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Artículo Original

Occurrence of aflatoxin M_1 and its main determinants in raw cow milk the Chiriquí in Panama farms

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Abstract: Occurrence of aflatoxin M_1 and its main determinants in raw cow milk the Chiriquí in Panama farms. Introduction: Aflatoxin contamination and its effects on human health have been a global concern and a research subject in developed countries for many decades. However, data generated from Latin America are scarce. **Objective.** To evaluate the occurrence of aflatoxin M_1 (AFM_1) and to identify its determinants in raw cow milk under current farm feeding practices in Panama. **Materials and Methods.** The study included 32 raw milk samples and 79 dairy cattle feed samples from 32 farms suppliers for a Panama dairy processing company. Contents of aflatoxin B_1 (AFB_1) and AFM1 were determined using HPLC-FLC and immunoaffinity columns. The carry-over of AFB1 to AFM_1 was determined according to aflatoxin contents in feed and raw milk. A survey was also applied to all dairy farmers in the study regarding the explanatory variables associated with AFM_1 occurrence in milk. **Results.** The overall prevalence of AFB_1 in feeds was 39.2%, whereas the highest prevalence was for concentrate feed (74.3%) and the lowest (3.70%) for fresh forages. The occurrence of AFM_1 in milk was 46.8%. The carry-over from AFB_1 to AFM_1 averaged 2.36%, which is characteristic of low-producing cows. **Conclusion.** The incidence and concentration of AFB_1 in dairy cattle feed observed in the present study resulted under the maximum levels set by the U.S. Food and Drug Administration (FDA) and the European Union Commission Regulation (EC). Under current feeding practices, silage and haylage fed are determinants of AFM_1 occurrence in tropical raw cow milk. **Arch Latinoam Nutr 2025; 75(2): 97-107.**

Keywords: Aflatoxin B_1, cattle feed, management practices, HPLC

Resumen: Presencia de aflatoxina M_1 y sus principales determinantes en la leche cruda de vaca en las fincas de Chiriquí, Panamá. **Introducción:** La contaminación por aflatoxinas y sus efectos en la salud humana ha sido de preocupación global y un tema de investigación en los países desarrollados durante muchas décadas, pero los datos generados desde América Latina son escasos. **Objetivo.** Evaluar la presencia de aflatoxina M_1 (AFM_1) e identificar sus determinantes en la leche cruda de vaca bajo prácticas actuales de alimentación en Panamá. **Materiales y métodos.** El estudio incluyó 32 muestras de leche cruda y 79 muestras de alimento para ganado lechero de 32 fincas proveedoras de una empresa procesadora de lácteos en Panamá. Los contenidos de aflatoxina B_1 (AFB_1) y AFM_1 se determinaron utilizando HPLC-FLC y columnas de inmunoafinidad. Se calculó la tasa de transferencia de AFB_1 a AFM_1 de acuerdo a los contenidos de aflatoxina en el alimento y la leche cruda. También se aplicó una encuesta a todos los productores lecheros participantes en el estudio, sobre las variables explicativas asociadas con la ocurrencia de AFM_1 en la leche. **Resultados.** La prevalencia total de AFB_1 en los alimentos fue del 39,2%; la prevalencia más alta fue para los concentrados (74,3%) y la más baja (3,70%) para los forrajes frescos. La aparición de AFM_1 en la leche fue del 46,8%. La tasa de transferencia de AFB_1 a AFM_1 promedió 2,36%, característico en vacas de baja producción. **Conclusiones.** La presencia y concentración de AFB_1 en el alimento para ganado lechero observada en el presente estudio resultó por debajo de los niveles máximos establecidos por las regulaciones de la Administración de Alimentos y Medicamentos de los Estados Unidos (FDA) y la Comisión de la Unión Europea (EC). Según las prácticas de alimentación actuales, el tipo de ensilaje y el henolaje suministrado son determinantes de la ocurrencia de AFM_1 en la leche cruda de vaca en el trópico. **Arch Latinoam Nutr 2025; 75(2): 97-107.**

Palabras clave: Aflatoxina B1, alimento para ganado, prácticas de manejo, HPLC

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Introduction

Aflatoxins are secondary metabolites formed under fungi stress conditions and are mainly produced by the genus *Aspergillus spp: Aspergillus flavus* and *Aspergillus parasiticus*. Fungal growth can occur from plant growth to pre- and post-harvest. *Aspergillus flavus* is found in the soil, contaminates many crops, and can be found in several foods and feed (1-3). Aflatoxin is an essential concern in animal nutrition and human health (4), and there are many types of them, where AFB_1 and AFM_1 are the most toxic. Aflatoxin B_1 is metabolized to AFM_1 in the liver to be excreted through the urine and secreted through the milk. It causes acute aflatoxicosis, characterized by general gastrointestinal symptoms and liver, kidney, and brain failure (5,6). Aflatoxin B_1 also causes chronic aflatoxicosis upon regular ingestion of low doses, inducing the appearance of hepatocellular carcinoma due to carcinogenic, teratogenic, mutagenic, hepatotoxic, and immunosuppressive activities (4). Other effects of AFB_1-contaminated food ingestion in humans and animal feed intake include delayed growth, malnutrition, and immunomodulation (7). It has been documented in dairy cows that AFB_1 has an accumulative effect and may lead to digestive and reproductive harm, interfere with nutrient absorption, and affect the nervous system (8,9).

