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Assessment of Aflatoxins Content in Cow Feeds and Milk in some Cattle Farms in Katsina State, Nigeria

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ABSTRACT
Aflatoxins, potent mycotoxins produced by Aspergillus species, pose a significant risk to animal and human health. Contamination of dairy feed with Aflatoxin B1 (AFB1) leads to the secretion of its carcinogenic metabolite, Aflatoxin M1 (AFM1), into milk. This study assessed the prevalence and levels of aflatoxin contamination in cow feed and milk from selected cattle farms in Katsina State, Nigeria, and evaluated the associated human health risks. Twenty samples of cow feed and milk were collected from ten locations across Katsina State. The physicochemical parameters (crude protein, crude fibre, crude fat) of the samples were determined using standard methods. Aflatoxin B1 in feed and Aflatoxin M1 in milk were quantified using High-Performance Liquid Chromatography (HPLC). Health risks were assessed by calculating the Estimated Daily Intake (EDI), Margin of Exposure (MOE), and Cancer Risk (CR). All feed samples (100%) were contaminated with AFB1 at concentrations ranging from 25.31 to 74.44 µg/kg, with a mean of 52.65 µg/kg, vastly exceeding the FAO/Nigeria regulatory limit of 20 µg/kg. Consequently, 100% of the milk samples were contaminated with AFM1, with levels ranging from 5.86 to 90.28 µg/L. These values exceeded the stringent European Union safety limit (0.05 µg/L) by a factor of 117 to 1,806. The nutritional quality of both feed and milk was generally poor. Health risk assessment revealed alarmingly low MOE values (as low as 26.28) and significant cancer risks, particularly for individuals positive for Hepatitis B surface antigen (up to 0.9930 cases per 100,000 per year). The dairy production system in the studied region is severely compromised. The universal and extreme contamination of milk with AFM1 represents an acute public health crisis, necessitating immediate interventions including farmer education, improved feed storage, and stringent milk monitoring.

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INTRODUCTION

Aflatoxins are toxic secondary metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, which frequently contaminate food crops and livestock feeds in tropical and subtropical regions (Eskola *et al.*, 2020). Among these toxins, aflatoxin B_1 (AFB_1) is the most potent, exhibiting strong hepatotoxic, mutagenic, and carcinogenic effects that pose serious threats to both animal productivity and human health (Smith *et al.*, 2022). In dairy production systems, ingested AFB_1 is metabolized in the liver of lactating animals and excreted in milk as aflatoxin M_1 (AFM_1), a hydroxylated metabolite that remains stable during pasteurization and other heat-based processing techniques (Flores-Flores & González-Peñas, 2023). Consequently, the contamination of milk with AFM_1 represents a significant food safety concern, particularly for populations with high dairy consumption, infants, and individuals with underlying liver conditions.

Nigeria faces persistent aflatoxin challenges due to its warm climate, inadequate post-harvest handling, poor storage systems, and limited regulatory enforcement (Udovicki *et al.,* 2022). In northern regions such as Katsina State, rapid shifts from traditional grazing to more sedentary livestock systems have increased dependence on stored crop residues and commercial feeds, which are highly vulnerable to fungal colonization under local environmental conditions (Garba *et al.,* 2020; Adegbeye *et al.,* 2020). Common feed ingredients including maize, groundnut cake, millet, and cottonseed readily support the growth of aflatoxigenic fungi when drying, aeration, and storage practices are suboptimal (Mahato *et al.,* 2021). This increases the likelihood of AFB_1 contamination and subsequent AFM_1 transfer into milk.

Despite the well-established risks, aflatoxin surveillance across dairy value chains in Nigeria remains limited, and awareness of mycotoxin hazards among small-scale farmers is low (Alamu & Adesokan, 2023). Weak enforcement of feed and milk safety regulations further exacerbates exposure risks (Udomkun *et al.,* 2020). Although visual signs such as mould growth and discolouration may indicate fungal contamination in feed, aflatoxins themselves are invisible, and AFM_1 cannot be detected in milk without laboratory analysis (Ezekiel *et al.,* 2022; De Santis *et al.,* 2023). Analytical methods such as enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) are therefore essential for accurate quantification (Sipos *et al.,* 2021).

