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Occurrence Of Aflatoxins In Animal Feed And Aflatoxin M1 In Milk In The Peri-Urban Area Of Bamako, Mali

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Abstract: The dairy sector in the peri-urban area of Bamako plays a crucial role in supplying fresh milk to the local population. However, contamination of cattle feed with aflatoxins represents a major food safety concern. These toxins are metabolized into aflatoxin M1 (AFM1), which is subsequently excreted in milk, thereby exposing consumers, particularly infants and young children, to significant health risks. This study aimed to assess aflatoxin contamination in cattle feed and milk produced in the peri-urban area of Bamako, Mali.

A total of 58 animals feed samples and 155 milk samples were analyzed using enzyme-linked immunosorbent assay (ELISA), and the results were subsequently confirmed by high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD). The mean total aflatoxin concentrations in feed were 5,903.78 ng/kg by ELISA and 7,576.12 ng/kg by HPLC-FLD, both exceeding the European Union regulatory limit of 4,000 ng/kg. Likewise, AFM1 concentrations in milk exceeded the maximum permissible level of 50 ng/kg established by the European Union, confirming the transfer of aflatoxins from contaminated feed to milk.

These findings demonstrate a substantial contamination of cattle feed and milk within peri-urban dairy production systems in Bamako. The results highlight the urgent need to strengthen aflatoxin monitoring and control throughout the dairy production chain in order to improve milk safety and protect public health.

Keywords: Aflatoxins; Aflatoxin M1; Cattle feed; ELISA; HPLC-FLD; Milk.

1. Introduction

According to the Food and Agriculture Organization (FAO), approximately 25% of the world's agricultural crops are contaminated with mycotoxins during growth or storage ([1], [2]). The major mycotoxin-producing fungi belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium* ([3]). The principal mycotoxins include aflatoxins, fumonisins, zearalenone, ochratoxin, and deoxynivalenol ([4]). Among these, aflatoxins are considered the most toxic and frequently contaminate a wide variety of food and feed commodities, including maize, peanuts, cereals, nuts, dried fruits, meat, and dairy products ([5], [6]).

Aflatoxins (AFs) are among the most extensively studied mycotoxins and occur naturally as contaminants in food and feed worldwide ([7]). They are toxic secondary metabolites mainly produced by several fungal species, including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, which proliferate in major food crops under a broad range of climatic conditions ([8]). Aflatoxins are odorless, tasteless, fluorescent compounds that exhibit remarkable thermal stability. They withstand extremely high temperatures, even above 320°C; consequently, conventional food-processing methods such as cooking, fermentation, refrigeration, freezing, and pasteurization are ineffective in eliminating these toxins ([9]).

The toxic effects of aflatoxins extend far beyond their carcinogenic properties. Acute exposure may result in severe aflatoxicosis characterized by acute liver failure that can be fatal, whereas chronic exposure has been associated with immunosuppression,

impaired child growth, reduced animal productivity, reproductive disorders, and an increased risk of liver diseases, particularly among populations infected with hepatitis B virus ([10], [11], [12]).

The major aflatoxins produced by *A. flavus* and *A. parasiticus* include aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Among these, AFB1 is the most toxic, carcinogenic, and genotoxic compound. It commonly contaminates cereal grains and is metabolized in the mammalian liver to produce its principal hydroxylated metabolite, aflatoxin M1 (AFM1), which is subsequently excreted into milk following the consumption of contaminated feed by dairy animals. AFM1 has been classified in the Group 1 human carcinogen by the International Agency for Research on Cancer ([13], [14]).

Milk is an important dietary source of high-quality proteins, calcium, and essential micronutrients, particularly for infants, young children, and pregnant women. Consequently, the occurrence of AFM1 in milk represents a significant public health concern because these population groups are especially vulnerable to its toxic effects ([15]). Furthermore, climate change is expected to promote the geographical expansion of aflatoxin-producing fungi, thereby increasing the risk of aflatoxin contamination in animal feed and, consequently, in milk ([16], [11]).

