: Presence of aflatoxin M1 in different types of milk powder.

Milk types	No. of samples analyzed	Positive samples	Percentage of contamination %	AFM1 contamination (ng/kg)	
				Range	Mean ± SE
Al-mudhish	5	2	40	32-44	15.2±9.4 *bd
Angolac	4	2	50	80-160	60±38.2 d
Lona	5	5	100	10-270	106±47.1 d
Dielac	5	4	80	30-310	109.2±54.7 d
Nido	6	6	100	50-280	157.1±36.5 d
Melgro	5	5	100	135-534	297.4±68.3 acd
Multi	5	5	100	200-640	314.4±81.9 ad
Total	35	29	82.9	10-640	154.1±25.5

*Means with different letter are significantly different ($P < 0.05$).

In this study, the mean of AFM1 level for (Melgro and Multi) types differed significantly with Al-mudhish type of milk powder, and the level of AFM1 concentration in (Melgro and Multi) types was higher than Al-mudhish ($P<0.05$). As shown in (Table 2). The distribution of concentration of AFM1 in all types of positive milk powder samples were found to be 79.3% (23 out of 29) and was higher than the permissible limits according to the European Commission (50 ng/kg), while 6.8% (2 out of 29) of positive samples exceeded the prescribed limit of US regulations (500 ng/kg).

Table 2: Aflatoxin M1 contamination in milk powder exceeding European Communities and United State regulations.

Milk types	Samples analyzed	Positive samples	Exceeding EC/Codex regulations (50 ng/kg)			Exceeding US regulations (500 ng/kg)		
			Number	%	Range	Number	%	Range
Al-mudhish	5	2	-	-	-	-	-	-
Angolac	4	2	2	100	80-160	-	-	-
Lona	5	5	3	60	95-270	-	-	-
Dielac	5	4	3	75	76-310	-	-	-
Nido	6	6	5	83.3	90-280	-	-	-
Melgro	5	5	5	100	135-534	1	20	534
Multi	5	5	5	100	200-640	1	20	640
Total	35	29	23	79.3	76-640	2	6.8	534-640

Discussion

Milk powder is one of the most milk products, which enters in many food industries as sweets and ice cream, in addition, it is being considered a second source of infant formula after the mother's milk. Our study showed that the rate of contamination with AFM1 in milk powder was 82.8% which is similar to other reults (15) which recorded that the rate of contamination with AFM1 in India was 87.3%. In Korea, 85% infant formula samples were found to be contaminated with AFM1 (16). The reason for high contamination of milk powder samples in this study, is probably the lack of information on the quality of the fodder given to lactating farm animals. The fodder may be contaminated with AFB1, due to the unpredictable climatic and environmental conditions. 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 (17). Further, investigators have suggested that on an average 1.6% of AFB1 fed to lactating cattle is excreted in milk as AFM1 (18). Because of toxicity, carcinogenic properties and high melting point of AFM1 led to resistant to thermal inactivation, pasteurization, autoclaving and other varieties of food processing procedures (19). The incidence of milk contamination with AFM1 in Iran is higher than the results of our study, where the percentage reached 96.3% (20). Our results were higher than those obtained by others (21,22), who observed 67%, 66.7% of milk powder samples contaminated with AFM1 in China and Brazil, respectively. A survey from Italy reported the presence of AFM1 in 53% of milk powder samples (23). The occurrence of AFM1 at such low levels in European countries could be attributed to stringent regulation of AFB1 in complementary feedstuffs for dairy cattle (16). European Communities has fixed the limit to a maximum of 50 ng AFM1/kg.However, US has prescribed a limit of 500 ng/kg for AFM1 in milk and dairy products (12,13). In our results, almost 79.3% of positive samples exceeded the European Communities, while 6.8% of positive samples above the permissible limit of US regulation. These results are considered less than those reported by Indian studies where 99% of the contaminated samples exceeded the EC, whereas 9% of milk samples were above permissible limit of US regulation (15). From above,it appears to be an urgent requirement to establish tolerance levels for AFM1 for milk and milk products in Iraq.

Acknowledgment

Authors wish to thank the College of Veterinary Medicine, University of Mosul for supporting and providing facilities.

