Mycotoxicology Society of Nigeria

Mycotoxicology, *2014, 1: 57-63*

ORIGINAL RESEARCH

Aflatoxigenic moulds and aflatoxin contamination of retailed fishery products in Lagos markets

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ABSTRACT

Forty fishery products (27 fin fish, 4 shell fish and 9 fish feed samples) were obtained from markets in Lagos and analyzed for the presence of aflatoxigenic moulds and aflatoxins with the view of assessing the quality of these products. Mycological analysis was performed by the dilution plating technique while aflatoxin analysis was by an Enzyme-Linked Immunosorbent Assay method. *Aspergillus flavus* and *A. tamarii* were the only *Aspergillus* section *Flavi* species recovered from all fish feed, 50% of shell fish and 37% of fin fish samples. *Aspergillus flavus* occurred more frequently (p<0.05) than *A. tamarii* in each category of fishery product. The incidence of non aflatoxigenic *A. flavus* isolates was higher than that of aflatoxigenic *A. flavus* isolates in all categories of fishery products. All fish and fish feed samples contained aflatoxins at concentration ranges of 1.05–25.00 µg/kg. Fish feed samples contained significantly (p<0.05) more aflatoxins than the fish samples. Total aflatoxin levels exceeded 10 µg/kg and 20 µg/kg in 66.67% and 22.22% of fish feed respectively. Smoked-dried fin and shell fishes may therefore represent a safe food for human consumption in view of mycotoxin contamination. Furthermore, efforts should be intensified to lower aflatoxin levels in feed formula fed to fishes.

Key words: Aflatoxin, *Aspergillus* species, Fish feed, Food safety.

1.0 Introduction

Fish is an important source of dietary protein and minerals, and from the economic standpoint, it is a source of income to many people in developing countries. Fish is highly perishable due to its high moisture and fat content. According to Akande and Tobor (1992), freshly caught fishes in artisanal fishery are usually covered with damp sack and sometimes wet grass in order to maintain a conducive temperature and to delay spoilage prior to further processing. However, this practice can serve as a source of microbial contamination to the harvested fish if processing is delayed. Additional sources of contamination include poor sanitary condition of handlers and market environs, and improper storage conditions of the fishes. In developing regions such as in sub-Saharan Africa where electricity is unstable in rural areas, fish is preserved more often through a traditional process involving salting, sun drying, smoking and a further air drying during sale. The step-wise process reduces the moisture content, limits the range and quantities of microbial flora that invade and colonize the smoke-dried fishes, and imparts flavour on the fishes. This in turn contributes to the extension of the shelf-life of the fishes (Park, 2002). However, in humid conditions, smoke-dried fish can absorb moisture from the atmosphere and this could lead to the fungal contamination and subsequent mycotoxin formation, thereby posing a challenge to the consumers of the product (Park, 2002).

Few studies have reported the presence of aflatoxins; highly toxic compounds naturally produced by *A. flavus*, *A. parasiticus* and some microsclerotial species of *Aspergillus* section *Flavi* (Pildain *et al*., 2008), in fish and fish feed (Adebayo-Tayo *et al*., 2008; Adejola, 2011; Almeida *et al*., 2011; Arowora *et al*.,

2012; Barbosa *et al*., 2013). In addition, the hazardous effects of aflatoxin contaminated feeds on fish health and production have been documented (Jantrarotai and Lovell, 1990; Hussein *et al*., 2000; Shehata, 2003; Abdelhamid *et al*., 2007; Zaki *et al*., 2008; Ruby *et al*., 2013). In spite of the available data on aflatoxin contamination of various foodstuffs and other livestock feeds, and the associated health implications, little or no data exists on the occurrence of *Aspergillus* species and aflatoxins in fin and shell fishes in Nigeria despite the fact that both fishes are widely consumed in Nigeria due to their high nutrient profiles. Adebayo-Tayo *et al*. (2008) had reported that fishes have the best sources of protein that is better digested than that of beef.

