OCCURRENCE OF AFLATOXIN IN THREE SELECTED SMOKED- DRIED FISH SPECIES AND ITS RISKS ON HUMAN HEALTH FROM NIGER SOUTH, NIGER STATE, NIGERIA

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ABSTRACT

Food contamination is a critical public health concern at the global level. Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus,* from fungi as their toxic secondary metabolites. Smoke-drying of fish is a popular traditional method and smoked-dried fish is mostly consumed in Nigeria. This study aimed to evaluate the occurrence of total aflatoxins in three selected smoked- dried fish species (*Clarias gariepinus*, *Prototems annectens* and *Oreochromis niloticus*) and the extent of the threat it might pose on human health. The study was carried out from three (3) riverine communities in Niger State, comprising of katcha, Muregi and Nupeko. A total of 18 samples comprising of *Clarias gariepinus*, *Prototems annectens* and *Oreochromis niloticus* intended for human consumption were analyzed based on seasons. The lowest aflatoxin concentration value obtained is (0.103 µg/kg) of *Clarias gariepinus* from Muregi and *Clarias gariepinus* from Nupeko have the highest aflatoxin value (3.59 µg/kg). The investigation was carried out using the enzyme-linked immunosorbent assay (ELISA) Kit. All the three (3) sampling stations show the presence of aflatoxin in all the fish samples from both seasons except the samples from katcha in dry season, which showed (0µg/kg). None of the value of Aflatoxin concentration from the fish samples exceeded the maximum level permitted (4µg·kg−1) by European Union, (20µg·kg−1) by Joint FAO/WHO Expert Committee on food additives (JECFA) and (15µg/kg) by United State and Food and Drug Administration. The risk calculated showed that the estimated daily intake (EDI) values of Aflatoxin was found higher in children (0.175 µg/kg/day, 0.196 µg/kg/day and 0,413 µg/kg/day) in Niger South (katcha, Muregi and Nupeko) followed by adolescent (0.098 µg/kg/day, 0.110 µg/kg/day and 0.232 µg/kg/day) and adult (0.075 µg/kg/day, 0.084 µg/kg/day and 0.177 µg/kg/day), the margin of exposure (MOE) values was lower in children (114.3, 102.0 and 48.4), followed by adolescent (204.1, 181.8 and 86.2) and adult (266.7. 238.1 and 113.0). All Moe values were < 10,000, which indicates possible health risk to consumers. The lower the value to 10,000, the higher the margin of exposure, the result showed children are more at risk compare to adult. According to the findings, the higher the degree of dryness in Smoked-dried fish the lower the chances of Aflatoxin contamination. Therefore, there is a need for the fishermen and the marketers to adopt a better method of preservation and storage and it will be necessary to estimate the assessment risk on infants, to understand the extent of aflatoxin contamination.

Keywords: Aflatoxin, Smoked-dried fish, *Clarias gariepinus*, *Prototems annectens*, *Oreochromis niloticus*, *Aspergillus.*

INTRODUCTION

Food safety and security remain a major concern in the sub-saharan Africa (Bankole *et al.*, 2006). Dry fish is the second most commonly consumed fish form in most Nigerian households and a preferred cheap method of preservation (Onyeneke *et al.*, 2020). Smoked fish products are the commonest form of fish product for consumption. Out of the total of 194,000 metric tons of dry fish produced in Nigeria, about 61% of it are smoked. One of the greatest problems affecting the fishing industry all over the world is fish spoilage, in high ambient temperature of the tropics, fresh fish have the tendency to spoil within 12 to 20 hours (Clucas and Ward, 1996). Ayeloja, *et al.*, (2018) opined that the major source of contaminants especially aflatoxins was as a result of poor handling, packaging and storage of fish and meat alike. Traditional methods are quite famous for fish processing, preservation, and storage in Nigeria, some of the most notable traditional methods used in fish processing and preservation include salting, smoke-drying and sun-drying. However, smoke-drying is the most popular method, and therefore a large volume of the fish is consumed in smoke-dried form. In rural areas where there are no refrigeration facilities, the smoke-dried fish happens to be the best option as the source of protein. The preference for the smoke-drying method lies in the fact of the availability and at low-cost of firewood. Reports have shown that mycotoxins can also occur in sun-dried or smoke-dried fish products, which are typically found in tropical and subtropical regions where high temperatures and humidity considerably influence fungal growth and toxin formation (Christane, *et al.*, 2020). Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus* and *A. parasiticus*. Rao *et al.*, (2021) highlighted that they are comparatively the most known mycotoxins owing to their ubiquitous nature. There are different types of aflatoxins, in which AFB1 and AFB2 is the most potent, among the to both humans and animals (Agriopoulou *et al.*, 2020). Due to these predicaments posed by mycotoxins (aflatoxins limits are usually set by countries/regions; e.g. European Union (4μg/kg), Food and Drug Administration of Unite State (15μg/kg) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) (20μg/kg) to regulate th permissible levels to safeguard consumers from cancer attributable to Aflatoxin and some other negative health effect:

