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Presence of Aflatoxin M1 in Milk Samples Collected from Jeddah, Saudi Arabia

Fardos Bokhari[1], Magda Aly[1,2*], Amany Al Kelany[1] And Samar Rabah[1]
[1]Faculty of Sciences, Biology Department, King Abdulaziz University,
P. O. Box 12161, Jeddah 21473, Saudi Arabia, [2] Botany Department, Faculty of Science, Kafr El-Sheikh University, Egypt.

Abstract: *Although rumen flora protects dairy animals against exposure to mycotoxins, various mycotoxins can pass this barrier to the animal milk. The major metabolite excreted with milk in dairy sheep, cows and other ruminants is Aflatoxin M1 (AFM1). In this connection, 160 milk samples of camel, cow milk, goat, sheep and pasteurized milk samples were collected from different farms and supermarkets of Jeddah, Saudi Arabia. For mycotoxins detection, all milk samples were screened for Aflatoxin M1 using immunoaffinity columns coupled with a Fluorometer. Out of 160 tested milk samples, 74 (47%) were contaminated with AFM1and the contamination level was less than 0.5 ppm. The less milk contaminated samples with AFM1 were camel milk samples< pasteurized milk< goat milk< sheep milk< cow milk. Out of 32 camel milk samples, 10(31%) were* contaminated with *AFM1*. The quantity of *AFM1* detected in camel milk was ranged from *0.017 -0. 140 ppb with mean value of 0.046 ppb which is lower than that of USA recommended limit (0.5 ppb). Statistical analysis showed that camel milk samples were significantly less contaminated compared to other milk samples. On conclusion, all examined milk samples collected from Jeddah were contaminated with AFM1 and the contamination levels were not exceed the USA limit, thus milk is a save food for consummation by human and infants.*

Keywords: *Aflatoxin M1, milk, cow, camel, mycotoxins, immunoaffinity*

I. INTRODUCTION

Aflatoxins are the most intensively studied mycotoxins in dairy cattle as the excretion of AFM1 in dairy milk is of public health concern (Fink-Gremmels, 2008). After ingestion of aflatoxin-contaminated feeds, a part of the ingested aflatoxin B1 is degraded in the rumen and by passive diffusion the remaining part is absorbed in the digestive tract and is hydroxylated to AFM1in the liver (Kuilman *et al*., 2000). Aflatoxin M1 can bond to glucuronic acid and was excreted through the bile or reach the systemic circulation and either was excreted in the urine or appeared in milk. Initially, 1–2% of the ingested aflatoxin B1 was excreted as AFM1in milk of dairy cows (Van Egmond, 1989) and various physiological and nutritional factors including feeding regimens, rate of ingestion and digestion, animal health, capacity of hepatic biotransformation, and actual milk production affect the transfer rate from feed to milk. The rate of aflatoxins absorption and AFM1 excretion in milk varies between individual animals, from day to day and consumption of significantly higher amounts of concentrated feeds by high-yielding cows might result in high level of aflatoxin M1 in milk.

Many authors also reported higher concentration of AFM1 in cold seasons as compared to hot seasons (Bilandzic *et al.*, 2010) and AFM1 has carcinogenic potency as high as that of aflatoxin B1 (Henry *et al*., 2001), thus many countries have set maximum acceptable levels for AFM1in milk and dairy products. US Food and Drug Administration (USFDA) set a maximum permissible level for aflatoxin M1 in milk of 0.5 µg/Kg while in Europe and some Africa and Asia countries, the maximum acceptable level of aflatoxin M1 in milk is 0.05 µg/kg (Van Egmond *et al*., 2007). To achieve this objective, aflatoxin B1in feeds for dairy animals must be limited to keep levels of AFM1 in milk < 0.05 µg/Kg (Pettersson, 1998). Other aflatoxins originating from hepatic-biotransformation reactions of other natural aflatoxins may be excreted with milk including Aflatoxicol which is the major metabolite of aflatoxin B1 (Carvajal *et al*., 2003). Aflatoxicol level was not influenced by pasteurization and had carcinogenic potency, comparable with that of aflatoxin B1 (Hendricks, 1994, Carvajal *et al*., 2003)). Due to the common occurrence and harmful effects of aflatoxin contamination, there is a need for detection and quantification of aflatoxin M1 in milk, thus the present study has been designed to detect aflatoxin M1 in different types of milk samples, consumed in Jeddah using immunoaffinity column and Fluorometer.

