Prevalence OF Aflatoxin M_1 in Raw Milk Produced in Tropical State (Qena, Egypt) and Imported Milk Powder

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ABSTRACT

Aflatoxins are natural toxic compounds produced by fungi such as *Aspergillus flavus* and *Aspergillus parasiticus.* Due to higher temperature and humidity, fungi are continuously grown and contaminated the cereals and cereal by-products with aflatoxin B_1 (AFB_1). Dairy cows fed AFB_1 contaminated feeds can produce milk contaminated with aflatoxin M_1 (AFM_1). Therefore, the aim of this study was to investigate whether raw milk produced in the Qena Province, Egypt, is contaminated with AFM_1. Raw milk samples (n=48) were collected from various dairy farms in the Qena and investigated for the presence of AFM_1. Additionally, 30 dry powdered milk samples were also purchased from supermarkets in the Qena, Egypt. The concentration of AFM_1 samples was determined with enzyme linked immune sorbent assay (ELISA). Results showed that the occurrence of AFM_1 was 97.92 % (47 samples out of 48 samples were positive) and the mean level of AFM_1 was 62.81±32.10 ng/L ranging from 2 ng/L to 110 ng/L. The level of AFM_1 in 53.19 % of raw milk samples was higher (79.85 ± 17.30 ng/L) than the maximum tolerance limit (50 ng/L) established by European Union (EU). According to the Egyptian regulation, the amount of AFM_1 in the positive samples (47 from 48 samples, 97.92 %) goes beyond the regulations, suggesting that the contamination of raw milk is very high, probably due to the higher contamination of cattle feeds with AFB_1 in the study area. On the other hand, only 18 samples (60.0%) dry powdered milk were positive for AFM_1 with mean level of 1.81±1.02 ng/Kg. In conclusion, high prevalence of AFM_1 contaminated in raw milk produced at the tropical conditions poses the potential risk for consumer health. Strict regulations for mycotoxins levels is not only important in human foods but also in animal feeds with special focus on occurrence of AFB_1 in the feed offered to dairy cows.

Key words: *Aflatoxin M_1, milk, occurrence, Qena.*

INTRODUCTION

Aflatoxins are natural toxic compounds produced mainly by *Aspergillus* species of fungi and commonly found in the tropical and subtropical feeds. These are extremely toxic, immunosuppressive, carcinogenic, mutagenic and teratogenic substances and are known to induce hepatic carcinogenesis in humans. The most frequent aflatoxins are B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) is especially the common one and has been reported as the most powerful natural carcinogen in human and animals (Hussain et al., 2008; Tokar and Vengust, 2008; Awad et al., 2012). Furthermore, it was shown that AFB_1 had negative effects on intestinal morphology and barrier function (Yunus et al., 2010). Aflatoxin M_1 (AFM_1) or milk toxin is a hydroxylated metabolite of aflatoxin B_1 and secreted in milk of dairy cattle after consumption of feed contaminated with aflatoxin B_1 (Dashti et al., 2009; Fallah 2010a.b; Iha et al., 2013). It has been shown that AFM_1 could quickly appeared (within 12 hour) after feeding of AFB_1 to lactating cows (Fallah, 2010a) and its concentration decreased gradually to be under the limit of detection within 72 h after removal of AFB_1 contaminated feeds (Rahimi and Karim, 2008). It seems likely that there is a positive correlation between the level of AFB_1 in the feed and AFM_1 in the milk of dairy cattle consumed AFB_1 contaminated feeds (Fallah, 2010a). In this scenario, it can be assumed that level of AFM_1 is high in the milk of dairy cows when the climatic conditions favourite the growth of fungi that produced AFB_1.

Qena Province, south of Egypt, has a hot desert climate with very hot summers and very little precipitation year round. Due to the higher temperature (39- 41˚C) and humidity (58-61%), it can be predicted that the amount of AFB_1 is high in the animal feeds. It is well known that the incidence and the level of AFM_1 contamination are variable due to the variations in the sources of AFB_1

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contamination of dairy feeds (Prandini et al., 2009). The problem can be further complicated when infected batches of cereal grains are fed to dairy cows. The limited information about the level of AFB_1 in animal feed, the lack of exact regulation for both AFB_1 and AFM_1 and less public concern about mycotoxin contamination in Egypt in general can be resulted in a higher AFM_1 contamination of milk and its by-products.

