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Occurrence of Aflatoxin B_1 in Fresh and Boiled Liver Broiler Chickens in Zaria, Nigeria

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ABSTRACT

There is an increasing concern for the contamination of poultry products with Aspergillus strains, responsible for the aflatoxin production. Therefore, the persistence of aflatoxin B_1 was analyzed in 60 fresh liver divided into two-part one remains fresh and the other part boiled from live bird markets in Zaria, Nigeria, using indirect competitive enzyme link immunosorbent Assay (iELISA). A total of 66.7% of fresh liver and 16.7% of boiled liver were found to be contaminated with aflatoxin B_1 above maximum detection limits. The levels of aflatoxin B_1 contamination varied in decreasing order as follows: Sabon Gari, Zaria city, Dan Magaji and Tudun wada had aflatoxin level of 66.40 µg/kg, 41.45 µg/kg, 35.25 µg/kg and 29.80 µg/kg, respectively in fresh liver samples. The highest level of aflatoxin B_1 in boiled liver was 29.02 µg/kg in Sabon Gari. The persistence of boiling aflatoxin B_1 was significant ($P < 0.05$) in the liver of broilers. The occurrence of aflatoxin B_1 in the liver of slaughtered broilers is dangerous and may adversely affect human health. It was concluded that the high level of AFB_1 in birds sold in live-bird markets, meant for slaughter for human consumption may have adverse effects on human health. Strict measures should be adopted to monitor and regulate aflatoxin level in feeds and, subsequently, in edible tissues.

Keywords: AFB1, ELISA, Chickens and Liver.
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INTRODUCTION

Mycotoxins are poisonous chemical compounds and secondary metabolites produced by fungi (Tola and Kebede, 2016). These secondary metabolites, are produced by members of various mould genera, have relatively low molecular weight, and are heat-stable (Binder, 2007; Valchev et al., 2014; Iqbal et al., 2014a). Mycotoxins in feeds and food are deleterious to the health of animals as well as human because they exert carcinogenic, toxic, mutagenic and teratogenic effects (Royer et al., 2004). Mycotoxins of significant importance in terms of occurrence and toxicity are aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone (Herebian et al., 2009; Iqbal et al., 2014b). Aflatoxins are polyketide secondary metabolites, which are toxic phytopathogens, produced by toxigenic strains of Aspergillus, Penicillium, Fusarium and Alternaria (Da Costa et al., 2010; Valchev et al., 2014). Chemically, they are difuro-coumorins, soluble in chloroform and methanol (Enyiukwu et al., 2014). Aflatoxins are among the most toxic mycotoxins (Molina and Gianuzzi, 2000; Passone et al., 2010; Sardiňas et al., 2011). These potent mycotoxins, produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus,* are of major health concern in poultry production (Magnoli et al., 2011). The toxicity of aflatoxins in broilers has been widely investigated for their carcinogenic, mutagenic,

teratogenic and growth-inhibitory effects (Oğuz et al., 2003; Sur and Celik, 2003). In Nigeria, limited reports have been documented in the presence of aflatoxin B_1 in broiler meat. However, various surveys have documented the presence of mycotoxin in poultry feed.
In Nigeria, mycotoxins were discovered by Obi and Ozugbu (2007) Osho et al. (2007) Uwaezuoke and Ogbulie (2008) Habib et al. (2015) Aliyu et al. (2016). Spain by Moreno and Suarez (1986) Abarc et al. (1994) Bragulat et al. (1995) Argentina by Oliveira et al. (2006) Brazil by Rosa et al. (2006) Simas et al. (2007) and Pakistan by Saleemi et al. (2010) Somashekar et al. (2004) have reported the contamination of poultry feed with *Aspergillus* section *flavi* that are capable to produced aflatoxin B_1. The international agency for research on cancer has placed aflatoxin in group 1 carcinogen (IARC, 1993, 1999). Aflatoxin B_1 is the most toxic and acute class of aflatoxin (Pittet, 1998), which can be found as the major residue in broiler liver after exposure (Fernandez et al., 2000; Oliveira et al., 2000; Denli et al., 2009). Aflatoxins can pass to the by-products of poultry such as egg and meat, thus having a negative effect on human health (Trucksess and slotoff, 1981; Trucksess et al., 1983). Numerous studies have shown the presence of mycotoxins in poultry feeds (Zinedine et al., 2007; Astoreca et al., 2011; Bryden, 2012). Animals are exposed to mycotoxins through consumption of contaminated feeds (Bryden, 2012). Live bird markets are located in the urban and semi-urban areas where they receive poultry from larger poultry industry (FAOUN, 2008). The birds on showcase are local breeds, spent layers, local chicken and broilers. The management system is extremely inadequate especially the basic hygienic practices are very poor, sales points, and in particular slaughtering point, through lack of knowledge or constraints to their implementation (FAOUN, 2008). Birds receive water and feed, generally from containers, giving rise to considerable spillage of water and loss of feed, some of the dispersed feed may be an attractant to pest and disease condition, given a short life span to the birds (FAOUN, 2008). This study was aimed at determining the level of aflatoxins B_1 residues in both fresh and boiled broiler livers in live-bird markets in Zaria, Nigeria.