II. EXPERIMENTAL

Material and Methods

Standard of AFM1 was purchased from Sigma-Aldrich (St. Louis MO, USA), immunoaffinity columns, AflaM1 TM, were from Vicam (USA) and methanol were from Merck (Germany).

Milk sample collection

Raw camel, cow, goat and sheep milk samples were collected from 40 private farms during 2013 in sterile plastic bottles, while pasteurized milk were purchased from supermarkets in Jeddah. From each type of milk 32 samples were collected and frozen at –20°C until analysis.

Extraction and determination of aflatoxin M1

Aflatoxin M1 was extracted after fat removal of the milk using centrifugation at 4°C and 5000 rpm for 20 min as described by Ruangwises *et al*. (2011). After fat layer removal, the resulting skimmed milk was then allowed to flow through immunoaffinity column, 1 ml/min. washing of the column by water, AFM1 was eluted from the column with 1.25 ml of acetonitrile/methanol (3/2 v/v) and 1.25 ml of dist. water. The eluate was filtered using membrane filter (pore size, 0.45 µm) and AFM1 in the filtrate was quantified using a Fluorometer (Jenway, 2600). Immunoaffinity columns coupled with a Fluorometer were used as it is quick and specific method for routine mycotoxins analysis (Scott and Trucksess, 1997; Shim *et al*., 2004).

Statistical analysis

The means of variable ± SD were recorded and all data was subjected to statistical analysis using SPSS 16, and the differences between mean values as determined by Student's t-test were considered significant at $P < 0.05$.

III. RESULTS

Aflatoxin M1 concentration in raw and pasteurized milk, collected from Jeddah, Saudi Arabia, were determined and compared to US tolerance limit and European Union limit for milk. The major sources of milk are camel, cow, goat, or sheep milk. **Table 1** gives the minimum, maximum, mean and standard deviation of AFM1 in milk samples collected from Jeddah during the year 2013. Out of 160 tested milk samples, 74 (47%) were contaminated with AFM1and the contamination level was less US tolerance limit for AFM1 in milk (0.5 ppb). Out of 32 camel milk samples, 10 (31%) were contaminated with AFM1while 24 (75%) samples were contaminated in case of cow milk samples. Concerning cow milk, 95 % of samples were contaminated and the quantity of AFM1was ranged from 0.09- 0.65 ppb with mean value of 0.04 ppb which is lower than the Euro-limit (0.05ppb) for milk while 6 samples exceed USA limit (0.5 ppb). No cow milk samples exceed the USA regulatory limit but 50% of samples exceed the Euro-limit. Moreover, 13 (40%), 20 (62%) and 7 (32%) samples of goat, sheep and pasteurized milk samples were contaminated with AFM1 and the contamination ranges of AFM1 were 0.041-0.06, 0.04-0.27 and 0.002-0.093 ppb with mean values of 0.270 ± 0.110, 0.29 ± 0.094 and 0.071 ± 0.009, respectively. Statistical analysis indicates significant ($p < 0.05$) difference in AFM1 concentration among milk types. The quantities of AFM1 detected in all examined milk samples were lower than recommended in USA (0.5 ppb). Moreover, 6 (22%), 16 (50%), 8 (25%), 10 (31%) and 7 (22%) of camel, cow, goat, sheep and pasteurized milk samples were exceed European recommended limit (Euro-tolerance, 0.05 ppb) for AFM1 in milk **(Table 1)**.