This survey work was therefore embarked upon to assess the presence and quantities of aflatoxigenic fungi and aflatoxins in some common smoked-dried fish and fish feeds sold in Lagos markets. This study will be useful for fish farmers and consumers as well as public health specialists.

2. 0. Materials and methods

2.1 *Sample collection and preparation*

Forty fishery products (27 fin fish, 4 shell fish and 9 fish feed samples) were obtained from fish retailers in Lagos markets. The fish samples had been smoked-dried and only fish and feed samples that showed no trace of visible mouldiness were purchased. The fish samples were randomly purchased from three markets (Oto, Mile 12 and Lagos Island) while the feed samples were obtained from Oko-oba market, Lagos. Each sample was collected as a bulk 1.5 kg representative sample obtained from several parts of the retailers' stock, placed in sterile polythene bags, labelled appropriately and taken to the laboratory for storage at 4^{o}C.

Fungal and aflatoxin analyses were performed on all samples within 48h of collection. Prior to analyses, each sample was ground into fine powder using a high speed blender and batched into two parts: part A for fungal analysis and part B for aflatoxin analysis. Each batch weighed 25 g as a representative sub-sample; this was taken for mycological and aflatoxin examination.

2.2 *Isolation and characterization of Aspergillus section Flavi from fishery products*

Mycological analysis was carried out on all part A sub-samples to assess the incidence of species belonging to *Aspergillus* section *Flavi*. Moulds were isolated from the samples by the dilution plating technique (Samson *et al*., 1995) and characterized by the phenotyping method for *Aspergillus* section *Flavi* described by Ezekiel *et al*. (2014a). Ten grams of each ground sub-sample was diluted with 90 ml of 0.1 % sterile peptone water, vortexed for 2 min and 1 ml aliquot of the sample was inoculated on triplicate plates of acidified ¼ strength Potato Dextrose Agar (PDA). Acidification of PDA was by addition of 0.01% lactic acid per litre of PDA. The inoculated plates were incubated at 30 ^{o}C for 3–5 days.

All isolates that bore a resemblance to *Aspergillus* section *Flavi* species were transferred from the PDA plates to neutral red desiccated coconut agar (NRDCA) plates and incubated at 30^{o}C for 5 days. The phenotyping of all isolates was performed by making (1) three-point inoculation/plate of each isolate in order to examine consistency in morphological (macroscopic and microscopic) characters, and (2) single-point inoculation/plate of each isolate for qualitative assessment of aflatoxigenicity (Ezekiel *et al*., 2014a).

2.3 *Qualitative determination of aflatoxigenic potential of isolates*

The isolates were also qualitatively screened for their ability to produce aflatoxins by observing the obverse and reverse sides of each single-point inoculated NRDCA plate at 24-hour interval under long wave UV light (365 nm) until the third day for maximum fluorescence (Ezekiel *et al*., 2014a). Pigmentation, fluorescence and characteristic colour of fluorescence were used as indices to evaluate the ability of each isolate to produce aflatoxins (Ezekiel *et al*., 2014a). The identity of each isolate was confirmed to belong to one of the following species of *Aspergillus* section

Flavi: *A. flavus* L-strain, *A. minisclerotigenes*, *A. parvisclerotigenus* and *A. parasiticus*. For confirmation of *A. tamarii* isolates, suspected isolates on NRDCA were transferred to 5/2 agar (5% V-8 juice and 2% agar, pH 5.2) and incubated at 30 ^{o}C for 5 days (Diedhiou *et al*., 2011).

2.4 *Analysis of fishery products for aflatoxins*

Assessment of fish and fish feed samples for levels of aflatoxin contamination was carried out by an Enzyme-Linked Immunosorbent Assay (ELISA) method in which free aflatoxin in the samples and control compete with enzyme-labelled aflatoxin conjugate for the antibody-binding site. This was performed as follows using the R-BIOPHARM (Darmstadt, Germany), -Ridascreen Aflatoxin Total Test kit (Art. No. R4701).