Despite the known risks, there is a scarcity of recent and comprehensive data on the prevalence and levels of aflatoxin contamination in the cow feed-milk continuum in Katsina State. Furthermore, a formal health risk assessment for the local population consuming these dairy products is lacking.

This study was therefore designed to determine the nutritional (physicochemical) quality of cow feed and milk in selected cattle farms in Katsina State; quantify the levels of AFB1 in feed and AFM1 in milk using High-Performance Liquid Chromatography (HPLC); and assess the potential human health risks associated with the consumption of contaminated milk using standard risk indices, including Estimated Daily Intake (EDI), Margin of Exposure (MOE), and Cancer Risk (CR).

MATERIALS AND METHODS

Study Area and Sample Collection

The study was conducted in Katsina State, located in north western Nigeria. A total of twenty samples each of cow feed and raw cow milk were purposively collected from ten different Local Government Areas (LGAs): Dutsin-ma, Daura, Kurfi, Kankia, Dutsi, Charanchi, and Musawa. Sampling was carried out during the dry season. The predominant feed types identified across the sampling locations included maize bran, groundnut cake, cottonseed cake, rice bran, and sorghum bran. Dutsin-Ma and Kurfi locations presented mixed feed compositions (maize bran and cottonseed cake), while Daura and Dutsi predominantly used groundnut-based feeds. The feed samples were collected in sterile black doubled-nylon bags, while milk samples were collected aseptically in sterile screw-capped bottles. The milk samples were immediately transported to the laboratory in ice-packed boxes and stored at -20°C until analysis.

Physicochemical Analysis of Feed and Milk

The proximate composition of the feed and milk samples was analyzed in triplicate. Crude protein was determined using the standard Kjeldahl method (AOAC 980.21). Crude fat was extracted using a Soxhlet apparatus with petroleum ether as the solvent (AOAC 920.39). Crude fibre was determined by the acid and alkali digestion method (FAO 2003).

Analysis of Aflatoxin M1 in Milk

Milk sample (15 mL) was mixed with 40 mL of chloroform and 3 mL of a sodium chloride solution in a separating funnel. The mixture was shaken and allowed to separate. The chloroform layer was collected, evaporated to dryness, and the residue was dissolved in acetonitrile, defatted with petroleum ether. The samples were reconstituted in 2mL methanol, further purified with n-hexane twice, before being injected into HPLC (Aginent 1260 Infinity) for Aflatoxin M1 analysis.

Analysis of Aflatoxin B1 in Feed

Feed samples were analysed for AFB1 according to the protocol provided by Helica Biosystems Inc. Feed sample (20 g) was digested with 100 mL of 70% (v/v) methanol for 30 minutes. The extract was filtered, and the filtrate was used for HPLC analysis under the same conditions as for AFM1.

Health Risk Assessment

Estimated Daily Intake (EDI)

The EDI of AFM1 was calculated using the following formula:

$$EDI(ng/kgbw/day) = \frac{(CXD)}{BW}$$

Where:
C = Mean concentration of AFM1 in milk (μg/L)
D = Daily milk consumption (L/day). An average daily consumption of 0.5 L for adults (60 kg body weight) based on local consumption patterns.
BW = Average body weight (60 kg).

Margin of Exposure (MOE)

The MOE was calculated as the ratio of the Benchmark Dose Lower Confidence Limit (BMDL10) for hepatocellular carcinoma to the EDI

$$MOE = \frac{BMDL_{10}}{EDI}$$

A $BMDL_{10}$ value of 870 ng/kg bw/day for AFM1 was used as established by the European Food Safety Authority (EFSA, 2020). An MOE value of 10,000 or higher indicates a low public health concern.

