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Original Article

Human Health risk Assessment of AflatoxinM1 in Cow milks from Selected Local Government Areas of Kano state, Nigeria

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A B S T R A C T

This study was carried out as part of environmental assessment in Kano state to determine contamination of cow milks by AflatoxinM1 from three local government areas of Kano state (Bichi, Rano and Nassarawa), and to correlate this concentrations to some risk indices, so as to analyze potential effects on consumers. The results obtained showed moderate contamination by AflatoxinM1 in the three local locations. The concentration of AFLM1 from Bichi local government ranges from 0.117 – 0.291µg/kg, while that of Nassarawa ranges from 0.095 – 0.283 µg/kg, and of Rano from 0.259 – 0.287µg/kg. Estimated daily intake (EDI) of AFLM1 and total hazard index (THI) for children within 1- 12years of age were calculated based on the data obtained therein.. The EDI of AFLM1 in the selected study area(s) was also determined and found to be within the range of 3.604 - 6.179 ng/kg.b.w/day in Bichi, 3.451 – 5.915 ng/kg.b.w/day in Nassarawa and 4.697-8.053ng/kg.b.w/day in Rano, all for children of age 1- 12 years. All hazard indices calculated for AFLM1 were below 1. Although the results of this investigation showed low risk of cancer, the variability in cow feeds and climatic conditions might influence contaminations, most especially AFLB1 contamination of feeds and consequently AFLM1 contamination of milk.

Introduction

Mycotoxins are a group of naturally occurring secondary metabolites which are mainly produced by the filamentous fungi (Iqbal *et al*., 2011).. Among the various classes of mycotoxins, aflatoxins (AFs), mostly produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and rarely by *Aspergillus nomius* and *Aspergillus niger* tends to be the most toxic and carcinogenic class. Varieties of fruits, vegetables cereals and animal feeds were also known to be contaminated with this mycotoxin (Asi *et al*., 2012). AFs are associated with the incidence of certain types of cancer which poses a global concern over food and feed safety (Gong *et al*., 2004; Turner *et al*., 2003) The four major naturally known aflatoxins produced by the *Aspergillus* species of mold include AFB1, AFB2, AFG1 and AFG2. AFM1 and M2 are metabolic products of AFB1 and B2, they are mostly secreted in milk of dairy animals. Countries in the Asian and sub Saharan Africa where poor agricultural practices coupled with sub-standard mitigation strategies are the worst heat by mycotoxicosis and its sttendant consequencies. More so, the tropical nature of the areas promotes to a large extent, the growth and proliferation of the fungi species (Thrasher, 2012; Wu, 2011).

Two main ways through which aflatoxins contaminate milk and dairy products are: Consumption of contaminated feed by the lactatng animals in which the aflatoxins B1 and B2 is transformed into aflatoxin M1 and M2 after being metabolized in the animal's body. Alternatively, contamination occurs when aflatoxin producing fungi in the environment, gain access to milk in the course milking or processes involved in the transport and storage of milk (Celik *et al*., 2005). Depending on geographic location, agricultural and agronomic practices, the occurrence of aflatoxins may vary in different food products. Moreover, fungal attack is thought to be prevalent in the entire process of food formulation ranging from pre-harvest to the last stage of processing (Thrasher, 2012; Wu, 2011). Various classes of food products such as: Cereals (pearl millet, maize, wheat and rice); spices (chillies, coriander, black pepper, turmeric), oil seeds (groundnut, cotton seeds and soybean) consumed

by the animals also contribute immensely in the introduction of aflatoxins B1 and B2 that metabolically get transformed into the MI and M2 (Lopez, 2002).

First isolated from milk of lactating animals fed on Moldy grains contaminated with aflatoxins, aflatoxins M1 and M2, are highly oxygenated heterocylic compounds that are as toxic as the aflatoxins B1 and B2 (Bennet and Klich, 2003). Contamination of feeds by aaflatoxins in Nigeria and hence its appearance as aaflatoxin M1 and M2 in the mikl of such animals has been reported by Oyeyipo *et al*. (2017), in the south western Nigeria and Makun *et al*. (2016), in Minna, North cental part of Nigeria.

Aflatoxins being lipophilic compounds, are readily absorbed through the gastrointestinal tract and respiratory tract into blood stream from the site of exposure (Agag,2004). Ingestion of other contaminated animal tissues may also lead to contamination of a healthy animals tissue with AFM1 (Makun *et al*., 2016) or by inhalation of dust particles of aflatoxins either B1 or B2 in contaminated foods in industries and factories (Coulombe, 1994).