Various factors, including climate conditions, farming intensity, storage conditions, feed type, source, and quantity fed, influence the presence of AFB_1 in dairy cattle feed. It has been reported that silage, forages, cottonseed, and commercially processed feed have the highest AFB_1 contamination (10,14). AFM_1 has been reported in dairy cattle milk after consuming feed contaminated with AFB_1. A portion of AFB_1 consumed by the cow is reduced to aflatoxicol in the rumen. The remaining portion is absorbed in the small intestine and transported to the liver, where different aflatoxin metabolites are synthesized (15). In the liver, AFM_1 is the primary hydroxylated metabolite of absorbed AFB_1, with a carry-over in cattle milk varying between 0.2 and 6% (11,16).

According to current regulations by the EU and FDA, maximum permitted limits have been set to monitor AFB_1 levels in animal feed and AFM_1 in human food. The EU has set an AFB_1 limit of 5 µg/kg for cattle feed, while the FDA has 20 µg/kg. Regarding fluid milk destined for human consumption, the EU has established an AFM_1 limit of 0.05 µg/L, while the FDA has 0.5 µg/L.

Only a few studies, including a total diet study, have been done in Panama since 1988 to monitor aflatoxin contamination levels and other mycotoxins in foods for human consumption (17-20). While the available information about these toxins is known nationally, there has been no joint approach for studying AFM_1 occurrence in cow milk, contamination paths, and risk factors associated with milk production in Panama. Seeing that there is a high incidence of cancer in the Panamanian population and aflatoxins are highly carcinogenic, it is necessary to monitor the occurrence of AFM_1 in milk. Outputs might contribute to identifying determinants associated with AFM_1 contamination in milk and scientifically justify good feeding management practices on tropical dairy farms aiming to reduce AFM_1 occurrence in milk (21). The present work was an exploratory study to evaluate the occurrence of AFM_1 and to identify its determinants in raw cow milk under current farm management practices in Panama.

Materials and methods

Standards and reagents

The analyses were performed using AFB_1 and AFM_1 standards (Trilogylab; Washington, USA), methanol and acetonitrile (Merck, Germany), AFB1 immunoaffinity column (Afla-OtaCLEAN; LCTech; Germany), AFM_1 column (AFLAPREP® M; R-Biopharm; Germany), and type 3 purified water (Smart-N Series water purifying system; Heal Force, China).

Sample preparation

Raw milk samples were thawed to 37°C in a water bath, and the fat layer was dispersed using a stir bar. Thawed samples were centrifuged at 2000 x g (Rotina 38R, Hettich Zentrifugen, Germany) to separate and discard the fat and filtered through Whatman #1 filter paper. A volume of 50 to 100 mL of the filtered residual was collected. An aliquot of 50 mL of filtered residual was passed through

the immunoaffinity column with a flow of 2-3 mL/min applying vacuum. The column was washed with 20 mL of water and dried with a nitrogen flow to eliminate the remaining water, and the AFM_1 was slowly eluted with 4 mL of acetonitrile. The acetonitrile was left in contact with the column for at least 60 seconds, evaporated using a nitrogen flow, and diluted in the mobile phase.

Concentrate feed, fresh and conserved forage samples were processed in amounts of 20 g, extracted with 100 mL of methanol at 80%, agitated at high speed using a vortex (MS-X, Scilogex, USA) for 5 min, and filtered through a Whatman #1 filter paper. A filtrate aliquot of 14 mL was taken and diluted with 84 mL of a phosphate buffer solution with 7.2 pH. Then, 50 mL of the diluted extract was passed through the immunoaffinity column at a flow no greater than 2 mL/min, using vacuum or syringe suppression. The column was washed with 10 mL of distilled water, and the residual water was removed. Aflatoxins were eluted with 1 mL of methanol, and this step was repeated twice, leaving it to act in the gel for 5 min. After this, it was dried with nitrogen and diluted in the mobile phase.

Equipment and chromatographic conditions

Concentrations of AFM_1 and AFB_1 were determined using a high-resolution liquid chromatograph (HPLC-FLD; Agilent Technologies; series 1260 Infinity chromatograph, USA). Chromatographic conditions were set for a 100-sample auto-sampler, injection volume of 100 µL, with a quaternary pump system, thermostatized column compartment, and fluorescence detector. A post-column UVE photochemical derivatizer (LCTech, Germany) was used for the aflatoxin derivatization.

Aflatoxin B_1

A modification of the methodology proposed by Xiao Y and R, Huber U. (22) was used to determine AFB1. Concentrate feed, fresh, and conserved forage samples were analyzed according to the AOAC Official Method 2003.02 (23), specifically, AFB_1 in Cattle Feed Immunoaffinity Column Liquid Chromatography Method First Action 2003 (Applicable for the determination of aflatoxin B_1 > 1 ng/g in cattle feed). Chromatographic separation for AFB_1 was done using a Poroshell 120 EC-18 column (4.6 x 50 mm, 2.7 µm) with a mobile phase composed of water/methanol (65/35) and an isocratic flow of 1.2 mL/min. The injection volume was 100 µL, column temperature was 30°C, excitation wavelength was 365 nm, emission wavelength was 440 nm, and run time was 15 min. The calibration curve was prepared for quantification in 16 to 26 µg/Kg with a sample detection limit of 0.083 µg/Kg. Methodology validation included verification of quantification (LQ) and detention (LD) limits, % recovery, linearity and precision, and the calibration curve for AFB_1 concentration calculated from the analyzed samples. For AFB1 concentration, the detection limit was 0.083 µg/Kg, and the quantification limit was 0.232 µg/Kg, with an R2 of 0.999.