Cancer Risk (CR)

The potential cancer risk was estimated using the potency factors for AFM1, which are significantly higher for individuals positive for Hepatitis B surface antigen ($HBsAg^{+}$) (Kew *et al*..,2013).

$CR\ (HBsAg^{+}) = EDI \times 0.03$ cases per 100,000 per year
$CR\ (HBsAg^{-}) = EDI \times 0.001$ cases per 100,000 per year

Statistical Analysis

All analyses were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Correlation analysis among the physicochemical parameters was performed using Pearson's correlation coefficient. Statistical significance was set at $p < 0.01$.

RESULTS AND DISCUSSION

Physicochemical Properties of Feed

The proximate analysis of cow feed samples revealed substantial variation (Table 4.1). Crude protein (CP) content ranged from 6.00% (Kurfi 1) to 14.17% (Charanchi), with most samples falling below 10%, which is critically low for lactating dairy cattle. Crude fibre (CF) content was extremely variable, from 8.00% (Daura 2) to 49.20% (Kurfi 2), indicating a reliance on high-forage, low-energy diets. Crude fat content ranged from 1.70% to 6.00%.

Table 1: Physicochemical Parameters of Cow Feed Samples

Sample	Crude Fat (%)	Crude Fibre (%)	Crude Protein (%)
Dutsin-ma 1	2.00 ± 0.10	20.33 ± 0.58	10.00 ± 0.72
Dutsin-ma 2	2.33 ± 0.40	20.00 ± 1.00	11.00 ± 0.62
Daura 1	2.00 ± 0.17	22.50 ± 0.87	9.00 ± 0.50
Daura 2	1.70 ± 0.10	8.00 ± 1.00	12.00 ± 6.00
Kurfi 1	3.00 ± 0.10	31.00 ± 1.00	6.00 ± 0.20
Kurfi 2	6.00 ± 0.10	49.20 ± 1.11	8.00 ± 0.50
Kankia	4.73 ± 0.21	47.00 ± 1.00	7.50 ± 0.30
Dutsi	4.00 ± 0.92	42.60 ± 1.44	7.07 ± 0.23
Charanchi	1.80 ± 0.26	11.30 ± 0.26	14.17 ± 0.49
Musawa	3.00 ± 0.10	34.20 ± 1.11	6.50 ± 0.62

Values are mean ± SD of triplicate analyses.

Physicochemical Properties of Cow Milk Samples

The analysis of milk samples also showed significant variation (Table 2). Protein content ranged from 1.20% to 7.30%, with most samples below the typical range of 3.0-3.5% for bovine milk. Fat content varied from 1.50% to 3.80%, with several samples below the typical benchmark of 3.5-4.0%.

Table 2: Physicochemical Parameters of Cow Milk Samples

Sample	Crude Protein (%)	Crude Fat (%)
Dutsin-ma 1	3.25 ± 0.50	2.60 ± 0.26
Dutsin-ma 2	3.20 ± 0.10	2.00 ± 0.10
Daura 1	3.20 ± 0.10	3.80 ± 0.10
Daura 2	7.30 ± 0.10	1.50 ± 0.40
Kurfi 1	2.07 ± 0.06	2.00 ± 0.17
Kurfi 2	1.20 ± 0.01	3.00 ± 0.10
Kankia	2.90 ± 0.10	2.67 ± 0.27
Dutsi	2.30 ± 0.01	2.40 ± 0.20
Charanchi	1.95 ± 0.50	2.50 ± 0.10
Musawa	1.99 ± 0.01	3.00 ± 0.10

Values are mean ± SD of triplicate analyses.

Correlations analysis among physicochemical parameters of cow feeds

Correlation analysis revealed a strong positive correlation between crude fat and crude fibre in feed (r = 0.917, p<0.01), and strong negative correlations between crude protein and both crude fibre (r = -0.775, p<0.01) and crude fat (r = -0.570, p<0.01).

