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Detection of total aflatoxin in chicken eggs from four Local Government Areas in Kaduna State, Nigeria.

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ABSTRACT

In today's changing world, food safety and security have generally remained basic human needs. Among chemical hazards of concern, the contamination of food and feed by mycotoxins, have been characterized as significant sources of food-borne illnesses. The objective of this study was to determine the presence of residues of aflatoxins in chicken eggs and risk factors that lead to contamination. In this study, 39 and 5 pooled egg albumin samples from the four local government areas (LGAs) and major egg marketers in Kaduna Metropolis respectively were analysed for total aflatoxins (B1,B2,G1,G2) using ELISA. Of the 39 pooled egg albumin samples from Igabi, Chikun, Kaduna North and Kaduna South Local Government Areas, total of 28 samples had aflatoxin concentrations ranged from 0.44 - 6.13 µg/kg , 1.14 - 8.75 µg/kg , 5.25 -14.08 µg/kg and 3.52 -10.94 µg/kg respectively. Four pooled egg albumin sampled from major egg marketers in Kaduna Metropolis revealed total aflatoxin concentrations of 3.06 - 17.5 µg/kg. There was no significance (P >0.05) difference in aflatoxin concentrations in sampled egg albumin from the four LGAs. Some of the predisposing risk factors that lead to the contamination of poultry by aflatoxin include: poor biosecurity practices by subsistent farmers, deep litter system of housing for birds may contaminate the feeds and grain which are sourced from the open markets. In conclusion, aflatoxin concentrations of 0.44 – 14.08 were found in egg albumin and this is of concern because of the health hazards it presents to the value chain actors.

Keywords: *Poultry farms, Mycotoxins, Food value chain, Health hazards*

1.0. INTRODUCTION

In today's changing world, food safety and security have generally remained basic human needs (van Egmond *et al.,* 2007). Ensuring the safety of food has been a major focus of international and national action over the last years. Both microbiological and chemical hazards are of concern (van Egmond *et al.*, 2007). Among chemical hazards, the contamination of food and feed by mycotoxins (toxic metabolites of fungi), fishery products by phyetotoxins (toxins produced by algae) and edible plant species by their plant toxins have been recently characterized by the World Health Organization (WHO) as significant sources of food-borne illnesses (WHO, 2002a). Of these three categories of natural toxins, most attention has been directed to mycotoxins until now. In several parts of the world, mycotoxins currently represent a major food safety issue (van Egmond *et al.*, 2013). General interest in mycotoxins was on the increase since 1960 when a feed-related mycotoxicoses called turkey X disease, which was later proved to be caused by aflatoxins, appeared in farm animals in England. Subsequently it was found that aflatoxins are hepatocarcinogen in animals and humans, and this stimulated research on mycotoxins (Peraica *et al*., 1999). Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in Cultures as well as in food products. The transfer of aflatoxin from feed to poultry products including meat and eggs has been investigated and it was found to be quite low (Makun *et al*, 2010). Poultry are generally very sensitive to aflatoxin and the effects vary by species and sex (Li *et al*., 1999). Small quantities of aflatoxin in feed (tens to hundreds µg/kg) can cause increased mortality, reduction in weight gain, feed intake, egg production, egg weight, and profit. Common symptoms of mycotoxicoses in poultry are reduced feed consumption, poor growth, reduced egg production, and reduced feed conversion efficiency, increased susceptibility to diseases, increased mortality, poor egg shell quality, reduced fertility, leg problems and carcass condemnation (Haladi, 2007). The most important single factor allowing anaerobic or micro-aerophilic organisms to flourish in body tissues is a low

oxygen tension, which is usually the result of a compromised blood supply and poor tissue perfusion (Linda *et al.*, 1994). Pathogenic *Aspergillus* species appear to be capable of growing at very low oxygen concentrations. However, they are clearly not capable of anaerobic growth. The growth rate is slowed at low oxygen concentrations and conidiation is delayed or absent (Linda *et al.*, 1994). Conidiation varied with the medium used. Isolates that did not grow under experimental conditions subsequently grew when incubated in air, indicating that a low oxygen tension is not harmful to the conidia of *Aspergillus* species (Linda *et al.*, 1994). Thus oxygen can be considered an essential element for the growth but not survival of *Aspergillus* species (Linda *et al.*, 1994).