Aflatoxin M_1

The concentration of AFM_1 was determined according to the AOAC Official Method 2000.08 (23) for Aflatoxin M_1 using Liquid Chromatography First Action 2000D.1. Chromatographic separation for AFM1 was done using a Porochell 120 EC-18 column (4.6 x 50 mm, 2.7 µm), with a mobile phase composed by water/acetonitrile (75/25) in an isocratic flow of 0.8 mL/min. The autosampler injection volume was 100 µL, column temperature was 25°C, excitation length was 365 nm, emission length was 435 nm, and run time was 15 min. The calibration curve for AFM_1 was prepared in a range of 0.6 – 4.0 µg/L with R2 of 0.999. The AFM_1 detection limit was 0.066 µg/L, and in the sample was 0.003 µg/L; the AFM_1 quantification limit was 0.173 µg/L and in the sample was 0.009 µg/L.

Sample analysis and chromatograms

Raw milk samples were analyzed in triplicates in HPLC-FLC, injecting 100 µL of the sample under a 15-minute run sequence and the chromatographic conditions previously described. The resulting chromatograms were individually examined to identify the presence or absence of AFM_1. Similarly, all concentrate feed, fresh, and conserved forage samples were analyzed in triplicates, and resulting chromatograms were analyzed to identify AFB_1 levels. Subsequently, a database was developed for statistical analysis.

Carry-over of AFB1 to AFM_1

The carry-over (CO, %) of AFB1 to AFM1 was calculated according to the following equation:

$$CO\ (\%) = \frac{(\text{AFM1 in milk (µg/cow/d)})}{(\text{AFB1 ingested (µg/cow/d)})} \times 100\% \quad (16)$$

The amount of AFM_1 in milk was quantified as the product of the concentration of AFM1 in the milk sample (µg/L) multiplied by the daily milk volume produced per cow (L/cow). The amount of AFB_1 ingested with the feed was quantified as the product of the concentration of AFB_1 in each type of feed (µg/Kg) on a dry matter basis multiplied by the amount of dry matter intake (DMI) of each feed ingested by the cows according to the feeding scenario of the farm. An average body weight of 450 kg was used to estimate DMI along with the average daily milk production per cow for each farm (DMI, kg/d = (2.6 × 450 kg)/100 + (0.186 kg × kg of milk production).

Statistical analysis

After examining the survey applied to the farms, a univariate analysis was done through a generalized linear model (GLM) to identify potential explanatory variables associated with AFM1 in milk. Variables with $p \leq 0.15$ were selected for later multivariate analysis. Multivariate logistic regression analyses were done using a generalized linear mixed model (GLMM) with dairy farms as a randomized effect to evaluate the effect of the chosen explanatory variables. When the response variable was considered continuous (e.g., AFM_1 concentration in milk), it was assumed that it followed a Gamma or Binomial distribution when the response variable was categorized as presence or absence according to the analytical techniques' detection limits and/or the limits set by food safety regulatory entities. A manually conducted backward elimination strategy was followed, excluding one variable with the highest *p*-value at a time. With each variable deleted from the model, the coefficient was verified for the significant variables, which resulted in a changeover of 20% on the estimations. All statistical analyses were done with InfoStat software (24).

Results

The average milk yield of the studied farms was 804 L/day (range = 120 – 3,300 L/day) with an overall daily milk production of 12 L/cow (range = 6.60 – 21.5), and the average lactation herd size was 64 cows (range = 12 – 180 cows) during the study. Generally, the volume of milk produced in these farms represents 4.71% of the national daily milk production and 11.3% of the daily production in Chiriqui. Farm feeding management practices, level of feed intake, and available feed type were variable among these dairy farms. Estimated DMI averaged 13.9 kg/d (range = 12.9 – 15.7).

Aflatoxin B_1 in dairy cattle feed

The occurrence of AFB1 in the various feeds given to dairy cows was 39.2%, with an overall mean concentration of 0.205 ± 0.206 µg/Kg (Table 1). Concentrates were the feeds with the highest AFB1 occurrence (74.3%), and the average concentration was 0.513 ± 0.851 µg/Kg of dry matter. The incidence of AFB1 in fresh forage was 3.70%, and the average concentration was 0.079 ± 0.016 µg/Kg of dry matter.

Table 1. Aflatoxin B1 concentration (µg/Kg) in feed samples collected from 32 dairy farms in Chiriquí, Panamá.

Dairy cattle feed type	Sample size	[1]Positive samples	Aflatoxin B1 concentration (µg/Kg)	
			[2]Range	[3]Mean ± SD
Concentrate feed	35	26	<DL - 4.976	0.513 ± 0.851
Fresh forage	27	1	<DL - 0.165	0.079 ± 0.016
Corn silage	7	2	<DL - 0.367	0.125 ± 0.107
Grass silage	8	2	<DL - 0.243	0.104 ± 0.079
Haylage	2	0	<DL - >DL	[4]ND
Overall	79	31	<DL - 0.205	0.205 ± 0.206

[1]Positive samples > detection limit (DL) / Muestras positivas > límite de detección (LD); [2]Min-Max / Rango mínimo y máximo; [3]Mean sample ± standard deviation / Media muestreal ± desviación estandard; [4]Not determined / No determinado; DL= 0.0834 µg/Kg of dry matter in HPLC-FLD / LD = 0.0834 µg/Kg de materia seca en HPLC-FLD.