MATERIALS AND METHODS

Fresh Broiler Liver Samples

A total of 60 fresh broiler liver samples, were collected from six live-bird markets in Zaria, Nigeria, from August 2015 to February 2016. The samples were collected in a sterile polythene bag and stored in ice box during transportation to prevent enzymatic reaction until analysis.

Boiled Broiler Liver Samples

A sub-section of the 60 fresh broiler liver samples were boiled for 90 min at 100°C from the same six live-bird markets.

Indirect Competitive Enzyme Link Immunosorbent Assay (iELISA)

The standard aflatoxin (ELISA plates, ELISA reader, incubator, orbital shaker, Elisa plate shaker, pH meter, fume hood, vortex mix waring blender with mini jars, a set of micro-pipettes including 12 channel) were purchased from (Nunc, Maxisorp. San Diego, USA) used in the present study, Bovine serum albumin conjugate (Sigma-Aldrich A6655), Phosphate-saline buffered with Tween PBS-T (Sigma-Aldrich P1379), P-nitrophenyl-phosphate (Sigma-Aldrich N9389), Diethanol amine (Sigma-Aldrich 31590), Potassium chloride (Sigma-Aldrich 60130), Methanol (BDH, 86542, Chemical Limited Poole, England), Bovine serum albumin (Sigma-Aldrich A7906), Anti-Rabbit IgG (Sigma-Aldrich, A9919) were purchased from www.icrisat.org/aflatoxin, while distilled water and other chemicals and reagents used were of analytical grade.

Detection Procedure for Aflatoxin

Detection procedure of AFs from meat samples was performed according to the methods (Sarimehmetoghlu et al., 2004; Rosi et al., 2007) with some modifications. The whole liver was placed into a Ziploc polythene bag aseptically and transported to the Mycotoxin Laboratory, Department of Crop Protection Laboratory. Ahmadu Bello University, Zaria. Samples were weighed and 1 g of subsamples was homogenized (HGBTWT, USA), with 100 ML of 70% Methanol (v/v 70 ML methanol and 30 ML distilled water) and 0.5% of KCl blended to mixed thoroughly, in a conical flask and shaken in an orbit shaker (Benchmark®) for 30 min at 150 rpm, the extract was filtered using Whatman paper. A total of 150 µL of Bovine Serum Albumin conjugate (AFB1-BSA) and carbonate buffer were filled into lane B-G boarder wells (lane A and H, well B-G, 1and 2) was filled with distilled water and plate was incubated in a shaker incubator (Technel and Technel, USA) at 37°C for 1 h. The plate was removed and the toxin washed with PSB-T 3 times, and allow 3 min for each wash. A total of 150 µL of 0.2% BSA was added to each well, while the border well was again filled with distilled water and incubated at 150 rpm, 37 °C for 30 min. The plate was then removed and the solution was discarded, then was washed with PBS-T, 3 times, allowing 3 min for each wash. Exactly 150 µL of goat anti-rabbit IgG-ALP was added to all wells, with the exception of border wells which were filled with distilled water, and incubated for 1 h. The plate was washed as

Table 1. Occurrence of aflatoxin B_1 in fresh liver samples of live-bird markets in Zaria, Nigeria (Mean ± S.D).

