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Full Length Research Paper

Evaluation of aflatoxin content of naturally occurring molds from poultry feeds

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Five different samples of poultry feeds were obtained from two different markets in Ibadan, Oyo State. These are broiler starter, broiler finisher, grower mash, layer mash and chick mash. The mycological analyses were carried out for the identification of molds naturally contaminating different poultry feeds. Also, aflatoxin concentration was determined. The fungi isolated include *Aspergillus flavus* (L-strain), *Aspergillus parasiticus, Aspergillus tamarii,* and *Rhizopus* specie. *A. parasitcus* was significantly higher in aflatoxin B_1 (35499.24 µg/kg) than *A. flavus* B_1 (13.27 µg/kg). For aflatoxin B_2, *A. flavus* was significantly higher in concentration (4450.19 µg/kg) while *A. parasiticus* was significantly lower in concentration (1285.55 µg/kg). For aflatoxin G_1, *A. parasiticus* was significantly higher (967.60 µg/kg) than *A. flavus* which is significantly lower in concentration (757.98 µg/kg). *A. parasiticus* produced 1373.12 µg/kg quantity of Aflatoxin G_2. Hence there is an increase of aflatoxicosis in poultry feeds that has been contaminated with molds.

Key words: Poultry feeds, fungi, aflatoxin.

INTRODUCTION

Poultry feeds are food materials used in raising poultry birds. Poultry includes all domesticated birds that can be used as a source of egg or meat production for human consumption (Obi and Ozugbo, 2007). Such birds are chicken, turkey, duck and guinea fowl which are generally acceptable among domesticated fowls quite popular in the Western nations. Poultry feeds are referred to as complete feeds as they are designed to contain all the nutritional materials needed for proper growth, meat and egg production in birds. There are various types of poultry feeds (grower mash, finisher mash, layer mash, starter mash and chick mash) depending on the functions they perform in the birds. Recently, nutritional factors and antibiotics such as bacitracin, tetracycline, oxytetracycline, chlorotetracycline have been incorporated into poultry feed formulations usually at low (prophylatic) level to prevent minor diseases and enhance efficient growth (Obi and Ozugbo, 2007). The raising of poultry birds on commercial level requires large scale use of commercially prepared poultry feeds so as to satisfy the teeming population of humans that depend to great extent on the poultry products as a source of protein (Uwazuoke and Ogbulie, 2008).

Poultry feeds have been implicated in several poultry diseases with varied pathological manifestations. These diseases are viral (avian influenza, Newcastle disease), bacterial (Salmonellosis, infectious Coryza), and fungal in nature (*Aspergillosis, Fusarium* spp and *Penicillium* (Jordan and Pattison, 1999). The involvement of poultry feeds in the transmission of aflatoxins is the most prevalent and economically significant mycotoxin and is of great health concern to the poultry farmers and consumers (Jordan and Pattison, 1999). Aflatoxins are known to be present in poultry eggs and human diseases like traveller's diarrhea and salmonella para-typhoid fever and these have been associated with the consumption of poultry birds that contacted the infection from contaminated poultry feeds (Truckess et al., 1983; Christopher and Yun 2010). Fungal occurrence and growth on foods and feeds is one of the major threats to human and animal health.

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Besides their negative impacts on nutritional and organoleptic properties, moulds can also synthesize different mycotoxins (Benkerroum and Tantaoui-Elaraki 2001).

Growth of commonly occurring filamentous fungi in foods may result in production of mycotoxins, which can cause a variety of ill effects in humans from allergic responses to immunosuppression and cancer. Aflatoxins are potent carcinogens, produced by *Aspergillus flavus* and *Aspergillus parasiticus* in peanuts, maize and some other nuts and oil seeds (Horn et al., 1995). The production of poultry feeds for local and commercial farmers in the developing countries like Nigeria requires above average microbiological safety regulations to avoid toxigenic fungal contamination of the product. The aim of this research therefore is to evaluate aflatoxin content from naturally contaminating molds from different types of poultry feeds used in Ibadan, Nigeria.

MATERIALS AND METHODS

Collection of samples

Five different types of poultry feeds which includes broiler starter, broiler finisher, grower mash, layer mash and chick mash were purchased and aseptically collected from two major markets in Ibadan city, Nigeria. Sample collection was done using clean nylon bag and all the samples were aseptically transported to the laboratory where mycological analysis were carried out within 24 h of purchased. The experiment was carried out in four different locations namely Olabisi Onabanjo University, Ago-Iwoye; University of Ibadan, Ibadan; International Institute of Tropical Agriculture (IITA) Ibadan; and Kappa Biotechnologies Ibadan.

