

AFLATOXIN M₁ RESIDUES IN RUMINANTS MILK IN LUXOR GOVERNORATETOHAMEYA A. HUSSIEN¹; ABDEL-LATIEF SH. SEDDEK¹ and DIEFY A. SALEM²¹ Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine,
South Valley University² Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Assiut University**Received:** 18 January 2017; **Accepted:** 6 March 2017**ABSTRACT**

This study was carried out to investigate aflatoxin M₁ (AFM₁) in raw milk of ruminant animals (cows, buffaloes, sheep, goats and camels) in Luxor Governorate to know any of these species milk is contaminated by the toxin and to determine its concentration to avoid its harmful effect on consumers' health. A total number of 165 milk samples were collected from various villages at the main three cities in Luxor Governorate (Esna, Armant and Luxor cities) in winter season 2015-2016 (11 milk samples from each species per city) and the samples had been analyzed by ELIZA test kits. The obtained results revealed that AFM₁ levels were lower than previous surveys in Egypt. The percent of positive milk samples in all species were 32.7, 58.18 and 56.36% from Esna, Armant and Luxor cities, respectively. AFM₁ could not be detected in sheep and goat samples from Esna, camel and goat samples from Armant and camel samples from Luxor city. Overall, the percent of positive milk samples in all cities from Luxor Governorate were 66.6% (22 out 33) in cows, 63.6% (21 out 33) in buffaloes, 15.2% (5 out 33) in camels, 66.7% (22 out 33) in sheep and 33.3% (11 out 33) in goats. AFM₁ mean values in milk samples of cows, buffaloes, camels, sheep and goats were 4.518, 1.951, 0.091, 2.966 and 0.582 ng/l respectively. The highest mean value of AFM₁ (10.953 ng/l) was found in cow's milk from Armant followed by sheep milk from Luxor (6.811 ng/l) then buffaloes milk from Armant (4.005 ng/l). The highest value of AFM₁ (14.307 ng/l) was detected in cow's milk from Armant city followed by (13.177 ng/l) in buffaloes milk from Luxor. Concerning the health hazard for consumers, no milk samples exceeded the permissible limits of the US regulations (500ng/l) and the European Commission regulations (50ng/l), while all positive samples of raw milk are exceeding Egyptian regulations (free from AFM₁). In conclusion, high prevalence of AFM₁ in milk from Luxor Governorate indicated that the contamination of raw milk is very high and this due to the contamination of feedstuffs of these animals with AFB₁. Because of these findings, we need to survey aflatoxins incidence and levels in feedstuffs and milk during all seasons of the year in this areas.

Key words: Aflatoxin M₁, Residues, Ruminants Milk, Luxor Governorate.

INTRODUCTION

Mycotoxins are products or metabolites produced by fungi which are harmful to other's life. In order to allow mycotoxins production, three factors should be involved: (1) The presence of mycotoxinogenic fungi, (2) The presence of substrate, and (3) the optimal environmental conditions eg. optimal temperature and relative humidity for example aflatoxins are mostly present in Africa because of the optimum temperature and high relative humidity (Phillips, 1999). Therefore, these factors lead to variations in the geographical distribution of mycotoxins, mycotoxicosis lead to

various hazard effects in animals starting with emaciation, loss of production and ending with mortality (Kiessling *et al.*, 1984). Moreover, mycotoxicosis has public health importance because of transmission to humane via milk, eggs, and meat (Manal *et al.*, 2012).

Aflatoxins (AFs) are a major class of mycotoxins (Creppy, 2002). Aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂) are the major classes of AFs (Sweeney and Dobson, 1998). Prolonged drought, high temperatures, substrate composition, storage time and storage conditions play an important role in fungal growth and the synthesis of AFs (Stack and Carlson, 2003). Aflatoxin B₁ is the most toxic, carcinogenic, teratogenic and mutagenic of AFs (Iqbal *et al.*, 2010). AFB₁ is a group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2002; Iqbal *et al.*, 2014) Aflatoxin M₁ (AFM₁) is a hydroxylated

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metabolite of AFB₁ (Asi *et al.*, 2012). AFM₁ is excreted in milk in the mammary glands of both humans and lactating animals (Fallah *et al.*, 2009). 0.3-6.2% of AFB₁ is converted into metabolized AFM₁ and excreted in milk, depending on the genetics of the animals, seasonal variation, the milking process and the environmental conditions (Unusan, 2006). Presence of AFM₁ in milk and milk products is a health damage because every age group regularly consumed milk and milk products in their daily diet (Fallah *et al.*, 2009). International Agency for Research on Cancer (IARC) has positioned AFM₁ with AFB₁ as a Group 1 carcinogen (IARC, 2002). AFM₁ is very stable at high temperatures (Oruc, 2006).