The presence of AFM1 in milk, particularly cow's milk, poses a potential health risk owing to its widespread consumption, especially by children ([17], [18]). For this reason, the European Union and many other countries have established maximum permissible limits for AFM1 in milk (50 ng/kg), cheese (250 ng/kg), and infant foods (25 ng/kg) ([19], [20]). Another aflatoxin occasionally detected in milk and dairy products is aflatoxin M2 (AFM2), a hydroxylated metabolite of aflatoxin B2 (AFB2) ([21]). However, no regulatory limit has yet been established for AFM2 in milk.

The present study aimed to evaluate aflatoxin contamination in animal feed and milk produced in the peri-urban area of Bamako using both ELISA and high-performance liquid chromatography (HPLC).

2. Materials and Methods

2.1. Sampling of animal feed and milk

Animal feed samples were collected from dairy farms and feed retail outlets located in the peri-urban area of Bamako along the major transportation corridors (50km radius), namely Bamako–Segou, Bamako–Guinea, Bamako–Sikasso, Bamako–Koulikoro, and Bamako–Kayes. To ensure representative sampling, a total of 58 animal feed samples were collected for total aflatoxin analysis. Milk samples were subsequently collected from the same dairy farms and retail outlets across the six administrative districts of Bamako for the determination of aflatoxin M1 (AFM1). Overall, 155 milk samples were collected, including 85 samples from dairy farms and 70 samples from retail outlets.

2.2. ELISA Analysis

2.2.1. ELISA Kits and Instrumentation

ELISA analyses were performed using an HF4500 microplate reader capable of measuring absorbance at 450 nm, the wavelength required for the enzymatic reactions involved in mycotoxin quantification. Additional laboratory equipment included a vortex mixer for sample homogenization, a high-precision analytical balance for sample weighing, and a centrifuge for phase separation during extraction procedures.

The ELISA kits contained total aflatoxin and AFM1 calibration standards, enzyme conjugates, substrates A and B, specific antibodies, and stop solution for quantitative determination of aflatoxins in the different sample matrices. Sample handling was performed using 10-mL graduated pipettes and single- and multi-channel micropipettes covering volume ranges of 10–100 µL, 30–300 µL, and 100–1000 µL.

2.2.2. Preparation of animal feed samples

Approximately 5.0 ± 0.02 g of finely ground feed was accurately weighed into a 50-mL centrifuge tube.

Aflatoxins were extracted with 25 mL of water – methanol (40:60, v/v). The suspension was vortex-mixed at 2,500 rpm for 5 min,

homogenized for 10 min at room temperature, and centrifuged at 4,000 rpm for 5 min. Subsequently, 1 mL of the supernatant was transferred into a new tube, diluted with 4 mL of deionized water, and thoroughly mixed.

An aliquot of 50 µL of the diluted extract was dispensed into each ELISA microplate well, followed by the addition of 50 µL of calibration standards, enzyme conjugate, and specific antibodies. The plate was incubated at 25°C for 30 min, emptied, and washed four to five times with 250 µL of washing buffer. Subsequently, 50 µL each of substrate's A and B were added, and the plate was incubated for an additional 15 min at 25°C. After adding 50 µL of stop solution, absorbance was measured at 450 nm using the HF4500 microplate reader.

2.2.3. Preparation of milk samples

For AFM1 analysis, 50 µL of raw milk or AFM1 calibration standard was added to each ELISA microplate well. Subsequently, 50 µL of enzyme conjugate and 50 µL of the specific antibody solution were added to each well, and the plate was gently shaken to ensure homogeneous mixing.

The plate was incubated at 25°C for 30 min. Following incubation, the wells were washed four to five times with 250 µL of washing buffer to remove unbound material and then dried carefully on absorbent paper to eliminate residual liquid.

Next, 50 µL of substrate A followed by 50 µL of substrate B were added sequentially, and the plate was gently mixed before a second incubation at 25°C for 15 min. Finally, 50 µL of stop solution was added, and absorbance was recorded at 450 nm using the microplate reader.

2.3. High-Performance Liquid Chromatography (HPLC)

2.3.1. HPLC-FLD Instrumentation

High-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) was used as the reference analytical method. Analyses were performed using a Waters Alliance™ 2695 HPLC system equipped with a Waters 474 Scanning Fluorescence Detector for sensitive detection of fluorescent aflatoxins.

