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ORIGINAL ARTICLE

Prevalence of Bacillus Cereus Virulence Genes in Some Dairy Desserts Assessment of Aflatoxins in Milk and Animal Feed in Kuwait

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Abstract

This study evaluated the prevalence and levels of total aflatoxin (TAF) in animal feed and aflatoxin M1 (AFM1) in milk in Kuwait, focusing on public health and economic impacts. Ninety-six raw milk samples were collected from cows, camels, sheep, and goats during winter 2017. The mean AFM1 concentrations were 0.122, 0.084, 0.056, and 0.051 mg/kg, respectively, with 100% of cow and camel samples, 70.8% of sheep samples, and 58.3% of goat samples exceeding the European Union's safety limits. Additionally, 100 animal feed samples were analyzed, revealing mean TAF concentrations of 13.2 to 14.56 mg/kg, with 8% to 16% of samples surpassing the EU's maximum recommended limits. The findings highlight significant aflatoxin contamination in Kuwait's animal feed and milk, indicating the need for enhanced monitoring and stricter regulations to safeguard public health and reduce economic losses.

Keywords: Aflatoxins, Camel milk, Dairy cattle, Feeds, Goats, Sheep

1. Introduction

Aflatoxins are a group of naturally occurring toxins produced by toxigenic molds, such as *Aspergillus flavus* [1]. There are several types of aflatoxins, including aflatoxins B1, B2, G1, G2, and aflatoxin M1 (AFM1) with aflatoxin B1 (AFB1) being the most toxic to both animals and humans [2].

Mycotoxins can be toxic when ingested by humans and animals. Although the rumen is supposed to be a barrier against mycotoxins, some studies have demonstrated that carryover of mycotoxins into milk is possible. Different studies have reported mycotoxin levels in animal milk, mainly related to contaminated feed for ruminants. AFM1 is the most studied mycotoxin in milk, and its levels that exceed the EU maximum level in this matrix (0.050 μg/kg) have been found. The maximum levels of other mycotoxins in milk have not been established; however, ochratoxin A, aflatoxins G1, G2, B1, B2, and M2, fumonisin B1, cyclopiazonic acid, zearalenone and its metabolites, and deepoxy-deoxynivalenol have also been found in milk samples. Considering that multi-exposure to mycotoxins is the most likely scenario, the co-occurrence of mycotoxins could affect their toxicological effects in humans and animals [3].

Food contamination with AF is of particular importance today and in global organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), and Codex (the Codex Alimentarius Commission) that have determined the maximum level of contamination of various foods. The US Food and Drug Administration (USFDA) has set the action level for AFM1 in milk and total AF in animal feed to be 0.5 mg L^{-1} and 20 mg kg^{-1}, respectively. Since the first of January 1999, EU-wide uniform residue limits for AFs have been established. For AFM1, the limit has been fixed at 0.05 μg/L (50 ppb).

AFM1 (AFM1) is a metabolite of AFB1, once AFB1 is ingested by the animal from contaminated feed, it

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is rapidly absorbed from the intestinal tract and is transformed into AFM1, which enters the blood within 15 min and then is secreted in milk [4].

AFM1 is a monohydroxylated derivative of AFB1, which is metabolized by the cytochrome P450 system in the liver and excreted in the milk of lactating cows fed an AFB1-contaminated diet [5]. AFs are highly toxic, teratogenic, carcinogenic, and mutagenic compounds implicated in human hepatocarcinoma [6–8]. Furthermore, mycotoxins exhibit a variety of biological effects in animals: liver and kidney toxicity, central nervous system effects, and estrogenic effects, to name a few. Some mycotoxins such as aflatoxin, fumonisin, and ochratoxin are carcinogenic. Aflatoxins may cause both acute and chronic diseases in both animals and humans, including liver cirrhosis, acute liver damage, and tumor induction [9].

Because of potential public health concerns, the maximum residue level (MRL) of AFM1 in milk and dairy products has been regulated worldwide to protect consumers. The MRL varies from 0 to 50 ng/kg depending on the country [10]. AFM1 is a very stable aflatoxin, so it is not destroyed by storage or processing, such as pasteurization, autoclaving, or other methods used in the production of fluid milk; if present in raw milk, it may persist in final products for human consumption [11].

To reduce AFM1 contamination, regulatory authorities in most countries have established permitted limits for AFM1 in milk and milk products ranging from 50 ng/L (EU) to 500 ng/L in the United States and China [12].

Owing to public health concerns and the consequent economic losses due to the presence of AFs in foods, the objective of this study was to determine the prevalence and quantify the levels of TAF and AFM1 in animal feeds and milk, respectively, in the state of Kuwait. To assess the knowledge and practices of dairy farmers and feed millers regarding AF in feed and milk.

