Survey for Aflatoxin in the Eggs of chickens and risk factors that may lead birds to aflatoxin exposure in Kaduna Metropolis, Nigeria

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Families bring out their grains from storage to clean up and if the grains change color or smell due to poor storage, they are sold to especially poultry toll feed millers either as whole grains or grinded fine particles, at cheap prices. The objective of this study is to determine the occurrence of residues of aflatoxin in eggs. Eggs from Igabi (A), Chikun (B), Kaduna North (C), Kaduna South (D) local government areas had some levels of aflatoxin concentrations of up to 6.13 ug/kg, 8.75 ug/kg, 14.08 ug/kg and 10.94 ug/kg respectively. Eggs sampled from major egg marketers in Kaduna metropolis revealed aflatoxin concentrations of up to 17.5%. The likely human exposure to mycotoxins are birds and eggs. The implication is that the birds would consume aflatoxin contaminated feed which they may pass to eggs and meat, which eventually human being would consume and become toxicated. Some of the predisposing risk factors that may have led 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. It is recommended that there should be adequate ventilation in live bird markets to reduce the multiplication of mycotoxin producing Aspergillus species.

Keywords: mycotoxin, poultry products, risk factors.

INTRODUCTION

Safe food is an imperative in food production worldwide. Poultry meat, eggs, and poultry products derived from them are crucial in safe food chain. As far as safety is concerned, special attention is directed towards possible contamination of food and poultry feed with fungi and to the risk of mycotoxin contamination (Radmilla et al., 2009).

In contrast, mycotoxins direct detection and quantification in the birds themselves (in the plasma, liver, kidneys and muscles) confirms the existence of the problem and minimizes the errors involved in feed sampling and analysis (Furlan et al., 2001).

The toxic effects of mycotoxins on animal and human health is referred to as mycotoxicosis, the severity of which depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed to. The chemical structures of mycotoxins vary considerably, but they are all relatively low molecular weight organic compounds (Peraica et al., 1999). A practical definition of a mycotoxin is a fungal metabolite that causes undesirable...
effects when animals or humans are exposed to through consumption of contaminated feedstuffs or foods. Mycotoxicosis are diseases caused by exposure to foods or feeds contaminated with mycotoxins (Nelson et al., 1993).

The fungal toxins are chemically diverse, representing a variety of chemical families, and range in molecular weight from about 200 to 500 Da (Pitt, 2000). There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis that are commercially available. The primary classes of mycotoxins are aflatoxins of which aflatoxin B1 (AFB1) is the most prevalent, zearalenone (ZEA), trichothecenes, primarily deoxynivalenol (DON) and T-2 toxin (T-2), fumonisins, ochratoxins (OTA) and the ergot alkaloids (Whitlow et al., 2010). Mycotoxins exhibit a variety of biological effects in animals: liver and kidney toxicity, central nervous system effects and estrogenic effects, to name a few. Some mycotoxins, i.e., aflatoxin, fumonisin and ochratoxin are carcinogenic (Whitlow et al., 2010).

General interest in mycotoxins rose in 1960 when a feed-related mycotoxicosis 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).

Mycotoxins cause illness and mortality in domestic animals fed moldy feedstuffs. These acute intoxications can have devastating effects and are difficult to diagnose and treat because the suspect feed may be consumed before it can be tested (CAST, 2003). It has been estimated that more than five billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shepherd, 2005; Strosnider et al., 2006).

In many parts of Africa, the need to eat outweighs other considerations such as food safety, and as such, this has made food-borne intoxications to be a serious problem in many respects. It is reported that about 40% of the productivity in birds is lost to diseases in developing countries but is most likely due to diseases exacerbated by aflatoxins (Bennett, 2003).

Regrettably, many of the people in the developing countries are not even aware of the adverse and detrimental effects of consuming moldy products. Due to the poor education levels and other socio-economic factors, even if steps are taken to make food products safe, the consumers will be unwilling to pay the extra costs, and still prefer to buy cheap commodities (Bennett, 2003).

Literature abound on information on mycotoxins from all over the world and work done in Nigeria have focused on their effects on human populations (Atanda and Akpan, 2005; Bankole and Kpodo, 2005; Fandohan et al., 2005). Okoli (2005) emphasised that literature is largely silent on fungal and mycotoxin contamination of feedstuff and mycotoxicses in farm animal, and in livestock production research issues in Nigeria. The only toxin that has gained prominence in scientific literature in food products from the West African sub-region is aflatoxin, while there are few studies conducted on poultry food chain (Bankole, 2003; Atanda et al., 2005, Akpan et al., 2003).