Exposure to aflatoxins in the diet is considered an important risk factor for the development of primary hepatocellular carcinoma, particularly in individuals already exposed to hepatitis B. In classical epidemiology, several studies have linked liver cancer incidence to estimated aflatoxin consumption in the diet (Li *et al*., 1999). Aflatoxins in humans are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver. The evaluation of epidemiological and laboratory results carried out in 1987 by the International Agency for Research on Cancer (IARC) found that there is sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins, which are therefore classified as Group 1 carcinogens, except for aflatoxin M1 (Group 2B), which is possibly carcinogenic to humans (IARC, 1993). The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish Regulations on mycotoxins in food and feed in the last decades to safeguard the health of humans, as well as the economic interests of producers and traders (van Egmond *et al.*, 2007). Setting mycotoxin Regulations is a complex activity, which involves many factors and interested parties. The first limits for mycotoxins were set in the late 1960s for the aflatoxins. By the end of 2003, approximately 100 countries had developed specific limits for mycotoxins in foodstuffs and feedstuffs, and the number continues to grow (van Egmond *et al.*, 2007). Bennet (2003) stated that mycotoxin exposure is more likely to occur in parts of the world where poor methods of food handling and storage are common, where malnutrition is a problem, and few Regulations exist to protect exposed population. In Nigeria, there are no set recommended guidelines on the issue of Regulatory limits of mycotoxins in grains. Other nations have established guidelines for safe levels of mycotoxins in grains. National Agency of Food and Drug Administration and Control whose responsibility is to ensure that food is safe for Nigerians, lack the capacity for monitoring and detection technology of stored grains in villages or open markets in place to control the menace caused by mycotoxins on daily basis. Okoli (2005) emphasised that literature is largely silent on fungal and mycotoxin contamination of feedstuff and mycotoxicoses in farm animal, and in livestock production research issues in Nigeria. It is of concern that little or no effort has been made to study mycotoxins in food value chain in Nigeria, considering the public health implication. It is expected that this research will directly translate to a better understanding of the extent of contamination of chicken eggs with aflatoxins. The hazard assessment approach does not apply for toxins where carcinogenicity is the basis for concern as the case with the aflatoxins (van Egmond *et al*., 2007). Assuming that a no-effect concentration limit cannot be established for genotoxic compounds, any small dose will have a proportionally small probability of inducing an effect (van Egmond *et al*., 2007). In addition to information about toxicity, exposure assessment is another main ingredient of the risk assessment. Reliable data on the occurrence of mycotoxins in various commodities and data on food intake are needed to prepare exposure assessment (van Egmond *et al.*, 2007). The quantitative evaluation of the likely intake of mycotoxins is quite difficult. The aim and objective of this study was to determine the occurrence residue of aflatoxin in chicken eggs in Kaduna state.

2.0. MATERIALS AND METHODS

2.1. Collection of Eggs

Seventy two commercial poultry farms: Backyard (>500 birds), (small >500-999 birds), large (1000 birds and above) in Kaduna Metropolis and four commercial egg marketers (selling >10,000 eggs per day) were selected using simple random sampling method. The sample size was calculated using an online calculator (HyLown, USA) standard deviation of 0.5, confidence level of 95 % and confidence interval (margin of error) of 11.6 %. Information on source of poultry feed was collected using different structured questionnaires that were distributed to farmers and marketers. 5 eggs per batch were selected once from each of the farms within three months. Total of 72 batches equal 360 eggs. Five eggs from different batches were collected randomly from major egg sellers within three months. The major egg sellers were visited weekly and information on source of eggs was collected. The total eggs collected were 480 from. Samples of 480 eggs collected were cleaned using sterile water and wiped with 70 % alcohol (14 parts of 95 % ethyl alcohol to 5 parts of