The correlation analysis confirms that the nutrient composition of cow feed across the studied locations is directly influenced by the nature of locally available ingredients. These findings highlight the critical need to balance fat, fibre, and protein in ration formulation to optimize nutritional value and digestibility for cattle.

Table 3: Correlations analysis among physicochemical parameters of cow feeds

	Crude fat Feed	Crude Fibre feed	Crude Protein Feed
Crude fat Feed	1.000	.	
Crude Fibre feed	0.917**	1.000	
Crude Protein Feed	-0.570**	-0.775**	1.000

** Correlation is significant at the 0.01 level (2-tailed).

Correlations analysis among physicochemical parameters of cow milk

The correlation between crude fat and crude protein contents in the milk samples is presented in Table 4. A moderate negative correlation was observed between these parameters (r = –0.493, p < 0.01), indicating that as milk fat content increased, protein content tended to decrease. This inverse relationship suggests that milk with higher fat levels generally contained lower protein concentrations, possibly due to differences in nutritional intake, breed composition, or stage of lactation (Ng-Kwai-Hang et al., 2002).

Table 4: Correlations analysis among physicochemical parameters of cow milk

	Crude fat Milk	Crude Protein milk
Crude Fat Milk	1.000	.
Crude Protein Milk	-0.493**	1.000

** Correlation is significant at the 0.01 level (2-tailed).

Aflatoxin B1 contamination levels in cow feeds samples

The results for aflatoxin contamination were alarming. All of the cow feed samples were contaminated with AFB1 at levels exceeding the FAO/Nigeria regulatory limit of 20 µg/kg as shown in Table 5. The concentrations ranged from 25.31 µg/kg (Kurfi 2) to 74.44 µg/kg (Daura 1), with a mean concentration of 52.65 µg/kg. The highest concentration was found in Daura 1 (74.44 ng/g), followed by Dutsin-ma 1 (72.82 ng/g) and Dutsin-ma 2 (69.62 ng/g), while Kurfi 2 (25 ng/g) recorded the lowest level. The results suggest poor handling and storage conditions of feed ingredients, which promote fungal growth and toxin formation, increasing the likelihood of aflatoxin transfer from feed to milk.

Table 5: Aflatoxin B1 Concentration in Cow Feed Samples

Sample ID	AFB1 Concentration (µg/kg)	Status (FAO-20 µg/kg Limit)
Daura 1	74.44	Exceeds
Daura 2	40.13	Exceeds
Musawa	50.32	Exceeds
Kankiya	38.16	Exceeds
Dutsi	44.06	Exceeds
Kurfi 1	61.65	Exceeds
Kurfi 2	25.31	Exceeds
Charanchi	49.94	Exceeds
Dutsin-ma 1	72.82	Exceeds
Dutsin-ma 2	69.62	Exceeds

All analyzed feed samples contained AFB_1 levels exceeding the EU regulatory limit of 20 µg/kg, confirming widespread contamination across all locations as shown in figure 1. The highest concentration was detected in Daura 1 (73 µg/kg), followed by Dutsin-ma 1 (72 µg/kg) and Dutsin-ma 2 (70 µg/kg), while Kurfi 2 (25 µg/kg) had the lowest concentration. These results point to inadequate handling and storage conditions of feed ingredients, which promote fungal growth and aflatoxin production, thereby increasing the risk of toxin transfer into the milk chain.