Sample extraction and clean-up were carried out using immunoaffinity columns (IAC), including AFLAPREP® M WIDE and AflaTotal® (R-Biopharm®, Germany), containing monoclonal antibodies immobilized on a solid polymeric support for selective capture of target mycotoxins. These columns effectively removed matrix interferences from feed and milk samples before chromatographic analysis.

The mobile phases consisted of HPLC-grade methanol, acetonitrile, and Milli-Q water. Certified aflatoxin reference standards (AFM1, AFB1, AFB2, AFG1, and AFG2) supplied by Libios, Sigma-Aldrich, and R-Biopharm were used for calibration and quantitative determination.

2.3.2. Chromatographic Conditions

Chromatographic separation was achieved under isocratic elution using a mobile phase composed of water (A), acetonitrile (B), and methanol (C) at a volumetric ratio of 68:14:18 (v/v/v). The flow rate was maintained at 1.0 mL/min, and analysis were performed at room temperature. Fluorescence detection was carried out with excitation and emission wavelengths set at 360 and 440 nm, respectively.

2.3.3. Sample Preparation for HPLC-FLD Analysis

2.3.3.1. Animal feed samples

Approximately 1–5 g of feed sample was mixed with 0.1 g 1 M (1M molar) of NaCl and extracted with 10 mL of methanol–water (80:20, v/v). The mixture was homogenized three times for 15 s at 3,000 rpm using an Ultra-Turrax homogenizer and filtered through Whatman filter paper.

One milliliter of the filtrate was diluted with 1 mL of HPLC-grade water before being loaded onto an AflaTotal immunoaffinity

column at a flow rate of 1–2 drops/s. The column was washed twice with 1 mL of HPLC-grade water. Total aflatoxins (AFB and AFG) were eluted using two 0.5-mL portions of methanol followed by 1 mL of HPLC-grade water (50:50, v/v). The eluate was centrifuged at 10,000 rpm for 3 min, and 800 µL was evaporated to dryness using a SpeedVac concentrator. The residue was reconstituted in 120 µL of the mobile phase (water/acetonitrile/methanol, 68:14:18, v/v/v), and 50 µL was injected into the HPLC system.

2.3.3.2. Milk samples

One gram of freeze-dried milk was reconstituted with 10 mL of Milli-Q water preheated to 50°C and transferred into a 30-mL centrifuge tube. After vortex mixing for 30 s, samples were centrifuged at 10,000 × g for 20 min at 4°C. The skimmed milk fraction was filtered through Whatman filter paper, and an additional 2.5 mL of Milli-Q water was added to maximize AFM1 recovery.

Approximately 11 mL of filtrate was collected and 3 mL was loaded onto an AFM1 immunoaffinity column at a flow rate of 1.5 mL/min. The column was washed twice with 3 mL of Milli-Q water, and AFM1 was eluted with 3 mL of acetonitrile–methanol (25:75, v/v). The eluate was evaporated to dryness using a SpeedVac concentrator, and the residue was reconstituted in 120 µL of the mobile phase (water/acetonitrile/methanol, 68:14:18, v/v/v). Finally, a 50-µL aliquot was injected into the HPLC system for AFM1 quantification.

3. Results

3.1. Detection of aflatoxins in animal feed

The average concentrations of total aflatoxins (AFT) in animal feed samples collected from dairy farms and retail outlets along the five major road corridors in the peri-urban area of Bamako were determined using both ELISA and HPLC-FLD.

The overall contamination rate determined by ELISA was approximately 76%. Of the 58 feed samples analyzed, 80% exceeded the European Union maximum limit of 4,000 ng/kg, whereas only 20% complied with the regulatory standard. HPLC-FLD analysis revealed contamination in all feed samples (100%). Among the 58 samples, 44.83% exceeded the EU regulatory limit, while 55.17% remained below the maximum permitted level.

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Figure 1 presents the mean total aflatoxin concentrations obtained by ELISA and HPLC-FLD across the five sampling corridors.