MATERIALS AND METHODS

Study Area

The study was carried out in three (3) riverine communities in Niger state comprising of Muregi in Mokwa Loca Government areas (LGA), Nupeko in Lavun Local Government area and Katcha Local Government area in North Central, Nigeria. Niger State has a tropical climate consisting of two seasons, i.e., rainy season (April to September) and dry season (October to March) with annual rainfall varying from 1100 to 1600 mm (Niger State Bureau of Statistics, NSBS, 2012). The people in these communities are mostly artisanal fishermen and rice farmers, The sampling location for Muregi in Mokwa LGA is located approximately on the latitude of 8⁰45'15" N and longitude 5⁰49'58" E, the sampling location for Nupeko under Lavun LGA is located on the latitude 8⁰50'41" N and longitude 5⁰24'27" E and that of Katcha LGA is on latitude 8⁰45' 38" N and Longitude 6⁰18' 45" E.

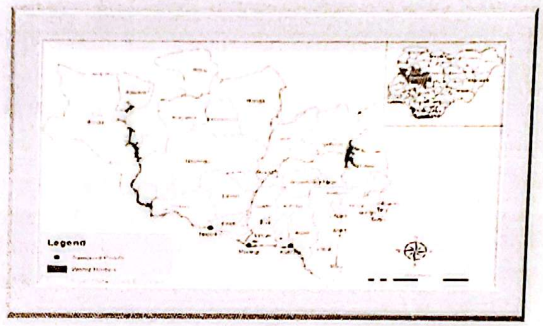

Fig. 1 Map of the three (3) sample areas

Collection of fish samples

The three species of smoked dried fish (*Oreochromis niloticus*, *Clarias gariepinus*, and *Prototems annectens*) were sampled randomly and purchased at each market. The fishes were identified using fish chart guide and by asking fish mongers for the local name. The fish samples were collected twice for a period of eight months from (May 2023 to December 2023). The first set of the smoked-dried fish samples were collected during mid raining season (July) and the second set were collected during early dry season (December).

Bio-Shield Total 5 ELISA kit test Method

It is a quantitative test based on ELISA, it allows the user to obtain exact concentrations of Aflatoxin in part per billion (ppb) or micro-gram per kilogram (μg/kg). The wells of the microtiter strips are coated with total aflatoxin specific antibodies. Toxins are extracted from a ground sample with 70% methanol. Aflatoxin standards or samples and Aflatoxin-HRP conjugate (detection solution) are added into the coated wells. Aflatoxin-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by Aflatoxin of standard or samples. Any unbound Aflatoxin-HRP conjugate of detection solution is removed in a washing step. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the detection antibody. The color development is then stopped by the addition of acid, turning the resultant final product yellow. The measurement is made photometrically at 450nm and the intensity of the produced colored complex is indirectly proportional to the concentration of total Aflatoxin present in the samples and standards (Eva Engvall and Peter Perlman, 1971).

Risk Assessment of exposure to Aflatoxin via consumption of Smoked-dried fish (Nile Tilapia, *Clarias gariepinus* and Africa Lungfish)

Estimated Daily Intake (Exposure)

The daily estimated intake (EDI) of Aflatoxin for adults, Adolescent and children were determined using the formula below and expressed in μg/kg of body weight/day (dos Santos *et al.*, 2013, EFSA, (2020).