The goal of the present work is to determine the prevalence of AFM₁ in raw milk samples collected from various ruminant species (cows, buffalos, sheep, goats and camels), in winter season 2015-2016 at various geographical areas from Luxor Governorate (Esna, Armant and Luxor cities) to estimate its levels to evaluate health risks for human consumers.

MATERIALS AND METHODS

Sampling:

In order to study prevalence of AFM₁ and its level in raw milk of different animal species in Luxor Governorate, a total number of 165 samples were collected from some villages at the three main cities (Esna, Armant and Luxor). The samples were collected from five species of ruminant animals (Cows, Buffaloes, Camels, Sheep and Goats). The total number of samples from each city was 55 samples (11 samples from every species). Milk samples were randomly collected during the duration between December 2015 and February 2016 (winter season). The samples were kept frozen till analysis.

Methods:

Aflatoxin M₁ was measured in milk samples using a commercially available ELISA test kit (REAGENTM, Product Code: RNM 98001, United states).

Aflatoxin M₁ ELISA Test Kit

AFM₁ ELISA Test Kit is competitive enzyme immunoassay for the quantitative analysis of AFM₁ in milk and milk powder. The sample can be directly used for the ELISA plate without extraction and with high sensitivity (0.005 ng/g or ppb) and low detection limit in milk (0.005ppb). The method is based on a competitive colorimetric ELISA assay. The AFM₁ antibody has been coated in the plate wells. During the analysis, sample is added to the wells for incubation. After washing the plate, the AFM₁-horseradish peroxidase (AFM₁-HRP) conjugate is added to the wells for incubation. If the AFM₁ residue is present in the sample, it will compete for AFM₁ antibody, thereby preventing the AFM₁-HRP

from binding to the antibody attached to the well. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the aflatoxin M₁ residue concentration in the sample.

AFM₁ in milk samples was measured according to the instructions of the manufacturer using the following standards (0.0, 0.005, 0.015, 0.03, 0.09 and 0.27 ng/ml). Briefly, 200 uL of each AFM₁ standard and sample were added in duplicate into different wells. The plate was incubated for 60 minutes in the dark at room temperature (20–25°C). The plate was washed 3 times with 250 uL of 1X wash solution. After the last wash, the plate was inverted and gently taped the plate dry on paper towels. Immediately after plate washings, 100 uL of AFM₁-HRP conjugate was added to each well. The plate incubated for 15 minutes at room temperature. Washing procedure was repeated again and 100 uL of TMB substrate was added to each well. After incubation for 15 minutes at room temperature (20–25 °C), 100 uL of stop solution was added to each well to stop the enzyme reaction. AFM₁ was measured on micro plate reader (Stat Fax 2100 Reader, USA) with 450 nm wavelength against the air blank.

Aflatoxin M₁ concentration calculations:

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/ml on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)} \times 100}{\text{absorbance zero standard}}$$

Statistical analysis:

The statistical software package SPSS version 16 was employed. Data are presented as mean \pm standard deviation (SD) and the range (minimum to maximum).

RESULT

The obtained results were presented in Tables (1, 2, 3 and 4) and Figure 1. The data showed that milk samples from all species were contaminated with different values and percent of AFM₁ which could not be detected in sheep and goat samples from Esna, and also in camel and goat samples from Armant and camel samples from Luxor city. The percent of positive samples represented 32.7, 58.18 and 56.36% of all tested species in Esna, Armant and Luxor cities, respectively. Overall, the highest mean value of AFM₁ (10.953 ng/l) was found in cow's milk from Armant followed by sheep milk from Luxor (6.811) then buffaloes milk from Armant also (4.005 ng/l). The highest value of aflatoxin M₁ (14.307 ng/l) was detected in cow's milk from Armant city followed by (13.177 ng/l) in buffaloes milk from Luxor.