Identification of *Aspergillus* species and strains

Culture on potato dextrose agar media were inoculated on to prepared 5/2 agar media (5% v8 juice and 2% agar, pH 5.2). All single spore colonies of *Aspergillus* were grown in petri dishes containing 5/2 media for screening and identification. The identification of *Aspergillus* and strains were done with the aid of microscope. After 5 days of incubation, isolates were classified into species and strains by observing colony characteristics and conidial morphology as described by (Cotty, 1989; Klich and Pitt, 1988).

The culture were transferred inside test tubes of 38 ml capacity containing10 ml of sterile distilled water each, 10 g of maize was weighed inside long tube each and were sterilized, after sterilization, they were left to cool. 1 ml of water was later dispensed into each long tube, after then each tube was inoculated with 500 µl of each inoculum, the tubes were shaken and incubate for 7 days.

Determination of aflatoxin content in maize kernels

This was determined according to the method of Atehnkeng et al. (2008). 50 ml of 80% methanol was added to 10 g of inoculated maize each and were grinded into fine particles using a high-speed blender for 3 min; they were transferred back into conical flask and shake for 30 min on a shaker. The mixture was then filtered through Whatman Paper No.1 and the extract collected in a 250 mlseparatory funnel, 20 ml of distilled water was added to ease separation. 15 ml of dichloromethane was also added and was shaken for proper mixture. Following separation, the dichloromethane layer was filtered out through 20 g of anhydrous sodium sulphate to remove residual water. The extraction was repeated the second time by adding 10 ml of dichloromethane into the separatory funnel and the separation was also done using the previous method. The extract was collected in a polypropylene cup and evaporated to dryness in a fume hood. The residue was re-dissolved in 1 ml of dichloromethane. Aflatoxin standards and extracts were separated on thin layer chromatography plate. Aflatoxin plate was observed under long wavelength U.V. light fitted in a black cabinet, and quantified using CAMAG TLC Scanner 3 with winCATS 1.4.2 software (Camag AG, Muttenz, Switzerland), (Atehnkeng et al., 2008).

Quantitative analysis of aflatoxins

Quantitative analysis of aflatoxins was done using the method of Kulwart et al. (1991). One milliliter of dichloromethane was added to the dried aflatoxin extract which was stored in small screw cap vial. Using capillary tube of 4 µl, 4 µl of this dichloromethane extract was spotted on the base line of thin layer chromatography (TLC) plate (1 cm). The spots should not be bigger than 0.5 cm in diameter. 4 µl of the aflatoxin standard was spotted on either side of the previous spots (G and I standards). The concentration of the standard aflatoxin should be 0.5 µg/ml which was used as a control. The spotted plate was developed in a developer (distilled water, diethylether and methanol 96.3.1v/v) solvent system for approximately 20 min so that the solvent front moves about 10 cm. The level of the solvent in the developing tank should not be more than 0.5 cm.

The plate was dried and was observed under long wavelength U.V light fitted in a black cabinet. The blue fluorescent spot indicate the presence of aflatoxin B and greenish-blue indicate aflatoxin G. The quantitative estimation was done by comparing the intensity of florescence produced in spots of sample with that of standard aflatoxin spots. Record the volume of the matching spots of the sample with that of the standard. If the intensity of the fluorescence of smallest spot of sample is too intense to match with the standard, the sample extract should be diluted and re-chromatographed.

The concentration of the aflatoxin is the sample (µg/kg) is calculated by the formula:

$$\frac{S \times Y \times V}{W \times Z}$$

S = Volume of aflatoxin B_1 standard, in µl, of equivalent intensity to Z µl of sample

Y = Concentration of aflatoxin B_1 Standard in µg/ml

V = Volume of solvent, is µl, required to dilute final extract

Z = Volume of sample extract, in µl, required to give fluorescence intensity comparable to that of S µl of the B_1 standard.

W = Weight of original sample, in g, contained in final extract.

The statistics employed

The data obtained from aflatoxin analysis were subjected to analysis of variance and means were separated by Duncan Multiple Range Test. The probability level is $p < 0.05$

RESULTS

Morphological characteristics of the fungal isolates

Four different organisms, *A. flavus, A. tamarii, A.*

Table 1. Occurrence of fungi isolates according to the sample.