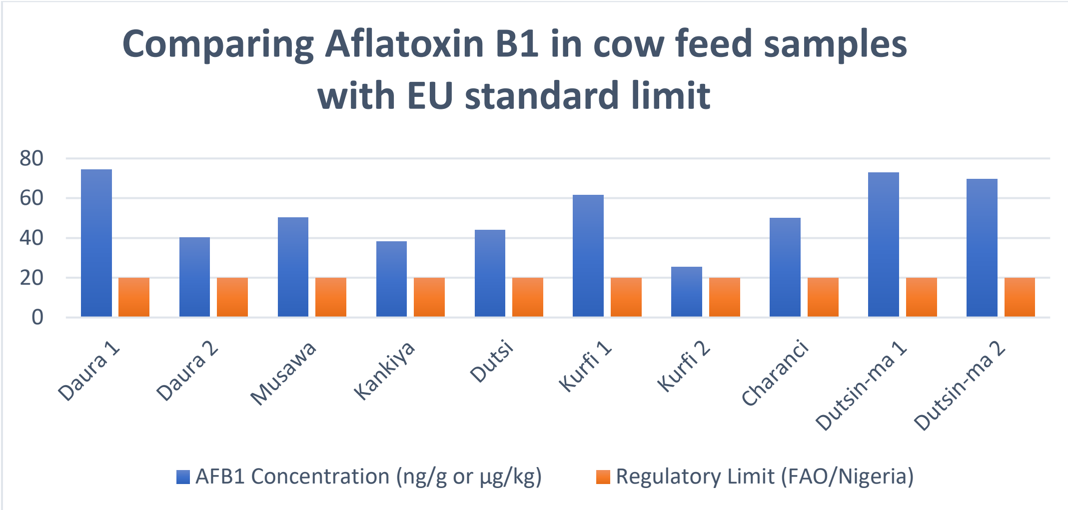

Figure 1: Comparison of Aflatoxin B1 concentrations in cow feed samples with EU standard limit

Aflatoxin M1 Concentration in Cow Milk Samples

The concentration of Aflatoxin M_1 (AFM_1) in cow milk samples collected from various locations compared with the EU regulatory limit of 0.05 ng/mL as shown in Table 6 below. All milk samples exceeded the permissible limit, indicating significant contamination. The highest AFM_1 concentration was found in Kurfi 1 (90.28 ng/mL), followed by Daura 1 (86.47 ng/mL) and Dutsi (70.31 ng/mL), while Musawa (6.42 ng/mL) and Dutsin-ma 2 (5.86 ng/mL) recorded the lowest values.

Table 6: Aflatoxin M1 Concentration in Cow Milk Samples

Sample ID	AFM1 Concentration (µg/L)	Status (EU Limit (0.05 µg/L)
Kurfi 1	90.28	Exceeds
Kurfi 2	10.28	Exceeds
Musawa	6.42	Exceeds
Kankiya	38.97	Exceeds
Daura 1	86.47	Exceeds
Daura 2	7.00	Exceeds
Charanchi	6.54	Exceeds
Dutsi	70.31	Exceeds
Dutsin-ma 1	7.19	Exceeds
Dutsin-ma 2	5.86	Exceeds

The elevated levels in Kurfi 1, Daura 1, and Dutsi as shown in Figure 2 below correspond to locations where highly contaminated feed ingredients such as *cottonseed, corn fibre,* and *millet stake* were used, suggesting a strong feed-to-milk carry-over effect. The results confirm that poor feed quality and storage conditions significantly influence AFM_1 contamination in milk, posing potential health risks to consumers.