Results of AFM₁ in milk samples from Esna city were reported in Table 1 and Figure1. Cow's milk contained the highest concentration of AFM₁ followed by camel's milk then buffalo's milk. AFM₁ was not detected in sheep and goat's milk from Esna.

Results of AFM₁ in milk samples from Armant city were reported in Table 2 and Figure1. AFM₁ was detected in all species milk except camels and goats.

Cow's milk was contained the highest mean values followed by buffalo's and sheep milk.

Results of AFM₁ in all milk samples from Luxor Governorate were reported in Table 4 and Figure1. AFM₁ mean value in cow's milk was the highest followed by sheep then buffalos and finally camel's milk.

Table (1): AFM₁ concentration (ng/l) in milk of different animal species from Esna city.

Parameter	Animal Species				
	Cow	Buffalo	Camel	Sheep	Goat
Mean	2.464	0.171	0.274	0.000	0.000
S. D.	1.583	0.197	0.341	0.000	0.000
Minimum	0.0	0.0	0.0	0.000	0.000
Maximum	3.388	0.376	0.753	0.000	0.000
Percent of positive samples	72.7 (8/11)	45.5 (5/11)	45.5 (5/11)	0 (0/11)	0 (0/11)
Exceeding ER	8	5	5	0	0
Exceeding EC	0	0	0	0	0
Exceeding US FDA	0	0	0	0	0

ER: Egyptian regulations, (1990), the limit in milk is 0 ng/L.

EC: European Commission, (2006), the limit in milk is 50 ng/L.

US FDA: US FDA, (2011), the limit in milk is 500 ng/LFDA: Food and Drug Administration.

Table (2): AFM₁ concentration (ng/l) in milk of different animal species in Armant city.

Parameter	Animal Species				
	Cow	Buffalo	Camel	Sheep	Goat
mean	10.953	4.005	0.000	2.088	0.000
S. D.	4.150	1.158	0.000	1.109	0.000
Minimum	0.000	2.635	0.000	0.753	0.000
Maximum	14.307	6.777	0.000	3.0120	0.000
Percent of positive samples	90.9 (10/11)	100 (11/11)	0 (0/11)	100 (11/11)	0 (0/11)
Exceeding ER	10	11	0	11	0
Exceeding EC	0	0	0	0	0
Exceeding US FDA	0	0	0	0	0

ER: Egyptian regulations, (1990), the limit in milk is 0 ng/L.

EC: European Commission, (2006), the limit in milk is 50 ng/L.

US FDA: US FDA, (2011), the limit in milk is 500 ng/LFDA: Food and Drug Administration.

Table (3): AFM₁ concentration (ng/l) in milk of different animal species in Luxor city.

Parameter	Animal Species				
	Cow	Buffalo	Camel	Sheep	Goat
Mean	0.137	1.677	0.000	6.811	1.746
S. D.	0.190	3.914	0.000	2.198	2.043
Minimum	0.000	0.000	0.000	0.753	0.376
Maximum	0.376	13.177	0.000	8.659	4.894
Percent of positive samples	36.4 (4/11)	45.5 (5/11)	0 (0/11)	100 (11/11)	100 (11/11)
Exceeding ER	4	5	0	11	11
Exceeding EC	0	0	0	0	0
Exceeding US FDA	0	0	0	0	0

ER: Egyptian regulations, (1990), the limit in milk is 0 ng/L.

EC: European Commission, (2006), the limit in milk is 50 ng/L.

US FDA: US FDA, (2011), the limit in milk is 500 ng/LFDA: Food and Drug Administration