The mechanism of carcinogenicity of both AFB1 and AFM1 is through metabolism by cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8, 9-epoxide, an active form that binds to DNA to form DNA-Aflatoxin adduct or to albumin in the blood serum, and hence causing the formation of DNA heat labile sites which leads to its breakage or damage (Wild and Montesano, 2009); Wu and Khlangwiset, 2010).

Contamination of foods and feeds with aflatoxin have dire consequences on human and animal health. More than 5 billion people in developing countries were estimated to be at the at risk of chronic aflatoxin exposure mainly due to consumption of aflatoxin contaminated foods.; four billion people or more develop hepatocellular carcinoma, as an aflatoxin related liver cancer (Strosnider, 2006; Liu, 2012; Shephard 2008; Williams, 2004).. It is safe to assume that the quantity of aflatoxin consumed is proportional to the mutagenic, carcinogenic, teratogenic, immunosuppressive effects in the body and stunted growth in children (Agag, 2004; USAID, 2012; Barret, 2005). An increase in circulating alpha tumor necrosing factor (α-TNF), as reported by some studies is aptly suggestive of the fact that these mycotoxins are also immunotoxic in humans and animals. Additionally, due to its body immunosuppressant effect, it has been associated with HIV and tuberculosis (Groopman *et al*., 2008; Liu and Wu, 2010). Aflatoxins also pose a threat to developing fetuses and they are transferred from mother to infant in breast milk. Aflatoxins have been reported to be associated with a Reye-'s syndrome in different countries such as Thailand, New Zealand, Czechoslovakia, the United States, Malaysia, Venezuela, and Europe (Thrasher and Crawley, 2009). These effects and its impacts on economic indices cannot be overridden.

Therefore, this study determined the concentration of AFLM1 in cow milk within the three studied local government areas of: Bichi, Nassarwa and Rano of Kano state and hence the EDI coupled to the derived hazard risk in consumers.

Materials and Methods

Background of study site

Kano state bounded by the latitude $12^{o}\ 00^{1}\ 0.43^{II}$ N and longitude $8^{o}\ 31^{I}\ 0.19^{II}$ E is located in the northern part of Nigeria, it is in the sahelian geographical region, south of the Sahara, it is one of the highly populated states in Nigeria after Lagos with an estimate of about 9,383,682 people (census 2006). Nassarawa is a local governments of Kano, situated centrally within Kano municipal, it has an area of 34 Km^2 and its densely populated, whereas, Bichi and Rano local governments located within the northern and the southern part of Kano respectively.

Sample collection

Eighty one (81) samples of cow milks were purposively sampled and analysed for AFLM1. These samples were collected from three identified herders sites called "Ruga" in the local dialect (Hausa), in each of the three selected study areas of Nassarawa, Bichi and Rano which are emirates that form the Kano central, north and south senatorial districts respectively. Nine (9) samples were collected from three(3) identified locations thus,. making a total of 27 samples from each local government.

Quantitative Determination of Aflatoxins

Sample collection and lyphophilization

Samples were collected in a clean and sterilized plastic containers and were freeze-dried prior to the experiment to prevent increase in moisture content.

Extraction of AflM1

The validated method of Asi *et al*. (2012). with some modifications was employed in the extraction of AflatoxinM1 from the milk samples. Briefly, the milk samples were defatted by centrifugation for 10 min at 3000 ×g. The supernatant (fat layer) was removed using spatula. The defatted samples were then used for the determination of AFM1 using ELISA kits.

Analysis of AflatoxinM1

The ELISA Kit protocol

The ELISA kit (Solarbio inc, China), operating manual guidelines for AflatoxinM1 determination was followed. All reagents and samples were brought to room temperature (20-25^{0} C) before use, defatted samples and standard solutions (100 µL each) were added in duplicate into the corresponding microwells of the Eliza plates, 50ul of diluted HRP (horse radish peroxidase) conjugate (prepared just before use) was added immediately to each well, the microplate was then covered with new adhesive foil and briefly shakened for seconds after which it was incubated for 30minutes at room temperature protected from light, each well was then aspirated and washed four times, washing was done by filling each well with 300ul of wash buffer(1x) and then left to stand for 30 seconds, 100ul of the mixture of substrate solution A and B(prepared just before use in 1: 1) was then dispensed into each well, and then incubated for 10 minutes at room temperature, 50ul of stop solution was then pipetted into each well and the optical density of each well was then recorded using a microplate reader set at 450nm and 630 nm

$$HI = EDI\ (ng/kg\ b.w./day)\ /(100ng/kg\ b.w)$$

Health Risk assessment

The estimated daily intake **(EDI)** was determined by the method of Fakhri *et al* (2019). While the HQ was determined based on the method described by Kuiper-Goodman (1990).