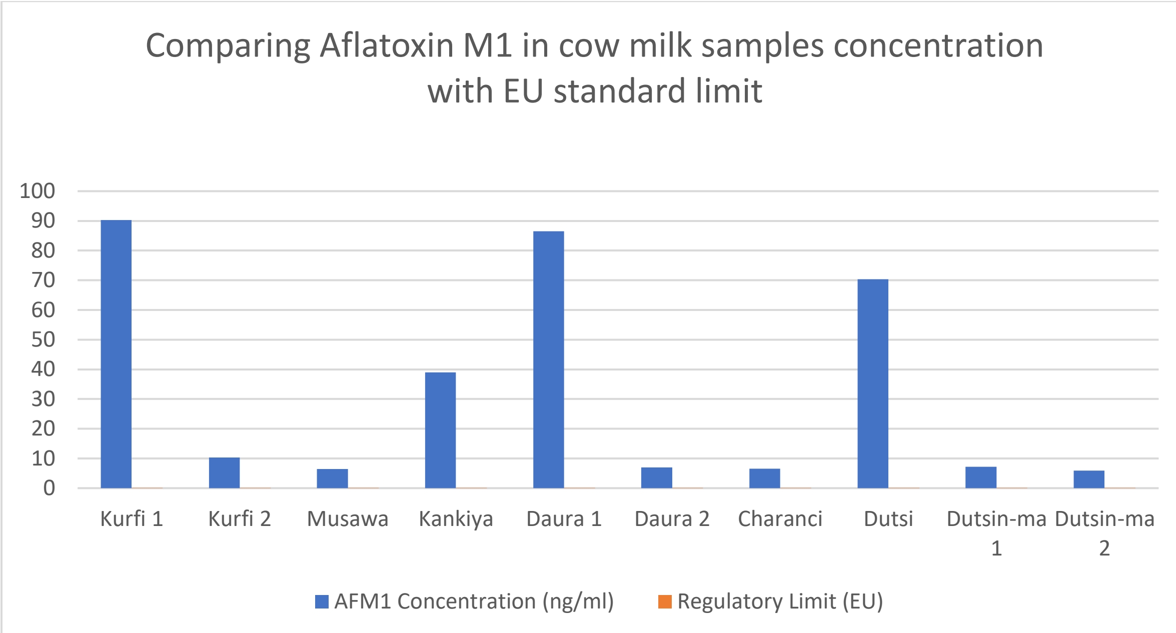

Figure 2: Comparison of Aflatoxin M_1 concentration in cow milk samples with EU standard limit

Health Risk Assessment

Estimated Daily Intake (EDI) and Margin of Exposure (MOE)

The health risk assessment confirmed a severe public health concern (Table 7). The Estimated Daily Intake (EDI) of AFM1 ranged from 2.15 to 33.10 ng/kg bw/day.

The Margin of Exposure (MOE) values, calculated using the $BMDL_{10}$ of 870 ng/kg bw/day, ranged from 26.28 (Kurfi 1) to 404.65 (Dutsin-ma 2). All MOE values were drastically below the safety threshold of 10,000.

Table 7: Estimated Daily Intake (EDI) and Margin of Exposure (MOE)

Sample	EDI (ng/kg bw/day)	MOE (= 870 / EDI)
Kurfi 1	33.10	26.28
Kurfi 2	3.77	230.77
Musawa	2.35	370.21
Kankiya	14.29	60.88
Daura 1	31.71	27.44
Daura 2	2.57	338.52
Charanchi	2.40	362.50
Dutsi	25.78	33.75
Dutsin-ma 1	2.64	329.55
Dutsin-ma 2	2.15	404.65

Cancer Risk (CR)

The calculated Cancer Risk (CR) was substantially higher for individuals positive for Hepatitis B (HBsAg+) than for those who were negative (HBsAg-). For HBsAg+ individuals, the CR ranged from 0.0645 to 0.9930 additional cases of liver cancer per 100,000 people per year. For HBsAg- individuals, the risk ranged from 0.00215 to 0.0331 cases per 100,000 per year (Table 8).

Table 8: Calculated Cancer Risk (CR) from AFM1 Exposure

Sample	EDI (ng/kg bw/day)	CR (HBsAg+) [cases/100k/year]	CR (HBsAg-) [cases/100k/year]
Kurfi 1	33.10	0.9930	0.03310
Kurfi 2	3.77	0.1131	0.00377
Musawa	2.35	0.0705	0.00235
Kankiya	14.29	0.4287	0.01429
Daura 1	31.71	0.9513	0.03171
Daura 2	2.57	0.0771	0.00257

Charanchi	2.40	0.0720	0.00240
Dutsi	25.78	0.7734	0.02578
Dutsin-ma 1	2.64	0.0792	0.00264
Dutsin-ma 2	2.15	0.0645	0.00215

Discussion

This study provides a stark and concerning assessment of the dairy value chain in some farms in Katsina State, Nigeria. The findings reveal a system plagued by nutritional deficiencies and severe, universal aflatoxin contamination, culminating in a significant public health threat.