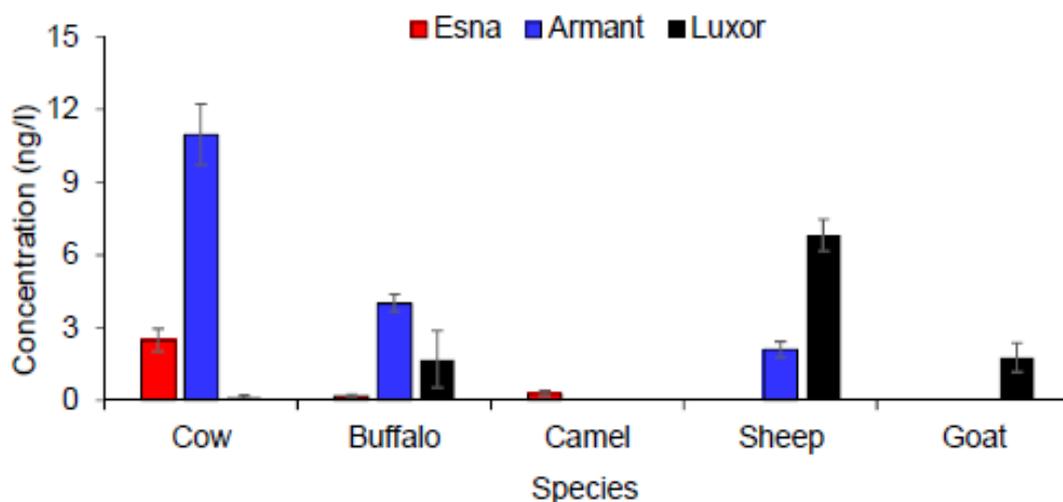
Table (4): AFM₁ concentration (ng/l) in milk of different animal species in Luxor Governorate.

Parameter	Animal Species				
	Cow	Buffalo	Camel	Sheep	Goat
Mean	4.518	1.951	0.091	2.966	0.582
S. D.	5.335	2.79	0.231	3.204	1.415
Minimum	0.000	0.000	0.000	0.000	0.000
Maximum	14.307	13.177	0.753	8.659	4.894
Percent of positive samples	66.7 (22/33)	63.6 (21/33)	15.2 (5/33)	66.7 (22/33)	33.3 (11/33)
Exceeding ER	22	21	5	22	11
Exceeding EC	0	0	0	0	0
Exceeding US FDA	0	0	0	0	0

ER: Egyptian regulations, (1990), the limit in milk is 0 ng/L.

EC: European Commission, (2006), the limit in milk is 50 ng/L.

US FDA: US FDA, (2011), the limit in milk is 500 ng/LFDA: Food and Drug Administration

**Figure 1:** AFM₁ mean values (ng/l) in different animal species and cities of Luxor Governorate.

DISCUSSION

Mycotoxins are toxic secondary metabolites of fungal origin and contaminate agricultural commodities before or under post-harvest conditions. They are mainly produced by fungi as the *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins affect a broad range of agricultural products including cereals, cereal based foods, dried fruits, wine, milk, coffee beans, meat products, which are the sources of the economies of many developing countries (Shephard *et al.*, 2012). They are one of the most important naturally occurring toxins in various foods stand in improper conditions. Meat, eggs, milk, and other palatable products from animals that consume mycotoxins contaminated feed are additional sources of potential exposure to these toxins (Report on Carcinogens, 2009). Milk is a highly nutritive food containing many macro- and micronutrients that are essential for the growth and maintenance of human health. The health of human populations is often reflected in the condition of their food-producing ecosystems. Moreover, the implementation of food regulations may be directly linked with the quantity and quality of available food. Therefore, consumers from developing countries, especially from rural areas, face problems related to food security and food safety because they depend on locally produced foods (Marroquín-Cardona *et al.*, 2014).

Many international studies reported AFM₁ with variable levels and percent in milk and milk products (Fallah *et al.*, 2009; Bilandzic *et al.*, 2010; Buket *et al.*, 2010; Golge, 2014; Iqbal *et al.*, 2014; Oluwafemi *et al.*, 2014 and Bilandzic *et al.*, 2015). In Egypt, There are limited surveys for AFM₁ in milk were carried in some governorates in lower and upper Egypt (Salem, 2002; Motawee *et al.*, 2004a and 2004b; Motawee *et al.*, 2009; Amer and Ibrahim, 2010; Ghareeb *et al.*, 2013; Shaker and El Sharkawy, 2014 and Abdallah, 2016). They found AFM₁ in milk with wide difference in its occurrence and levels depending on the geographical location and the environmental conditions of the area under research.

