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Detection of Aflatoxin M1 in the Milk of Naturally Grazed and on-farm-fed Camels

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Abstract

Aflatoxins (AFs) are mycotoxins produced by numerous species of Aspergillus. AFs contaminate agricultural commodities and thus feed and food including milk. So, this study aimed to assess the effect of the feeding type on the occurrence of AFM1 in camel milk. A total of 45 camel milk samples were obtained from the natural grazing herd in Shalatin (n= 20) and small scales farm breeding in Daraw (n= 25) in the period between September to December 2021. AFM1 levels were determined in these samples using the Vicam method. A significant difference was found between AFM1 levels in milk samples from herds of camels kept in a traditional environment (Natural grazing) and that in samples from camels in semi-intensive management systems (On-farm). Of note, all was samples obtained from the nomadic area were free from AFM1, whereas, in the camel milk samples collected from a semi-intensive farm, AFM1 was reported in eight samples (32%) with 2 (8%) samples exceeding the EU Limits of 0.05 µg/ kg and 6 (24%) samples below such EU Limits. In conclusion, there was high contamination of milk samples obtained from camels reared on-farm with AFM1, while milk samples of camels naturally grazed were negative for AFM1. Therefore, milk hygiene from the farm, chilling, and distribution should be evaluated to reduce AFM1 levels in milk.

KEYWORDS
Aflatoxin, Camel milk, Feeding, AFM1, Vicam, Grazing

INTRODUCTION

Camel is the ship of the desert, which is uniquely adapted to the hot environment and its ability to walk in the desert. Camel milk is white and opaque, with a slight but noticeable salty taste and a normal pH (6.2 to 6.5). Proteins in camel milk produce bioactive peptides when digested. Such bioactive peptides are performed as hypoglycemic, hypoallergic, immune stimulants, antimicrobial factors, and anti-carcinogenic (Khalifa and Zakaria, 2019).

Camel milk has a modest fat content, with 96 percent triglycerides and 30 mg/100 g dry matter cholesterol. Its fat contains fewer short-chain fatty acids than cow's milk and more unsaturated fatty acids (Ereifej *et al*., 2011; Khalesi *et al*., 2017). The average size of fat globules is smaller than those of other ruminant milk. Camel milk's unique protein and fat nature may explain its easy digestibility.

In contrast to all edible milk, camel milk is rich in iron and ascorbic acid, perhaps 3 to 5 times more than cow's milk (El-Hatmi *et al*., 2015). Owing to camel's milk's antimicrobial nature; it might resist bacterial and mold contamination and or chemical contaminants. (EL-Fakharany *et al*., 2012).

Aflatoxins are toxic secondary by-products of little molecular weight secreted by filamentous fungi which contaminate food and feedstuffs. Principally contaminate crops and animal feeds kept in humid moist climates (Khalifa and Shata, 2018).

Among aflatoxins the aflatoxin M1 (AFM1), a metabolic form of aflatoxin B1 (AFB1) which is classified as hepatocarcinogenic (IARC, 2012) can be rich to the consumer via consumption of milk from an animal fed on contaminated crops by aflatoxin B1. For detoxification of AFB1, it is biotransformed into aflatoxin M1 (AFM1) by the hepatic microsomal cytochrome P450 in a percentage of 1:3 and secreted with milk after 24 h of first animal ingestion (Forbisch *et al*., 1986).

The degree of aflatoxin elimination in milk varies greatly between animals, depending upon many factors, seasons, and conditions of concentrated feedstuffs for dairy animals could result in increased AFM1 levels in the milk. Bilandzic *et al*. (2017) reported that AFM1 in milk in winter higher than in summer.

Due to AFM1 carcinogenic opportunity many countries and governments across Europe, Africa, and Asia have set acceptable limits for AFM1 in milk and dairy below 0.05 µg/kg (Van Egmond *et al*., 2007).