The poor nutritional quality of the feeds, characterized by low crude protein and high crude fibre, indicates a reliance on low-quality forages and a lack of balanced feed formulation. This directly impacts animal health and productivity, as evidenced by the suboptimal protein and fat levels in the milk, which are below standard benchmarks for bovine milk (Heck et al., 2009; Alothman et al., 2019). The negative correlation between fibre/protein in feed suggests that nutrient-dense ingredients are being diluted by fibrous, low-quality materials, a common challenge in smallholder systems with limited resources (McDonald et al., 2019).

The most critical finding is the 100% prevalence of AFB1 in feed samples at levels that, on average, were 2.6 times the regulatory limit. This widespread and high-level contamination is a direct consequence of poor post-harvest management, including improper drying and storage of feed ingredients under the warm and humid conditions typical of Nigeria, which are ideal for *Aspergillus* growth and aflatoxin production. Similarly high levels of contamination have been reported in other parts of sub-Saharan Africa, highlighting a regional challenge (Matumba *et al*., 2020).

The efficient carry-over of AFB1 from feed to milk resulted in extreme contamination of milk with AFM1. The concentration in Kurfi 1 (90.28 μg/L) is over 1,800 times the EU's maximum limit. Such extreme levels suggest not just contamination from daily intake but potentially a "metabolic saturation" in the cattle, where chronic exposure leads to an accumulation of the toxin, overwhelming the liver's metabolic capacity (Britzi *et al.,* 2013). This situation poses an acute toxicological risk to consumers.

The health risk assessment quantifies this threat. The Margin of Exposure (MOE) approach is used for genotoxic carcinogens like AFM1 where no safe threshold has be established (EFSA, 2020). EFSA considers an MOE of 10,000 or higher to indicate a low health concern. The MOE values in this study, which were as low as 26.28, are astronomically below this threshold, indicating a severe public health risk.

The calculated cancer risks further contextualize this danger. While the absolute numbers per 100,000 may seem small, they represent a significant population-level burden in a region like Katsina with a high consumption of milk and a relatively high prevalence of Hepatitis B (8-12%) (Olayinka et al., 2016; Musa et al., 2021). The synergistic effect between aflatoxin exposure and Hepatitis B infection in dramatically increasing the risk of hepatocellular carcinoma is well-documented (Kew *et al*., 2013). This data shows that the cancer risk for HBsAg+ individuals consuming this milk is up to 30 times higher than for HBsAg- individuals. This underscores the urgent need for integrated public health interventions that address both mycotoxin control and HBV vaccination.

CONCLUSION

This study conclusively demonstrates that the dairy production system in the studied areas of Katsina State is severely compromised. Cattle are fed a nutritionally poor diet that is universally and heavily contaminated with aflatoxin B1. This contamination is efficiently transferred into milk as the highly toxic and carcinogenic aflatoxin M1, resulting in contamination levels that pose an acute and severe threat to public health. The consumption of this milk is associated with an unacceptably high risk of liver cancer, particularly for the vulnerable sub-population infected with Hepatitis B. The current state of the milk supply in this region is a public health crisis.

RECOMMENDATIONS

While this study has unequivocally documented the severe aflatoxin contamination in the feed-milk continuum within Katsina State, it also opens several critical avenues for future investigation. Subsequent research should focus on evaluating the efficacy and economic feasibility of locally available mycotoxin binders, such as certain clays and activated charcoals, in reducing the carry-over rate of AFB1 to AFM1 under typical smallholder farming conditions in Nigeria. Furthermore, to gain a more comprehensive understanding of the total toxic threat, future studies should expand the analytical scope to include a multi-mycotoxin panel, screening for other prevalent toxins such as ochratoxin A, fumonisins, and zearalenone in both feed and milk. Investigating the specific drivers of contamination, perhaps through a detailed survey correlating particular feed ingredients like maize or groundnut cake from specific sources with higher AFB1 levels, would help target intervention strategies more precisely.

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