The objective of this study is to determine any residues of aflatoxins in poultry eggs and risk factors that may lead birds to aflatoxin exposure in Kaduna metropolis, Nigeria

MATERIALS AND METHODS

Collection of Eggs

Seventy two commercial poultry farms (small (>1,000 birds), large (1,000 birds and above) and backyard (>500 birds) in Kaduna metropolis and four commercial egg marketers were selected using disproportional sampling method.

Collection of Liver samples

Thirty nine samples of liver from broilers at slaughter from both upgraded (supplying electricity and upgrading physical infrastructure such as tabletops, floors to improve bio-security and hygiene production) and local (low resource settings) live bird markets (LBM) were systematically randomly sampled.

Processing of Eggs Samples

Samples of 600 eggs were cleaned using sterile water and wiped with 24% ethanol. The eggs were grouped in pools of 5, after thorough disinfection, the eggs were cracked with sterile surgical forceps 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).

Processing of Liver Samples

Whole liver was weighed and placed into Ziploc bags aseptically before freezing. 1g of liver sample was macerated, homogenized and added to 9ml of methanol/distilled water (70:30v/v) in a test tube and mixed for 10 minutes at room temperature (20-25°C) using a shaker.
Determination of risk factors for contamination of eggs with aflatoxin

From a review of literature, a survey questionnaire was developed to collect data for risk factors for aflatoxins contamination. The questionnaires were issued to 30 farmers first on a pilot project basis. The purpose was to refine the questionnaires so that the respondents will have no problem in answering the questions and there will be no problem in recording the data. Modifications to the questionnaire were then effected and then the document issued out to the 180 respondents. The respondents (farmers and veterinary doctors) filled the questionnaires by ticking their responses while the samples were collected accordingly. The questions mainly focused on issues of level of farm management practices in the production, handling and storing of the poultry feeds, history of signs noticed in birds that were ill and other diseases.

Enzyme Linked Immunosorbent Assay

The enzyme linked Immunosorbent assay (ELISA) was carried out to detect aflatoxin in eggs 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°C) prior to the test.

Data analysis

Descriptive statistics was used to describe the basic features of the data in this study. Summaries were made in tables and presented using charts. Chi square and Fisher’s Exact Test were used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in all categories. Bivariate and multivariate analysis were undertaken using logistic and multiple regression respectively to examine interactions between possible risk factors.

RESULTS

The mean absorbance values of the standard and samples were analyzed using R sudden® computer program (R-Biopharm). Data obtained for the different treatments were subjected to ANOVA, followed by multiple regression. Significant was set at p < 0.05. For Kaduna south LGA, aflatoxin concentration in egg samples revealed 0 – 10.94 ug/kg, Kaduna North LGA revealed 0- 14.09 ug/kg, Chikun LGA showed 0 – 8.75, Igabi LGA 0 – 6.13 ug/kg and Major egg marketers 0 – 17.5 ug/kg.

Comparing aflatoxin concentration values in egg samples from four LGA

Aflatoxin concentration in sampled eggs from the four(Igabi, Chikun, Kaduna North and Kaduna South) different local government areas in Kaduna metropolis revealed value of 0.201 which is greater than the significance level of 0.05 (5%). This indicates that there was no significant difference between aflatoxin concentrations in eggs sampled from the four LGAs.

Relationship between risk factors and their impacts on the aflatoxin in eggs

The overall model fit was R² = 0.81 which implies that the independent variables (biosecurity practices) has explained the dependent variable at 80.7% leaving 19.3% unexplained (Table 1).

Responses of farmers to questionnaires

Figure 1 shows the years of experience of respondents in poultry farming or length of time individuals have actually engaged in poultry farming with 123 respondents representing 68.7 % indicating that they have been into poultry farming for about 5 years. Here also 55 respondents representing 30.2% revealed that they have been engaging in poultry farming between 10 to 25 years while the remaining 2 respondents representing 1.1% stated that they have been into poultry farming for over 15 years.

The issue of housing the birds in a safe and conducive environment is of paramount importance to the poultry farmer (Figure 2). From the analysis, majority of the respondents 89.0% revealed that they make use of the deep litter system of housing the birds while the
remaining 11.0% stated that they make use of the battery system. Although, the percentage of those who use deep litter is high, it does not necessary mean that they are the only set of people that are acquainted with better housing system. This simply implies that there is a tendency that poultry farmers in Kaduna State prefer the use of deep litter system for housing birds.