water). The eggs were grouped in pools of 5, after thorough disinfection, the eggs were cracked with sterile surgical forceps at the small end and the albumin of all 5 eggs was poured into small sterilized transparent polythene bags. The egg albumin sample was homogenized using blender (Master blender 1753, USA) (AOAC, 2000) and 1 ml of albumin was added to a test tube containing 9 ml of 10 % methanol (1-10 dilution) (Salem *et al.*, 2009) in test tubes and mixed for 10 mins at room temperature (20-25^0 C) by shaking the tubes vigorously. The entire extract was filtered using a filter paper, 100 µl of the filtrate was diluted with 600 µl distilled water. 50 µl per well was employed and the washing procedure was done as written in the owner's manual Radisson (r-Biopharm AG, Darmstadt, Germany). Absorbance was read within 30 mins at 450 nm.

2.2. Determination of risk factors for contamination with aflatoxins

A structured questionnaire was developed to collect data for risk factors for aflatoxins contamination. The questionnaires was pretested to validate respondents' knowledge of the problem. Modifications to the questionnaire were then effected then administered to 180 respondents (farmers and veterinary doctors) who were willing. The questionnaire was used to obtain information on farm management practices, handling and storing of the poultry feeds, history of symptoms noticed in birds that were ill and other diseases.

2.3. Determination of aflatoxin residues in eggs using enzyme linked immuno sorbent assay

The enzyme linked Immunosorbent assay (ELISA) was carried out according to the methods described by Radisson (r-Biopharm AG, Darmstadt, Germany, 2013). The reagents in the ELISA kit were adjusted to room temperature (25 ^{0}C) prior to the test. One milliliter of egg albumin was added to 10 % methanol and mixed for 10 minutes at room temperature (20-25 ^{0}C) using a shaker. The entire extract was filtered, using a filter paper and 100 µl of the filtrate was diluted with 600µl distilled water. Washing buffer was prepared by dissolving the entire buffer salt in the kit in 1 L of distilled water. 50 µl of the filtrate was employed per well in the assay. Prepared sample and 50 µl of standard solution was added to each well. Exactly 50µl of enzyme conjugate was added in the 96 well. Fifty microliters of the antibody solution was added to each well and mixed gently by shaking the plate manually and incubated for 30 min at room temperature (20-25 ^{0}C). The mixture was poured out of the wells with the micro well holder turned upside down and tapped vigorously three times in a row against absorbent paper to ensure complete removal of mixture from wells. The wells were filled twice with 250 µl washing buffer and the mixture was poured out and repeated twice. One hundred milliliter of substrate/chromogen was added to each well, mixed well by shaking the plate manually and incubated for 15 min at room temperature (20-25 ^{0}C) in the dark. One hundred microliter of the stop solution was added to each well, mixed gently by shaking the plates manually and absorbance measured within 30 min at 450 nm. The percentage absorbance was calculated manually using the formula:

$$\frac{Absorbance\ standard\ (of\ sample)}{Absorbance\ zero\ standard}\ x\ 100 = \%\ absorbance$$

The zero standard was made equal to 100 % and the absorbance values were quoted in percentage. The values calculated for the standard were entered in a system of coordinates in a semi logarithmic graph paper against the aflatoxin concentration (µg/kg). In order to obtain the aflatoxin concentration in µg/kg actually contained in a sample, the concentration read from the calibration curve was further multiplied by the dilution factor of 35 as outlined in the R-Biopharm manual.

2.3. Data analysis

Bivariate and multivariate analyses were undertaken using logistics and multiple regression respectively to examine interactions between possible risk factors. Cross tabulation of the biosecurity practice by farmers with respect to their LGA to check dependency on their knowledge of the various risk factors. Chi square was used to test independence.