2. Methods

2.1. Sample collection

A total of 100 animal feed samples (25 each of cow, shecamel, sheep, and goat) as well as a total of 96 raw milk samples (24 each of cow, shecamel, sheep, and goat) were randomly collected from different areas in the state of Kuwait, at winter season, 2017. All the samples were collected and stored at optimum temperature for each product until examination. AFM1 levels in raw milk and TAF levels in the feed samples were analyzed using enzyme-linked immunosorbent assay (ELISA).

2.2. Determination of aflatoxin M1

2.2.1. Extraction procedure of milk samples

For each sample, 5 ml of milk was placed in a test tube and incubated for 30 min at 4 °C, followed by centrifugation at $3000\times g$ for 10 min. About 0.4 ml of milk serum below the fat layer was mixed with 0.1 ml of 100 % methanol (the ratio between milk serum and methanol was 4:1), and the sample was ready for ELISA testing.

2.2.2. Aflatoxin detection in milk

A total of 100 milk samples were analyzed for AFM1 using the RIDASCREEN test kit (RIDASCREEN AFM1 (Art. No.: R11211, detection range, 5–80 ng/kg)) according to the manufacturer's instructions. The LOD was 0.005 μg/l, and solutions of 0, 5, 10, 20, 40, and 80 μg AFM1/l were used for quantification. 2.2.3. Carryover-rate calculations. The total amount of AFM1 excreted in milk was calculated considering the AFM1 concentration in milk (in μg/l milk).

2.3. Determination of total aflatoxin in feed samples

Two grams of ground feed sample were placed in a glass vial with a screw-on cap. Then, 10 ml of methanol and distilled water (70/30) were added and mixed at room temperature using a shaker. The mixture was filtered using filter paper, and 100 μL of the filtrate was diluted with 600ul of the sample dilution buffer. Then, 50 μL of the diluted sample was used for ELISA determination of total aflatoxin. Enzyme-linked immunosorbent assay (ELISA) was used in this study. By following the manual instructions, commercial ELISA kits were purchased from RIDASCREEN aflatoxin's total (Cat. No. R4701). The TAF concentration in the feed was calculated as the sum of the AFB1 concentrations in each ingredient (in μg/kg).

2.4. Statistical analysis

Values were statistically analyzed, and descriptive statistics were analyzed using SPSS version 20 and Excel. Numerical variable data are presented as frequencies, percentages, and means ± standard deviations. Bar charts were used to express the total average and percentage of noncompliant AFM or TAF samples exceeding the selected samples.

3. Results and discussion

In this study, the mean concentrations of AFM1 in dairy cow, camel, sheep, and goat milk samples were 0.122, 0.084, 0.056, and 0.051, respectively (Table 1, Table 2 and Fig. 2). It was observed that almost 100.0, 100.0, 70.8 %, and 58.3 % of the cow, camel, sheep, and goat milk samples collected from dairy farms situated in the state of Kuwait, respectively, were contaminated higher than the safe limit of EU less than 0.05 mg/kg. The most effective way to prevent AFM1 contamination in milk is to reduce AFB1 in food and supplements used in dairy cattle. Therefore, it is recommended that the feed available be monitored permanently for the amount of aflatoxin contamination. Storage and harvesting of forage and other feedstuffs should be technically and hygienically performed, and feeds susceptible to molding, especially flour, bread, pulverized sugar beet pulp, and wet and moldy fodder, should be removed from the diet of lactating animals.

This study was conducted on a hundred samples of animal feed randomly collected from different areas in the state of Kuwait (25 cows, camels,

Table 1. Descriptive statistics of total aflatoxin for animal milk samples.

	Maximum	Minimum	Mean	SD	SEM	Median	Mode
Dairy cow milk	0.152	0.097	0.122	0.012	0.002	0.119	0.112
Camel milk	0.114	0.057	0.084	0.017	0.003	0.084	0.100
Sheep milk	0.074	0.039	0.056	0.009	0.002	0.055	0.049
Goat milk	0.072	0.028	0.051	0.011	0.002	0.050	0.050

Table 2. Occurrence of total aflatoxin for animal milk samples.

Type of samples	Source of milk	No. of samples	No. of samples above EC limit °	No. of samples below EC limit °	Range (ug/kg)
Dairy cow milk	Local	24	24 (100 %)	0	0.097–0.152
Camel milk	Local	24	24 (100 %)	0	0.057–0.114
Sheep milk	Local	24	17 (70.8 %)	7 (29.2 %)	0.039–0.074
Goat milk	Local	24	14 (58.3 %)	10 (41.7 %)	0.028–0.072

° The regulatory limit for aflatoxin in the European Union (EU) is 0.05 ug/kg.