The EDI, was calculated thus:

$$EDI\ (ng/kg\ b.w./day) = DMI \times C/BW \quad (1)$$

Where, EDI is Estimated Daily, DMI, daily milk intake (ml/d); C, mean AFM1 concentration (ng/l); and BW as mean body weight (kg)

Estimated hazard index (HI)

Estimated hazard index (EHI) at TD_{50} which is the carcinogenic risk was estimated thus:

$$HI = EDI\ (ng/kg\ b.w./day)\ /TD50(100ng/kg\ b.w)$$

TD_{50}, is the dose at which 50% of test animals would have malignant tumors, with a safety factor of 50,000, which has been suggested as 100 ng/kgbw/day (Brera *et al*., 2015; Fakhri *et al*., 2019).

The value of the HI/TD50 ratio grater than 1, is indicative that, consumers are exposed to considerable liver carcinogenic risk (Kuiper-Goodman, 1990; Brera *et al*., 2015).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) in SPSS statistical package (version 20; SPSS, Chicago, IL).

Results and Discussion

Concentration and Exposure Assessment of AflatoxinM1

AflM1 was detected in all local governments studied. There is significant difference between the mean value of AflM1 obtained and the NAFDAC, maximum permissible limit (MPL) (used as a reference value.

Table 1. The occurrence of AFM1 in three different locations of Kano.

Location	N	positives (%)	Concentration of AFM (µg/kg)					
			Range (min. - max.)	Mean ± Sd	MPL	t	p	Above MPL(%)
Bichi	27	66.67	0.117 – 0.291	0.211 ± 0.066	0.5	-12.35	<0.01	none
Rano	27	44.44	0.259 – 0.287	0.275 ± 0.007	0.5	-93.66	<0.01	none
Nassarawa	27	88.89	0.095 – 0.283	0.193 ± 0.057	0.5	-15.00	<0.01	none

MPL = Maximum permissible limit (by NAFDAC), N = number of samples

Significant difference between values and the maximum permissible limits of Aflatoxins in milk samples was determined using One sample t-test

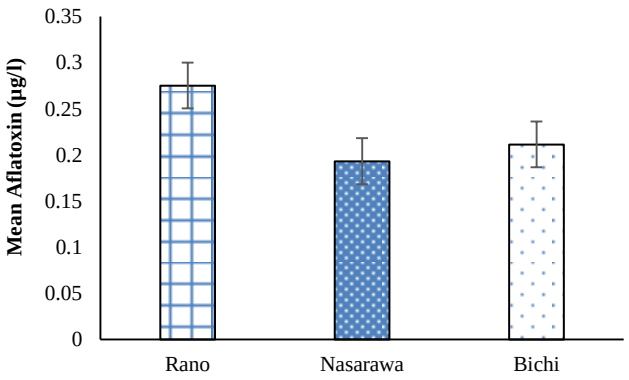

Fig 1. Comparison of the level of AflatoxinM1 concentration across the three local governments, bars with * are statistically significant at P ≤ 0.05.

The mean concentration of AflatoxinM1 detected from Bichi, Rano and Nassarawa local governments areas with values of: 0.211, 0.275 and 0.202 µg/kg respectively, is a clear indication that concentrations wise, Rano > Bichi > Nassarawa (Table 1). Despite the fact that, the values were below the MPL set by NAFDAC at 0.5 µg/kg, it is above the limit set by European union at 0.05 µg/kg . Though the values were within the acceptable limit in the Nigerian concept, possibility of trade on this commodity will be hampered between the country and the EU countries as it does not conform to the standard of the latter. The percentage abundance though averaged at 66.67% significant variation was noticed between local government studied. More so, the contamination level and the mean concentration are comparable the result obtained by Oyeyipo *et al*. (2017), in milks from south western Nigeria where he recorded a range value of 0.05 – 0.48 µg/kg, similarly, the percentage abundance obtained in this study can be compared with the result obtained by Makun *et al*. (2016), that recorded a value of 80% contamination in cow milks from Minna, in the North central Nigeria. Also, studies carried out in the Asian countries by Shahzad and Muhammad (2013); from Punjab, India, other parts of Pakistan Hussain and Anwar (2008), reported almost a mean concentration (212.2 ±11.9 ng/l) , a result quite similar to the one obtained in this as this study. It's well documented that fungi growth varies with ecological condition and climate Makun *et al*. (2010). Therefore the findings in this study, might be attributed climatic nature of the region that favours growth and proliferation of this fungi species., variation in feed type and aseptic conditions during sampling. At this juncture, it suffice to caution the personnel working in the milk and dairy product value chain on the need to adhere strictly to the Good Manufacturing Practices (GMP), since contamination of milk and other dairy products by aflatoxin M1 is still a major concern for the producer, consumer and regulatory bodies (Rahmani *et al*., 2018).