IV. DISCUSSION

Concentration of AFM1 was determined by Fluorometer with a prior clean-up step with immunoaffinity columns which have been successfully used in the analysis of aflatoxins in food and feed during the last few years (Shim *et al*., 2004). Immunoaffinity columns in combination with HPLC or Fluorometer were used for the analysis of aflatoxins (Gurbay *et al.*, 2006). Aflatoxin B1 and AFM1 in pig liver were determined using Immunoaffinity columns in combination with Fluorometer (Chiavaro *et al.*, 2005). The previous method was used in this study to evaluate the contamination level of AFM1 in 160 raw and pasteurized milk samples. In this study, 31% of camel milk samples were contaminated with AFM1while 75% cow milk samples were contaminated. On contrast, AFM1 contamination has not been detected in the camel milk by Hussain *et al*., (2010). Similarly, contamination of camel milk with AFM1 was recorded by Rahimi *et al*. (2010) and many studies determine AFM1 in cow milk (Boudra *et al*., 2007, Gurbay *et al*., 2006, Elzupir and Elhussein, 2010). Occurrence of aflatoxin M1 in goat milk was confirmed (Finoli and Vecchio, 2003, Virdis *et al.*, 2008, Ruangwises *et al*., 2011). Many studies declared contamination of AFM1 in sheep milk (Battacone *et al.*, 2005). In present study, the contamination level of AFM1 (74%) in milk samples was found to be higher as compared to the results of earlier studies (Hussain *et al*., 2010; Hussain *et al.*, 2008). Out of 120 cow milk samples, 52.5% were contaminated by AFM1 with the mean value of 0.027 ppm (Hussain *et al*., 2008).

In general, regardless of the camel milk, all samples were below the USFDA borderline limit (0.5 ppm), 22.0% of the samples had concentration of AFM1 which exceeded the Euro- tolerance. The European Community and Codex Alimentarius Commission advise that the maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 0.05 ppm (Codex Alimentarious Commission, 2001; Creppy, 2002). In Switzerland and Austria the maximum level AFM1 in milk was reduced to 0.01 ppm for infant food commodities (European Commission Regulation, 2004).

Results of the present study were compared with those of other studies made. In Morocco, 54 samples of pasteurized milk produced by five different dairies were surveyed for the presence of AFM1 and 88.8% of the samples were contaminated with AFM1; 7.4% being above the maximum level of 0.05 ppm set by the Moroccan and European regulations for AFM1 in liquid milk (Zinedine *et al.*, 2007). In Iran, of the 111 samples, 85 (76.6%) were found contaminated with AFM1 in concentration between 0.015 and 0.28 ppm (Kamkar, 2005). Out of 40 milk samples were analyzed in Italy for AFM1, 30% of milk samples had levels ranging from 0.004 to 0.023 ppm and no contaminated samples exceeded the legal limit of 0.05 ppm. Although USFDA regulations allowed AFM1, 10 times higher than that of European Community, 3% Pakistani milk samples exceeded the maximum limit (Hussain *et al*., 2008). Similar to our study, 55, 40, 30, 24, and 20 samples of buffaloes, cows, goats, sheep, and camel milk were analyzed for AFM1 and contamination levels were 34.5%, 37.5%, 20%, and 16.7%, respectively (Hussain *et al*., 2010). In Abeokuta and Odeda, Nigeria, Atanda *et al*., (2007) found the AFM1 level in the range of 2.04-4.00 µg/l in milk and ice cream which indicated a high level contamination compared to African diet limit 0.002 ppb. Higher contamination level was found by El-Sayed Abd Alla *et al*. (2000) who found 3 of 15 cows' milk samples positive for AFM1 with mean value of 6.3 ppb and one sample of dried milk was positive (5 ppb). Recently, lower AFM1 level was recorded in Italy using ELISA, out of 1668 analyzed milk samples, 36 (2.2%) were positive with AFM1level ranged from 18 ± 2 to 208 ± 27 ppt (Belli *et al*., 2016). Thus, the concentration of mycotoxins, especially AFB1 in animal's feeds, which is transformed to AFM1 in milk, should be reduced by good manufacturing, storage and practices and there is a need for quality control during processing and distribution of these products.