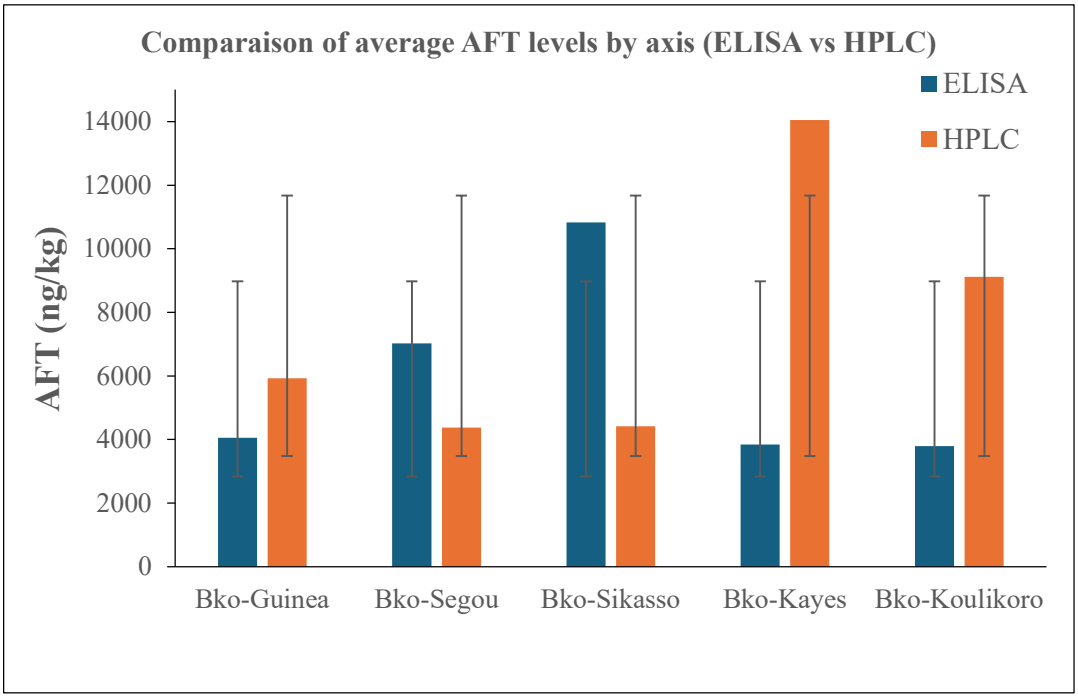


Figure 1: Average concentrations of AFT by ELISA and by HPLC on the 5 axes

Overall, the mean total aflatoxin concentration in animal feed was 5,903.78 ± 3,067.11 ng/kg by ELISA and 7,576.12 ± 4,097.75 ng/kg by HPLC-FLD. Both values exceeded the European Union regulatory limit of 4,000 ng/kg, indicating that dairy cattle in the peri-urban area of Bamako are substantially exposed to aflatoxins and are therefore at risk of transferring aflatoxin B1 (AFB1) into milk as aflatoxin M1 (AFM1).

These values exceeded the entire regulatory limit set by the European Union for the sum of aflatoxins (4000 ng/kg), indicating widespread contamination of food intended for dairy animals. The difference observed between the two methods highlights the importance to use precise analyses such as HPLC to assess the actual risk. These levels contamination levels suggest that dairy animals in the peri-urban area of Bamako are significantly exposed to aflatoxins through their diet, which involves an increased risk of transfer of AFB1 in milk as AFM1 and, consequently, a potential exposure of populations consuming milk and dairy products.

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3.2. Detection of AFM1 in milk collected from dairy farms

The average AFM1 concentrations measured in milk samples collected from dairy farms and analyzed by ELISA and HPLC-FLD across the five major road corridors are presented in (fig.2)

The overall contamination rate determined by ELISA reached approximately 91%. Among the 85 milk samples analyzed, 62.34% exceeded the European Union maximum permissible limit of 50 ng/kg, whereas 37.66% complied with the regulatory threshold. By contrast, HPLC-FLD detected AFM1 in 97.65% of the samples. Of these, 93.98% exceeded the regulatory limit of 50 ng/kg, while only 6.02% remained within the acceptable limit.

The figure 2 illustrates the mean AFM1 concentrations measured along each sampling corridor.