It is well known that AFM_1 has cytotoxic, genotoxic, and carcinogenic effects (Fallah 2010b; Awad et al., 2012). Therefore, the carcinogenicity of AFM_1 is reclassified by the International Agency for Research on Cancer (IARC) of World Health Organization (WHO) to be group 1 instead of group 2 (IARC, 2002). The danger of this natural toxin is coming from its stability and it cannot be removed by heating of milk (Prandini et al., 2009). The AFB_1 is patent molecules of AFM_1 and classified as group 1 of human carcinogen (IARC, 2002).

Several countries have set acceptable limits of AFM_1 in milk and its by-products to exclude the possible toxicity for humans. In the European Union, the maximum limit of AFM_1 in liquid milk and dried or processed milk products is set at 50ng/L (European Commission Regulation, 2001). In USA, the level of AFM_1 in milk should not be higher than 500ng/Kg (Stoloff et al., 1991). In Egypt, the Ministry of Health recognized that fluid milk and dairy products should be free from AFM_1 (Egyptian Regulations, 1990).

Very limited reports are available regarding the level of contamination of AFM_1 in milk and milk by-products in Egypt. Amer and Ibrahim (2010) showed that 38 % of raw milk samples collected from Alexandria city (north of Egypt) were positive for AFM_1 with a mean concentration of 49.74 ± 17.26 ng/L. This study also reported that all contaminated samples are exceeding the EU permissible limits. However, no study is available about the level of AFM_1 contamination in raw milk produced in Qena province (south of Egypt), as well as in the imported dry milk available in the supermarkets. Therefore, the present study was conducted to investigate the concentration of AFM_1 in the raw milk samples collected from dairy farms in Qena and also in the powdered milk presented in the supermarkets of Qena province.

MATERIALS AND METHODS

Sampling of milk

A total of 48 raw milk samples were collected from 48 lactating cows (one sample from each animal) of the individual dairy farms in the Qena Province (Dandara village, El-Jebal village and Qena city) and were processed for the presence of AFM_1 using ELISA technique. Additionally, 30 dry powdered milk samples (Nestle Nido dry milk, Holland) were purchased from supermarkets of Qena city and analysed with ELISA for occurrence of AFM_1. All the samples were collected during March-October, 2012. The samples of milk powder (10.0 g) were suspended in 100 mL of warm deionised water (20-25 °C) and were shaked for 10 min at the speed of 250 per minute. Afterwards, the samples (20 mL of milk) were centrifuged at 3,500 g for 10 minute at 10 °C. Upper creamy layer was completely discarded and the lower phase was used for ELISA.

Measurement of AFM_1 in milk samples

The AFM_1 was analyzed using a commercial ELISA kit (Ridascreen, aflatoxin M1 R-Biopharm, Product code, R1101, Darmstadt, Germany). Detection limit for milk samples were 5ng/L with recovery rate of 95%. AFM1 in skimmed milk samples was measured according to the instructions of the manufacturer using standards (0, 10, 20, 40, 50, and 80 ng/L). Briefly, 100 µL of skimmed milk samples plus 10 µL of standard solutions (80ng/L was used due to the detection limit of 5ng/Kg) were added to each well. The antibody binding sites were occupied proportionally to the AFM1 concentration during incubation of 60 min at room temperature (20–25 °C) in the dark. The liquid was poured out of the wells and the wells were washed 3 times using 250 µL of washing buffer per well. Afterwards, 100 µL of the enzyme conjugate was added and incubated for 60 minute at room temperature in dark. Washing procedure was repeated again and 50 µL of substrate solution and 50 µL of chromogen was added to each well, the plate was mixed and incubated for 30 minute at room temperature in the dark. At the end, 100 µL of the stop solution was added to each well and the plate was mixed. The AFM_1 was measured photometrical at 450 nm against the air blank of ELISA reader (Tecan Group Ltd, Switzerland).

Statistical analysis

The statistical program SPSS (version 20; SPSS GmbH, SPSS Inc., Munich, Germany) was used for data analysis. Data are presented as mean ± standard deviation (SD) and the range (minimum to maximum).