The consumption of milk contaminated by AFM1 residues is potentially a great health hazard, especially for those who use camel milk as a functional food, which reported by several studies to have carcinogenic (Cullen *et al*., 1987) and immunosuppressive effects (Luongo *et al*., 2013), in humans and animals. Owing to the mutual incidence and carcinogenicity of aflatoxin, the detection of AFM1 in milk is necessity, thus this study was

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performed to investigate the effect of the feeding nature on the incidence of AFM1 in camel milk and to determine the safety of the camel milk as a functional food commonly consumed raw.

MATERIALS AND METHODS

The vials of AFM1 Standard were purchased from Sigma-Aldrich (St. Louis MO, USA), immunoaffinity columns, AflaM1TM, were from Vicam (USA) and methanol was from Merck (Germany).

Sampling

In this study, 45 camel milk samples were obtained from a natural grazing herd (n= 20) in Shalatin village, Red Sea governorate, Eypt and small scales farm breeding (n= 25) in Daraw city, Aswan governorate, Egypt, during the period between September to December 2022 (sampling sites are shown in Fig. 1). Collected samples were kept at −18°C until analysis, using a VICAM Series-4EX fluorometer as described in the manufacturer's catalog.

Fig. 1. Egypt map showing the sampling site (Shalateen and Daraw).

Principles of Afla M1 FL+ Test

The milk samples underwent preparation by adding an extraction solution and then filtered to detect the levels of AFM1. The extract was then applied to the Afla M1 FL+ Test column, this column was injected with specific antibodies for AFM1. The column was washed to eliminate impurities then the AFM1 level was determined utilizing a calibrated fluorometer after the aflatoxin was eluted.

Vicam Afla M1 FL+ fluorometer procedure

The milk sample was centrifugated at 4°C and 5000 rpm for 20 min to remove fat then Aflatoxin M1 was extracted as described by Ruangwises *et al.* (2011). Afla M1 FL+ calibration was performed using Start Up Kit contains (QSO_4). Samples (50ml) were mixed with 5 g sodium chloride and blended with 100 ml methyl alcohol (80%) at high speed for 60 sec. The resulted mixture was then filtered by utilizing filter paper.

Ten ml of the resulted extract was added to 40 ml of purified water for dilution. through a 1.5 µm glass microfiber filters the obtained diluted extract was filtered. Then the Afla M1 FL+ affinity column was used to pass 10 ml of filtered extract at a flow rate of 2 drops/sec followed by water (10 ml, 2 drops/sec). Methanol (HPLC grade) was used to remove aflatoxins. (1 ml, 1 drop/sec) and composed in a clean cuvette. AflaM1 FL+ developer (1 ml) was mixed well with the elute and the obtained extract was submitted to the calibrated fluorometer.

Statistical analysis

SPSS 16 (IBM- Chicago, IL, US) was used to analyze the obtained data, and the differences between mean values were determined using Student's t-test. A result was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

A healthy human diet is based on milk and dairy products as many people, particularly the pediatric population, commonly consume them in the diet (Başkaya *et al.*, 2006). Parallel to the importance of milk and milk products as functional food, the studies on the safety and levels of contaminants including AFM1 in milk and dairy products have been increasing worldwide including in Egypt and Africa.

Levels of AFM among camel milk samples

Several factors influence the composition of milk: breed in front of it, feedstuffs, animal condition, housing system, and seasonal variations being of higher significance. Several types of research indicate the presence of AFM1 in milk and dairy products. Due to the carcinogenic effect of AFM1, there is an increasing need for accurate analytical methods for its trace-level detection. Of note, many studies determined AFM1 in camel milk (Abdel-El-Fatah and Daud, 2002).

The results of the current study (Table 1) revealed a significant difference between AFM1 concentrations in milk samples from herds of camels kept in traditional environments (Natural grazing on grasses and legumes) and camels in semi-intensive management systems (On-farm feeding on concentrates). All (100%) milk samples obtained from camels naturally grazing were nega-

Table 1. AFM1 µg/ kg levels in camel milk.