Aflatoxin M_1 in raw milk

Fifteen of 32 milk samples resulted positive for AFM_1 (<DL – 0.0143). The mean ± SD concentration was 0.004 ± 0.003 µg/L (DL = 0.003 µg/L in HPLC-FLD). None of these samples exceeded the regulation limits established by the EU and the FDA.

Carry-over of AFB_1 to AFM_1

The overall carry-over of AFB_1 to AFM_1 averaged 2.60%, ranging from 0.03 to 10.8% (Table 2); 12 farms rose above the average. For most farms, the carry-over was below 6%, except for farms 11, 21, and 32, averaging 10.8%, 6.92%, and 9.04%, respectively.

Table 2. Carry-over of aflatoxin B1 to aflatoxin M1 in dairy farms. Samples were collected from 32 dairy farms in Chiriquí, Panamá

Dairy farm	Aflatoxin B1 ingestion (µg/d)	Milk production (L/cow/d)	Aflatoxin M1 concentration in milk (µg/L)	Aflatoxin M1 secretion in milk (µg/d)	[1]Carry-over of aflatoxin B1 to aflatoxin M1, (CO %)
1	3.365	11.4	0.0068	0.0775	2.30
2	5.905	9.40	0.0028	0.0263	0.45
3	3.314	21.5	0.0068	0.1462	4.41
4	3.812	9.30	0.0068	0.0632	1.66
5	2.223	11.0	0.0068	0.0748	3.36
6	1.470	12.5	0.0011	0.0138	0.94
7	1.673	9.40	0.0031	0.0291	1.74
8	1.789	11.2	0.0027	0.0302	1.69
9	9.617	8.80	0.0057	0.0502	0.52
10	1.956	18.5	0.0005	0.0093	0.47
11	1.164	10.7	0.0117	0.1252	10.8
12	3.531	12.5	0.0082	0.1025	2.90
13	1.471	10.8	0.0052	0.0562	3.82
14	1.220	15.8	0.0031	0.0490	4.02
15	2.776	15.5	0.0022	0.0341	1.23
16	1.268	9.40	0.0013	0.0122	0.96
17	1.792	6.60	0.0045	0.0297	1.66
18	2.836	12.0	0.0086	0.1032	3.64
19	2.378	9.60	0.0045	0.0432	1.82
20	2.232	11.3	0.0082	0.0927	4.15
21	1.518	10.4	0.0101	0.1051	6.92
22	1.272	19.2	0.0012	0.0230	1.81
23	1.714	10.9	0.0018	0.0196	1.14
24	4.010	12.5	0.0001	0.0013	0.03
25	2.371	10.0	0.0048	0.0480	2.02
26	1.532	9.60	0.0015	0.0144	0.94
27	1.156	11.7	0.0029	0.0339	2.93
28	1.161	10.0	0.0004	0.0040	0.34
29	1.112	8.80	0.0004	0.0035	0.32
30	1.258	18.3	0.0022	0.0403	3.20
31	1.471	12.5	0.0024	0.0300	2.04
32	1.977	12.5	0.0143	0.1788	9.04

[1]Calculated as $CO\ (\%) = \frac{(\text{AFM1 in milk (µg/cow/d)})}{(\text{AFB1 ingested (µg/cow/d)})} \times 100\%$ (16)

Concentration of AFM_1 in milk and its relation with dairy farm management practices

According to the survey responses, explanatory variables of AFB_1 ingestion potentially associated with AFM_1 occurrence in raw milk were selected and examined for each farm (Table 3). From these variables, concentrate feed storage period on the farm (p = 0.002), amount of haylage fed (p = 0.005), silage type fed (p = 0.04), and concentrate feed characteristics (p = 0.09) resulted associated with AFM1 occurrence in milk using $p < 0.15$ as significance value. In contrast, all the other examined variables were not associated ($p \geq 0.17$) with AFM_1 concentration in raw milk.

Examining all identified explanatory variables associated with AFM_1 presence in milk by using a

Table 3. Definition of explanatory variables of aflatoxin B1 ingestion with potential association ($p < 0.15$) with aflatoxin M1 concentration in raw milk samples collected from 32 dairy farms in Chiriquí, Panamá