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LITERATURE

[1]. **Atanda O., A. Oguntubo, O. Adejumo, J. Ikeorah, I. Akpan. 2007**. Aflatoxin M1 contamination of milk and ice cream in Abeokuta and Odeda, local governments of Ogun State, Nigeria. *Chemosphere* 68(8):1455-1458.

[2]. **Battacone G., A. Nudda, M. Palomba, M. Pascale, P. Nicolussi, G. Pulina. 2005.** Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. *J Dairy Sci* 88:3063–3069.

[3]. **Bellio A., D.M. Bianchi, M. Gramaglia, A. Loria, D. Nucera, S. Gallina, M. Gili and L. Decastelli. 2016**. Aflatoxin M1 in Cow's Milk: Method Validation for Milk Sampled in Northern Italy. *Toxins* 8, 57; doi:10.3390/toxins8030057.

[4]. **Bilandzic N., I. Varenina and B. Solomun. 2010**. Aflatoxin M1 in raw milk in Croatia. *Food Control* 21:1279–1281.

[5]. **Boudra J., S. Barnouin, L. Dragacci and D.P. Morgavi. 2007**. Aflatoxin M1 and ochratoxin A in raw bulk milk from French dairy herds. *J Dairy Sci* 90:3197-3201.

[6]. **Carvajal M., F. Rojo, I. Mendez, A. Bolanos. 2003**. Aflatoxin B1 and its interconverting metabolite aflatoxicol in milk: the situation in Mexico. *Food Additives and Contaminants* 20:1077–1086

[7]. **Codex Alimentarius Commissions (CAC). 2001**. Comments supmitted on the draft maximum level of Aflatoxin M1 in milk. Codex Committee on foog Addites and Contaminant 33rd sessions, Hauge, the Netherlands ftp://ftp.fao.org/codex/ccfac33/fao120e.pdf

[8]. **Creppy E.E. 2002.** Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters* 127: 19-28.

[9]. **El-Sayed Abd Alla A.M., A. A. Neamat-Allah, E. Aly Soher. 2000**. Situation of mycotoxins in milk, dairy products and human milk in Egypt. *Mycotoxin Research* 16(2):91-100.

[10]. **Elzupir A.O. and A.M. Elhussein 2010.** Determination of aflatoxin M in dairy cattle milk in Khartoum State, Sudan. *Food Control* 21(6):945-946.

[11]. **European Commission Regulation. 2004**. No. 683/2004/EC of 13 April 2004, amending Regulation (EC) No. 466/2001 as regards aflatoxins and ochartoxin A in foods for infants and young children. *Official Journal of European Communities* L106, 3-5.

[12]. **Fink-Gremmels J. 2008**. Mycotoxins in cattle feeds and carry-over to dairy milk: A review. *Food Additives and Contaminants* 25(2): 172–180

[13]. **Finoli C. and A. Vecchio, 1997**. Aflatoxin M1 occurrence in goat dairy products. *Microbiologie Aliments Nutrition* 15: 47-52.

[14]. **Gurbay A., S. Aydin, G. Girgin, A.B. Engin and G. Sahin 2006**. Assessment of Aflatoxin M1 levels in milk in Akara, Turkey. *Food control* 17:1-4.

[15]. **Hendricks J.D. 1994**. Carcinogenicity of aflatoxins in nonmammalian organisms. In: Eaton D.L., J.D. Groopman, editors. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. San Diego (CA): Academic Press.

