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**DETERMINATION OF AFLATOXIN M<sub>1</sub> LEVELS IN  
BOVINE FARMED MILK WITH SPECIAL  
REFERENCE TO THE LEVELS OF  
AFLATOXIN B<sub>1</sub> IN THE ANIMAL FEED  
(With 3 Tables)**

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تحديد مستوى الأفلاتوكسين م ١ في ألبان المزارع البقرية مع الإشارة إلى  
مستوى الأفلاتوكسين ب ١ في أعلاف الحيوانات

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في دراسة لتحديد مستوى الأفلاتوكسين ب ١ & م ١ تم جمع ٤٠ عينة من أربعة مزارع  
مختلفة من مدينة بورسعيد بواقع ٢٠ عينة من كل من أعلاف الحيوانات وألبان  
الأبقار (٥ عينات ألبان و ٥ عينات أعلاف من كل مزرعة). ووجد ان متوسط مستوى  
الأفلاتوكسين ب ١ في عينات أعلاف الحيوانات كان  $41,38 \pm 9,6$  ،  $10,7 \pm 1,6$  ،  $7,48$  ،  
 $3,5 \pm 1,19$  و  $0,9 \pm 1,19$  جزء في البليون، بينما كان متوسط مستوى الأفلاتوكسين م ١ في  
عينات الألبان البقرية  $51,6 \pm 10,8$  ،  $21,48 \pm 4,5$  ،  $35,72 \pm 7,3$  و  $8,63 \pm 3,9$   
جزء في الترليون. وقد أسفرت النتائج عن وجود الأفلاتوكسين ب ١ و الأفلاتوكسين م ١ في  
عينات أعلاف الحيوانات والألبان البقرية بنسبة  $80\%$  (١٦) ،  $65\%$  (١٣) علي التوالي.  
وبمقارنة مستوى الأفلاتوكسين ب ١ & م ١ في العينات موضع الدراسة وجد ان  
 $25\%$  (٥) و  $20\%$  (٤) من كل من أعلاف الحيوانات والألبان البقرية علي التوالي قد تعدت  
الحد المسموح به طبقا لمنظمة FAO/WHO. وقد تم مقارنة مستوي الأفلاتوكسين ب ١  
والأفلاتوكسين م ١ في العينات موضع الدراسة وكذلك مناقشة تأثير الأفلاتوكسين م ١ علي  
صحة المستهلك.

## SUMMARY

A total of 40 samples of animal feed and milk (20 of each) were randomly collected from four small dairy farms and analyzed for aflatoxin B<sub>1</sub> and M<sub>1</sub> respectively. The mean average of AFB<sub>1</sub> and AFM<sub>1</sub> were  $41.38 \pm 9.6$ ,  $1.07 \pm 0.6$ ,  $7.48 \pm 3.5$  and  $1.19 \pm 0.9$  ppb;  $51.6 \pm 10.8$ ,  $21.48 \pm 4.5$ ,  $35.72 \pm 7.3$  and  $8.63 \pm 3.9$  ppt in the examined animal feed and milk samples respectively. The incidence of AFB<sub>1</sub> and AFM<sub>1</sub> was detected in 80% (16) and 65% (13) of the examined animal feed and milk samples respectively. On the other hand, 25% (5) and 20% (4) of the examined animal feed and milk samples were exceeded the limits recommended by FAO/WHO. The relationship between the levels of AFB<sub>1</sub> and AFM<sub>1</sub> in the examined animal feed and milk were discussed. Also the effect of AFM<sub>1</sub> on the human health was discussed. There is need to create awareness and establish routine monitoring of animal feeds and milk to reduce risk to animal and consequently human response.

*Key words: Aflatoxins, Aspergillus spp., milk, animal feed.*

## INTRODUCTION

Aflatoxins (AFs) are secondary metabolites produced by some species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. They are naturally contaminants of food and several feedstuffs, initiated under favorable conditions of temperature, relative humidity/moisture and poor storage conditions with the long storage under favorable conditions molds produce their metabolites in the form of fungal toxins (IARC, 2002; Strosnider *et al.*, 2006).

Aflatoxins are a group of toxins classified into aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. These toxins are fluorescent under the ultraviolet light and don't have flavor and scent. They are resistant to high temperatures up to 320°C thus didn't exterminate or fragment by boiling and pasteurization (Early, 2000). The potential danger of aflatoxins group is the transfer of these toxins to human through food chain (Rory and Enda, 2008).