Explanatory variables	Levels	Aflatoxin M1 concentration, µg/L (Mean ± SD)	*p*-value
Knowledge about mycotoxins	No	0.005 ± 0.001	0.74
	Yes	0.004 ± 0.001	
Carries out composition analysis of feed	No	0.004 ± 0.001	0.63
	Yes	0.005 ± 0.002	
Milk yield	<11 L/cow/d	0.005 ± 0.001	0.85
	>11 L/cow/d	0.004 ± 0.001	
Dairy cattle breeds	Pure breed	0.005 ± 0.002	0.47
	Crossbreed	0.004 ± 0.002	
Production system	Extensive	0.004 ± 0.001	0.47
	Intensive	0.007 ± 0.003	
	Semi-intensive	0.004 ± 0.002	
Feeding supplement	Before milking	0.005 ± 0.001	0.20
	During milking	0.003 ± 0.001	
	Before and during milking	0.006 ± 0.002	
Amount of concentrate feed fed	Kg/d	3.94 ± 1.43	0.61
Amount of silage fed	Kg/d	2.56 ± 2.55	0.83
Silage type fed	No silage used	0.004 ± 0.001	0.04
	Mixed corn and grass silage	0.003 ± 0.001	
	Corn silage	0.008 ± 0.002	
Amount of haylage fed	Kg/d	0.50 ± 1.97	0.005
Concentrate feed storage period on the farm	Below eight days	0.005 ± 0.001	0.002
	Above eight days	0.002 ± 0.001	
Concentrate feed characteristics	With sequestrants	0.005 ± 0.001	0.09
	W/O sequestrants	0.003 ± 0.001	
Concentrate feed type	Commercial	0.004 ± 0.001	0.98
	Farm-made	0.004 ± 0.002	
Farm storage type by infrastructure	Regular (roofed area, open-air)	0.004 ± 0.001	0.34
	Good (closed storage)	0.005 ± 0.002	

Data obtained from surveys applied to dairy farms (n = 32). Variables were grouped as nominal or scalar; nominal variables can mostly be categorized to rule out false positives. Levels were answer options. Means according to interviewees

Table 4. Final multivariate logistic regression model (backward selection) for aflatoxin M1 concentration in raw milk samples collected from 32 dairy farms in Chiriquí, Panamá.

Predictive variables	[1]Level	Aflatoxin M1 concentration, µg/L (Mean ± SD)	[2]p-value
Concentrate feed characteristics	With sequestrants	0.005 ± 0.001	0.06
	W/O sequestrants	0.003 ± 0.001	
Silage type fed	No silage used	0.003 ± 0.001	0.005
	Mixed corn and grass silage	0.002 ± 0.001	
	Corn silage	0.006 ± 0.002	
Amount of haylage fed	Kg/d	0.500 ± 1.967	<0.001

[1]Level = answer options / Nivel = opciones de respuesta; [2]*p*-value for a confidence value of 95%; n = 32 milk samples / *p*-valor para un valor de confianza de 95%; n = 32 muestras de leche.

multivariate logistic regression model indicated that concentrate feed characteristic (p = 0.06), silage type fed (p = 0.005), and amount of haylage fed (p < 0.001) to the cows are three significant variables associated with dairy farm management practices with direct impact on AFM_1 occurrence in milk (Table 4). In contrast, the concentrate feed storage period on the farm was not directly correlated.

Discussion

Dairy cattle feeds are often contaminated with mycotoxins (25), and aflatoxin's presence in milk is a human health issue that needs to be addressed (5). Aflatoxin contamination of feed under farm management practices has been amply discussed (26) and considered by the FDA and EU to establish regulations or recommendations limits in animal feeding. For most farms in this study, cattle feed is mainly based on fresh forage grazing, conserved forage, and commercial supplements of concentrate feed; in a few cases, a grain mix is prepared at the farm.

Aflatoxin B_1 in dairy cattle feed

Overall, AFB_1 values observed were below the EU and FDA norms for dairy cattle feed. The occurrence of AFB_1 in feed samples was lower than values reported in Argentina by Signorini *et al*. (2012) (27), who observed a general AFB_1 occurrence of 78.9% in 597 analyzed samples. Notwithstanding, these observed low AFB_1 values should not be ignored because the slightest toxins' presence in cattle feed and animal products represents a risk to animal and human health.

The occurrence of AFB_1 is more common in concentrate feed than forages (28,29). Concentrate feeds are primarily composed of grains and contain soluble carbohydrates, mainly starch, readily available energetic components. In contrast, forages predominantly comprise structural carbohydrates such as cellulose and hemicellulose. Feed sampling in the present study was carried out during the rainy season. During this period, the feeding strategy used by most dairy farms is pasture grazing with concentrate feed supplementation. The most used forage to concentrate feed ratio in these farms oscillates between 60:40 and 70:30.

According to the data in Table 1, concentrates are the dairy feeds with the highest AFB_1 prevalence (74.3%), with maximum values of 4.976 µg/Kg of dry matter. These values are still below the EU regulations and FDA limits. Corn silage, another commonly used feed in dairy cattle, also showed a maximum AFB1 concentration of 0.367 µg/Kg and a

prevalence of 28.6%. A review study about corn contamination with mycotoxins from 2005 to 2020 reported corn silage as the primary source of AFB_1 in dairy cattle feed (30). Grass silage had an AFB_1 prevalence of 25% but with maximum detected values of 0.243 µg/Kg. Although most fungi do not tolerate some chemical changes during corn or grass ensiling, bad practices during the ensiling process may contribute to AFB_1 occurrence.

Aflatoxin M_1 in raw milk

Occurrences of AFM_1 in milk have been associated with the presence of AFB_1 in feeds (31). Mean AFM_1 variations are explained by dietary differences, feed source availability in each farm, and the level of feed intake. None of the samples with positive results for AFM_1 had concentrations above the limits set by the EU (0.05 µg/L) and FDA (0.5 µg/L). The concentration of AFM_1 in raw milk samples was lower than the values reported in other studies (11-13,27,32,33), probably because all farms participating in this study are suppliers of a dairy processing plant that follows high-quality standards and has implemented strict monitoring programs. There are studies that have shown that even when aflatoxin concentrations in milk for consumption are low, they can accumulate, especially in populations for whom milk is a staple food in their diet, such as children and pregnant women (34,35).