The present study showed that AFM₁ levels were lower than previous surveys in Egypt. The percent of positive milk samples in all species were 32.7, 58.18 and 56.36% from Esna, Armant and Luxor cities, respectively. AFM₁ could not be detected in sheep and goat samples from Esna, camel and goat samples from Armant and camel samples from Luxor city. The highest mean value of AFM₁ (10.953 ng/l) was found in cow's milk from Armant followed by sheep milk from Luxor (6.811ng/l) then buffaloes milk from Armant also (4.005 ng/l). The highest value of aflatoxin M₁ (14.307 ng/l) was detected in cow's milk from Armant city followed by (13.177 ng/l) in buffaloes milk from Luxor.

Overall, the percent of positive milk samples in all cities from Luxor Governorate were 66.6% (22 out 33) in cows, 63.6% (21 out 33) in buffaloes, 15.2% (5 out 33) in camels, 66.7% (22 out 33) in sheep and 33.3% (11 out 33) in goats. AFM₁ mean values in milk samples of cows, buffaloes, camels, sheep and goats were 4.518, 1.951, 0.091, 2.966 and 0.582ng/l respectively.

The levels specially in cow and buffalo milk were lower than observed in Egyptian governorates although the prevalence were nearly similar when comparing the levels of AFM₁ in milk detected in this study with previous research (Salem, 2002; Motawee *et al.*, 2004a and 2004b; Motawee *et al.*, 2009; Amerand Ibrahim, 2010; Ghareeb *et al.*, 2013; Shaker and El Sharkawy, 2014 and Abdallah, 2016).

Amer and Ibrahim (2010) found that 38 % of raw milk samples collected from Alexandria city (north of Egypt) were positive for AFM₁ with a mean concentration of 49.74 ± 17.26 ng/L and all positive samples were exceeding the Egyptian regulations, while 52.6% of examined samples were exceeding European Commission regulation (50 ng/l or Kg) (European Commission Regulation, 2006); and all of them are with in the US regulations (500 ng/l or Kg) (FDA, 2011).

Ghareeb *et al.* (2013) reported that the occurrence of AFM₁ in milk samples from Qena province was 97.92 % (47 samples out of 48 samples were positive) and the mean level of AFM₁ was 62.81 ± 32.10 ng/L ranging from 2 ng/L to 110 ng/L. The level of AFM₁ 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 (European Commission Regulation, 2006). According to the Egyptian regulations (1990), the amount of AFM₁ 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 AFB₁ in the study area.

Shaker and El Sharkawy, (2014) found that all milk samples from Sohag and Assiut cities were positive for AFM₁. The mean concentration of AFM₁ in raw buffalo milk from Sohag was 64.49 ± 16.8 ng/L, with an average of 123.27 ng/L; 86.5% contained AFM₁ at levels higher than the maximum permissible limit of 50 ng/L set by the EU regulations (European Commission Regulation, 2006). In Assiut, the mean concentration of AFM₁ in raw buffalo milk was 130.6 ± 29.9 ng/L, with an average of 250.79 ng/L. All tested samples from Assiut were above the MRL set by the EU regulations (European Commission Regulation, 2006); but only one sample at the 500 ng/L maximum set by the FDA (2011).

Recently, Abdallah *et al.* (2016) detected AFM₁ in all the samples analyzed in a limited survey on raw milk

from local shops in Assiut. The range was (0.02-0.19 µg/kg) were lower than the incidence of AFM₁ reported by Shaker and El Sharkawy, (2014) and higher than Salem's study (Salem, 2002) in which up to 0.015 µg/kg in Assiut city was detected by ELISA. Their results showed also that 14 samples (70%) were above the maximum permissible level in the European Union which is 0.05 µg/kg (4). All samples were above the Egyptian regulation in 1990 (Egyptian Regulations, 1990) (milk sold in Egyptian markets should be free of AFM₁).

The obtained results showed that the occurrence of AFM₁ were 66.7 % (22/33) in cows and sheep milk, 63.6% (21/33) in buffalo's milk, 33.3% (11/33) in goat's milk and 15.2% (5/33 samples) in camel's milk, which are similar or lower than reported previously mentioned.

Occurrence and level AFM₁ in the raw milk produced in Luxor Governorate are lower compared with raw milk produced in a similar study (Motawee *et al.*, 2009) in the Ismailia in Egypt, during the summers of 2003 and 2004. They examined 175 milk samples (50 cows, 50 buffalos, 50 goats and 25 camels) and found that all samples were positive for AFM₁. Most milks (80%, 74%, 66% and 52% of the camel, goat, cow and buffalo milks, respectively) were below the European Union maximum of AFM₁ ≤50 ng/L and all milk samples were <500 ng/L.