The toxicity of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in animals is varied due to differences in susceptibility (Lanza *et al.*, 1982). The toxic dose for cattle has been shown to be from 300–700 ppb depending up on the individual animal. The adverse effects include low weight gain and

dietary intake causing growth impairment and depress the immune status (Raisuddin *et al.*, 1993).

In human, aflatoxin B<sub>1</sub> is the most toxic compound produced by some *Aspergillus* species and the most potent hepato-carcinogens. The International Agency for Research on Cancer of WHO cited that aflatoxin B<sub>1</sub> is carcinogenic of "Group 1" for humans (IARC, 2002). The long-term exposure to low levels of AFB<sub>1</sub> in the diet produced carcinogenic, mutagenic, teratogenic, oes-trogenic, neurotoxic and immunotoxic effect (Albert *et al.*, 2006).

In the liver of animal, AFB<sub>1</sub> was transformed biologically by hepatic microsomal cytochrome P450 into aflatoxin M<sub>1</sub> (AFM<sub>1</sub>). Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is the principal hydroxylated AFB<sub>1</sub> metabolite present in milk of cows fed with a diet contaminated with AFB<sub>1</sub> (Battacone *et al.*, 2005) and excreted within 12 hours after administration of contaminated feeds (Battacone *et al.*, 2003; Applebaum *et al.*, 2003). The US Food and Drug Administration (FDA) have indicated that aflatoxin is the only mycotoxin that currently warrants regulation in milk (Wood and Trucksess, 1998).

Milk containing violative levels of AFM<sub>1</sub> cannot be marketed. The action level for most feeds is 20 ppb; the action level for human food is also 20 ppb for total aflatoxins, with the exception of milk which has an action level of 0.5 ppb for aflatoxin M<sub>1</sub>. (FDA, 2005). the acute toxicity begins and seems to be similar or slightly less than that of aflatoxin B<sub>1</sub> but its carcinogenic potency is probably one or even two orders of magnitude lower than that of aflatoxin B<sub>1</sub> (Henry *et al.*, 2001).

This study was initiated to assess the levels of AFB<sub>1</sub> and AFM<sub>1</sub> in animal feeds and milk of different farms respectively, and the significant differences of parameters analyzed.

## **MATERIALS and METHODS**

### **1- Samples collection:**

#### **1-1: Milk:**

Milk samples (20) from four smallholder dairy farming households (5 of each) in Port-Said city were collected in sterile 15 milliliters tubes. All the samples were thoroughly identified and transported to the laboratory in cool boxes. The samples were analyzed as soon as possible and any delay, the samples should be frozen.

## **1-2: Animal feeds:**

From the same smallholder dairy farming households, about 500g of animal feed were taken and thoroughly identified (20 samples) transported to the laboratory and analysed as soon as possible.

## **2- Sample preparation:**

### **2-1: Milk:**

Individual milk sample was centrifuged at 3500 rpm for 10 min. at 10°C for a process of de-fatting. By using a Pasteur pipette, the upper cream layer was removed completely, stored in cool place and protect against light (A.O.A.C., 2000).

### **2-2: Cereals and feed:**

The cereal and feed sample was mixed and thoroughly ground. In a screw cap bottle 20 g of the mixed and ground sample was mixed with 10 ml of methanol: distilled water (70:30, v/v) by using shaker for 10 minutes at room temperature. The homogenate was filtered through Whatman® No. 1 filter paper. 100µ l of the sample filtrate was diluted with 600µ l of the sample dilution buffer. The diluted sample was stored in cool place and protect against light (Kang'ethe and Lang'a, 2009).

## **3- Enzyme immunoassay:**

### **3-1: Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) in milk:**

In each well, 100 µl of the skimmed milk sample were taken and analyzed directly using an ELISA kit for M<sub>1</sub> following the manufacturer's instructions (Ridascreen® Aflatoxin M<sub>1</sub>, purchased from r-biopharm, Germany). Samples were run in duplicates. The sample was diluted and re-tested when OD reading above the reading of 40 parts per trillion (ppt) standards. The kit had a sensitivity of 5ppt.

### **3-2: Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in cereals and feed:**

50µl per well were employed directly in the assay. Competitive enzyme immunoassay for AFB<sub>1</sub> was done using Aflatoxin kit following the manufacturer's instructions (Ridascreen® Aflatoxin, Commercial License Eliza Kit, obtained from r-biopharm, Germany). Any sample having more than 13.5 ppb was diluted further (sample dilution buffer containing 10% methanol) and re-tested. This concentration was the upper limit of the sensitivity of the standard curve. The kit had a sensitivity limit of 1.8 ppb.