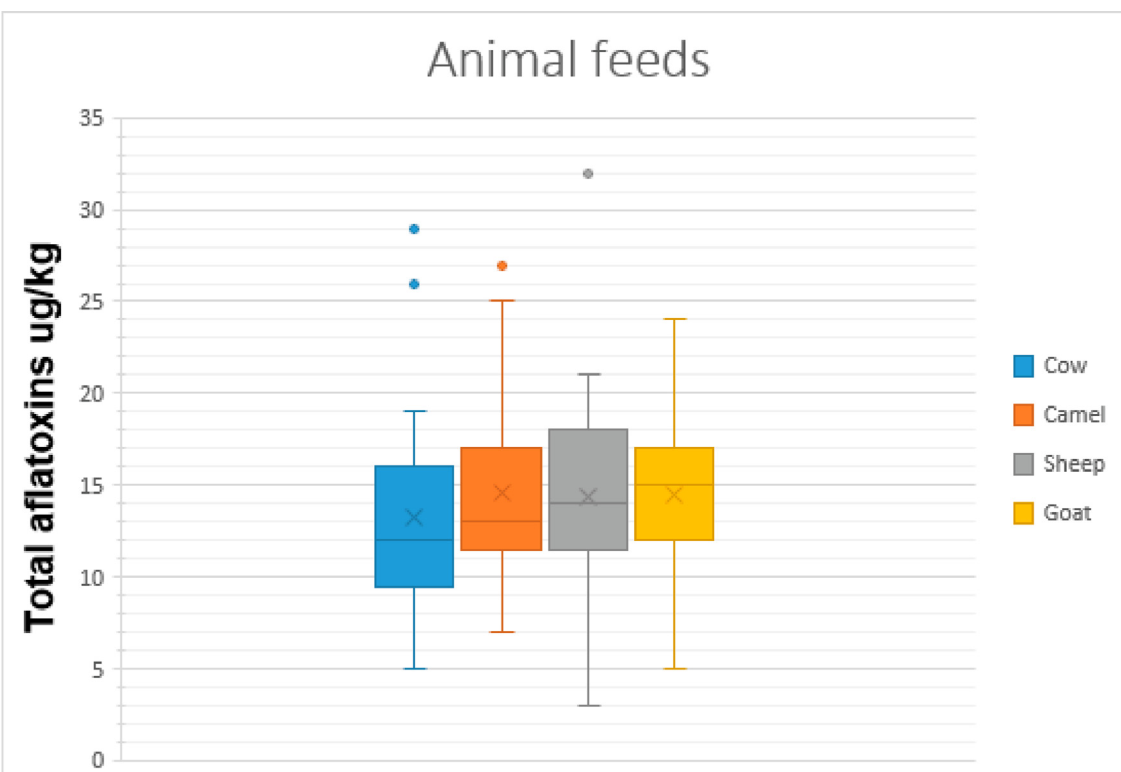

Fig. 1. Different types of feed samples tested for total aflatoxins.