Samples	No. of samples	No & (%) of +ve	Over limits	Less limits
Natural grazing	20	0	0 (0%)	20 (100%)
On-farm	25	8 (32.00%)	2	23
		P = 0.005*	P=0.196	P = 0.196
Total	45	8 (17.77%)	2	43

None of the natural grazing milk samples were positive for AFM1. On the other hand, 8 on-farm milk samples were positive for AFM1. There was a statistically significant difference (P = 0.005) between natural grazing and on-farm milk samples regarding AFM1 levels.

tive for AFM1, whereas in the milk samples collected from camel rearing in on- farms; AFM1 was found in eight samples (32%) with 2 (8%) samples exceeding the EU Limits of 0.05 µg/ kg and 6 (24%) samples below such EU Limits (Table 2 and Fig. 2). The obtained results were comparable to those reported by Yousof and El Zubeir (2020) who analyzed camel and cow milk for M1 contamination.

Percentage of milk samples contaminated by AFM1

The results obtained in Table 3 revealed that 8 (32%) of examined milk samples from On-farm camels were contaminated by AFM1, and 6 (24%) samples exceeded the EU permissible limits. Compared to the milk from natural grazing camels that were 100% free from AFM1.

In another study, in Kosovo, Bosnia Rama *et al.* (2016) found that AFM1 was a low incidence in the examined samples and was detected only in 2.8%, but less than permissible limits. Low levels of AFM1 were also detected by Fallah *et al.* (2016) in camel milk in Iran. Their reported levels did not exceed ISIRI and EC limits (0.05 µg/kg). Bokhari *et al.* (2017) in Saudi Arabia determined AFM1 in 10 (31%) camel milk samples but non (0%) exceeded the permissible limit. While Motawee *et al.* (2009) in Egypt detected a high incidence of AFM1 56% of examined samples and Yosef *et al.* (2014) in Saudi Arabia found that 78% of camel milk samples were positive for AFM1. In Pakistan Far East, AFM1 exceeded the permissible levels set by the EU and Codex in camel's milk (Asi *et al.*, 2012; Asghar *et al.*, 2018). This may be due to climatic changes, individual variations, and/or feeding nature. Several factors could be influenced by these variations, on top of which the feed

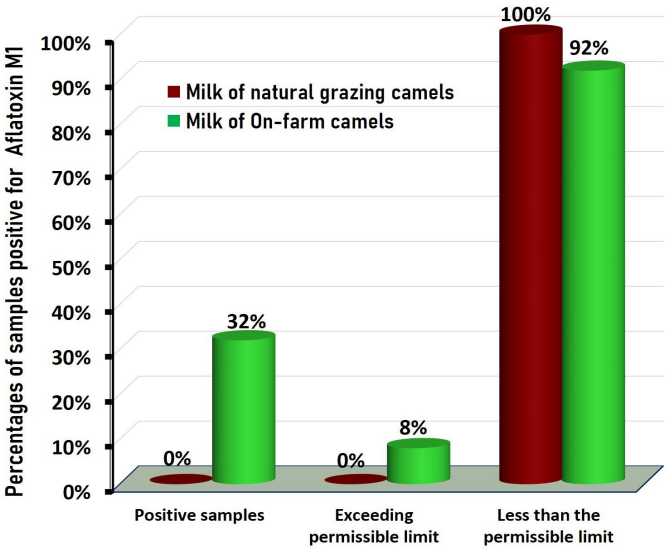

Fig 2. The incidence of camel milk samples positive for aflatoxin M1 (AFM1) and the percentages of samples exceeding the permissible limit. 100% of natural grazing milk samples were negative for AFM1 and thus were below the EU Limits and the US Limits. On the other hand, 8 On-farm milk samples were positive for AFM1 and only 2 of these positive samples (8%) exceeded the EU Limits of 0.05 µg/ kg while the other 6 samples were below the EU Limits. None of the on-farm camel milk samples exceeded US Limits of 0.5 µg/ kg.

