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High Presence of Toxigenic *Aspergillus* spp. in Commercial Poultry Feeds in Ilaro, Nigeria

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HIGHLIGHTS

- All feed samples (100%) from Ilaro, Nigeria were contaminated with *Aspergillus* spp.
- Out of 93 *Aspergillus* spp. isolates, *A. flavus* had the most prevalence, while *A. parasiticus* was the least.
- Totally, 15 out of 93 (16.1%) *Aspergillus* spp. strains showed toxin production potentials.

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Acronyms and abbreviations
AF=Aflatoxin
PDA=Potato Dextrose Agar

ABSTRACT

Background: Several health problems may be occurred due to consumption of mycotoxin-contaminated foods and feeds. The maize and oilseeds, as the main components of poultry feeds are susceptible to mould contamination and mycotoxin production. The aim of this study was to determine the presence of toxigenic *Aspergillus* spp. in poultry feeds from Ilaro, Nigeria.

Methods: A total of 60 poultry feed samples were collected from five (A-E) feed millers in Ilaro, Nigeria. The feeds were classified into four groups, including broiler super starter, broiler starter, boiler grower mash, and broiler finisher mash. Moulds were isolated by spread plate technique and were identified using the conventional morphological method. The toxigenic potentials of the isolates were determined by ammonia vapor test. Statistical analysis was carried out using SPSS version 20.

Results: The results showed that all feed samples (100%) were contaminated with *Aspergillus* spp. Out of 93 *Aspergillus* spp. isolates, *A. flavus* (40 of 93) had the most prevalence, while *A. parasiticus* (8 of 93) was the least. Totally, 15 out of 93 (16.1%) *Aspergillus* spp. strains showed toxin production potentials.

Conclusion: The presence of toxigenic *Aspergillus* in the feed leads to the secretion of hazardous toxins especially aflatoxins which can contaminate poultry meat endangering food chain. Consequently, there is an urgent need to create more awareness on the health implications of feeding poultry with mycotoxins-contaminated feeds in this region of Nigeria.

Introduction

Moulds are ubiquitous organisms that are found in various environments and items. Many of them are pathogenic in nature while others are saprophytes. Some moulds produce toxic secondary metabolites, especially, mycotoxins that are not essential for their normal function. Several health problems are associated with the consumption of mycotoxin-contaminated foods and feeds (Jard et al., 2011; Shephard, 2008). The high prevalence of mycotoxin-contaminated foods and feeds is due to favorable climatic and storage conditions in developing

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countries, most especially in tropical regions, that encourage fungi growth and mycotoxin development in cereal-based products (Krnjaja et al., 2008; Shephard, 2008). Human exposure to mycotoxin and mycotoxin-contaminated foods and feeds has dramatically reduced in developed countries due to the presence of well-established regulatory institutions or agencies saddled with such responsibilities (Stefi et al., 2016). However, mycotoxin-contaminated agricultural products induce serious problems in the developing countries because of over-reliance on subsistence farming and also unregulated local markets (Shephard, 2008). The extent to which mycotoxins affect health in developing countries is an important challenge to investigate due to lack of required capacity in the health sector and limited resources. Mycotoxins are produced by a wide variety of fungal species, although *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. are the primary producers (Hathout and Aly, 2014). Toxigenic moulds produce various mycotoxins such as aflatoxins (AFs), fumonisin, deoxynivalenol, ochratoxin A, citrinin, zearalenone, and cyclopiazonic acid (Shephard, 2008).

Aspergillus spp. produce some toxins in cereals and cereal products on the field and during storage. They are known as the source of AFs and cyclopiazonic acid (Habib et al., 2015; Sabry et al., 2016), although not all the species exhibit this characteristic. *A. flavus* and *A. parasiticus* primarily produce AFs. There are about 20 AFs that have been reported, but AFB_1, AFB_2, AFG_1, and AFG_2 are the major ones (Dimitrieska-Stojkovikj, 2018). The health consequences of AFs in contaminated foods and feeds include nutritional interference, acute illnesses, teratogenicity, cancer, immunological suppression, and death (Williams et al., 2004). On the other hand, after accumulation of AF in the body of farm animals, the toxin enters into the foods of animal origin endangering consumers' health. So, because of high toxicity and carcinogenicity of AF, its presence in the food chain is of great concern worldwide (Parviz et al., 2014).