[16]. **Henry S.H., T. Whitaker, I. Rabbini, J. Bowers, D. Park, W.D. Price, F.X. Bosch, J. Pennington, P. Verger, T. Yoshizawa, H. van Egmond, M.A. Jonker, R. Coker. 2001.** Aflatoxin M1. In: Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). FAO Food and Nutrition Paper No. 74. Rome (Italy): Food and Agriculture Organization of the United Nations.

[17]. **Hussain I., J. Anwar, M.A. Munawar, M.R. Asi. 2008**. Variation of levels of aflatoxin M1 in raw milk from different localities in the central areas of Punjab, Pakistan. Food Control; 19(12):1126-1129.

[18]. **Hussain I., A. Jamil, M.R. Asi, M.A. Munawar, M. Kashif. 2010**. Aflatoxin M 1 contamination in milk from five dairy species in Pakistan. *Food Control*, 21(2):122-124.

[19]. **Kamkar A. 2005**. A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab City of Iran. *Food Control*, 16: 593-9

[20]. **Kuilman M.E., R.F. Maas, J. Fink-Gremmels. 2000**. Cytochrome P450-mediated metabolism and cytotoxicity of aflatoxin B1 in bovine hepatocytes. *Toxicology In vitro* 14:321–327.

[21]. **Pettersson H. 1998**. Concerning Swedish derogation on aflatoxin. Complement to the Memo of 97-03-03 on 'Carry-over of aflatoxin from feeding stuffs to milk'. Uppsala (Sweden), Department of Animal Nutrition and management, Swedish University of Agricultural Sciences.

[22]. **Rahimi E., M. Bonyadian, M Rafei, H.R. Kazemeini. 2010**. Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. *Food Chem Toxicol*. 48(1):129-31.

[23]. **Ruangwises S., P. Saipan and N. Ruangwises. 2013**. Occurrence of Aflatoxin M1 in Raw and Pasteurized Goat Milk in Thailand. http://dx.doi.org/10.5772/52723, 2014.09.29.

[24]. **Ruangwises N., P. Saipan and S. Ruangwises. 2011.** Estimated Daily Intake of Aflatoxin M1 in Thailand. In: Guevara-González RG (ed.). Aflatoxins- Biochemistry and Occurrence of Aflatoxin M1 in Raw and Pasteurized Goat Milk in Thailand. *Molecular Biology*. www.intechopen.com/articles/show/title/estimated-daily-intake-of-aflatoxin-m1 -in-thailand.

[25]. **Scott P. and M.W. Trucksess. 1997.** Application of Immunoaffinity Columns to Mycotoxin Analysis. *Journal of AOAC International* 80: 941-949.

[26]. **Shim W.B., A.Y. Kolosova, Y.J. Kim, Z.Y. Yang, S.J. Park, S.A. Eremin, I.S. Lee, and D.H. Chung. 2004**. Fluorescence polarization immunoassay based on a monoclonal antibody for the detection of OTA. *International Journal of Food Science and Technology* 39: 829-837.

[27]. **Van Egmond H.P., R.C. Schothorst, M.A. Jonker. 2007.** Regulations relating to mycotoxins in food: perspectives in a global and European context. *Analytical and Bioanalytical Chemistry* 389:147–157.

[28]. **Van Egmond H.P. 1989**. Aflatoxin M1: occurrence, toxicity, regulation. In: Van Egmond H.P., ed., *Mycotoxins in dairy products*. London (UK): Elsevier Applied Science.

[29]. **Virdis S., G. Corgiolu, C. Scarano, A.L. Pilo, E.P.L. De Santi. 2008**. Occurrence of Aflatoxin M1 in tank bulk goat milk and ripened goat cheese. *Food Control*,Vol. 19(1): 44–49.

[30]. **Zinedine A., L. González-Osnaya, J.M. Soriano, J.C. Moltó, L. Idrissi and J. Mañes. 2007**. Presence of aflatoxin M1 in pasteurized milk from Morocco. *International Journal of Food Microbiology*, 114: 25–29.