Five grams of each part B sub-sample was weighed into an extraction bottle and 25 ml of 70 % methanol added. The mixture was shaken vigorously for about 10 min and allowed to settle. The supernatant was filtered through Whatman No.1 filter paper and the filtrate was collected into a collecting tube. About 100 µL of the filtrate was then diluted with 600 µL distilled water and 50 µL of this mixture was taken for the assay. About 50 µL each of standard solution, enzyme conjugate and antibody solution were successively added to 50 µL of the samples in duplicate microtiter wells. The mixture was gently shaken and incubated in the dark at ambient temperature for 30 min. After incubation, the microtiter wells were emptied of the mixture and washed three times with washing buffer. About 100 µL of the substrate/ chromogen was then added to each well, mixed gently and incubated in the dark at ambient temperature (30^{o}C) for 15mins. This was followed by the addition of 100 µL of the stop solution to each well and subsequent measurement of the absorbance at 450 nm using Convergys ELR 96X ELISA Reader (Convergent Technologies GmbH & Co. KG, Germany).

The results were then extrapolated from a standard curve using RIDA SOFT Win (Art. No. Z999) software and reported as µg/kg total aflatoxin of the samples.

2.5 *Data analysis*

All the data obtained were analyzed by the SPSS® 14.0 Windows version (SPSS, IL, ISA). Means were calculated, tested for significant difference at α = 0.05 using the one-way ANOVA and separated by the Duncan's Multiple Range Test.

3.0 Results

3.1. *Incidence of Aspergillus section Flavi isolates*

The incidence of *Aspergillus* section *Flavi* isolates in the fishery products from Lagos markets are shown in Table 2. All fish feed, 37% of fin fish and 50% of shell fish samples were contaminated with *Aspergillus* section *Flavi* isolates. A total of 109 isolates (fish feed-59, fin fish-41 and shell fish-9) were isolated. There was no significant difference in the microbial load (cfu/g) of the *Aspergillus* section *Flavi* isolates in the fishery products although the load was highest in the fish feed samples. In particular, only *A. flavus* and *A. tamarii* species were found in the samples.

Table 1: Incidence of *Aspergillus* section *Flavi* isolates in fishery products from Lagos state, Nigeria

Fishery product	*N*[a]	Mean CFU/g[b]	*N*[c]	Incidence[d] (%) of species	
				A. flavus	*A. tamarii*
Fish feed	9/9	590a	59	49 (83.05)a	10 (16.95)b
Fin fish	10/27	380a	41	36 (87.80)a	5 (12.20)b
Shell fish	2/4	450a	9	9 (100.00)a	0 (0.00)b

[a]Number of samples contaminated with propagules of *Aspergillus* section *Flavi* isolates.
[b]Means of colony forming unit per gram of fishery products analyzed.
[c]Number of *Aspergillus* section *Flavi* isolates obtained from each fishery product.
[d]Incidence of species with different alphabets in a row are significantly different (p<0.05).

Table 2: Aflatoxin contamination of fishery products from Lagos state, Nigeria

Fishery product	N^a	Min	Max	Mean[b] ± SD	N^c (%) >10	N^c (%) >20
Fish feed	9/9	4.97	25.00	13.69a ± 7.18	6 (66.67)	2 (22.22)
Fin fish	27/27	1.05	10.00	5.40b ± 2.40	0 (0.00)	0 (0.00)
Shell fish	4/4	4.23	5.90	5.23b ± 0.77	0 (0.00)	0 (0.00)

[a]Number of samples contaminated with aflatoxins.
[b]Mean values with different alphabets are significantly different (p<0.05).
[c]Number of samples positive for aflatoxins above the specified limit of 10 µg/kg and 20 µg/kg.

The incidence of *A. flavus* (83.05–100.00%) was significantly (p<0.05) higher than that of *A. tamarii* (12.20–16.95%) in the fishery product. Only 20.40% , 11.11% and 22.22% of *A. flavus* isolates in the fish feed, fin fish and shell fish samples, respectively, produced aflatoxins on NRDCA during phenotyping while all *A. tamarii* isolates did not produce any aflatoxin (data not shown).