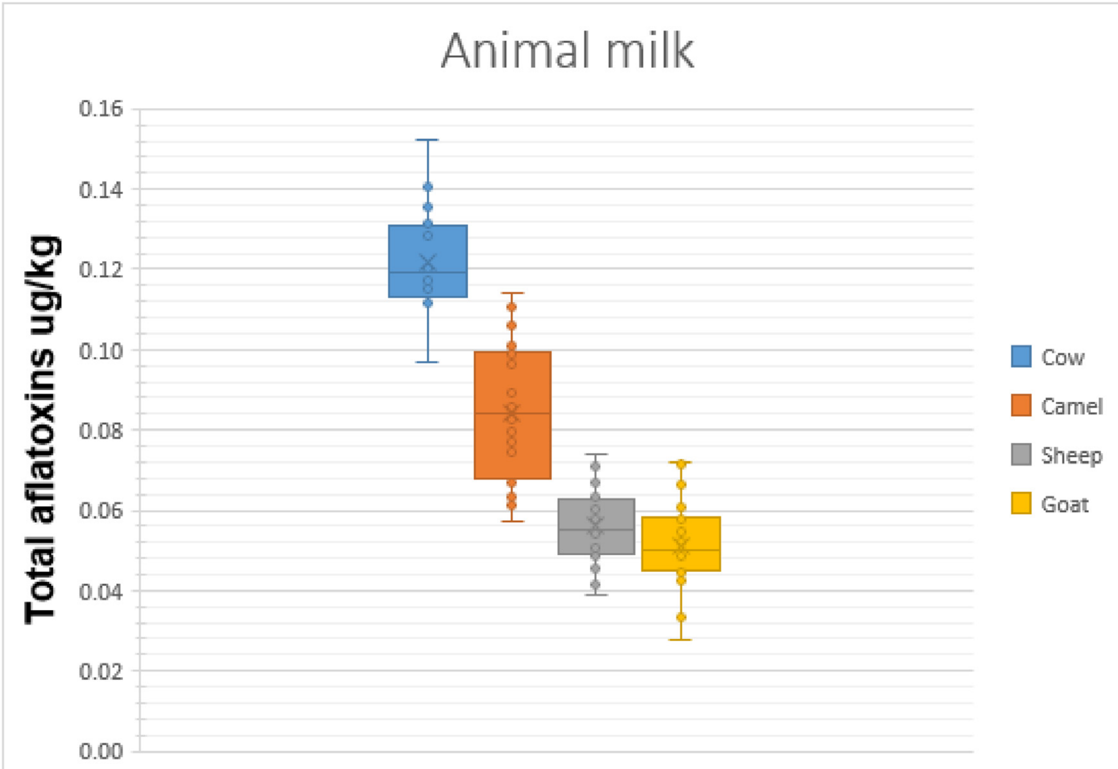

Fig. 2. Types of feed samples tested for total aflatoxins.

sheep, and goats), and TAF contamination was detected. The mean concentrations of TAF in dairy cow, camel, sheep, and goat feed samples were 13.2, 14.56, 14.36, and 14.36, respectively (Table 3, Table 4 and Fig. 1). A total of 8, 16, 8, and 4 % of the cow, camel, sheep, and goat samples, respectively, had higher levels than the maximum recommended limits by the European Union for feed samples.

In similar studies in Pakistan, AFM1 contamination was observed the highest in Eastern cluster (0.59 ± 0.03 μg/L) followed by Northern (0.51 ± 0.30 μg/L), Western (0.51 ± 0.30 μg/L), and Central (0.46 ± 0.30 μg/L) cluster, while Southern cluster (0.46 ± 0.30 μg/L) remained lower throughout the year as compared with the Eastern cluster. The average AFM1 contamination levels and the number of samples exceeding US permissible limits decreased from

Table 3. Descriptive statistics of total aflatoxin for animal feed samples.

Types of feed	Maximum	Minimum	Mean	SD	SEM	Median	Mode
Dairy cow feed	29	5	13.2	5.72	1.14	12	12
Camel feed (Alfa Alfa hay pellets)	27	7	14.56	5.39	1.08	13	12
Sheep feed	32	3	14.36	5.63	1.13	14	14
Goat feed	24	5	14.52	3.98	0.80	15	15

Table 4. Occurrence of total aflatoxin for animal feed samples.

Type of samples	Source of feeds	No. of samples	No. of samples above EC limit °	No. of samples below EC limit °	Range (ug/kg)
Dairy cow feed	local	25	2 (8 %)	23 (92 %)	5–29
Camel feed (Alfa Alfa hay pellets)	local	25	4 (16 %)	21 (84 %)	7–27
Sheep feed	local	25	2 (8 %)	23 (92 %)	3–32
Goat feed	local	25	1 (4 %)	24 (96 %)	5–24

° The regulatory limit for aflatoxin in the European Union (EU) is 20 ug/kg.

north to south as topography, temperature, rainfall, humidity, and weather conditions varied considerably. However, the average AFM1 values of all clusters were not statistically significant. The current findings are similar to those of Jawaid, Talpur, Nizamani, and Afridi (2015) [13], who reported 96.43 % AFM1-contaminated samples with an average contamination level of 0.38 μg/L. Other studies from Greater Addis Ababa found that 26.3 % of milk samples collected from farms were above the US permissible limits [14]. Similar findings were reported by Iqbal and Asi (2013) [15], where 71 % of milk samples were found contaminated [16]. Aflatoxin contamination of raw, pasteurized, and powdered milk samples from the Syrian market was reported to be 22, 32, and 58 % above the permissible limits of America, Syria, and Europe, respectively. Many studies from Pakistan reported raw milk contamination as 58 % [15], 72 % for buffalos [17], 53 % [18], 42 % of milk samples from urban areas, and 27 % from rural areas [15], which were well above the limits permitted by the European Union (EU). A study from India reported that 99 % of fresh milk samples exceeded Codex limits [16].

4. Conclusion

The levels of contaminated animal feeds and milk mentioned in this study with TAF and AFM1 suggest the importance of implementing Good Practices in obtaining feed for dairy cows, and strict monitoring of raw materials and feed samples to prevent cattle exposure to aflatoxin-contaminated feeds, which would lead to the excretion of AFM1 in milk and eventually pose a risk to the animals themselves, as well as humans, through consumption of contaminated milk (Tables 3 and 4 and Fig. 2).

Ethical approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

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Data access statement

Data are provided within this study are available from the NN repository located at www.NNN.org/download/.

Author contributions

All authors equally contributed to this study conception, implementation, analysis, and writing.

Conflicts of interest

The authors declare that they have no conflict of interest.

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