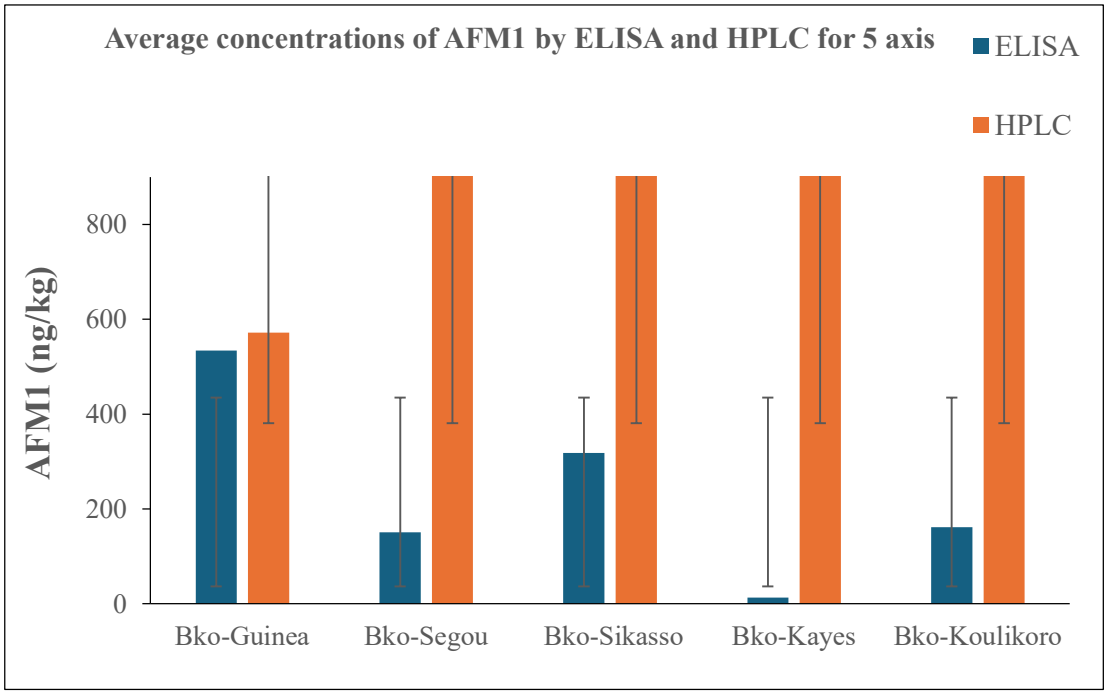


Figure 2: Average concentrations of AFM1 by ELISA and by HPLC on the 5 axis

Across all sampling sites, the mean AFM1 concentration in raw milk reached 235.5 ± 199.12 ng/kg by ELISA and 2,011.38 ± 1,630.80 ng/kg by HPLC-FLD. Both values were substantially higher than the European Union maximum limit of 50 ng/kg, indicating severe contamination of raw milk and suggesting a high level of consumer exposure.

We notice that these levels of contamination in AFM1 show high exposure of population by the contamination of milk and dairy products on all axes in the area peri-urban Bamako.

3.3. Detection of AFM1 in milk collected from retail outlets

The average AFM1 concentrations measured in milk samples collected from retail outlets across the six administrative districts of Bamako are presented in figure 3.

The overall contamination rate determined by ELISA was 97.14%. Among the 70 samples analyzed, 89.70% exceeded the European Union regulatory limit of 50 ng/kg, whereas only 10.30% complied with the standard.

The HPLC-FLD analysis revealed contamination in 100% of the milk samples, with every sample exceeding the maximum permissible level established by the European Union.