	Feed samples						
Fungi Isolates	**Starter**	**Finisher**	**Grower**	**Layer**	**Chickmash**	**Number of occurrence**	**Percentage occurrence**
A. flavus	-	2	1	1	2	6	50
A. tamarii	1	-	-	-	-	1	8.3
A. parasiticus	1	1	-	-	-	2	16.7
R. species	1	1	1	-	-	3	25

Table 2. Macroscopic and microscopic characterization of fungi isolates.

Isolate codes	Macroscopic	Microscopic	Probable identity
ST1, FN1, GR1	Whitish fluffy with dark spores on it and the reverse is creamish	The young mycelium at the border of the colony consists of characteristically branched cells of large diameter. The sporangiophores arise in groups of two or three from a tangle of rhizoids.	*Rhizopus* sp.
ST2	The colony is rusty brown and creamish brown at the reverse	The stripe is long and rough. The head is partly globular and the conidia is thick and strongly roughened	*Aspergillus tamarii*
ST3, FN3	Obverse, the colony is dark green in colour. Yollowish-green at initial stage reverse is creamish-yellow	It produced rough conidia, conidial head with phialides	*Aspergillus parasiticus*
FN2, GR2, GR3, CM1, CM2, LA1	Obverse yellowish-green becoming light-green with age, the reverse is creamish-yellow	It has smooth conidia surface. The head is radiating and becoming loosely columnar with age, the stripe is long, verrucose and has a small metulae	*Aspergillus flavus*

ST1, ST2, ST3- Starter (plate 1, 2 & 3). FN1, FN2, FN3- Finisher (plate 1, 2 & 3). GR1, GR2, GR3- Grower (plate 1, 2 & 3). CM1, CM2- Chickmash (plate 1 & 2)
LA1- Layer (plate 1).

parasiticus and *Rhizopus* were identified from the poultry feed samples. It was observed that *A. flavus* was the dominant organism identified and the least organism identified was *A. tamarii* (Table 1). The percentage occurrence of the fungal isolates was as follows: *A. flavus* was 50% of the total fungal isolates followed by *Rhizophus* sp which constitutes 25%. *A. parasitcus* was 16.7% while *A. tamarii* was 8.3% of the total isolates.

Table 1 shows the number of fungi isolates from the poultry feed samples; it was observed that *A. flavus* had the highest number of occurrence which was a total number of 6, while *A. tamarii* had the lowest number of occurrence which was 1.

The descriptions of the fungi isolates were shown in (Table 2) indicating their texture, colour and their microscopic characteristics. Two major morphological parameters were indicated in the microscopic examination which is the smoothness and roughness of the spores which actually indicate the type of strains of the organisms. Macroscopically, some of the isolates were whitish fluffy and were creamish at the reverse, microscopically, the sporangiophores arise in groups of two or three from a tangle of rhizoids which indicates *Rhizopus* specie.

It was also observed that some were yellowish green and with age becoming green, the reverse was creamish yellow macroscopically while microscopically it has smooth conidial surface, the head radiating and becoming loosely columnar with age, this was identified as *A. flavus.* Similarly, macroscopically for *A. parasiticus* initially, it was yellowish green and later became dark green and at the reverse creamish yellow while micro-scopically it produced rough conidia, conidial head with phialides. As for *A. tamarii* macroscopically the colony was rusty brown and creamish brown at the reverse while microscopically the stripe is long and rough, the head partly globular.

Table 3 shows the identification of the fungal strains. There are two major types of *Apsergillus* strains which are L-strain and S-strain. The microscopic examination

Table 3. Classification of *Aspergillus* species to strain Level.

Isolates codes	Fungal isolates	Isolate strain
ST2	*A. tamarii*	*Tamarii*
ST3	*A. parasiticus*	*parasiticus*
FN2	*A. flavus*	L-strain
FN3	*A. parasiticus*	*parasiticus*
GR2	*A. flavus*	L-strain
GR3	*A. flavus*	L-strain
CM1	*A. flavus*	L-strain
CM2	*A. flavus*	L-strain
LA1	*A. flavus*	L-strain

Table 4. The quantity of aflatoxin produced by the fungal isolates.