3.0 RESULTS

The results of this analysis showed that all the different pools of eggs had aflatoxin at various concentrations (Figure 1) among the four Local Government Areas examined. The aflatoxin concentration in sampled eggs from the four local government areas in Kaduna Metropolis did not show any significance difference, but the concentrations of aflatoxin in eggs across the four LGAs (Table 1).

3.1. Distribution of poultry farms based on production systems, type of birds raised and sources of feed ingredients

On the basis of management system, 89 % of the respondents reared their birds on deep litter system while the remaining 11 % made use of battery cage system (Table 2). The predominant type of birds reared was the layer which accounted for 83 % as only sold bird on such farms.

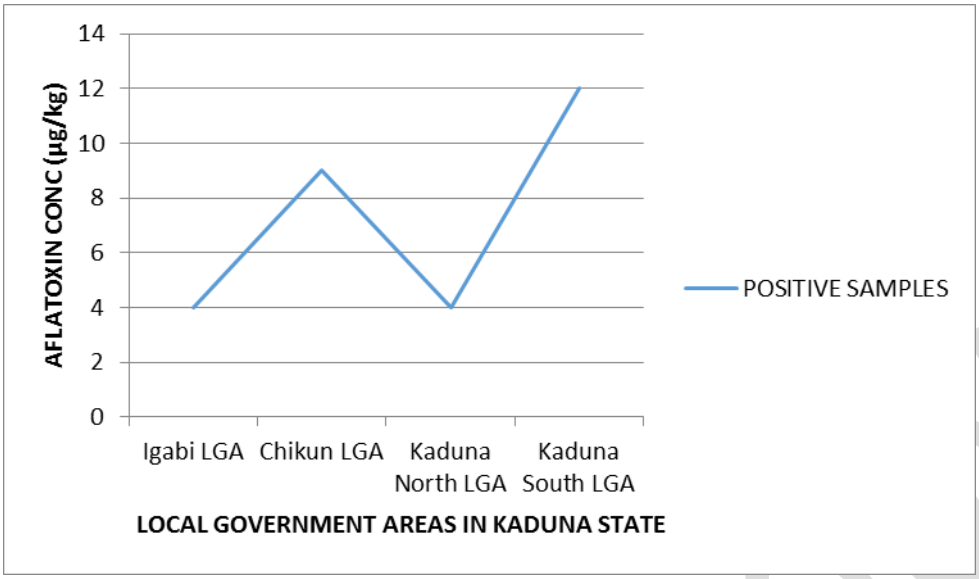

Figure 1: Aflatoxin concentrations in chicken eggs from four local government areas in Kaduna Metropolis.

Table 1: Results of aflatoxin concentration in eggs from four LGA in Kaduna Metropolis.

Local Government Area	Number Positive egg samples	Number Negative egg samples	Range of aflatoxin concentration in eggs ug/kg	(%) Positive aflatoxin in eggs Ug/kg
Igabi	4	1	0-13. 12	80
Chikun	9	2	0-8.75	82
Kaduna north	4	2	0-14.00	66.67
Kaduna south	12	1	0-10.94	92.31

The results further revealed that others were broilers, quails, turkey in combination with layers as 13 %, 2.7 % and 1 % respectively (Table 3). From the analysis (Table 4), 53 respondents representing 30 % revealed that they bought their grains they use for feed production from the market and another 13 respondents (7 %) revealed that they purchase direct from the farmers while the remaining 6 (3 %) respondents said that they use the grains from their farms. Majority of the respondents representing 26 % revealed that they make use of sacs to store the grains they purchased or farmed for feed production and 4 respondents representing 2 % indicated that they make use of polythene bags to store their grains. The result also showed that 4 (2 %) of the respondents use of both methods of storage. The remaining 124 respondents did not respond (Table 5). Forty two percent of the respondents were found to always inspect their feeds for dampness while 33 (18 %) respondents indicated that they inspect their feeds for dampness. Others 34 (19 %) other respondents stated that they do not inspect their feeds for dampness as they believed that the feeds were safe and free from fungal contamination. The data revealed that the remaining 21 % did not respond (Table 6). The respondents revealed that they made use of organic acids representing 23 % while another 5 respondents representing 3 % they combine organic acids with salts. The remaining 8 % of the respondents stated that they do not made use of these agents (Table 7). The analysis presented revealed that 51% of the respondents cleaned the feeding and handling equipment weekly while 30 % do so monthly (Table 8).