## **4- Calculation:**

Special software, the RIDA®SOFT win (Art. No. Z9999) is available for evaluation of the RIDASCREEN® enzyme immunoassays.

The course of the slandered curve is shown in the quality assurance certificate enclosed in the test kit.

Remark for the calculation without software:

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

### 5- Sensitivity:

The mean lower detection limit of the RIDASCREEN ® Aflatoxin total assay is about 50 ng/kg (0.5 ppb) for milk. According to the test preparation record, the detection limit is (1.75ppb) for cereals and feed.

### 6- Statistical methods:

Data were submitted to one-way ANOVA to test differences between treatments, and linear regression analysis was carried out to test AFM<sub>1</sub> and AFB<sub>1</sub> (SPSS, 2007).

## RESULTS

**Table 1:** Quantitative estimation of aflatoxins B<sub>1</sub> and M<sub>1</sub> in the examined animal feed and milk samples in different farms (n=5).

No. of sample	Farm 1		Farm 2		Farm 3		Farm 4	
	AFLB <sub>1</sub> (ppb)	AFLM <sub>1</sub> (ppt)	AFLB <sub>1</sub> (ppb)	AFLM <sub>1</sub> (ppt)	AFLB <sub>1</sub> (ppb)	AFLM <sub>1</sub> (ppt)	AFLB <sub>1</sub> (ppb)	AFLM <sub>1</sub> (ppt)
1	26.43	53.01	3.55	41.12	1.05	27.76	1.794	22.04
2	12.29	54.61	1.750	23.35	1.259	30.46	0.038	15.31
3	45.20	58.03	N/D	19.19	12.048	42.24	N/D	N/D
4	90.20	58.08	0.039	23.74	20.770	44.61	0.116	5.78
5	32.8	34.37	N/D	N/D	2.276	33.53	N/D	N/D

ppb = part per billion

ppt= part per trillion

N/D=not detectable

**Table 2:** Statistical analytical results of aflatoxins B<sub>1</sub> and M<sub>1</sub> in the examined animal feed and milk samples in different farms (n=5).

Farms	Statics	AFLB <sub>1</sub> (ppb)	AFLM <sub>1</sub> (ppt)
Farm1	Min.	12.29	34.37
	Max.	90.20	58.08
	Mean ± SE	41.38 ± 9.6	51.6 ± 10.8
Farm2	Min.	0.039	19.19
	Max.	3.55	41.12
	Mean ± SE	1.07 ± 0.6	21.48 ± 4.5
Farm3	Min.	1.05	27.76
	Max.	20.770	44.61
	Mean ± SE	7.48 ± 3.5	35.72 ± 7.3
Farm4	Min.	0.038	5.78
	Max.	1.794	22.04
	Mean ± SE	1.19 ± 0.9	8.63 ± 3.9

**Table 3:** Incidence of aflatoxin B<sub>1</sub> and M<sub>1</sub> in the examined animal feeds and milk samples in different farms. (n=5).

Farm No.	AFLB <sub>1</sub> in animal feed						AFLM <sub>1</sub> in milk					
	Contaminated samples		Samples exceed PMIs		Samples (ND)		Contaminated samples		Samples exceed PMIs		Samples (ND)	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
1	5	100	4	80	0	0	5	100	4	80	0	0
2	3	60	0	0	2	40	1	20	0	0	1	20
3	5	100	1	20	0	0	5	100	0	0	0	0
4	3	60	0	0	2	40	2	40	0	0	2	40
Total	16	80	5	25	4	20	13	65	4	20	3	15