In Nigeria, there is a concerted effort towards boosting agriculture in order to diversify the economy. The federal government provides soft loans for poultry farmers and also offers extensive services to increase their productivity. This government aspiration can only be achieved when factors militating against high yield are addressed. Feeds play a vital role in poultry yield regarding the quality of eggs and meat (Arotupin et al., 2007; Osho et al., 2007). The maize and oilseeds, as the main components of poultry feeds, are susceptible to mould contamination. There is a necessity to emphasize food safety in order to reduce the incidence of mycotoxins in foods and poultry feeds. Screening for toxigenic *Aspergillus* spp. in the poultry feeds is one of the appropriate methods for ascertaining their safety. Therefore, the current study was designed to find the toxigenic *Aspergillus* spp. in commercial poultry feeds in Ilaro, Nigeria.

Materials and methods

Collection of poultry feed samples

A total of 60 commercial poultry feed samples were randomly collected from five feed millers (A-E) located in Ilaro, Nigeria, from May to September, 2017. The feeds were classified into four groups, including broiler super starter, broiler starter, boiler grower mash, and broiler finisher mash. Each feed miller was sampled thrice with sterile polyethene bags. Each sample was labeled accordingly, transported immediately to Microbiology Laboratory of the Department of Science Laboratory Technology, Federal Polytechnic, Ilaro and analyzed within 6 h of collection.

Isolation and morphological identification of moulds

The feed samples were aseptically plated on Potato Dextrose Agar (PDA; Merck, Germany) supplemented with chloramphenicol, using spread plate technique. Briefly, the homogenate was made by transferring of 1 g sample into 9 ml sterile distilled water and thoroughly mixed using stomacher (Giorni et al., 2007). A ten-fold serial dilution was carried out, and 0.1 ml aliquots of appropriate dilutions were spread on plates containing PDA media under aseptic condition using sterile bent glass rod. The plates were subsequently incubated at 27±2 ^{o}C for 72 h in the dark. Then, the isolates were enumerated and subcultured on PDA media to obtain pure cultures. The pure cultures were maintained on PDA slants and kept in the refrigerator at 4 ^{o}C pending further analyses (Oliveira et al., 2006).

The conventional morphological method was adopted for the identification. Their cultural and microscopic characteristics were analyzed for identification of the isolates. Colonial characteristics were examined such as mycelia color and reverse color of the freshly subcultured isolates (3 days old). The mycelium was stained with lactophenol cotton-blue before microscopic observation. The microscopic properties were studied such as conidial heads**,** stipe, color, length, vesicles shape and serration, metulae, conidia shape as well as texture. Fungal taxonomic descriptions, identification keys, and atlas were used as references (Adeniran and Abiose, 2009; Giorni et al., 2007; Rodrigues et al., 2007; Sabry et al., 2016).

Determination of toxigenic Aspergillus spp.

In order to determine toxin producing *Aspergillus* spp. strains, the ammonia vapor test method was done as

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described previously by Stefi et al. (2016). The organisms were centrally inoculated on PDA plates. The plates were incubated in the dark at a temperature of 27±2 °C for 3-7 days. After 3 days of incubation, a set of plates were inverted over 2 ml ammonium hydroxide for 10-15 min. The second sets of the plates were also exposed to ammonia vapor after 7 days of incubation of the same duration. A change in color was used as a parameter to determine the toxigenic potentials or otherwise of the isolates. Those strains having reverse turned pink or red color were recorded as positive (toxins producing strains). However, those without color change were recorded as negative (non-toxigenic isolates).

Statistical analysis

Statistical analyses were performed using SPSS version 20 at significant level of $p<0.05$.

