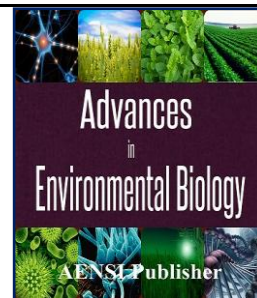




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ABSTRACT

Aflatoxin M₁ (AFM₁) is an important mycotoxin frequently found in milk and dairy products. In this study, one-hundred samples (fifty each) of raw goat and camel's milk collected from Cairo and Giza governorates, Egypt were examined for aflatoxin M₁ by Enzyme Linked Immuno-Sorbent Assay (ELISA). AFM₁ was found in 54% and 18% by the mean level of 0.05 and 0.04ug/kg for raw goat and camel's milk respectively. Eight % and 4% of the examined goat and camel's milk samples were higher than maximum tolerance limit accepted by European Union (EU) and Codex Alimentarius Commission (0.05ug/kg) respectively.

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INTRODUCTION

Goat's milk is of particular economic interest in the developing countries. The production of this type of milk has to be a useful strategy to tackle the problems of under nutrition in Asia and Africa [1]. Camel's milk represents one of the basic ingredients of human food in many parts of the world, especially in the arid and semi-arid zones. Camels, even under extreme hostile conditions of high temperatures, drought, lack of pastures and lack of water, can survive and produce good quality milk. Despite the low percentage of camel's milk in the total milk production in Egypt, camel's milk has attracted the attention of researchers over the past few decades [2].

Aflatoxins, a group of highly toxic secondary fungal metabolites produced by some *Aspergillus* species, are found in a wide variety of foods and feeds around the world. Aflatoxin B₁ (AFB₁) is the most frequent form present in contaminated foods and feeds. Ingestion of aflatoxin B₁ contaminated feed by lactating animals lead to excretion of aflatoxin M₁ (AFM₁), 4 hydroxylated metabolite of AFB₁, in milk. Although children are the major milk and dairy products consumers, the quality of milk products has a strong influence on the health of people in various age brackets. AFM₁ is known to be hepatotoxic and carcinogenic. The World Health Organisation changed its classification from group 2 to group 1 [3,4,5].

Aflatoxin M₁ is formed in the liver. It is excreted in the milk from the mammary glands of animals fed with feeds contaminated with AFB₁, these toxins are considered the most potential threat for humans especially for children and elderly people, who are milk and milk derivatives consumers [6,7].

The toxic effects include acute toxicity [8], carcinogenicity [9,10], mutagenicity [11], teratogenicity [12] in animals and humans at different levels of contamination.

Several countries have regulated the maximum permissible levels of AFM₁ in milk to protect consumers specially children. Several researchers have been reported of potential hazardous human exposure to AFM₁ through milk [13,14]; so many countries to reduce this risk proposed legal regulations for AFM₁ levels in milk. In addition, these regulations vary in different countries due to economic considerations. A maximum acceptable limit of European Union (EU) and Codex Alimentarius Commission (0.05ug/kg) [15]. However, acceptable limit level of AFM₁ set by the Egyptian Standards [16] was 0.5ug/kg, which is the same as US Food and Drug Administration [17] accepted level.

The stability of AFM₁ is not affected appreciably neither by heat treatments used in dairy industry, i.e. pasteurization and sterilization nor during processing and storage of various dairy products. Due to the widespread consumption of milk, presence of AFM₁ in these milks has become a worldwide concern [6,18].

Therefore, our work was planned to study, the incidence of AFM₁ in the milk of desert animals.

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MATERIALS AND METHODS

Collection of samples:

A total of 100 samples (fifty each) of raw goat and camel's milk were collected from dairy farms located in Cairo and Giza Governorates, Egypt. All of milk samples were transported at 2–4°C in an icebox and analyzed by indirect competitive enzyme Linked Immune-Sorbent Assay (ELISA) for presence of AFM₁.