N/D =not detectable

PMIs =Permissible limits

## DISCUSSION

A total of 20 animal feeds samples were collected from 4 farms (5 of each). The results tabulated in Table 1 showed that the highest recorded levels of aflatoxin B<sub>1</sub> and M<sub>1</sub> were 90.20 ppb and 58.08 ppt in the examined feed animal and milk samples of farm 1 respectively, while the lowest levels were 0.038 ppb and 5.78 ppt in feed animal and milk samples of farm 4 respectively. The results of feed animal samples were agree with the results recorded by Karki and Sinha, (1989) but not agreed with the result of Abdel-Fattah and Yacoub, (2008) who stated that aflatoxin not detected in animal feed. The variation in the levels of aflatoxin B<sub>1</sub> between the different samples and farms may be attributed to the differences in storages facilities of animals' feeds, temperature, humidity and high insect activity (Strosnider *et al.*, 2006). On the other hand, milk samples results were agree with the results of Battacone *et al.* (2003) but disagree with the results recorded by Westlake *et al.* (1989). Also, Table 1 showed a significant relationship between the levels of aflatoxin B<sub>1</sub> and M<sub>1</sub> in animal feed and milk samples respectively. Thus Aflatoxin M<sub>1</sub> used as possible marker of exposure to aflatoxin B<sub>1</sub>, as the amount of AFB<sub>1</sub> ingested increased the AFM<sub>1</sub> concentration in milk rose. These results agree with the result of Veldmann *et al.* (1992); Michael *et al.* (2002) but not agree with that of Frobish *et al.* (1986). The significant relationship between the levels of aflatoxin B<sub>1</sub> and M<sub>1</sub> may be regards to that the considerable part of the ingested aflatoxin B<sub>1</sub> is degraded in the rumen and extensively metabolized in the liver, resulting predominantly in aflatoxin M<sub>1</sub>. Circulating aflatoxin M<sub>1</sub> can be excreted via the kidneys and appears in milk. The excreted amount of aflatoxin M<sub>1</sub> in the milk of dairy cows was estimated to represent 1-2 % of the ingested aflatoxin B<sub>1</sub>. Also the changes in the plasma- milk barrier result in a higher carry over rate of aflatoxin M<sub>1</sub> into milk (Munksgaard *et al.*, 1987; Veldmann *et al.*, 1992).

As shown in Table 2 the mean levels of aflatoxin B<sub>1</sub> and M<sub>1</sub> in the examined animal feed and milk samples were 41.38 ± 9.6, 1.07 ± 0.6, 7.48 ± 3.5 and 1.19 ± 0.9 ppb; 51.6 ± 10.8, 21.48 ± 4.5, 35.72 ± 7.3 and 8.63 ± 3.9 ppt, respectively in the four farms. The variation between the levels of aflatoxin B<sub>1</sub> in different farms and within the same farm is regarded to the variation of the feeds nutrient content of the different farms i.e. feed manufactured from grains is considered the major source for aflatoxin B<sub>1</sub> contamination that find their way to animal feed. Also

high temperature, humidity and moisture content induce the mold growth and aflatoxin B<sub>1</sub> production in animal feed (IARC, 2002).

The obtained results recorded in Table 3 showed that 80% (16) and 65% (13) of the examined animal feeds and milk samples were contaminated with aflatoxin B<sub>1</sub> and M<sub>1</sub> respectively. This result is nearly agreed with the results recorded by Rory and Enda (2008), they reported that eighty six percent of the feed samples from farmers were contaminated with aflatoxin B<sub>1</sub>. Contamination of animal feeds with AFB<sub>1</sub> was prevalent throughout the farms. 20% (4) of samples were free from AFB<sub>1</sub>, Meanwhile, 25% (5) of the contaminated samples had aflatoxin B<sub>1</sub> levels above FAO/WHO limits (20 ppb), and also 20% (4) of contaminated milk samples had aflatoxin M<sub>1</sub> levels above FAO/WHO limits 0.05 µg/Kg (50 ppt). (FAO/WHO, 2002). These results confirm unavoidable contaminants in food and feed, so AFB<sub>1</sub> level must be lowered 20 ppb of total aflatoxin in animal feed to avoid its harmful effects for the animals or human who consume its products. Total rations containing aflatoxin at levels greater than 20 ppb certainly should not be fed to milk cows. In this concern, the Food and Agriculture Organization (1996) reported that 25% of the world's food crops are affected by mycotoxins.

## CONCLUSION

These results suggest that presently the contamination of milk with AFM<sub>1</sub> does not appear to be a serious health problem in Port-said, and may be a direct and immediate correlation between the presence of AFB<sub>1</sub> in feedstuff and the detection of AFM<sub>1</sub> in milk from the same farm. It is necessary to highlight the procedures in place for minimizing potential risks to animals and subsequent risks to consumers from animal derived food products. Aflatoxin M<sub>1</sub> contamination of milk cannot be completely prevented because AFB<sub>1</sub> does occur naturally in grains. It is not practical to completely eliminate AFB<sub>1</sub> from feeds or AFM<sub>1</sub> from milk. However, it is possible to control the amount of AFM<sub>1</sub> present in milk by limiting the amount of aflatoxin in animal feeds. The FDA has established specific guidelines on acceptable levels of aflatoxins in human food and animal feed by establishing action levels that allow for the removal of violative lots from commerce (Smith, 2005). Nevertheless, a continuous surveillance programme may be warranted to monitor regularly the occurrence of aflatoxin in the animal feeds



responsible for current limited contamination and to note rapidly any worsening in the situation that may depend on market changes or on unfavorable climatic developments

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