Location	Number of Liver Samples	Concentration (µg/Kg).
Zaria City	10	41.45 ± 29.55
Sabon Gari	10	66.40 ± 22.28
Samaru	10	10.00 ± 1.74
Tudun Wada	10	29.80 ± 17.16
Dan Magaji	10	35.25 ± 8.14
Kwangila	10	9.90 ± 6.50
Total	60	192.80 ± 85.37

*µg/kg = Microgram per kilogram.

Table 2. Occurrence of aflatoxin B_1 in boiled liver samples of live-bird markets in Zaria, Nigeria (Mean ± S.D).

Location	Number of Boiled Samples	Concentration (µg/Kg)
Zaria City	10	8.22 ± 5.05
Sabon Gari	10	29.02 ± 9.53
Samaru	10	5.22 ± 5.10
Tudun Wada	10	4.46 ± 3.35
Dan Magaji	10	0.60 ± 0.32
Kwangila	10	1.80 ± 0.77
Total	60	49.42 ± 24.12

µg/kg = Microgram per kilogram.

Table 3. Occurrence of aflatoxin B_1 in live-bird markets in Zaria, Nigeria.

Location	Fresh Liver Samples (µg/Kg)	Boiled Liver Samples (µg/Kg)	Mean Reduction (%)
Zaria city	41.4 ± 29.5^a	8.2 ± 5.0^b	79.7
Sabon Gari	66.4 ± 22.2^a	29.0 ± 9.5^b	53.6
Samaru	10.0 ± 1.7	5.2 ± 5.1	45.9
Tudun wada	29.8 ± 17.1^a	4.5 ± 3.3^b	83.3
Dn Magaji	35.2 ± 8.1^a	0.6 ± 0.3^b	98.4
Kwangila	9.9 ± 6.5^a	1.8 ± 0.7^b	76.1

* Values with different superscript letters are significantly ($P < 0.05$) different, % = percentage, µg/kg = Microgram per kilogram.

removed and the solution was discarded, then was washed with PBS-T, 3 times, allowing 3 min for each wash. A total of 150 µL of P-nitrophenyl-phosphate in 10% diethanol amine buffer was dispensed into each of the wells, with the exception of border wells which was filled with distilled water and incubated for 30 min. The plate was removed and read in ELISA reader using the microplate reader of 405 nm (As recommended by Manufacturer).

Statistical Analysis

The data obtained from both fresh and heated samples were analyzed and presented as mean ± standard deviation (SD). They were analyzed using Graphpad prism version 5.0 (San Diego, California, USA) and subjected to a repeated-measures analysis of variance (ANOVA), followed by Tukey's *post-hoc* test to compare mean values among different groups. Paired *t*-test was used to determine the effect of heat treatment on aflatoxin residues. Values of $P < 0.05$ were considered significant (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The result of 60 fresh liver samples analyzed for the occurrence of aflatoxin B_1 from slaughter broilers in the live-bird market is shown in Table 1, while that obtained from 60 boiled, broiler liver samples analyzed for the persistency of aflatoxin B_1 residues using one-way ANOVA is shown in Table 2. Table 3 shows the comparison between fresh and boiled liver sample using paired *t*-test. In the present study, 66.7% (60) samples of liver were positive with AFB_1 contamination. The highest level of AFB_1 was found in Sabon Gari and Zaria City with 66.40 ± 19.09 and 41.45 ± 24.11 µg/kg, respectively. The occurrence of aflatoxin in boiled broiler liver samples is

presented in Table 2. The result showed that 16.7% (60) of the boiled liver samples were found contaminated with AFB_1. The highest level of AFB_1 contamination was found in Sabon Gari, with mean value of 29.02 ± 9.53 µg/kg. The aflatoxin residue was higher than that of maximum limit level of set by the standard organization of Nigeria (NAFDAC, 2003). The aflatoxin residue obtained in the present study was more than the level reported by Bintvihok et al. (2002), in the liver of domestic fowls, Zaghini et al. (2005), in liver tissue of laying hens following feeding of 2500 ppb AFB_1. Herzallah (2009) documented maximum concentration of AFB_1, AFB_2, AFG_1 and AFG_2 levels in analyzed food products, which are 0.15 to 6.36 µg/kg in imported and fresh meat samples. Hussain et al. (2010), documented 6.97 ± 0.08 ng/g, following increased in AFB1 in dietary feed with aflatoxin level 1600, 3200 and 6400 µg/kg. Markov et al. (2013) documented that maximum aflatoxin concentration in commercial sausage sample was 3.0 µg/kg, which represented 10% (90) of the sausage sample analyzed. Iqbal et al. (2014a), obtained maximum aflatoxin B_1 concentration of 2.64 ± 0.58 µg/kg in broiler liver.