RESULTS AND DISCUSSION

Results showed that the occurrence of AFM_1 in the raw milk produced in Qena was 97.9% as only one sample was found negative with mean concentration of 62.9 ± 32.1 ng/L, ranging between 2–110 ng/L (Table 1). Occurrence and level AFM_1in the raw milk produced in south part of Egypt (Qena) are higher compared with raw milk produced in Alexandria city (38% and 49.7 ± 17.3 ng/L respectively) as reported by (Amer and Ibrahim, 2010) indicating that milk produced in south of Egypt is

more contaminated with AFM_1 than that produced in north of Egypt. In the present study, the level of AFM_1 in raw milk samples was higher than the maximum tolerance limit (50 ng/L) established by European Union (European Commission Regulation, 2001) and the amount was ranged from 51–110 ng/L with a mean value of 79.9 ± 17.30 ng/L (Table 2). The higher concentration of AFM_1 in the milk samples might be due to the feeding of AFB_1contaminated feeds to the animals in south Egypt, a region of higher temperature and humidity. Interestingly, the detection level of AFM_1 in the milk powder purchased from Qena markets was low compared with the permissible level AFM_1 in the milk powder according to EU regulations (Table 2).

In the present study, the number of dry milk samples for AFM_1 determination (30 samples) was comparable to or higher than the number of samples investigated in previous reports (Dashti et al., 2009; Iha et al., 2013). The results showed that the level of AFM_1 in dry powdered milk samples was 1.8 ± 1 ng/Kg ranging from 0.5–4 ng/Kg, being occurrence of 60% (Table 1). The level of AFM_1 in milk powdered samples was low compared with raw milk produced in Qena. This indicates that dry milk imported from the EU countries is less contaminated with AFM_1 because AFM_1 in liquid milk and dried or processed milk products is set at 50ng/L (European Commission Regulation, 2001). In addition, in Europe, the cool temperature and other climatic conditions do not favourite the growth of aflatoxins producing fungi and consequently the low level of AFB_1 in animal feeds is expected. This may explain the low occurrence and low level of AFM_1 in dry milk samples compared with raw milk samples in the current study.

It is well known that people consume a high amount of milk or milk products in their diets. For this reason, milk can have a significant potential health risk for human due to the AFM_1 contamination. Consequently, AFM_1 remains a permanent health hazard issue worldwide. Qena province is characterized by high temperature and humidity. At this favourite climatic condition for growth of aflatoxins producing fungi, farmers in this part of Egypt harvest hay in the summer and keep it for cattle feeding during the winter. Fungi may easily produce toxins in an inappropriate storage conditions. Following the consumption of AFB_1 contaminated feed by dairy cattle, AFB_1 is converted to AFM_1 in the liver and resulted in contamination of the milk of lactating cows with AFM_1. The higher prevalence rate along with higher contamination level of AFM_1 (97.9%) in the raw milk samples indicate that lactating cows in Qena are fed with AFB_1-contaminated feeds. However, the level of AFB_1 in the feed of dairy cattle could not be measured in the present study. It has been reported that environmental temperature, humidity, and moisture content of the feed as well as pH and mechanical damage to cereal grains resulted in higher AFB1 in animal feeds (Amer and Ibrahim, 2010). Aflatoxin-contaminated food tolerates the thermal inactivation, pasteurization, autoclaving and other food processing procedures (Park, 2002). To produce high quality milk, it is essential to keep feeds of lactating cows free from AFB_1 contamination.

In conclusion, results revealed that the presence of food borne Aflatoxin (AFM_1) in the examined milk appeared to be a serious public health hazard, indicating that a need for the periodical monitoring of AFM_1 in the raw milk produced in Qena province. Further studies are also required to measure the level of AFB_1 in the rations of lactating cows in relation to the level of AFM_1 in milk.

Table 1 Occurrence of AFM_1 in the raw milk and dry milk samples

Sample	Number of samples examined	Positive samples		AFM_1 contamination (ng/L-ng/Kg)	
		Number	(%)	Mean ± SD	Range (minimum-maximum)
Raw milk	48	47	97.9	62.81 ± 32.10	2-110
Dry milk	30	18	60.0	1.81 ± 1.02	0.5-4

Table 2 AFM_1 contamination of raw milk samples exceeding the EU regulation

Sample	Positive samples	Exceeding EU regulation [a]			
		Number	(%)	Mean ± SD (ng/L)	Range (ng/L) (minimum-maximum)
Raw milk	47	25	53.19	79.85 ± 17.30	51-110

Percentage of positive samples for raw milk samples that exceed EU regulation

[a] EU Regulation 466/2001 (the limit is fixed for AFM_1 at 50 ng/L for raw and liquid milk)

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