EDI = (Daily Intake (food) x mean level of total aflatoxin)/ average body weight ……. (1)
Daily intake of smoked dried fish in Nigeria according to Assogba *et al.* (2019) is approximately 18.2g/day (6.643kg/yr). The different age categories used in this study were done as follows; children – 26 (24-28) kg (WHO, 2006, Biritwum *et al.*, 2005), adolescents – 46.25 (38.5-54) kg (Afrifa–Anane *et al.*, 2015), Adults – 60.7kg (Walpole *et al.*, 2012).

Margin of Exposure of Aflatoxin
MOE calculate the risk by the ratio of carcinogenic dose to population intake. In this study, MOE was calculated by dividing benchmark dose limit (BMDL) of total aflatoxin by toxin exposure (Adetunji *et al.*,2018) as expressed in equation below;

MOE = BMDL/Toxin Exposure (EDI) ……………………..……………………………… (2).

Estimated liver cancer risk due to consumption of Smoked-dried fish
The cancer risk can be calculated using the below formula (Kortei *et al.*, 2021);

Cancer Risk = Exposure (EDI) x Average potency ……………...……………………… (3).

According to the FAO/WHO, the population risk for primary liver cancer can be estimated with the assumption of 25% carriers of hepatitis B in developed countries (Duarte *et al.*, 2013). The potencies of hepatitis B virus (HBV) infection and HBV non-infection values are 0.3 and 0.01 respectively, estimated from animal and epidemiological studies (Duarte *et al.*, 2013). Hence, the potency of liver cancer in Nigeria population can be estimated using the following equation;

Average potency $= (0.01 \times 75\%) + (0.30 \times 25\%) = 0.0825$

Questionnaire
A total of one hundred and fifty (150) well-structured validated questionnaires of 15 items each were prepared and administered in an interview form and answered by fifty (50) fish processors each from the three communities comprising of male and female adults. An assistant field officer from the communities with at least O'Level qualification and also understood the local language helped in administering and collection of answered questionnaires.

Data Analysis
Data were analyzed using SPSS (IMB, version 29.0) packages. The mean (estimates) and standard errors of the aflatoxin levels in the various smoked-dried fish species were calculated. Significance of difference in aflatoxin levels between various fish species, the sampling area, weight, length, seasons etc. was examined using one-way variance of analysis (ANOVA). For each aflatoxin, ($p < 0.05$) mean levels between various fish species, weight, and seasons were investigated using Pearson Chi square Tests. Deterministic risk assessment models were used; dietary exposure (Estimated Dietary Intake), MOE values, Average potency and cancer risk.

RESULTS

Table 1: Concentration of Aflatoxin in smoked dried fish for both seasons.

Sample name	Sample Conc. (µg/Kg)	
	Rainy season	Dry season
KCat	0.273	0
KLung	0.295	0
KTilap	0.158	0
MCat	0.141	0.103
MLung	0.379	0.224
MTilap	0.484	0.417
NCat	0.368	3.59
NLung	0.451	0.25
NTilap	0.545	0.385

Keys: K – Katcha, M – Muregi, N – Nupeko, Cat – *Clarias gariepinus*, Lung – Lungfish, Tilap – Tilapia.

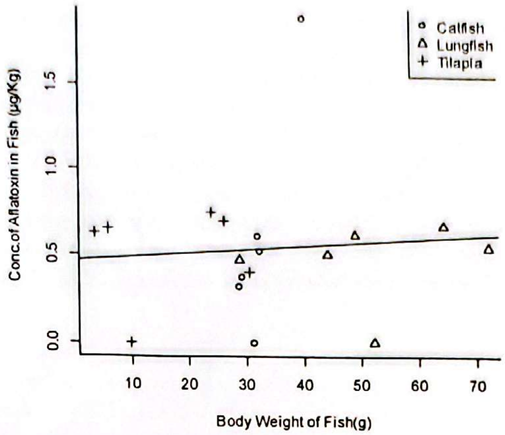

Figure 2: The relationship between aflatoxin and body weight of fishes

Table 2: Socio-demographic characteristics of the 150 surveyed consumers in the study area

	Gender		Age					Education			
Respondent	Male	Female	25-29	30-34	35-39	40-45	45 >	Primary	Secondary	Tertiary	None
(%)	48.34	51.66	4.64	35.10	27.15	17.22	15.89	50.99	45.70	1.32	1.99

Table 3: Risk Assessment for Total Aflatoxin via Consumption of Smoked-dried fish.