Results

The present study showed that all feed samples (100%) were contaminated with *Aspergillus* spp; totally, 93 *Aspergillus* spp. were isolated (Table 1). *A. niger*, *A. flavus*, *A. tubingensis*, and *A. parasiticus* were recorded in the poultry feeds; these four *Aspergillus* species were isolated in all the feed millers except miller B where *A. parasiticus* was not isolated. Out of 93 *Aspergillus* spp. isolates, *A. flavus* (40 of 93) had the most prevalence, while *A. parasiticus* (8 of 93) was the least.

The average loads of *Aspergillus* spp. were 5.25, 3.50, 4.25, 4.50, and 5.75 log for A, B, C, D, and E, respectively in different feed groups. The feed produced by miller E showed significantly ($p<0.05$) higher *Aspergillus* spp. load when compared with other millers. However, there was no significant ($p>0.05$) difference between rate of *Aspergillus* spp. among four feed groups, including broiler super starter, broiler starter, boiler grower mash, and broiler finisher mash.

The results of toxigenic potentials of the *Aspergillus* spp. associated with Ilaro commercial poultry feeds are shown in Table 2. Totally, 15 out of 93 (16.1%) *Aspergillus* spp. isolates showed toxin production potentials.

Discussion

The high contamination rates of *Aspergillus* spp. recorded in Ilaro poultry feed samples indicate probable feed contamination due to poor handling and unhygienic processing actions. This mould load also could be due to contamination of the various constituents used for the feed formulation. On the other hand, such high rate of *Aspergillus* spp. contamination may be resulted from the process and post-process contaminations of the feeds. Likewise, the tropical climatic condition of Nigeria may play an essential role in the growth of mesophilic moulds. Our finding is in agreement with the work of Azarakhsh et al. (2011) that reported high incidence (92%) of *Aspergillus* spp. in Iranian broiler feeds. The high incidence of *Aspergillus* spp. (54.3%) was also observed in Serbia commercial feedstuffs (Krnjaja et al., 2008). The high *Aspergillus* spp. load recorded in our investigation is in agreement with similar studies earlier reported in other areas within and outside Nigeria (Cegielska-Radziejewska et al., 2013; Habib et al., 2015; Omojasola and Kayode, 2015; Shareef, 2010; Ukaegbu-Obi et al., 2017); however, some other researchers found *Penicillium* as the most prevalent mould genus in feed samples (Labuda and Tancinova, 2006; Oliveira et al., 2006; Stefi et al., 2016).

In the present research, the significant difference observed among the *Aspergillus* spp. loads from the various millers could be due to different sources of raw materials, process, and storage techniques. The high mould counts recorded in this study were not so surprising; the regulatory agency, saddled with such oversight responsibility, pays little attention to poultry feeds quality, but rather undue emphases are directed toward human foods. With this shortcoming, the feed millers also might not pay required attention to the quality of raw materials since it would not be consumed directly by humans. The mould loads could also be due to contamination of cereals and oilseeds that are the major constituents used for poultry feeds formulation since these agricultural products are usually dried by subsistent farmers on bare rock/floor and roadside (Habib et al., 2015). Thus, most poultry feed millers depend on these farmers for their raw materials. Such high *Aspergillus* spp. contamination recorded from miller E, could be due to a long period of storage under a poor condition as a result of low demand by poultry farmers. More so, it could be attributed to the prevailing water activity of the feed that favors the growth of the organism. The feed miller might have stored the lot in high humid environment that allows absorption of moisture. High water activity (0.95≤) has been reported to favor colonization and growth of *Aspergillus* spp. on sesame seeds by Sabry et al. (2016). Favorable temperature and humidity encourage fungal growth and mycotoxins development in feeds (Krnjaja et al., 2008).