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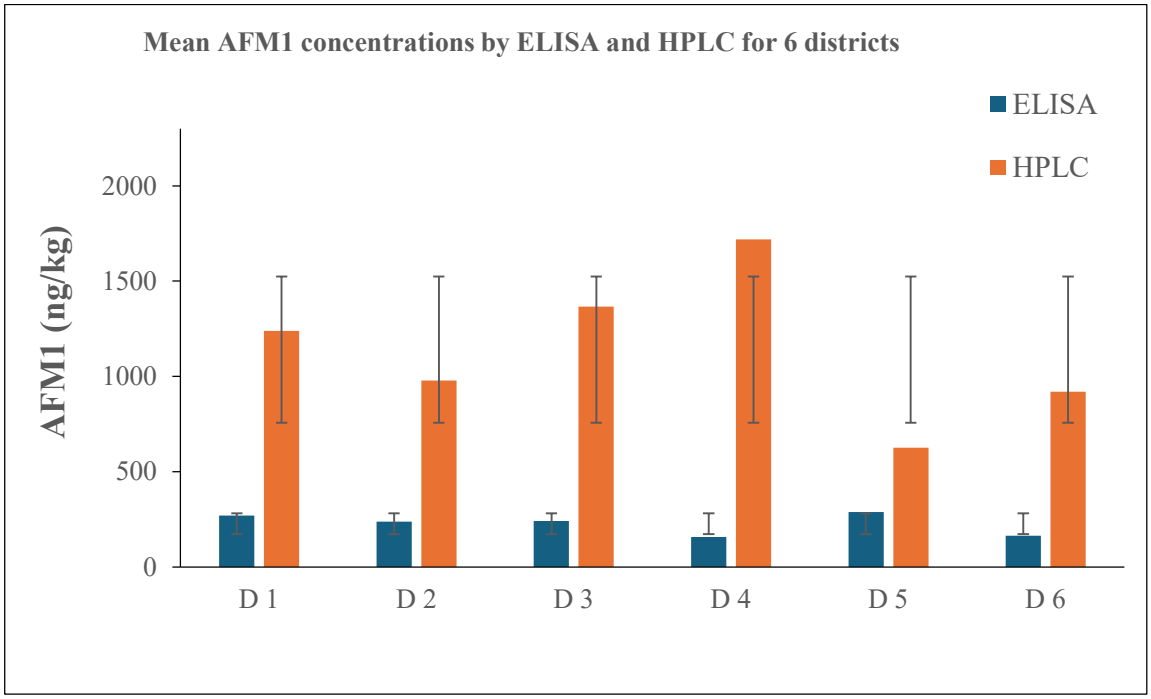


Figure 3: Average concentrations of AFM1 by ELISA and by HPLC in the 6 districts

Across the six districts of Bamako, the mean AFM1 concentration in milk marketed at retail outlets was 227.18 ± 54.42 ng/kg according to ELISA and 1,141.58 ± 383.87 ng/kg according to HPLC-FLD. These concentrations consistently exceeded the European Union regulatory limit, in some cases by several orders of magnitude. Districts 1, 2, 3, and 4 exhibited particularly high AFM1 concentrations together with large standard deviations, indicating marked variability among samples and the occurrence of dangerously elevated contamination levels. These findings clearly demonstrate that consumers purchasing milk from retail outlets are exposed to a substantial health risk.

3.4. Comparison between ELISA and HPLC-FLD methods

In some sampling areas, considerable differences were observed between the two analytical methods. In several cases, ELISA yielded higher aflatoxin concentrations than HPLC-FLD, suggesting substantial heterogeneity among the analyzed samples. ELISA is generally considered a highly sensitive screening method; however, its specificity may be affected by cross-reactivity with structurally related compounds or other matrix interferences, potentially resulting in an overestimation of the actual aflatoxin concentration. In contrast, HPLC-FLD remains the reference analytical technique because its superior specificity, accuracy, and reliability for quantitative aflatoxin determination, particularly when coupled with fluorescence or mass spectrometric detection.

4. Discussion

The analysis of animal feed samples collected in the peri-urban area of Bamako revealed mean total aflatoxin (AFT) concentrations of 5,903.78 ng/kg by ELISA and 7,576.12 ng/kg by HPLC-FLD, confirming the exposure of dairy cattle to aflatoxin B1 (AFB1) and, consequently, the potential transfer of its hydroxylated metabolite, aflatoxin M1 (AFM1), into milk. These findings demonstrate a considerable level of aflatoxin contamination in cattle feed, which may adversely affect the microbiological and chemical safety of dairy products.