LGAs	Age category	Avg. Body Wgt. (kg)	EDI (µg/kg/day)	MOE	Population risk for Liver Cancer (Cases/100,000person/yr)
Katcha (0.25µg/kg)	Children (36months-10yrs)	26 (Benford *et al.* 2010)	0.175	114.3	0.014
	Adolescents (11-17yrs)	46.25 (Tongo *et al.* 2018)	0.098	204.1	8.1×10^{-3}
	Adults	60.7 (Tongo *et al.* 2018)	0.075	266.7	6.2×10^{-3}
Muregi (0.28µg/kg)	Children (36mths-10yrs)	26	0.196	102.0	0.016
	Adolescents (11-17yrs)	46.25	0.110	181.8	9.1×10^{-3}
	Adults	60.7	0.084	238.1	6.9×10^{-3}
Nupeko (0.59µg/kg)	Children (36months-10yrs)	26	0.413	48.4	0.034
	Adolescents (11-17yrs)	46.25	0.232	86.2	1.91×10^{-2}
	Adults	60.7	0.177	113.0	1.46×10^{-2}
Average					**0.014**

Margin of Exposure-MOE. Estimated Daily Intake-EDI. average potency of aflatoxin= 0.0825 Average Body weights were obtained from the different ranges referenced by the authors. 1µg = 1000ng.

DISCUSSION

The three (3) fish samples (*Oreochromis niloticus*, *Clarias gariepinus*, and *Protoptems annectens*) tested, shows the presence of Aflatoxin within the acceptable range. The number of Smoked-dried fish samples contaminated with Aflatoxin is presented in Tables 1. The level of occurrence of the Aflatoxin ranged between 0 and 3.59µg/kg. The *Clarias gariepinus* from Muregi showed the lowest Aflatoxin concentration (0.103µg/kg) in dry season, *Clarias gariepinus* from Nupeko had the highest aflatoxin concentration (3.59μg/kg) in dry season. All fish samples from rainy season shows the presence of aflatoxin except three (3) species show zero (0) aflatoxin concentration in dry season. The mean recorded 0.25, 0.28, and 0.59 μg/kg for Katcha, Muregi, and Nupeko region, respectively, and showed statistical differences ($P < 0.05$) (fig 1), when the Relationship between Aflatoxin concentration in fish samples from three regions was evaluated. Furthermore, when the effect of season on the concentration of aflatoxin was evaluated, the linear model results revealed no significant difference in aflatoxin ($P > 0.05$) between the wet and dry season and as such no seasonal effect. Despite the non-significant relationship, the Aflatoxin concentration was slightly higher in the wet season than dry season. The concentration of aflatoxin in catfish increased with an increase in body weight, while in contrary, the concentration of aflatoxin in Tilapia decreased with an increased body weight. However, the response of Lungfish to this relationship was apt, in that heavy bodied Lungfish had a decreased level of aflatoxin (Figure 2).