Carry-over of AFB_1 to AFM_1

Carry-over estimation of AFB_1 to AFM_1 in dairy cows is essential in determining the acceptable AFB_1 intake in feed (36). The average carry-over is similar to the results reported by Britzi *et al*. (2013) (36) in low and high-producing dairy cattle under controlled experimental conditions. Rodríguez-Blanco *et al*. (2020) (37) reported a carry-over of 0.6 to 6% in dairy cows, and Costamagna (2018) (11) studies in Argentina reported an average of 0.84% (range 0.05 to 5.93%). Observations suggest that the carry-over of AFB_1 to AFM_1 in dairy cows may be related to the level of intake and milk yield. Farms with higher AFB_1 ingestion rates above the observed average intake (2.386 µg/d) resulted from fed concentrate contaminated with AFB_1 above the observed average concentration.

According to Britzi *et al*. (2013) (36), AFM_1 appears to be correlated with milk yield and days of lactation. Around 1 to 2% of the ingested AFB_1 has been reported to be metabolized to AFM_1 in cows producing under 30 L/d, and up to ~6% in cows producing more than 30 L/day, cows milked twice daily. Dairy farms with high milk yield tend to feed more concentrate to meet cow production requirements (38).

Studies by Armorini *et al*. (2016) (39) and Costamagna (2018) (11) reported higher levels of AFM_1 in milk from high-production than from low-production dairy cattle. The no association of milk yield as an explanatory variable observed in this study could be related to inferior feed DMI and the national low daily average milk yield in tropical Panama (6 L/cow) compared to countries like Argentina, whose daily average production is 30 L/cow. In the present experiment, cows had a lower level of feed intake than in Costamagna's (11) study (13.9 vs 23.5 kg DMI/day). Besides this, differences in study design may also affect the carry-over and the difference in cattle breed, health, AFB_1 ingestion from the diet, AFB_1 dose, and exposure time (40).

Examining the data used for carry-over estimation of AFB_1 to AFM_1, we observed that the increased carry-over for farms 11, 21, and 32 was related to the more significant AFM_1 presence in milk (above 0.01 µg/L) and not associated with AFB_1 content in feed. A common characteristic in these three farms is that the milk collection truck picks up every two days, unlike the other farms where the collection is done daily or every other day. In this way, any change in the components of the diet during those days prior to collection could have affected the concentration of aflatoxins in the milk stored in the bulk tank.

Concentration of AFM_1 in milk and its relation with dairy farm management practices

Farm management practices related to feeding feeds contaminated with AFB_1 had been studied to examine their association with the occurrence of AFM_1 in milk. As observed in this study, the type of production system, concentrate feed type, amount

of concentrate feed fed, and farm storage type by infrastructure were not associated with AFM_1 occurrence in milk. In contrast, the concentrate feed storage period on the farm, amount of haylage fed, silage type fed, and concentrate feed characteristics were associated. Similar variables were used in Costamagna (2018) (11), Njombwa et al. (2021) (33), and Signorini et al. (2012) (27) studies and their outputs showed that the presence of AFM_1 in milk is predisposed by several factors related to poor farm management practices.

Outputs from the final multivariate logistic regression model showed a lower association between haylage feeding and corn silage feeding amount. Commonly, corn silage is more susceptible to AFB_1 contamination than haylage (41), and the type of carbohydrates present in these feeds explains it. Therefore, corn silage feeding in dairy farms is directly related to the content of AFM_1 in milk (30,37). Levels of AFB_1 in the analyzed forage samples were relatively low, and cows from sampled farms were mainly under a semi-intensive production system on pasture, grazing large amounts of forage.

The association of feeding commercial concentrate feed with mycotoxin sequestrants added with AFM_1 occurrence in milk may be due to the poor storage conditions at the farm level, where it became easily contaminated with fungi. Concentrate feeds are composed of grains containing highly soluble fermentative carbohydrates that predispose to fungi growth under poor storage conditions, representing a potential risk factor associated with AFM_1 in milk.

Conclusions

None of the samples with positive results for AFM1 had concentrations above the limits set by the EU (0.05 µg/L) and the FDA (0.5 µg/L). Although AFM1 levels are low and within the range permitted by FDA and EU regulations, there is a cumulative effect of this mycotoxin in vulnerable populations, such as children and pregnant women, due to its consumption as part of the daily diet. Therefore, it is recommended to implement a good practices plan on farms to control the presence of these contaminants in milk. The concentration of AFB_1 in cattle feed observed in the present study resulted under the maximum levels set by FDA and UE regulations. Concentrate feed characteristics, concentrate feed storage period on the farm, silage type, and amount of haylage fed to the cows were shown to be determinants of AFM_1 occurrence in raw cow milk . Outputs from this study might scientifically justify adequate feeding practices based on current dairy farm management practices.

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Interests conflict

The authors declare no conflicts of interest.