Sample codes	Isolates	Aflaxtoxin concentration µg/kg			
		B_1	B_2	G_1	G_2
ST2	*A. tamarii*	0.00^g	0.00^d	0.00^c	0.00^b
ST3	*A. parasiticus*	15857.30^c	1285.55^c	0.00^c	0.00^b
GR2	*A. flavus*	13.27^f	0.00^d	0.00^c	0.00^b
GR3	*A. flavus*	0.00^g	0.00^d	0.00^c	0.00^b
CM1	*A. flavus*	0.00^g	0.00^d	0.00^c	0.00^b
CM2	*A. flavus*	599.44^d	0.00^d	0.00^c	0.00^b
FN2	*A. flavus*	28030.28^b	4450.19^a	757.98^b	0.00^b
FN3	*A. parasiticus*	35499.24^a	3790.78^b	967.60^a	1373.12^a
LAI	*A. flavus*	58.31^e	0.00^d	0.00^c	0.00^b
STI	*Rhizopus* sp	0.00^g	0.00^d	0.00^c	0.00^b
FNI	*Rhizopus* sp	0.00^g	0.00^d	0.00^c	0.00^b
GRI	*Rhizopus* sp	0.00^g	0.00^d	0.00^c	0.00^b
S.E		2049.16	259.41	54.83	64.15

Means with the same letter in the same column are not significantly different at $P < 0.05$.

reveals that most of the fungi isolates were of L-strains which were *A. flavus*. Those with smooth conidial surface were identified as L-type *A. flavus* which was light green colonies on 5/2 media. Isolates that had dark green colonies on 5/2 media and produced rough conidia were considered *A. parasiticus*. Isolates that produced only B – aflaxtoxins with smooth surface conidia were considered as *A. flavus* and placed into either the S – or L – type on the basis of sclerotial morphology on 5/2. Isolates exhibiting *A. flavus* conidial morphology and colony characteristics with smooth conidia but not producing aflaxtoxin were classified as putative atoxigenic strains of *A. flavus.*

Aflatoxin concentration from the fungal isolates

Isolates with brown to yellow – brown colonies on 5/2 agar were classified as belonging to either *A. caelatus* or *A. tamarii* both of which do not produce aflaxtoxins (Table 3). Fungi isolates were screened for aflatoxin production. All four aflaxtoxins B_1, B_2, G_1 and G_2 were detected from the isolates of poultry feed samples. The highest concentration of aflatoxin B_1 was detected in *A. parasiticus* FN3 (35499.24 µg/kg) followed by *A. flavus* FN2 (28030.28 µg/kg) and the lowest concentration of aflatoxin B_1 was 13.27 µg/kg which was detected from *A. flavus* GR2 (Table 4). It was also observed that the highest concentration of aflatoxin B_2 was detected from *A. flavus* FN2 (4450.19 µg/kg) followed by *A. parasiticus* FN3 with 3790.78 µg/kg and the lowest concentration of aflatoxin B_2 was detected from *A. parasiticus* ST3 with 1285.55 µg/kg.

The highest value of aflaxtoxin G_1 concentration was detected from *A. parasitcus* FN3 (967.60 µg/kg) while the lowest concentration was detected from *A. flavus* FN2 (757.98 µg/kg). Aflatoxin concentration (Figure 1) for G_2 was from *A. parasitcus* FN3 (1373.12 µg/kg). It was observed that aflatoxin concentration was not detected from some isolates; this happened as a result that some