Table 2: Distribution of Poultry Farms According to type of house in Kaduna Metropolis.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Battery	20	11.0	11.0	11.0
	deep litter	160	88.5	89.0	100.0
	Total	180	99.5	100.0	

Table 3: Distribution of Poultry Farms According to type of Birds in Kaduna Metropolis.

Types of birds	Frequency	Percent	Valid Percent	Cumulative Percent
Layers	150	83.0	83.0	83.0
layers and broilers	23	13.2	13.2	96.2
layers, broilers and quail	5	2.7	2.7	98.9
layers, broilers and turkey	2	1.1	1.1	100.0
Total	180	100.0	100.0	

Table 4: Distribution of Poultry Farms According to Source of Grains

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Buy from grain market	53	29.7	74.0	74.0
	Buy direct from farmers	13	7.1	17.8	91.8
	Farm myself	6	3.3	8.2	100.0
	Total	73	40.1	100.0	
	Not Applicable	108	59.9		
Total		180	100.0		

3.2. Biosecurity Practices by Farmers with Respect to their Local Government Area to Check Dependency on their Knowledge of the Various Risk Factors

Knowledge of biosecurity practices revealed that 91 % of respondents in Igabi LGA had adequate knowledge while 9 % had inadequate knowledge of biosecurity (Table 9). In Chikun LGA, of the 45 farmers, (89 %) had adequate knowledge while (11 %) have inadequate knowledge of biosecurity. Kaduna north LGA, of the 46 farmers, (95.7 %) have adequate while (4.3 %) have inadequate knowledge of biosecurity. In Kaduna south LGA, of the 43 farmers, (88.4 %) have adequate while 5 (11.6 %) have inadequate knowledge of biosecurity. The result did not show any significant difference (P>.05) as far as knowledge of biosecurity practices is concern among the LGAs.

Table 5: Distribution of Poultry Farms According to type of Grain Storage container

Storage	Frequency	Percent	Valid Percent	Cumulative Percent
Sacs	47	26.1	26.1	26.1
polythene	4	2.2	2.2	28.3
Both	4	2.2	2.2	30.6
NA	125	69.4	69.4	100.0
Total	180	100.0	100.0	

Table 6: Distribution of Poultry Farms According to time of inspection for dampness of feed in Kaduna Metropolis**.**

Response	Frequency	Percent	Valid Percent	Cumulative Percent
Never	34	18.7	23.8	23.8
Sometimes	33	18.1	23.1	46.9
Always	76	41.8	53.1	100.0
Total	143	78.6	100.0	
No response	37	21.0		
Total	180	100.0		

4.0. DISCUSSION

The occurrence residue of aflatoxin in chicken eggs and eggs from Igabi, Chikun, Kaduna North, Kaduna South local government areas had some levels of aflatoxin concentrations of 0 - 6.13 µg/kg , 0 - 8.75 µg/kg , 0 - 14.08 µg/kg and 0 - 10.94 µg/kg respectively. Eggs sampled from major egg marketers in Kaduna Metropolis revealed aflatoxin concentrations of 0 - 17.5 %. These aflatoxin concentrations may be due to transfer from birds, the excretion of aflatoxin residues in hens' eggs might occur at 1relatively low concentrations under conditions of long term exposure of laying hens to low level of aflatoxin in naturally contaminated feed (Aly and Answer, 2009), physical handling, water used for washing eggs, crates that are used to package egg or during transportation of egg where by the vehicle could have been contaminated by aflatoxins.