Table 2. Aflatoxin M1 (AFM1; µg/ kg) levels in camel milk*

N	Natural grazing camel	On-farm camel	Exceeding EU Limits 0.05 µg/ kg	Exceeding US Limits 0.5 µg/ kg
1	0	0.02		0
2	0	0.05		0
3	0	0.09	x	0
4	0	0.01		0
5	0	0.00		0
6	0	0.03		0
7	0	0.1	x	0
8	0	0.05		0
9	0	0		0
10	0	0		0
11	0	0		0
12	0	0		0
13	0	0		0
14	0	0		0
15	0	0		0
16	0	0		0
17	0	0		0
18	0	0		0
19	0	0		0
20	0	0		0
21	-	0		0
22	-	0		0
23	-	0		0
24	-	0		0
25	-	0		0
Total	20	25	2	0

All natural grazing milk samples were negative for AFM1. On the other hand, 8 on-farm milk samples were positive for AFM1 and only 2 of these positive samples exceeded the EU Limits of 0.05 µg/ kg, however, none of them exceeded US Limits of 0.5 µg/ kg

Table 3. The incidence of AFM1 in camel milk

Origin	N	Positive	Less limits	Exceeding EU Limits 0.05 µg/ kg	Exceeding US Limits 0.5 µg/ kg
Natural grazing	20	0	0	0	0
On-farm camel	25	8 (32%)	6 (24%)	2 (8%)	0
Total	45	8 (17.7%)	6 (13.3%)	2 (4.4%)	0

quality, if feedstuffs were not stored hygienically, they will be susceptible to mold growth and the formation of Aflatoxin B which is ingested by animals and converted in the liver to AFM1 then excreted with milk (Fallah *et al*., 2016).

AFM1 levels recorded in this work could be linked to the type of feed utilized by the dairy camels. Concentrate feeds are considered the major source of aflatoxins. This was supposed previously by several studies (Elzupir and Elhussein, 2010; Elteib *et al*., 2012; Ali *et al*., 2014; Fadlalla *et al*., 2020). These studies recommended that these differences in AFM1 concentrations between the examined milk samples could be due to the nature and quality of feed, camel's diet, breeds of dairy camels, and geographic and seasonal variations. As a result, and to decrease AFM1 levels in milk, the European Commission recognized a limit for AFB1 of 15 µg/kg in dairy camel feed (European Commission, 2006).

In the present study, Absence of AFM1 in all (100%) examined samples of milk from camel naturally grazing could be due to the nature of desert climate and plants that are grown in dry non humid media and thus they are less susceptible to mold growth and mycotoxin formation. In contrast to long-stored concentrates used in on-farm camel feeding (Hussain *et al*., 2010; Fallah *et al*., 2016).

Public health significance of AFM133

Aflatoxins are toxic. When ingested, aflatoxins can exert toxicity by affecting intestinal integrity or modifying the expression of cytokines which can be so dangerous, especially for immune suppressors Liver can convert aflatoxin by specific p450 enzyme to a reactive form of Aflatoxin-8-9-epoxide which attaches the hepatocytes leading to its failure, or it may bind to DNA, leading to hepatocellular carcinoma. Liver cirrhosis is another complication of aflatoxicosis leading to death in both humans and animals at high doses. Long exposure to AFs is maximizing the risk of liver malignancy, (IARC 2012) immune suppression, an increased vulnerability to conditions like HIV and malaria, as well as a potential reduction in the effectiveness of vaccine. (Luongo *et al*., 2013).

CONCLUSION

The current study revealed high contamination of milk samples obtained from camel rearing in semi-intensive farms with AFM1, while milk samples of camel feed from natural yards were negative for AFM1, which could promote it as safe food. Therefore, hygienic measures during milking, feeding, transportation, chilling, and storage must be improved to reduce AFM1 concentrations in camel milk. It is recommended that periodical monitoring of aflatoxins should be applied for all feed, feeding practices, and milk to secure the production of safe milk that rises to be organic food.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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