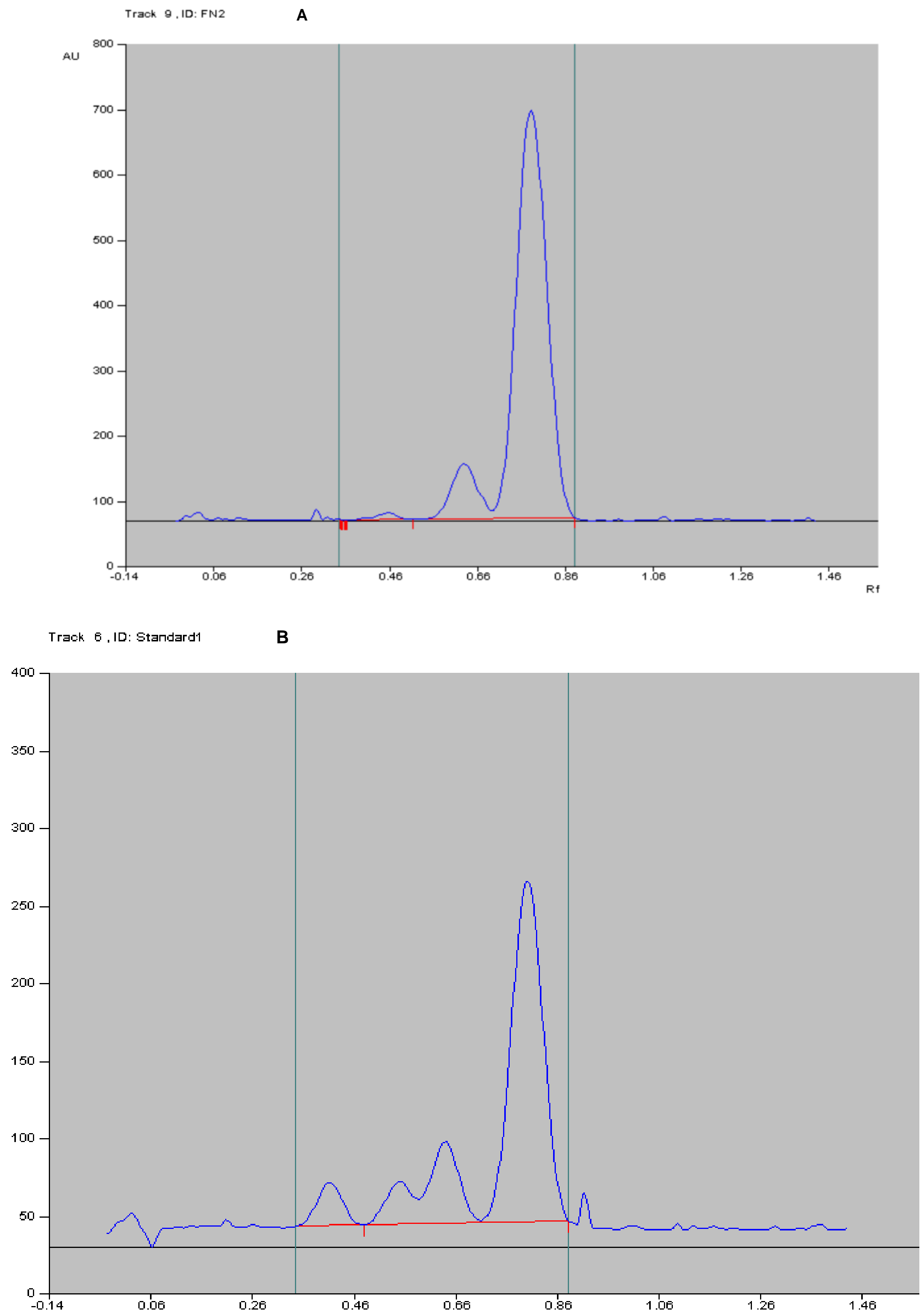

Figure 1. a. Graph of Aflatoxin concentration (Laboratory Standard G). b. Graph of Aflatoxin concentration (from the isolate). Sample code: FN2.

isolates do not produce aflatoxins, for example organisms like *A. tamarii*, *Rhizopus* species and some strains of *A. flavus* which were classified as putative atoxigenic strains of *A. flavus*.

The laboratory G standard was compared to *A. flavus* FN2 code, it was observed that *A. flavus* FN2 was a perfect concentration in comparison to G standard.

DISCUSSION

The importance of aflatoxin content associated with poultry cannot be overemphasized. *Aspergillus flavus* was the predominant species isolated from the poultry feed samples in this study, the fungal isolated from the poultry feed samples were *A. flavus, A. tamarii, A. parasiticus* and *Rhizopus* species. The presence of *Aspergillus* in the feed samples is of great health and economic importance due to involvement in the production of aflaxtoxins which are powerful mycotoxins that have been found to be carcinogenic, teratogenic and mutagenic in humans and birds (Prescott et al., 2005). *A. flavus* and *A. parasiticus* are toxigenic, so the presence of these in the poultry feeds are of great concern. L-strain of *A. flavus* accounted for 50% of the total isolates. Atehnkeng et al. (2008) reported that *A. flavus* appears to be the most important causal agent of contamination of maize which is the basic constituent of poultry feeds.