Referencias

1. Jallow A, Xie H, Tang X, Qi Z, Li P. Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. CRFSFS. 2021; 20(3):2332-2381. https://doi.org/10.1111/1541-4337.12734
2. Caceres I, Khoury AA, El Khoury R, Lorber S, Oswald IP, El Khoury A, et al. Aflatoxin biosynthesis and genetic regulation: A review. Toxins. 2020;12(3):150. doi:10.3390/toxins12030150.
3. Rushing BR, Selim MI. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem. Toxicol. 2019; 124: 81–100. https://doi.org/10.1016/j.fct.2018.11.047.
4. Espinosa-Plascencia A, Bermúdez-Almada MDC. Las aflatoxinas, un tóxico que continúa presente en los alimentos y sus efectos biológicos en los humanos y en los animales. Tip Rev Espec Cienc Quím Biol. 2023;26:1-16. doi:10.22201/fesz.23958723e.2023.553.
5. Ahlberg S, Grace D, Kiarie G, Kirino Y, Lindahl J. A risk assessment of Aflatoxin M1exposure in low and mid-income dairy consumers in Kenya. Toxins. 2018; 10(9): 348. https://doi.org/10.3390/toxins10090348.

6. Min L, Fink-Gremmels J, Li D, Tong X, Tang J, Nan X, et al. An overview of aflatoxin B1 biotransformation and aflatoxin M1 secretion in lactating dairy cows. Anim Nutr. 2021;7(1):42-8. doi:10.1016/j.aninu.2020.11.002.
7. Min L, Li D, Tong X, Sun H, Chen W, Wang G, et al. The challenges of global occurrence of aflatoxin M1 contamination and the reduction of aflatoxin M1 in milk over the past decade. Food Control. 2020; 117:107352. https://doi.org/10.1016/j.foodcont.2020.107352.
8. Gonçalves BL, Corassin CH, Oliveira CAF. Mycotoxicoses in dairy cattle: A review. Asian J Anim Vet Adv. 2015;10(11):752–60. https://doi.org/10.3923/ajava.2015.752.760.
9. Amaro FX, Jiang Y, Arriola K, Pupo MR, Agustinho BC, Bennett SL, et al. The Effects of Incremental Doses of Aflatoxin B1 on In Vitro Ruminal Nutrient Digestibility and Fermentation Profile of a Lactating Dairy Cow Diet in a Dual-Flow Continuous Culture System. Toxins. 2023;15(2):90. https://doi.org/10.3390/toxins15020090.
10. Michlig N, Signorini M, Gaggiotti M, Chiericatti C, Basílico JC, Repetti MR, et al. Risk factors associated with the presence of aflatoxin M1 in raw bulk milk from Argentina. Food Control. 2016;64:151–156. https://doi.org/10.1016/j.foodcont.2015.12.025.
11. Costamagna DA. Contaminantes en leche, productos y subproductos lácteos: evaluación de factores de riesgo y estrategias de intervención. Rafaela, Buenos Aires: Universidad Nacional del Litoral Facultad de Ingeniería Química, 2018. Tesis Doctoral.
12. Admasu FT, Melak A, Demissie B, Yenew C, Habtie ML, Bekele TT, et al. Occurrence and associated factors of aflatoxin M1 in raw cow milk in South Gondar Zone, North West Ethiopia 2020. Food Sci Nutr. 2021;9(11):6286–6293. https://doi.org/10.1002/fsn3.2589.
13. Costamagna D, Gaggiotti M, Signorini ML. Quantitative risk assessment for aflatoxin M 1 associated with the consumption of milk and traditional dairy products in Argentina. Mycotoxin Res. 2021; 37(49): 315-325. https://doi.org/10.1007/s12550-021-00444-w.
14. Ajmal M, Bedale W, Akram A, Yu JH. Comprehensive review of aflatoxin contamination, impact on health and food security, and management strategies in Pakistan. Toxins. 2022;14(12):845. doi:10.3390/toxins14120845.
15. Tolosa J, Rodríguez-Carrasco Y, Ruiz MJ, Vila-Donat P. Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: A review. Food Chem. Toxicol. 2021; 158(6): https://doi.org/10.1016/j.fct.2021.112661.
16. Zentai A, Jó´zwiak Á, Süth M, Farkas Z. Carry-Over of Aflatoxin B1 from Feed to Cow Milk—A Review. Toxins. 2023;15(3):195. https://doi.org/10.3390/toxins15030195.
17. Franco H, Vega A, Reyes S, De León J, Bonilla A. Niveles de Ocratoxina A y Aflatoxinas totales en cafés de exportación de Panamá por un método de ELISA. Arch Latinoam Nutr. 2014;64(1):42-9.
18. Troestch J, Reyes S, Vega A. Determination of Mycotoxin Contamination Levels in Rice and Dietary Exposure Assessment. J Toxicol. 2022; 2022(1): 3596768 https://doi.org/10.1155/2022/3596768.
19. Him JJ, Tuñón D, Rodríguez Y. Detección de aflatoxinas en maní y maíz para palomitas en tres marcas locales en Panamá. Rev. Col. Ciencia. 2022;3(1):22–9. ISSNL 2710-7434.
20. Chong V, De La Cruz A, Cedeño J, Vargas L. Flora fúngica y niveles de aflatoxinas en frutas y hortalizas frescas. Cienc Agropecu. 2023; 36: 75-95. http://www.revistacienciaagropecuaria.ac.pa/index.php/cienciaagropecuaria/article/view/60
21. Alameri MM, Kong ASY, Aljaafari MN, Ali HA, Eid K, Sallagi MA, et al. Aflatoxin contamination: An overview on health issues, detection and management strategies. Toxins. 2023;15(4):246. doi:10.3390/toxins15040246.
22. Xiao Y, An R, Huber U. Sensitive fluorescence analysis of aflatoxins using post-column UV derivatization on the Agilent 1260 Infinity LC. Application Note Food Testing & Agriculture. 2013. Publication No.: 5991-1217EN.
23. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of AOAC International. 18th ed. AOAC International; Maryland: 2010.
24. Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW. Infostat (Versión (2020) [Programa de Computadora]. [Internet]. Córdoba; 2020. https://www.infostat.com.ar/index.php?mod=page&id=15.
25. Smaoui S, D'Amore T, Tarapoulouzi M, Agriopoulou S, Varzakas T. Aflatoxins contamination in feed commodities: From occurrence and toxicity to recent advances in analytical methods and detoxification. Microorganisms. 2023;11(10):2614. https://doi.org/10.3390/microorganisms11102614.
26. Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF. Review on mycotoxin issues in ruminants: Occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins. 2015; 7(8): 3057–3111. https://doi.org/10.3390/toxins7083057.
27. Signorini ML, Gaggiotti M, Molineri A, Chiericatti CA, Zapata de Basílico ML, Basílico JC, et al. Exposure assessment of mycotoxins in cow's milk in Argentina. Food Chem. Toxicol. 2012;50(2):250–257. https://doi.org/10.1016/j.fct.2011.09.036
28. Granados-Chinchilla F, Molina A, Chavarría G, Alfaro-Cascante M, Bogantes-Ledezma D, Murillo-Williams A. Aflatoxins occurrence through the food chain in Costa Rica: Applying the One Health approach to mycotoxin surveillance. Food Control. 2017;82:217– 226. https://doi.org/10.1016/j.foodcont.2017.06.023
29. Ferrari L, Rizzi N, Grandi E, Clerici E, Tirloni E, Stella S, et al. Compliance between food and feed safety: eight-year survey (2013–2021) of aflatoxin M1 in raw milk and aflatoxin B1 in feed in northern Italy. Toxins. 2023;15(3):168. doi:10.3390/toxins15030168.
30. Leite M, Freitas A, Silva AS, Barbosa J, Ramos F. Maize food chain and mycotoxins: A review on occurrence studies. Trends Food Sci. Technol. 2021; 115(2): 307–331. https://doi.org/10.1016/j.tifs.2021.06.045.