Exposure Assessment

This study focuses more on exposure effects to children rather than adult, this is because Infancy is a critical period of human life and a stage of exponential growth rate, and also because of the higher growth rate and lower body weight of infants, detoxification rates are lower than those of adults (Eaton *et al.*, 1994; Wild and Montesano, 2009). Therefore, children are more susceptible than adults to AFM1 danger (WHO, 2006).

The estimation of Hazard Index (HI) was as per described by Kuiper-Goodman (1990), which was reported also by Shundo *et al*. (2009) . In more details, estimated daily intake (EDI) was computed using mean values of AFM1 residues in positive samples and the data from milk consumption daily. The consumption of milk for the age of 1 was estimated to be 250 ml, for the ages of 3, 5 and 7 is 400 ml and 800 ml for the age of 12 (Tsakiris *et al*., 2013). The exposure assessment were developed for the ages of 1, 3, 5, 7 and 12 with a body weight of 10, 14, 19, 24 and 37 kg respectively (based on Greek pediatric development normograms).

Table 2. EDI (µg/kg body weight/ day) of Children from Bichi, Rano and Nassarawa LGA.

Location	EDI (ng/kg body weight/ day)				
	1	3	5	7	12 (years)
Bichi	5.486	6.179	4.553	3.604	4.680
Rano	7.150	8.053	5.934	4.697	6.095
Nassarawa	5.252	5.915	4.359	3.451	4.476

Table 2 is an estimate of the daily intake of toddlers in the ages stated provided the assumed quantity of milk is ingested per day, The EDI for Rano is the highest in the range of 4.697 – 8.053 ng ingestion per day, with children within 1-3 years having the highest daily intake, The EDI values for children decreased with increasing body weight. Bichi's EDI also ranges between 3.604 - 6.179,and Nassarawa having close range to Bichi, between 3.451 – 5.915, in all these children, at the age of 1-3 years the highest contamination is recorded. A study by Fakhri *et al*. (2019) reported EDI for male and female infants as 0.02 to 5.57 and 0.02 to 3.68 ng/kgbw/day similar to the present study, Additionally, EDI values for AFM1 from Spain, Argentina, Thailand, Brazil, and Pakistan in human breast milk, as reported by Alonso *et al*. (2010); Cano-Sancho *et al*. (2010); Ishikawa *et al*. (2016); Ismail *et al*. (2016); Ruanwises *et al*.,(2011). were similar to our findings in the range of 0.018 to 5.45 ng/kgbw/day. Due to the fact that aflatoxins are carcinogenic, international expert commitees (JECFA) did not specify a tolerable daily intake for these substances and concluded that daily exposure as low as < 1ng/kg b.w contributed to the risk of liver cancer, it was therefore recommended that levels should be reduced to as low as reasonably achievable [43].however the present study have EDI even upto 8ng/kg b.w. which translates to high contamination to the consumers and in essence predisposition to liver cancer.

HAZARD INDEX

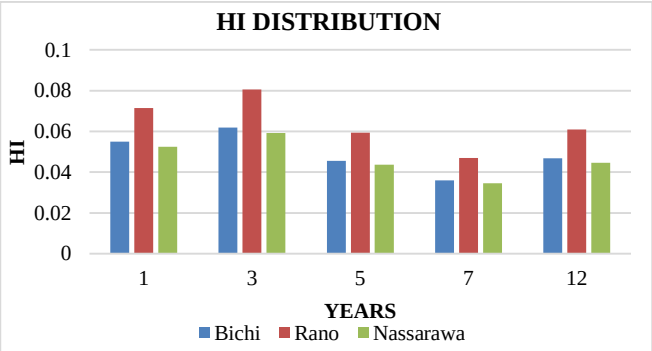

Fig 2. Hazard index distribution.

From fig. 2, The rank order of age of inhabitants based on EDI and harzard index(HI) values is 3yrs > 1yr > 12yrs > 5yrs > years, these ranks irrespective of local governments are the same, indicating a higher carcinogenic risk for younger infants from AFM1 in cow milk. The highest carcinogenic risk due to consumption of raw milk was detected in children from Rano. However, the consumers of cow milk in Nassarawa are not as exposed as those from Rano to a risk of AFM1 carcinogenesis, even though risk tends not to be so high, reduction plans are required to be implemented for reducing the concentration of AflatoxinM1 in cow milk.

Conclusion

AflatoxinM1 were detected from the selected local governments understudy and levels were higher than the permissible limits set by European union (0.2) and below that set by National agency for food and drug administration agency(NAFDAC), therefore levels detected were considered to be a source of risk for hepatotoxicity, and also due to elevated hazard index, it present a level of concern, therefore it is advisable that consumers are to take caution, and concern agencies should take proper actions in reducing or eliminating these risks.

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