High level of *Aspergillus* species have previously been reported in Nigeria in pre-harvest maize (Bankole and Mabekoje, 2003). It is likely that pre-harvest infections greatly influence the mycoflora in storage (Hell et al., 2003). In reference to Murphy et al. (2006), the presence of *Aspergillus* calls for attention in the storage strategies employed by the poultry feed manufacturers, the ware house condition distributions and sellers. The high level of *A. flavus* compared to other members of *Aspergillus* can be explained by the occurrence of correspondingly high levels of *Aspergillus* section flavi resident in the soil, plant debris and insects (Atehnkeng et al., 2008) which acts as the reservoir of inoculum for infection of kernels in the field. In the course of study, it was observed that not all L-strain of *Aspergillus flavus* can produce aflatoxins. In addition, in this study it was observed that *A. tamarii* did not produce aflatoxin, likewise, according to Atehnkeng et al. (2008) it was observed that *A. tamarii* do not produce aflatoxins. Although *Rhizopus* species was isolated but it is naturally known that it does not produce aflatoxins only some L-strain *A. flavus* and *A. parasitcus* produced aflatoxins.

Conclusion

Contamination of poultry feeds could be as a result of pre-harvest infection it was observed in this study that some isolates does not produce aflatoxin and those that produced are still within the safe level. In conclusion, government and public health parastatals should raise public awareness on the human diseases that could be possibly contracted from poultry birds that consume contaminated feeds and the much needed microbial safety in commercial poultry farms. Effort should continue in determining the occurrence of mycotoxins in feed through diagnostic procedure, surveys and epidemiology studies. Imperative to the tasks of minimizing mycotoxins production and contamination of the feed supply is the development of the newer methods to detoxify or de-contaminate the affected commodities.

It is recommended that producers of poultry feed should provide service points where subsistence poultry farmers can purchase wholesome feeds without the risk of contamination. It is advisable that both the producers and the retailers are to be well educated on proper handling of poultry feeds. The retailers should caution by not exposing the feeds to contaminants like flies and dust particles gaining access to the exposed feeds. Lastly, there should be good agricultural/manufacturing practices.

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REFERENCES

Atehnkeng J, Peter S O, Matthias D, Ikotun T, Richard AS, Peter JC, Ranajit B (2008). Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. Int. J. Food Microbiol., 122: 74-84.

Bankole SA, Mabekoje OO (2003). Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. Food Additives Contaminants. 2: 251-255.

Benkerroum S, Tantaoui-Elaraki A (2001). Study of toxigenic moulds and mycotoxins in poultry feeds. Revue Med. Vet., 152(4): 335-342.

Christopher PW, Yun YG (2010). Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis, 31:1.

Cotty PJ (1989). Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathologyy, 79: 808-814.

Hell K, Cardwell KF, Poehling HM (2003). Distribution of fungal species and aflatoxin contamination in stored maize in four agro-ecological zones in Benin, West Afr. J. Phytopathol., 151: 690-698.

Horn BW, Greene RL, Dorner JW (1995). Effect of corn and peanut cultivation on soil populations of *Aspergillus flavus* and *A. parasiticus* in Southwestern Geogia. Appl. Environ. Microbiol., 61(7): 2472-2475.

Jordan FTW, Pattison M (1999). Poultry disease. WB sounder, Publishers, London, pp 24-50.

Klich MA, Pitt JI (1988). Differenciation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Transactions of the British Mycological Society. 91: 99 - 108.

Kulwart S, Jens C, Frisvad U, Mathur SB (1991). An illustrated manual on identification of some seed-borne.

Obi CN, Ozugbo IJ (2007). Microbiological Analyses of Poultry Feeds sold in Umuahia main Market, Abia State, Nigeria. Res. J. Appl. Sci., 2(1):22-25.

Murphy PA, Hendrich S, Landgren C (2006). Food Mycotoxins, J. Food Sci., pp.13-23

Prescott LM, Harley JP, Klein DA (2005). Microbiology. McGraw Hill., P. 992.

Truckess MW, Stoloff L, Young K (1983). Aflatoxicol an Aflatoxins B_1 and M_1 in Eggs and Tissues of Laying Hens Consuming Aflatoxin Contaminated Feed.

Uwazuoke JC, Ogbulie JN (2008). Microbiological quality commercially available poultry feeds sold in parts of Eastern Nigeria. J. Appl. Sci. Environ. Manage., 12(1): 113 – 117.