The finding of this study agreed with Oyero and Oyefolu (2010), that aflatoxin B_1 are thermostable, physical treatment by heat or sun-dried results in small changes in their levels. However, the result of the present study agreed with those of Binder (2007) and Iqbal et al. (2014a) that aflatoxins are usually heat-stable compounds, which cannot be destroyed totally by any thermal processing. The high aflatoxin residue recorded in this study could be as a result of consumption of aflatoxin-contaminated animal feeds, which could be as a result of storage condition and handling of the product by those feeding the birds. The high concentration of AFB_1 residue observed in this study may be as a result of long-time ingestion of AFB_1, variation in the dose of AFB_1 and duration of ingestion of AFB_1 toxin in feed which is similar findings of Hussain et al. (2010). The result of the study agreed with that of Perši et al. (2014) that feeds contaminated with aflatoxin ultimately contaminate the meat Iqbal et al. (2014) showed that the presence of aflatoxin in meat may not only affect their organoleptic properties but also produce mycotoxin and pose a potential health hazard to the consumers. The transfer of aflatoxin contamination from feed to animal by-products could result in health and economic implication on both animals and humans. Epidemiological studies have correlated the incidence of liver cancer in animals and humans to the consumption of aflatoxin in contaminated cereals (maize or sorghum) from cereals to hens, and consequently to humans, and the effects of AF were increased by hepatitis B viral infection (Henry et al., 1999). The traces of AFB_1 have been found in animal liver and eggs used for human consumption (Park and Pohland, 1986). Tedesco et al. (2004) indicated that AFB_1 contamination in poultry possesses a public risk due to high consumption of contaminated animals. Bintvihok and Kositcharoenkul (2006) found higher levels of AFB_1 and AFM_1 in hen liver than in muscle in broilers, fed a diet containing low levels of aflatoxin B_1. The European community and many other countries have imposed 2 ng/g AFB1 maximum tolerance level in human food products (Anonymous, 2004).

The differences in AF susceptibility make the extrapolation of data from animals to humans difficult because human fatalities due to AF acute toxicity are less common. Some adult humans can consume anywhere from 2 to 6 mg of AF per day (Krishnamachari et al., 1975), and lethal dosages for adult humans have been calculated to be in the range of 10 to 20 mg of AF (Pitt, 2000). The secondary exposure to aflatoxin through consumption of chicken liver is derived from the poultry-fed aflatoxin-contaminated feed, which may pose a risk to human health due to acute aflatoxicosis, hepatocellular carcinoma, hepatitis B virus infection and growth impairment in the world, especially African countries (Wild and Gong, 2010). When animals consume AFB_1, their livers tend to detoxify it, forming the less toxic hydroxylated metabolites, AFM_1, AFP_1, and AFL, since there is a health risk for humans that ingest them, it is meaningful to measure all AF metabolites. Aflatoxin M1 was the most abundant AF hydroxylated metabolite; it is frequently found in human urine and has a direct association with increased incidence of hepatitis, cirrhosis, and hepatocellular carcinoma (Sun et al., 1999). In this study, the high aflatoxin B_1 residue could be caused by the feeding practice and type of poultry feed. The result obtained from the study may be beneficial in increasing the awareness of the public health hazard associated with the toxin.

CONCLUSION

The level of aflatoxin B_1 in chicken meat is important for the health as well as the economy of the country. Aflatoxin residues were found in edible tissues both before and after heat treatment. Therefore, there is need to regulate and control aflatoxins in chicken meat products, and the maximum limits of meat should be implemented to avoid consumption of toxins. There are high levels of AFB_1 in the liver of broilers liver meant for human consumption in Zaria, Nigeria, which may affect adversely the health status and economy trade.

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