Occurrence of Aflatoxin

Smoked-dried fish is consumed by most Nigerians across certain age categories; children, adolescents and Adults (Bellio *et al.*, 2016). The results obtained from this study show a considerable presence of aflatoxins in Smoked-dried fish sold in Katcha, Nupeko, and Muregi markets of Niger state, Nigeria due to presence of moisture i.e the degree of dryness in the fishes were poor. Although, the concentration levels are below the permissible limit but continued exposure can have a severe effect on human health. Singh and Nsokolo, (2020), reported similar results of Aflatoxin concentration ranging from 1.3ppb – 3.84pbb when working on the prevalence of Aflatoxin on smoked-dried and fresh fish in Zambia. Akinyemi *et al.* (2011) reported much lower aflatoxin concentrations from 0.030 ppb to 1.150 ppb from smoked-dried Fish sold in Abeokuta, Ogun State, South-West Nigeria. The present study had lower aflatoxin contamination compared with previous studies on dried fish and smoked fish from Zambia and Nigeria (Kachapulula *et al.*, 2018). The high aflatoxin contamination of *Clarias gariepinus* observed in the present study may be attributed to these practices, especially poor storage and improper drying. Another reason for possible continuous high contamination observed in the rainy season values might be because of the presence of moisture (humidity) in air, which increases the chances of fungal growth and mycotoxin production. Often, retailers display the smoked-dried fish samples in trays or table openly, and this favors fungal attack and production of toxins (Eyo, 1992. Bukola *et al.*, 2008). The results obtained from the questionnaire showed that females 155 (51.7%) were more involved in smoked-drying of fish than the males with 145 (48.3%). The age of the fish processors determined their acceptability and willingness, Age range of 30 – 34years and 35- 39years had the maximum while age range of 25 – 29years and 45years>had the minimum involvement. The educational level of the fish processors has affected the improvement of traditional method to the new techniques of processing fish, fish processors with primary education and secondary education has the maximum participation with tertiary education level been the lowest. This present observation on the sex, age and educational level participation in fish processing is in accordance with report of Davies α Davies, (2009).

Risk Assessment

The estimated mean Aflatoxin 0.413 μg·kg−1·BW·day−1 (Table 4) exposure obtained from the present study was much higher than those reported in Ghana and Nigeria, with the mean dietary intake of aflatoxin in rice and peanuts (0.013 and 0.17) ng·kg−1·BW·day−1 respectively (Oyedele *et al.*, 2017, Korley Kortei *et al.*, 2019). Similarly, the estimated daily intake of aflatoxins through the consumption of smoked dried fish from this study was higher than that of Orony *et al.*, (2015).

Several studies previously used the margin of exposure (MoE) approach for risk description of genotoxic and carcinogenic mycotoxins like AFs. We adopted the same approach in this study. It is reported that an MoE value of ≥10000 should be considered as "safe," while an MoE value ≤ 10000 could cause a potential risk to public health, and the lower the value, the higher the risk (Benford *et al.*, 2010, Heshmati *et al.*, 2017, Cartus α Schrenk, 2017). As shown in Table 4.7, the MoE values obtained from this study is <10000, indicating a potential risk to the consumers of Smoked-dried fish. Previous studies reported that MoE values for the babies and toddlers were the lowest, which is not different from the present study. This indicates that children might have the highest risk of being exposed to Aflatoxin (Wang *et al.*, 2018, Zhang *et al.*,2020).

In Nigeria, cases of liver cancer have been increasing with an age standardized incidence rates (ASR) of 7.7 and 2.1 (male and female) per 100,000 population (Kew, 2013). Therefore, it is estimated from our results that consuming aflatoxin-contaminated Smoked-dried fish could be responsible for 0.18% and 0.67% representing male and female respectively, of all cancer cases of the three communities in Niger South of Niger state. This is minimal since it's not up to 1% but it should not be ignored due to bioaccumulation or since there is a greater contribution that may take place for the population consuming a daily amount of Smoked-dried fish above the national average.

CONCLUSION AND RECOMMENDATION

Although, the presence of Aflatoxin concentrations in Smoked-dried fish was below the limits acceptable by regulatory bodies in the three different regions in Southern Niger but continuous intake or Bioaccumulation of aflatoxins contaminated food may constitute a health hazard. However, it is evident from the results of these study that smoked-dried fish could also be problematic routes for exposure to aflatoxins, since presence of moisture, improper smoking, drying and poor storage of fishes may lead to insect infestation, fungal attack and degradation of smoked-dried fish. Considering the adverse health especially hepatocellular carcinoma (HCC) outcome of the health risk assessments in different age categories since the calculated margin of exposure (MOE) were less than 10,000. It is therefore important that adopting a better method of preservation by increasing the degree of dryness and proper storage of smoked-dried fish in lessening Aflatoxin contamination in processed fish.

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Data availability: The datasets generated during or analyzed during the current study are available from the corresponding author on reasonable request.

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