3.2. Occurrence of aflatoxins in processed fish and fish feed samples

Table 1 shows the total aflatoxin contamination of the fishery products. All fish and fish feed samples contained aflatoxins and the concentrations of total aflatoxins ranged between 1.05–25.00 µg/kg. The concentrations of aflatoxins in the fish feed samples (range = 4.97–25.00 µg/kg; mean = 13.69 µg/kg) were significantly (p<0.05) higher than the concentration in the fish samples [fin fish (range = 1.05–10.00 µg/kg; mean = 5.40 µg/kg); shell fish (range = 4.23–5.90 µg/kg; mean = 5.23 µg/kg)]. Total aflatoxin levels exceeded 10 µg/kg and 20 µg/kg in 66.67% and 22.22% of fish feed respectively.

4. Discussion

Consumption of aflatoxin contaminated feed has been a major challenge in fish farming as it causes reduced growth and several pathologies in different fish and shrimp species (Ruby *et al*., 2013; www.biomin.net). This study has shown that fishery products retailed in Lagos markets are contaminated with aflatoxigenic fungi and aflatoxin, albeit at low-to-moderate levels. Fungal propagules may have been introduced into the fish samples during handling or through improper storage of the fish or as a result of poor sanitary conditions of the markets. The recorded dominance of *A. flavus* over *A. tamarii* in the fish and fish feed samples conforms with previous studies on fishery products (smoked-dried fish and fish feed) by Wheeler *et al*. (1986), Atapattu and Samarajeewa (1990), Fafioye *et al*. (2002), Essien *et al*. (2005), Adebayo-Tayo *et al*. (2008), Almeida *et al*. (2011) and Barbosa *et al*. (2013) that isolated *A. flavus* as the predominant member of the *Aspergillus* section *Flavi* group. This possibly indicates that the nutrient profiles of fish, water activity levels, competition from related fungal species as well as other unknown factors may be responsible for the absence or non-recovery of other members of the section *Flavi* especially the highly toxigenic species (e.g. *A. minisclerotigenes* and *A. parvisclerotigenus*). However, this is the first report of *A. tamarii* occurrence in smoked fish in Nigeria.

The levels of aflatoxins in the present study is similar to that obtained from smoked-dried fish from Uyo (South-South Nigeria) (range = 1.5–8.1 µg/kg (Adebayo-Tayo *et al*., 2008) but higher than the levels in smoked-dried fishes from Abeokuta (South-Western Nigeria) (range = 0.03–1.15 µg/kg) (Adejola, 2011). Furthermore, aflatoxin levels in the smoked-dried fish were within the acceptable limit of 10 ppb. However, this does not guarantee the safety of the fishes for human consumption since (1) they are used on a daily basis for soups and stews in almost every home in Nigeria, and (2) continuous consumption of foods contaminated with low-to-moderate aflatoxin levels over a period of time can increase population exposure (Ezekiel *et al*., 2014b). This may further contribute to chronic aflatoxicosis which is characterized by hepatocellular carcinoma, severe immune

suppression and growth retardation especially in children (Eaton and Groopman, 1994; Turner *et al*., 2002, 2003). Therefore there is the need to implement good post-harvest practices for fish processing (e.g. application of longer heat- and air-drying) and to continuously monitor smoked-dried fishes and other fishery products retailed in local markets in Nigeria for aflatoxin levels so as to ensure their safety for consumption.

The higher level of aflatoxins in the fish feed samples as compared to the fish samples is supported by the fact that contamination of feed is more complex in nature (e.g. contamination from cereal adjuncts which are usually diverse in nature). Specifically, the feeds might have been contaminated with aflatoxin and aflatoxigenic moulds through the raw materials like maize, groundnut cake, soybean, and cassava used for feed formulation, during feed processing, handling and/or through inadequate storage conditions. Considering the complexity of fish feeds, likely higher contamination rates of feeds compared to contamination of fish products and possibility of horizontal transfer of aflatoxins from ingested feeds to fish, fish farmers who compound fish feeds are encouraged to source for healthy grain components as basal ingredients during feed formulation.