Reference

1. Tajkarimi M, Aliabadi-Sh F, Salah Nejad A, Poursoltani H, Motallebi AA, Mahdavi H. Aflatoxin M1 contamination in winter and summer milk in 14 states in Iran. Food Control. 2008;19:1033-1036.
2. Ji Eun L, Byung MK, Jang HA, Tae HJ. Occurrence of Aflatoxin M1 in raw milk in south Korea using an immunoaffinity column and liquid chromatography. Food Control. 2009;20:136-138.
3. Cassel EK, Campell B, Draper M, Epperson B. Aflatoxin hazards in grains aflatoxicosis and livestock. University of Maryland cooperative Extension service fact sheet. 2001:444- 445.

4. Zinedine A, Gonzalez-Osnaya L, Soriano JM, MOlto JC, Idrissi L, Manes J. Presence of Aflatoxin M1 in pasteurized milk from Morocco. Int J Food Microbiol. 2007;114:25-29.
5. Moschini M, Masoero F, Gallo A, Diaz D. Mucosal absorption of Aflatoxin B1 in lactating dairy cows. Ital J Anim Sci. 2007;6:324-326.
6. Henry SH, Whitaker TB, Rabbani I, Bowers J, Oark D, Price W.Aflatoxin M1, Chemical safety information from inter government organization.2001 Report 1021, Aflatoxin M1(WHO Additives, Series 47).Joint Export Committee on Food Additives(JECFA).
7. Yaroglu T, Oruc HH, Tayar M. Aflatoxin M1 levels in cheese samples from some province of Turkey. Food Control. 2005;16:883-885.
8. Cavaliere CH, Foglia P, Pastorini E, Samperi R, Lagana A. Liquid chromatography/tandem mass spectrometric confirmatory method for determining Aflatoxin M1 in cow milk: comparison between electrospray and atmospheric pressure photoionization sources. J Chromatography A. 2006;1101:69-78.
9. IARC. International Agency For Research on Cancer. ARC Monograph on the evaluation of carcinogenic risk ton humans: some naturally is occurring substance: Food Items and Constituent Heterocyclic Aromatic Amines and Mycotoxins. Lyon, 1993.
10. Nyikal J, Misore A, Nziok C, Njuguna C, Muchiri E. Outbreak of Aflatoxin poisoning eastern and central provinces. Kenya. Morbidity and Mortality weekly report. 2004;53:790-793.
11. Creppy EE. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters. 2002;27:19-28.
12. Codex Alimentarius Commission Comments submitted on the draft maximum level for Aflatoxin M1 in milk. Codex committee on food additives and contaminats, 2001;33 rd session. Hague, Netherlands.
13. Commission regulation (EC). Setting maximum levels for certain contaminants in food stuffs. Official Journal of European Union. 2006;1881:L364.
14. Petrie A, Watson P. Statistics for veterinary and animal science. Blackwell Science, London; 2003.
15. Shipra R, Premendra D, Subhash K, Mukul D. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA, Food Control.2004;15:287-290.
16. Kim EK, Shon DH, Ryu D, Park JW, Hwang HJ, Kim YB.occrrence of aflatoxin M1 in Korean dairy products determined by ELISA and HPLC. Food additives and contaminants. 2000;17:59-64.
17. Applebaum RS, Brackett RE, Wiseman DW, Marth EH. Aflatoxin toxicity in dairy cattle and occurrence in milk and dairy products.Food protection.1982;45:752-777.
18. Forbisch RA, Bradley BD, Wagner DD, Long-Bradley PE, Hariston H. Aflatoxin resides in milk of dairy cows after ingestion of naturally contamination grain.Food protection.1986;49:781-785.
19. Park DL. Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology. 2002;504:173-179.
20. Mohammad RO, Behrooz J, Naficed S, Mannan H, Azadeh N. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. Food Control. 2007;18:1216-1218.
21. Shi CP, Yuan YZ, Sergei AE, Won JL. Detection of aflatoxin M1 in milk products from China by ELISA using monoclonal antibodies.Food control. 2009;20:1080-1085.
22. Oliveira CAF, Ferraz JCO. Occurrence of aflatoxin M1 in pasteurized,UHT milk and milk powder from goat origin. Food control. 2007;18:375-378.
23. Galvano F, Galofaro V, Ritieni A, Bognanno M, De Angelis A, Galvano G. Surevy of occurrence of aflatoxin M1 in dairy products marketed in Italy: Second year of observation.Food additives and contaminants. 2001;18:644-646.