AFM₁ was detected in camel's milk from Esna city only with low level 0.274 ± 0.341 ng/L (and prevalence (5/33 samples) in comparison with that reported by Balata and Bahout (1996), who reported AFM₁ levels in Egyptian camel milk up to 850 ng/L and by (Motawee *et al.*, 2009), who found AFM₁ levels in camel milk up to 250 ng/L.

Several countries have set acceptable limits of AFM₁ in milk and its by-products to exclude the possible toxicity for humans. In the European Union, the maximum limit of AFM₁ in liquid milk and dried or processed milk products is set at 50ng/L (European Commission Regulation, 2006). In USA, the level of AFM₁ in milk should not be higher than 500ng/Kg (FDA, 2011). In Egypt, the Ministry of Health recognized that fluid milk and dairy products should be free from AFM₁ (Egyptian Regulations, 1990).

Concerning the health hazard for consumers, all positive samples of raw milk are exceeding Egyptian regulations (free from AFM₁), while no milk samples exceeded the permissible limits of the US regulations (500ng/l) and the European Commission regulations (50ng/l).

Prandini *et al.* (2009) reported that more than half of the milk samples are contaminated by AFM₁. The presence of AFM₁ in milk and dairy products is an important issue, especially for developing countries. AFM₁ is stable in kashar cheese for over 60 days and

in traditional white pickled cheese for over 90 days, also the toxin is stable during cheese storage and ripening (Govaris *et al.*, 2002). The mean level of AFM₁ in milk of Punjab, Pakistan was 0.323 mg/L (Sadia *et al.*, 2012). The levels of AFs in food vary from 0 to 50 mg/kg (FAO/WHO, 2009). The levels of AFM₁ in milk and dairy products in Ismailia, Egypt were 0.05 µg/L in Buffalo, 0.05µg/L in Cow, 0.05µg/l in Goat and 0.05µg/l in Camel (Motawee *et al.*, 2009). High rate of contamination were found in raw cow milk from North African countries where the level of AFM₁ ranging between 30 and 3130 ng/L (Elgerbi *et al.*, 2004). In Korea, the concentration of AFM₁ in raw milk was 57ng/L (Kim *et al.*, 2000). In Croatia, in 98.4% of raw milk samples, levels of AFM₁ were less than the maximum acceptance level of the European Union (Bilandzic *et al.*, 2010). AFM₁ concentration in cow's milk samples was 108.2 ng/L in Nigeria (Oluwafemi *et al.*, 2014).

AFM₁ has been detected in milk, which cannot be removed from milk by pasteurization, ultra-high temperature heat processing or other methods (Iqbal *et al.*, 2010). The AFM₁ molecule cannot be inactivated in the dairy industry (Fallah *et al.*, 2011). AFM₁ concentration in milk is related to seasonal variations, and AFM₁ contents in raw milk are the highest during cold seasons (Bilandzic *et al.*, 2015).

The level of AFM₁ in milk samples during winter is significantly higher than summer in all lactating species i.e., dairy cow, buffalo, goat, sheep and camel in Pakistan (Asi *et al.*, 2012). AFM₁ concentration in milk during winter exceeded the European Union limit level, with the maximal level of 1101 ng/L in Adana province of Turkey (Golge, 2014).

In conclusion, high prevalence of AFM₁ in milk from Luxor Governorate indicated that the contamination of raw milk is very high and this due to the contamination of feedstuffs of these animals with AFB₁. Because of these findings, we need to survey aflatoxins incidence and levels in feedstuffs and milk during all seasons of the year in this areas.