A. niger, *A. flavus*, *A. tubingensis*, and *A. parasiticus* were the four *Aspergillus* species isolated from our poultry feed samples, and their presence could be due to the poor quality of the raw materials and condition of storage of the feeds. Our results are in agreement with findings of Habib et al. (2015) who recorded the presence of *A. fumigatus*, *A. parasiticus*, *A. flavus*, *A. niger*, and *A.*

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Table 1: Frequency of *Aspergillus* spp. in poultry feed samples obtained from different millers of Ilaro, Nigeria

Feed miller	*Aspergillus* spp.	Number (Total No. = 93)
A	*A. niger*	4
	A. flavus	12
	A. tubingensis	2
	A. parasiticus	2
B	*A. niger*	4
	A. flavus	8
	A. tubingensis	2
	A. parasiticus	0
C	*A. niger*	3
	A. flavus	7
	A. tubingensis	7
	A. parasiticus	1
D	*A. niger*	3
	A. flavus	7
	A. tubingensis	6
	A. parasiticus	2
E	*A. niger*	4
	A. flavus	6
	A. tubingensis	10
	A. parasiticus	3

Table 2: The toxigenic potential of *Aspergillus* spp. isolated from poultry feeds samples of Ilaro, Nigeria

Aspergillus spp.	Ammonia vapor test		Toxigenic potential
	Initial color	Color change	
A. tubingensis EC	Cream	-	-
A. flavus ED	Cream	Pink	+
A. tubingensis ED	Cream	-	-
A. tubingensis DD	Cream	-	-
A. flavus EB	Cream	Pink	+
A. tubingensis DA	Cream	-	-
A. flavus EA	Cream	Pink	+
A. tubingensis BB	Cream	-	-
A. parasiticus AD	Yellow	Pink	+
A. tubingensis AD1	Cream	-	-
A. parasiticus EC	Cream	Red	+
A. niger EB	Yellow	-	-
A. niger BB	Yellow	-	-
A. niger CC	Yellow	-	-
A. tubingensis AD2	Cream	-	-
A. flavus DA	Cream	Deep pink	+
A. flavus DD	Cream	Deep pink	+
A. flavus CC	Cream	Pink	+
A. flavus CD	Cream	Pink	+
A. parasiticus DD	Yellow	Pink	+
A. niger AA	Yellow	-	-
A. niger DC	Yellow	-	-
A. niger ED	Yellow	-	-
A. flavus AA	Cream	Pink	+
A. flavus AD	Cream	Pink	+
A. parasiticus CC	Yellow	Pink	+
A. parasiticus EA	Yellow	Pink	+
A. parasiticus ED	Cream	Red	+
A. niger CD	Yellow	-	-
A. niger BD	Yellow	-	-

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terreus in poultry feeds from Kaduna State, Nigeria. In another study, Stefi et al. (2016) isolated *Penicillium* spp., *A. niger*, *A. fumigatus*, *A. flavus*, *Mucor* spp., *Rhizopus* spp., and *Fusarium* spp. from livestock feeds sampled from Kerala, India. This variation might be due to different types and sources of raw materials used, different climatic condition and also mode of handling the finished product; since moulds are ubiquitous organisms.

In this study, strains of *A. flavus* and *A. niger* were implicated as toxin producers which were previously recorded by some authors (Azarakhsh et al., 2011; Labuda and Tancinova, 2006; Stefi et al., 2016), and could also be due to similar climatic and storage conditions. The presence of these toxigenic organisms in the feeds makes them unsafe for the birds and will indirectly impact negatively on the health of the consumers of the poultry meat in this area (Parviz et al., 2014).

Conclusion

All the feeds obtained from the various feed millers showed the presence of toxigenic *Aspergillus* spp. at the unsafe levels. The presence of toxigenic *Aspergillus* spp. in the feeds leads to the secretion of hazardous toxins especially AFs which can contaminate poultry meat endangering food chain. Consequently, there is an urgent need to create more awareness on the health implications of feeding poultry with mycotoxins-contaminated feeds in this region of Nigeria.

Author contributions

F.F. designed the study; F.F. and A.E.A. conducted the experimental work, analyzed the data, and wrote the manuscript. Both authors revised and approved the final manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

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