From the analysis (Figure 3), respondents revealed their source of grains for feed production. 36 respondents representing 20% revealed that they buy the grains they use for feed production from the market and another 13 respondents revealed that they purchase direct from the farmers while the remaining 6 respondents stated that they farm the grains they use for their feed production by themselves. Here also in the table, 125 respondents did not respondent to the question asked as they do not formulate the feeds they use for their birds.

The respondents from the analysis (Figure 4) revealed that they always inspect their feeds for dampness accounting for 41.8% and another 33 respondents indicated that they inspect their feeds for dampness sometimes while 34 other respondents stated that they do not inspect their feeds for dampness as they believed that the feeds are safe from fungal growths. The remaining 39 respondents representing 21.4% did not respond.

Sequel to the previous analysis, Figure 4 revealed respondents’ response to the inspection of feeds for mould or mouldy growths on feeds. 33 respondents revealed that they do not inspect their feeds for mould growth and another 9 respondents stated that they do so sometimes. Here also, majority of the respondents revealed that they inspect their feeds always accounting for 50.5% of the population while the remaining 26.4% did not respond.

Here presented in Figure 4 is the respondents’ affirmation of inspecting their poultry feeds and consequently protecting them against infestation as revealed by 51.1% and 11.5% of the respondents who attested that they inspect their feeds always and sometimes respectively, while 18 other respondents representing 9.9% stated that they do not.

DISCUSSION

Persons working with feeds in feed mills or poultry farms, farmers during planting, harvesting, drying, winnowing, storage, milling of grains, may be affected and also those selling or buying grains, consumption of poultry and

Table 1. Relationship between risk factors and their impacts on the aflatoxin concentration in the sampled eggs.

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.807a</td>
<td>.652</td>
<td>-.392</td>
<td>2801.24192</td>
</tr>
</tbody>
</table>

**Figure 1.** Pie chart showing frequency distribution of duration of activity of 180 poultry farms sampled in Kaduna metropolis.

**Figure 2.** Pie chart showing frequency distribution of types of bird house of 180 poultry farms sampled in Kaduna metropolis.
poultry by-products may be exposed to aflatoxin at the various points of food chain (conception to consumption of grains).

The initial cost to start a poultry farm using cages is expensive so deep litter is preferred by poultry farmers because it is cheaper than battery cages. Environment, birds, feed and eggs are dirty on deep litter. The environment becomes good medium for mycotoxin production and birds, eggs and feed may likely be contaminated with mycotoxins producing fungi.

Layers are reared for table eggs. The likely human exposures to mycotoxins are birds and eggs. Poisoning may likely come from eggs that have mycotoxin residues such as aflatoxin and ochratoxins. Small amounts of aflatoxins are found in the eggs and this study suggests that a program to control the negative impact of aflatoxins on the poultry industry might include assessing mycotoxin levels in tissues and organs of chicken. Determining the level of mycotoxin in eggs is a useful way of predicting exposure of chickens to mouldy feeds and also suggests that residues will be found in poultry tissues and organ.

Eggs from Igabi, Chikun, Kaduna North, Kaduna South local government areas had some levels of aflatoxin concentrations of up to 6.13 ug/kg, 8.75 ug/kg, and 14.08 ug/kg and 10.94 ug/kg respectively. Eggs sampled from major egg marketers in Kaduna metropolis revealed aflatoxin concentrations of up to 17.5%. 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 class.

The difference in concentration from the four local government areas and the eggs from the egg marketers, may be due to the heterogeneous nature of Aspergillus in feeds. 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) they 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 of 4-5 days after feeding and remained relatively constant throughout aflatoxin feeding period.

Most farmers said that, they have started checking their stored feed and grains for dampness, no more keeping of feed on the bare floor and they remove mouldy parts of the feed. The farmers do same for mould and mouldy feeds. To keep insects’ away, phosphine tablets are kept in parts of the store. Insect infestations create conducive environment for the growth of fungi. The reasons are of economic incentive to the farmers. The farmers are afraid of losing their investment as some may not be aware of mycotoxin problems.

**Conclusion**

Aflatoxin residues surveyed in eggs range from 0-17.5 ug/kg levels that represent a health hazard for both chicken consuming aflatoxin contaminated feeds and humans consuming such eggs. Some of the predisposing risk factor that may lead to the contamination of poultry by aflatoxin include:

i. Poor biosecurity practices by subsistent farmers
ii. Deep litter housing for birds
iii. Sources of grain are from the open markets.

**REFERENCES**