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بقايا الأفلاتوكسين م₁ في ألبان المجترات في محافظة الأقصر

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تهدف هذه الدراسة إلى الكشف عن معدل تواجد السم الفطري (الأفلاتوكسين م₁) في ألبان المجترات من أبقار وجاموس وجمال وأغنام وماعر في محافظة الأقصر ومعرفة تركيزاته المختلفة لبيان خطورته على صحة الإنسان المستهلك لهذه الألبان وذلك من خلال التحليل المعمل لعدد 165 عينة تم جمعها خلال فصل الشتاء 2015-2016م من مدن محافظة الأقصر (إسنا - أرمنت - الأقصر) بواقع 55 عينة لكل مدينة حيث تم جمع 11 عينة لكل نوع من هذه المجترات. وقد تم التحليل باستخدام كواشف خاصة بالسم الفطري بواسطة جهاز قارئ الإليزا. تبين من النتائج تواجد الأفلاتوكسين م₁ بمعدل 66,7 و 63,6 و 15,2 و 66, و 33,3% في ألبان الأبقار والجاموس والجمال والأغنام والماعز في جميع مدن محافظة الأقصر على التوالي وبمتوسط تركيز بلغ 4,518 و 1,951 و 0,911 و 2,966 و 0,582 جزء في الترليون. وأحتوى اللبن البقري على أعلى تركيز (4,31 جزء في الترليون) تلاه اللبن الجاموسي (13,177) ثم لبن الأغنام (8,66) جزء في الترليون. اظهرت النتائج ان ألبان كل الحيوانات في مدينة اسنا بها أفلاتوكسين م₁ بمستويات ومعدلات متفاوتة ما عدا عينات البان الماعز والأغنام التي لم يوجد بها السم، ووجد أن أعلى تركيز من الأفلاتوكسين م₁ كان في عينات ألبان الأبقار والأقل منها الجمال ثم الجاموس وكانت نسبة العينات الايجابية في ألبان الأبقار 72,7% وألبان الجاموس 45,5% وألبان الجمال 45,5% وكانت نسبة العينات الايجابية بجميع الحيوانات للأفلاتوكسين م₁ في مدينة اسنا 32,7%. وفي مدينة الأقصر دلت نتيجة التحليل أن ألبان كل الحيوانات بها أفلاتوكسين م₁ ما عدا عينات البان الجمال، ووجد أن أعلى تركيز من الأفلاتوكسين م₁ في عينات ألبان الأغنام والأقل منها الماعز ثم الجاموس وأخيراً عينات الأبقار. وكانت نسبة العينات الايجابية في ألبان الأبقار 36,4% وألبان الجاموس 45,5% وألبان الأغنام والماعز 100%. وبلغت نسبة العينات الايجابية للأفلاتوكسين م₁ في مدينة الأقصر 56,36%. أما في مدينة أرمنت، فقد احتوت ألبان الأبقار والجاموس والأغنام على الأفلاتوكسين م₁ بنسبة 90,9% و 100% و 100% على التوالي، أما عينات ألبان الجمال والماعز فكانت سلبية. وكان أعلى تركيز من الأفلاتوكسين م₁ في عينات ألبان الأبقار وتلاها ألبان الجاموس ثم الأغنام ونسبة العينات الايجابية للأفلاتوكسين م₁ في مدينة أرمنت 58%. وبذلك تكون أعلى نسبة ايجابية للأفلاتوكسين م₁ في مدينة أرمنت تليها مدينة الأقصر ثم مدينة إسنا. وكان تركيز الأفلاتوكسين م₁ في عينات ألبان أبقار أرمنت الأعلى ثم إسنا ثم الأقصر. وتبين أن تركيز الأفلاتوكسين م₁ في عينات جاموس أرمنت أعلى من الأقصر وإسنا. وكان تركيز الأفلاتوكسين م₁ في عينات أغنام الأقصر أكبر من أرمنت. وكانت عينات ألبان الجمال في أرمنت وعينات ألبان الماعز في الأقصر سلبية للأفلاتوكسين م₁. وعلى الرغم من النسبة العالية للعينات الموجبة والتي قد تدل على زيادة معدل تلوث الأغذية الخاصة بهذه الحيوانات بالسم الفطري الأفلاتوكسين م₁ والذي يعتبر المصدر الرئيسي للأفلاتوكسين م₁ فإن أياً من هذه التركيزات التي وجدت في جميع العينات لم تتعد الحدود المسموح بها لهذا السم الفطري بالألبان في كل من الاتحاد الأوروبي والولايات المتحدة الأمريكية مع العلم بأن جميع تركيزاته كانت أعلى من المواصفة المصرية والتي تنص على خلو الألبان ومنتجاتها من هذا السم الفطري تماماً الأمر الذي يتطلب زيادة البحث والمراقبة لهذه السموم في منتجات هذه الحيوانات بهذه المناطق الجغرافية.