Table 7: Distribution of Poultry Farms According to Antimold agents added to feed

	Response	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	None	15	8.2	24.6	24.6
	Salts of organic acids	5	2.7	8.2	32.8
	Organic acids	41	22.5	67.2	100.0
	Total	61	33.5	100.0	
No response		119	66.5		
Total		180	100.0		

Table 8: Distribution of Poultry Farms According to time of Cleaning of feed handling equipment.

	Response	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Monthly	54	30.0	36.5	36.5
	Weekly	92	51.0	63.5	100.0
	Total	146	81.0	100.0	
No response		34	18.7		
Total		180	100.0		

In most countries, Regulations are established to control the contaminants in foodstuffs to protect human health; it may include specific maximum limits for several contaminants for different foods. Nigeria is yet to established permissible limits in animal tissues. The difference in aflatoxin concentrations µg/kg from the four Local Government Areas and the eggs from the egg marketers, may be due to the heterogeneous nature of *Aspergillus* in feeds whereby some birds eat more contaminated feed than others while some may be lucky not to feed or by given the contaminated parts of the feed to eat. The difference may also be due to different feed sources, management system, biosecurity practice and sources of birds. The study also agrees with the findings of Oliveria *et al*. (2000) where the result of their findings emphasized the importance of controlling aflatoxin levels in rations of laying hens. Wolzak *et al*. (1986) stated that transfer of aflatoxin to eggs occurred rapidly reaching maximum levels 4-5 days after feeding and remained relatively constant throughout aflatoxin feeding period. Studies by Sager (2013) showed that birds fed with B1 contaminated feed reduced egg production by 30 %. This may be the reason for inability of layer bird farms to reach 100 % production in Nigeria. The study has shown that eggs may play a major role in human aflatoxicosis because of its cumulative (Kelly *et al.* 1997). Bufalo, (2000) showed that eggs are responsible for an estimated 230,000 case of foodborne illnesses. Aflatoxin concentration in eggs obtained from the four local government areas revealed that there was no difference in quality of eggs or aflatoxin contaminated eggs from the four LGAs. This may be due to the same sources of feeds for the birds. Layers birds are for table eggs. The likely human exposure to aflatoxin is birds and eggs. Poisoning may likely come from eggs that have mycotoxin residues such as aflatoxin.

Table 9: Comparison of knowledge of biosecurity practices among farmers in four local government areas in Kaduna Metropolis.

		Biosecurity practice		
		Inadequate knowledge	Adequate knowledge	Total
LGA	Igabi LGA	4	41	45
	Chukun LGA	5	40	45
	KAD North LGA	2	44	46
	KAD south LGA	5	38	43
Total		16	163	179

The initial cost to start a poultry farm using cages is expensive so deep litter was preferred by poultry farmers because it is cheaper than battery cages. Proper sanitation is usually difficult on deep litter. The environment becomes good medium for aflatoxin production and birds, eggs and feed may likely be contaminated with mycotoxins producing fungi.Most feed millers buy their grains from the subsistence grain farmers. The risk of grain production is left to the subsistent farmers. The methods of drying and storage of grains by subsistence farmers are still traditional ways. They dry their grains by the road side, on bare floor in their compounds and they also leave the corn still on stalk on farms until dried. These methods can cause mechanical damage to the grains as well as promotes favourable conditions for mould growth. The

feed millers buy the grains only during dry season and carryout quality check for moisture (maximum of 13 %) content in grains and also mouldy grains before buying. The knowledge of farmers was adequate on biosecurity practices and most likely influenced by previous outbreak of Avian Influenza.

CONCLUSION

Varying levels of aflatoxin residues were detected in chicken eggs ranging from 0-17.5 µg/kg. Some of the risk factors that led to the contamination of poultry by aflatoxin were poor biosecurity level practices by subsistent farmers, the management system (the use of deep litter instead of cages) and Sourcing grains directly from the open markets.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest as regard the results presented in this article.

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