Conclusion

This study has shown that smoked-dried fin and shell fishes in Lagos markets are contaminated by low-to-moderate levels of aflatoxin although fish feeds contained higher aflatoxin levels. Since aflatoxin-contaminated foods are consumed daily by the populace in developing countries as the food are being transported to local markets without proper checks by regulatory agencies, the findings of the present study therefore raise serious threats to the health of consumers. Hence, it is imperative that policies and enforcement of measures for the control and management of aflatoxins in fishery products, be set in place. In addition, more awareness is needed on the dangers and effects of feeding fishes with aflatoxin-–contaminated feeds.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgement

The authors would like to thank the management of the Nigerian Institute for Oceanography and Marine Research (NIOMR), for providing the funds for this project. We also acknowledge the support of all members of staff of the Department of Fish Technology and Product Development, NIOMR.

References

Abdelhamid, A.M., Salem, M.F.I., Mehrem, A.I. and El-Shaarawy, M.A.M. (2007). Nutritious attempts to detoxify aflatoxic diets of tilapia fish. *Egyptian Journal of Nutrition and Feeds*, 10(1): 205- 223.

Adebayo-Tayo, B.C., Onilude, A.A. and Ukpe, G.P. (2008). Mycoflora of smoke-dried fishes sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Sciences*, 4(3): 346-350.

Adejola, Q.A. (2011). Presence of aflatoxins in smoked-dried fish sold in Abeokuta, Ogun State, South-Western Nigeria. *B.Sc project submitted to the Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta*, pp. 35.

Akande, G.R. and Tobor, J.G. (1992). Improved utilization and increased availability of fishing products as an effective control of aggravated animal protein deficiency induced malnutrition in Nigeria. *Proceedings of the 10th annual conference of the Fisheries Society of Nigeria*, pp. 18-31.

Almeida, I.F.M, Martins, H.M.L., Santos, S.M.O., Freitas, M.S., da Costa, J.M.G.N. and Bernardo, F.M.A. (2011). Mycobiota and aflatoxin B1 in feed for farmed Sea Bass (*Dicentrarchus labrax*). *Toxins*, 3: 163-171.

Arowora, K.A, Abiodun, A.A, Adetunji, C.O, Sanu, F.T., Afolayan, S.S. and Ogundele, B.A. (2012). Levels of Aflatoxins in Some Agricultural Commodities Sold at Baboko Market, Ilorin, Nigeria. *Global Journal of Science*

Frontier Research Agriculture and Veterinary Sciences, 12(10): 30-33.

Atapattu, R. and Samarajeewa, U. (1990). Fungi associated with dried fish in Sri Lanka. *Mycopathologia*, 111: 55-59.

Barbosa, T.S., Pereyra, C.M., Soleiro, C.A., Dias, E.O., Oliveira, A.A., Keller, K.M., Silva, P.P.O., Cavaglieri, L.R. and Rosa, C.A.R. (2013). Mycobiota and mycotoxins present in finished fish feeds from farms in the Rio de Janeiro State, Brazil. *International Aquatic Research*, 5: 3. doi:10.1186/2008-6970-5-3.

Diedhiou, P.M., Bandyopadhyay, R., Atehnkeng, J. and Ojiambo, P.S. (2011). *Aspergillus* colonization and aflatoxin contamination of maize and sesame kernels in two agroecological zones in Senegal. *Journal of Phytopathology*, 159: 268–275.

Eaton, D.L. and Groopman, J.D. (1994). The Toxicology of Aflatoxins, Academic Press, New York, NT, pp. 383-426.

Essien, J.P., Ekpo, M.A. and Brooks, A.A. (2005). Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (*Chlamydoselachus anguincus*). *Journal of Applied Science and Environmental Management*, 9 (3): 53- 57.