The analysis of animal feed revealed heavy contamination, affecting up to 100% of samples analyzed by high-performance liquid chromatography (HPLC), with a substantial proportion exceeding the European regulatory limit of 4,000 ng/kg for total aflatoxins

(AFT). This occurrence of aflatoxins in feedstuffs is primarily attributed to environmental and microclimatic conditions characteristic of tropical zones (elevated temperatures and high relative humidity), combined with inadequate storage conditions along supply chains. Feed stored under warm and humid conditions provides a highly favorable substrate for the proliferation of mold species belonging to the genus Aspergillus, leading to high production of aflatoxin B1 (AFB1), which is subsequently metabolized by livestock into aflatoxin M1 (AFM1) and excreted into milk.

Downstream, the contamination of milk sampled both at dairy farms (97.65% by HPLC) and urban retail points (100% by HPLC) confirms the efficient carry-over of these mycotoxins into the final product. Mean concentrations measured across the six communes of Bamako systematically and significantly exceeded the European Union maximum limit of 50 ng/kg for AFM1. The persistence of markedly elevated AFM1 levels at the urban retail level is driven by the pooling of collected milk batches, the lack of strict sanitary quality control upon receipt, and the absence of thermal treatments capable of inactivating AFM1, given its thermostability. This situation directly exposes the population of Bamako to a high chronic toxicological risk, particularly hepato-carcinogenicity. Based on these findings, controlling the public health risk associated with aflatoxins in the peri-urban area of Bamako requires an integrated "One Health" approach. Consequently, it is necessary to strengthen feed management practices, improve production systems, establish upstream sanitary control mechanisms for livestock feed, build awareness among farmers regarding good storage practices on dairy farms, implement a regular regulatory surveillance program for commercialized milk, and sustainably support the development of the dairy value chain in Mali, particularly in urban centers such as Bamako.

The overall agreement observed between ELISA and HPLC-FLD supports the reliability of the analytical findings and confirms the suitability of both methods for routine aflatoxin monitoring in feed and dairy products. Although some discrepancies were observed between the two analytical techniques, these differences are likely attributable to the higher analytical specificity of HPLC-FLD and the potential cross-reactivity associated with ELISA.

The results of this study emphasize the urgent need to strengthen quality control measures for dairy cattle feed, improve storage and preservation practices for feed ingredients, and implement continuous monitoring of dairy products throughout the production and distribution chain. Such interventions would substantially reduce consumer exposure to aflatoxins and contribute to improving the microbiological and chemical safety of milk produced and marketed in Bamako. Overall, the findings highlight a major food safety issue associated with elevated aflatoxin contamination in both cattle feed and milk, exposing consumers to potentially serious health risks. Strengthening feed management practices, improving dairy production systems, and reinforcing food safety surveillance are essential to ensure milk quality and to promote the sustainable development of the dairy sector in Mali, particularly within the peri-urban area of Bamako.

5. Conclusion

The present study assessed the contamination of dairy cattle feed with total aflatoxins and the occurrence of aflatoxin M1 (AFM1) in milk produced on dairy farms and marketed through retail outlets in the district of Bamako, Mali. ELISA and high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) were employed to quantify aflatoxins concentrations in both feed and milk samples.

The results revealed substantial contamination of dairy cattle feed with total aflatoxins. The average concentrations reached 5,903.78 ng/kg by ELISA and 7,576.12 ng/kg by HPLC-FLD, both exceeding the European Union maximum regulatory limit of 4,000 ng/kg. Furthermore, AFM1 was detected in milk collected from both dairy farms and retail outlets at concentrations well above the European Union maximum permissible level of 50 ng/kg. The average AFM1 concentrations were 235.5 ng/kg and 2,011.38 ng/kg in farm milk, and 227.18 ng/kg and 1,141.58 ng/kg in retail milk, as determined by ELISA and HPLC-FLD, respectively.

These findings clearly demonstrate significant contamination throughout the dairy production chain, from contaminated animal feed to milk, indicating chronic exposure of dairy cattle to aflatoxins and highlighting a potentially serious public health risk for consumers. The study underscores the urgent need to strengthen surveillance and quality control of both animal feed and milk in order to reduce aflatoxin exposure and improve food safety within the dairy sector.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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