MILK CONTAMINATION WITH AFLATOXIN M1 A IN JEDDAH, SAUDI ARABIA

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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 of Jeddah and supermarkets for the pasteurized milk samples. All samples were screened for Aflatoxin M1 using immunoaffinity columns and Fluorometer for mycotoxin detection. Out of 32 camel milk samples, 10(31%) were contaminated 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 that recommended by FDA (0.5 ppb). 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 limit recorded 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. The less milk contaminated samples with AFM1 were camel milk samples< pasteurized milk< goat milk< sheep milk< cow milk. Statistical analysis showed that camel milk samples were significantly less contaminated compared to cow milk samples. On conclusion, all examined milk samples collected from Jeddah were contaminated with minimal level of AFM1 and not exceed the USA limit, thus milk is a save food for consummation by human and infants.

Key words- Aflatoxins, milk, cow, camel, mycotoxins

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. 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).

III. 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.

IV. 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).

V. 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 $P < 0.05$.

VI. RESULTS AND DISCUSSION

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 (Finoli and Vecchio, 2003). The major sources of milk are camel, cow, goat, or sheep milk. 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 (Scott and Trucksess 1997). 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. Out of 160 examined milk samples, 64 (40%) samples were contaminated with AFM1. 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 than 0.5 ppm which is US tolerance limit for AFM1 in milk. Out of 32 camel milk samples, 10 (31%) were contaminated with AFM1while 24 (75%) samples were contaminated in case of cow milk samples. On contrast, AFM1 contamination has not been detected in the camel milk by Hussain et al., (2010).

Moreover, 13 (40%), 20 (62%) and 7 (32%) samples of goat, sheep and pasteurized milk samples were contaminated with AFM1. The contamination ranges of AFM1 were 0.017-0.14, 0.022-0.041, 0.041-0.06, 0.04-0.27 and 0.002-0.093 ppb with mean values of 0.046 ± 0.029, 0.22 ± 0.094, 0.270 ± 0.110, 0.29 ± 0.094 and 0.071 ± 0.009 for camel, cow, goat, sheep and pasteurized milk samples, respectively. ANOVA indicates significant ($p < 0.05$) difference in AFM1 concentration among milk types. The quantities of AFM1 detected in all examined milk samples were lower that recommended by USFDA (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. Similarly, contamination of camel milk with AFM1 was recorded by Rahimi et al. (2002) 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 by Virdis et al. (2008). Many studies declared contamination of AFM1 in sheep milk (Battacone et al., 2005).

In present study the contamination level of AFM1 in raw milk samples was found to be low 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 level was 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 ppm. 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).

In conclusion, the concentration of AFB1 in feeds which is transformed to AFM1 in milk should be reduced by good manufacturing, good storage practices and there is a need for quality control during processing and distribution of these products.

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Table 1. The aflatoxins M1 contamination levels of five different types of milk samples collected from Jeddah, Saudi Arabia.

	Camel milk	Cow milk	Goat milk	Sheep milk	Pasteurized milk	Total milk samples
No samples	32	32	32	32	32	160
Lower contamination range	0.017	0.022	0.041	0.-040	0.002	0.002
Higher contamination range	0.140	0.401	0.260	0.270	0.093	0.270
Mean ±SD	0.046±0.029	0.22±0.094	0.170±0.110	0.19±0.094	0.071±0.009	0.183±0.01
Mean ±SE	0.046±0.009	0.22±0.005	0.170±0.014	0.19±0.010	0.071±0.011	0.183±0.009
Number of contamination sample (%)	10 (31%)	24 (75%)	13(40%)	20(62%)	7(32%)	74 (47%)
Number of clean sample (%)	22 (69%)	8(25%)	19 (80%)	12(34%)	25(78%)	86 (53%)
No samples exceed USA limit (0.5 ppb)	0.0(0.0%)	0.0(0.0%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
No samples exceed Euro-limit (0.05ppb)	6 (22%)	16 (50%)	8 (25%)	10 (31%)	7 (`32%)	47 (29.4%)

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