31. Sassahara M, Pontes Netto D, Yanaka EK. Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Paraná state. Food Chem. Toxicol. 2005;43(6):981–984. https://doi.org/10.1016/j.fct.2005.02.003.
32. Jafari K, Fathabad AE, Fakhri Y, Shamsaei M, Miri M, Farahmandfar R, et al. Aflatoxin M1 in traditional and industrial pasteurized milk samples from Tiran County, Isfahan Province: a probabilistic health risk assessment. Ital J Food Sci. 2021;33(Suppl 1):103. doi:10.15586/ijfs.v33iSP1.2054.
33. Njombwa CA, Moreira V, Williams C, Aryana K, Matumba L. Aflatoxin M1 in raw cow milk and associated hepatocellular carcinoma risk among dairy farming households in Malawi. Mycotoxin Res. 2021;37(1):89–96. https://doi.org/10.1007/s12550-020-00417-5
34. Mamiro AP, Martin HD, Kassim N. Risk of exposure to aflatoxin M1 through consumption of cow's milk among children in Magadu, Morogoro. Mycotoxin Res. 2024;40(2):245-53. doi:10.1007/s12550-024-00456-7.
35. Tadesse H, Tamene A, Dessie G. Aflatoxin M1 exposure and health risk assessment in children and adults due to pasteurized milk consumption in Addis Ababa, Ethiopia. Eur J Agric Food Sci. 2024;6(2):51-9. doi:10.24018/ejfood.2024.6.2.789.
36. Britzi M, Friedman S, Miron J, Solomon R, Cuneah O, Shimshoni JA, et al. Carry-over of Aflatoxin B1 to aflatoxin M1 in high yielding Israeli cows in mid- and late-lactation. Toxins. 2013;5(1):173-83. doi:10.3390/toxins5010173.
37. Rodríguez-Blanco M, Ramos AJ, Prim M, Sanchis V, Marín S. Usefulness of the analytical control of aflatoxins in feedstuffs for dairy cows for the prevention of aflatoxin M1 in milk. Mycotoxin Res. 2020;36(1):11–22. https://doi.org/10.1007/s12550-019-00362
38. Bervis N, Lorán S, Juan T, Carramiñana JJ, Herrera A, Ariño A, et al. Field monitoring of aflatoxins in feed and milk of high-yielding dairy cows under two feeding systems. Toxins. 2021;13(3):201. doi:10.3390/toxins13030201.
39. Armorini S, Altafini A, Zaghini A, Roncada P. Occurrence of aflatoxin M1 in conventional and organic milk offered for sale in Italy. Mycotoxin Res. 2016;32(4):237 246. https://doi.org/10.1007/s12550-016-0256-8.
40. Iqbal SZ, Jinap S, Pirouz AA, Ahmad Faizal AR. Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends Food Sci Technol. 2015;46(1):110–119. http://dx.doi.org/10.1016/j.tifs.2015.08.005 .
41. Vaičiulienė G, Bakutis B, Jovaišienė J, Falkauskas R, Gerulis G, Kerzien S, et al. Prevalence of mycotoxins and endotoxins in total mixed rations and different types of ensiled forages for dairy cows in Lithuania. Toxins. 2021;13(12):890. doi:10.3390/toxins13120890.

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