Ezekiel, C.N., Adetunji, M.C., Atanda, O.O., Frisvad, J.C., Houbraken, J. and Samson, R.A. (2014a). Phenotypic differentiation of species from *Aspergillus* section *Flavi* on neutral red desiccated coconut agar. *World Mycotoxin Journal*, DOI: 10.3920/WMJ2014.1727.

Ezekiel, C.N., Warth, B., Ogara, I.M., Abia, W.A., Ezekiel, V.C., Atehnkeng, J., Sulyok, M., Turner, P.C., Tayo, G.O., Krska, R. and Bandyopadhyay, R. (2014b). Mycotoxin exposure in rural residents in northern Nigeria: a pilot study using multi-urinary biomarkers. *Environment International*, 66: 138–145.

Fafioye, O.O., Efunloye, M.O. and Osho, A. (2002). Studies on the fungal infestation of five traditionally smoked-dried fresh water fish in Ago-Iwoye, Nigeria. *Mycopathologia* 154: 177-179.

Goncalo, S. (2013). Prevelence of Mycotoxins in Aqua Feedstuffs. www.biomin.net. Accessed on 19/12/2013.

Hussein, S.Y., Mekkawy, I.A.A., Moktar, Z.Z. and Mubarak, M. (2000). Protective effect of *Nigella sativa* seed against aflatoxicosis in *Oreochromis niloticus*. *Proceedings of Conference of Mycotoxins and Dioxins and the Environment, Bydgoszcz*, pp. 109-130.

Jantrarotai, W. and Lovell, R.T. (1990). Subchronic toxicity of dietary aflatoxin B1 to channel catfish. *Journal of Aquatic Animal Health* 2: 248- 254.

Park, D.L. (2002). Effect of processing on aflatoxin. *Advances in Experimental Medicine and Biology*, 504: 173-179.

Shehata, S.A., Mohamed, M.S. and Mohamed, G.A. (2003). Reducing the toxicity of aflatoxin B1 by different adsorbents in fish. *Journal of Agricultural Science* 28(10): 7157--7167.

Pildain, M.B., Frisvad, J.C., Vaamonde, G., Cabral, D., Varga, J. and Samson, R.A. (2008). Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. *International Journal of Systematic and Evolutionary Microbiology*, 58: 725–735..

Ruby, D., Masood, A. and Fatmi, A. (2013). Effect of aflatoxin contaminated feed on growth and survival of fish Labeo Rohita (Hamilton). *Current World Environment*, 8(3): 479–482.

Samson, R.A., Hoekstra, E.S., Frisvad, J.S. and Filtenborg, O. (1995). Methods for the detection and isolation of food-borne fungi. In: Samson, R.A., Hoekstra, E.S., Frisvad, J.S. and Filtenborg, O. (eds) *Introduction to Foodborne Fungi*. Utrech, The Netherlands, Central Bureau voor Schimmel cultures, pp 235–242.

Turner, P.C., Sylla, A., Diallo, M.S., Castegnaro, J.J., Hall, A.J. and Wild, C.P. (2002). The role of aflatoxins and hepatitis viruses in the aetiopathogenesis of hepatocellular carcinoma: a basis for primary

prevention in Guinea-Conakry, West Africa. *Journal of Gastroenterology and Hepatology*, 17: S441–448.

Turner, P.C., Moore, S.E., Hall, A.J., Prentice, A.M. and Wild, C.P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*, 111: 217–220.

Wheeler, K.A., Hocking, A.D., Pitt, J.I. and Anggawati, A.M. (1986). Fungi associated with Indonesian dried fish. *Food Microbiology*, 3: 351- 357.

Zaki, M.S., Sharaf, N.E., Mostafa, H.S. and Fawzi, O.M. (2008). Diminution of aflatoxicosis in *Tilapia niloticus* fish by dietary supplementation with fix in toxin and *Nigella sativa* oil. *American-Eurasian Journal of Agriculture and Environmental Science*, 3(2): 211- 215.