Method for analysis of AFM₁:

The quantitative analysis of AFM₁ in the milk samples was performed by competitive enzyme immunoassay ELISA kit. The quantitative analysis of AFM₁ in the samples was based on competitive enzyme immunoassay using RIDASCREEN® Aflatoxin M₁ 30/15 (Art. No.:R1111, R-Biopharm, Darmstadt, Germany) test kit. Most of the used reagents were provided by the kit manufacturer. The other chemicals such as chloroform, dichloromethane, methanol and n-heptane were purchased from Merck. For conducting recovery study, AFM₁ standard was obtained from Sigma (Sigma–Aldrich, 6428). Stock solution of AFM₁ (50 mg/ml) was prepared in a methanol/chloroform mixture (81:19, v/v) and stored at –20 °C. Before using, it was diluted with methanol/chloroform (1:1, v/v) at appropriate concentrations [19].

Preparation of milk samples:

Preparation of samples was conducted according to the instructions of kit. Milk samples were chilled to 10°C and centrifuged at 2000g for 5 min. The upper creamy layer was completely removed by aspirating through a pasteur pipette and from the lower phase (defatted supernatant). 200 µl was directly used per well in the test.

ELISA test procedure:

ELISA test procedure was conducted according to the instructions of kit. 200 µl of standard solutions (were provided in 0, 5, 10, 25, 50 & 100 ng/l concentrations) and prepared samples were added into separate microplate wells and incubated for 30 min at room temperature (25°C) in the dark. The liquid was poured out and the wells were washed with washing buffer (250 µl) thrice. In the next stage, 200 µl of the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark. Again, the wells were washed thrice with washing buffer. Afterwards, 200 µl of substrate/chromogen was added, mixed gently and incubated in the dark at room temperature for 15 min. Finally, 50 µl of the stop reagent was added into the wells and the absorbance was measured at $k = 450$ nm in ELISA plate reader against air blank within 15 min. According to the Euroclone AFM₁ kit guidelines, the lower detection limit is 5 ng/l for milk.

Recovery study:

In order to validate our method, recovery study was performed by spiking known amounts (50, 150, 250 & 450 ng/kg) of AFM₁ into extracted milk samples just before the test. The preparation of the samples and ELISA test procedure were done as described above. All experiments were carried out using four samples per each treatment. Under these conditions, the mean recovery scores in spiked samples were 99% with a coefficient of variation of 8.5%. According to the instructions of the kit, the recovery rate in milk examined were approximately 100% with a mean coefficient of variation of 1.1%.

Statistical analysis according to SPSS [20]:

The statistical methods used in this study were based on normal confidence intervals and analysis of variance (ANOVA).

Results:

Table 1: Occurrence of aflatoxin M₁ in the examined samples.

Type of samples	Total no. of samples	Positive samples		Concentration (ug/kg)		Exceed permissible limit (0.05 ug/kg)	
		No.	%	Mean	Range	No.	%
Goat's milk	50	27 ^a	54.00	0.05	0.02- 0.150	4	8.00
Camel's milk	50	9 ^b	18.00	0.04	0.03- 0.09	2	4.00

Significant differences between the values had the different letter in each column ($p < 0.05$).

Table 2: Levels of aflatoxin M₁ in the examined samples.

Type of samples	Distribution of samples (%)											
	<0.050 ug/kg		0.05- 0.150 ug/kg		0.151- 0.250 ug/kg		0.251- 0.500 ug/kg		0.51- 1 ug/kg		1.1- 13 ug/kg	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Goat's milk	6	12.00	21	42.00	0	0.00	0	0.00	0	0.00	0	0.00
Camel's milk	7	14.00	2	4.00	0	0.00	0	0.00	0	0.00	0	0.00

Discussion:

The contamination of milk with AFM₁ displays variations according to geography, country and season [21]. The results of analysis of AFM₁ level (ug/kg) in raw goat and camel's milk are shown in **(Table1)**. The presence of AFM₁ was observed in 54% and 18% for goat and camel's milk samples respectively. The overall mean level of AFM₁ was 0.05 and 0.04 ug/kg for goat and camel's samples respectively. However, 8% and 4% of the goat and camel's milk samples were higher than the maximum international tolerance level of AFM₁ in liquid milk regarding international Codex Alimentarius Commission respectively. There was a significant difference ($p < 0.05$) was observed between samples of goat and camel's milk. The highest level of aflatoxins M₁ in the examined goat's milk lies within the range of 0.05- 0.150 ug/kg in a percentage of 42.00, while the highest level of aflatoxins M₁ in the examined camel's milk lies within the range of <0.050 ug/kg in a percentage of 14.00 **(Table2)**.

Higher incidence of AFM₁ contamination has been expressed by many researchers Fallah [5], Kamkar [22], Ayar *et al.* [23], Oveisi *et al.* [24], Hussain & Anwar [25], Tajkarimi *et al.* [26] and Ruangwises & Ruangwises [27]. Our results variations may be related to different reasons such as type of milk, geographical region, the country, the season and the analytical methods employed. According to results obtained, incidence and contamination levels of AFM₁ in goat's milk, seem to be a serious problem for public health. For this reason, milk of desert animals have to be inspected and controlled continuously for AFM₁ contamination, animal feeds should be checked regularly for AFB₁ and storage conditions of feeds must be taken under strict control.

The growth of fungi and the production of aflatoxins in natural substrates is influenced not only by the kind, moisture content of the substrate and by the fungi species, but also by the minerals content in the substrate and by different other factors e.g. humidity and temperature [28]. Depending on the particular combination of the external parameters for growth, the biosynthesis of the aflatoxins may be completely inhibited, even in conditions of normal development. Knowledge about these relationships may allow an assessment regarding the combinations of parameters that can control the biosynthesis of aflatoxins or the factors that are beneficial for the onset and production of aflatoxins [29].

The relationship between AFM₁ occurrence level in milk and AFB₁ content of feed was reported. Out-pasturing of milking camels was the most important factor in the low levels of aflatoxin in milk and findings of these researchers also demonstrated low levels or absence of AFM₁ [22,26]. Therefore it is possible to say that the results obtained in present study were parallel to the results of prior studies which could be related to specific feeding systems.

Aflatoxin M₁ contamination occurs as a consequence of feeds contaminated with AFB₁. Many researchers showed that there is no linear correlation between the quantity of AFM₁ in milk and the AFB₁ quantity in the feed intake of the animals [30]. Approximately a quantity in the range 0.3-6.2% of AFB₁ in the animal feed is transformed to AFM₁ in milk [31]. Moreover the results reported can be different from animal to animal, from one day to the other, as well as from one milking to the other [32].

Ingestion in a short time of large quantities of aflatoxins may lead to acute poisoning, as acute aflatoxicosis occurs, resulting in hemorrhages, acute liver failure and even death. The risk of human aflatoxicosis is high in the countries, where there are no strict regulations regarding the maximum level of aflatoxins allowed, and where the agricultural crops are not monitored [33].

Presence of AFM₁ in milk is a major risk to humans, since these milks are mainly consumed by children, who are considered to be much more sensitive to the adverse effects of AFM₁ [34].

Conclusion:

The results of the present study indicated that the AFM₁ levels in these milks consumed in Egypt were relatively high and it can provide a potential hazard for public health. The best way to deal with this problem is reducing the AFB₁ concentration in animal feed by improved processing and storage practices. At the same time, attention should be given to regular monitoring of aflatoxins in animal feed and dairy products. In addition, the governmental agencies should train the farmers, dairy companies and dairy products consumers on the potential health consequences of aflatoxins. Finally, milk contaminated with high levels of AFM₁ must be prohibited for human consumption by the public health authorities.

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