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Aflatoxin A Contaminant In Poultry Feed: Sources, Effect And Remediation For Optimum Poultry Production.

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ABSTRACT
This study aims at detecting the presence and level of aflatoxins in super starter feed and raw ingredients in poultry feed formulation with specifics on maize, groundnut cake and soya bean cake using ELISA method. A total of eight (8) samples: one sample of maize, one sample of soya bean cake, one sample of groundnut cake and one sample of poultry feed from FM 1 and FM 2 respectively now formed composite samples for analysis. The parameters were analyzed using one-way Analysis of Variance (ANOVA) implemented in R car (version 3.0-2) package to test the effect of feed materials on nutritional profile of feed. Significant differences were separated using Tukey test ($\alpha = 0.05$) for multiple comparisons through R lsmeans (version 2.30-0) and R multcomp (version 1.4-10) packages. Pearson correlations was used to test the relationship between total aflatoxin and proximate composition. There were significant ($P<0.05$) variations in the moisture content, crude protein and crude fibre of feed ingredients. The lipids, ash, NFE and pH were similar ($P>0.05$) across the dietary groups. SBC had significantly ($p<0.05$) highest concentration of total aflatoxin (37.3µg/kg), followed by GNC (33.8µg/kg), SS (20.3µg/kg) and maize (3.46 µg/kg). SBC had the highest contamination factor (9.32) while SS had the least (4.06). Total aflatoxin had positive, high and significant relationship with crude protein ($r=0.48$), crude fibre ($r=0.925$) and lipids ($r=0.631$). Total aflatoxin had negative, high and significant relationship with moisture, NFE and pH. The total aflatoxin level in GNC, soyabean and super starter is higher than 20µg/kg FDA acceptable level for poultry feed while that of maize was found to be within the range 4µg/kg of FDA acceptable level for maize. Aflatoxin management of groundnut cake, maize, and soyabean in this study is critically needed to attain food security and food safety in animal feed industry.
Keywords: *Aflatoxin, Contaminant, Poultry,Feed, Remediation,Production.*

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I. INTRODUCTION

Aflatoxin are groups of naturally occurring mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus Parasiticus* species of fungi that typically affect corn, soybean and groundnuts, which are ingredients that are used in both food and feed products (Williams *et al.*, 2004). Cereals and its by products have been widely reported to be prone to contamination by potentially toxigenic fungi. However, incidences of aflatoxins contamination of cereals and associated food products are more prevalent in developing countries such as Africa compared to Europe (EFSA, 2013). Aflatoxins are toxic chemical compounds produced in foods and food products by *Aspergillus flavus* and *Aspergillus parasiticus*. These mycotoxins from different research have been shown to induce both genotoxic and carcinogenic effects in humans (EFSA, 2007).

At least 15 different aflatoxins are produced in nature. Aflatoxin B1 is seen as the most toxic and is made by both *Aspergillusparasiticus* and *Aspergillus flavus*. Aflatoxin M1 is existent in the fermentation broth of *A. parasiticus*; it and aflatoxin M2 are also made when an infested liver metabolizes aflatoxin B1 and B2.
Aflatoxin B1 & B2, made by *A. flavus* &*A. parasiticus.*

- Aflatoxin G1 & G2, made by some Group II *A. flavus* &*A. parasiticus* (Geiser *et al.*, 2000).
- Aflatoxin M1, metabolic products of aflatoxin B1 in humans & animals (exposure in ng levels can come from a mum's milk)
- Aflatoxin M2, metabolic products of aflatoxin B2 in milk of cattle fed on polluted foods.
- Aflatoxicol
- Aflatoxin Q1 (AFQ1), main metabolic products of AFB1 in *in vitro* liver preparations of other greater vertebrates (Smith and Sivewright, 1991).

Aflatoxins are produced by both *A. flavus* and *A. parasiticus*, common forms of 'weedy' molds extensive in nature. The incidences of those molds do not always show that dangerous levels of aflatoxin are existent but does show a momentous risk. The molds can inhabit and infect foodstuff before harvest or throughout storage, principally following protracted exposure to high-humidity surroundings, or too stressful environments such as drought. The native habitat of *Aspergillus* is in the soil, decaying vegetation, hay, and a grain undergoing fungal deterioration, but it occupies all kinds of organic substrates when conditions are satisfactory for its progression. Favorable environments include high moisture (at least 7 percent) and high temperature.

Aflatoxins have been sequestered from all main cereal crops, and sources as varied as cannabis and peanut butter. The chief commodities regularly contaminated with aflatoxins include cassava, chilies, peanuts, rice, sorghum, sunflower, corn, cottonseed, millet, seeds, wheat, tree nuts, and a variety of spices anticipated for animal or human consumption. Aflatoxin makeover products are occasionally found in eggs, dairy products, and meat when animals are given contaminated grains (Fratamico *et al.,* 2008).

flavus and *A. parasiticus* grow very well at 28–30˚C and 25–35˚C, when conidia (spores) encounter a suitable nutrient source and favorable environmental conditions (hot and dry) the fungus rapidly colonizes and successfully produces aflatoxins. Its presence is enhanced by factors such as stress or damage to the crop due to drought before harvest, insect activity, soil type and inadequate storage conditions (Verma *et al.,* 2004). The occurrence of aflatoxins is common in wide varieties of food and feeds.

Aflatoxin toxicity is related to biochemistry, hematology, reproduction and poultry pathological changes (Ortatatli and Oguz, 2001).Previous research indicated that the reduced growth rate because of AF ingestion in the diet is usually due to the reduction in feed intake. If the feed is contaminated by multiple mycotoxins at the same time, AF can interact with other mycotoxins, such as ochratoxin A and T-2 toxin, to produce more severe effects on broiler performance than individual mycotoxins.

Andretta *et al*. (2011) concluded that an average AF concentration of 950ppb reduced both feed intake and daily weight gain by 11 percent, and worsened feed conversion by 6 percent.

According to (Ariyo, *et al.,* 2011) aflatoxins are the most toxic causing considerable economic losses to poultry industry and health problems due to the frequent contamination of feeds.Broilers are more susceptible to aflatoxin than layers (Rodrigues *et al.,* 2011). The negative effect of aflatoxin to birds is most significant in production aspects, such as weight gain, feed consumption, feed conversion ratio (FCR) andharvest (Hussain *et al.*, 2010).

In chickens, the effects of aflatoxins include liver damage, impaired productivity and reproductive efficiency, decreased egg production, inferior eggshell quality, inferior carcass quality and increased susceptibility to disease (Kamalavenkatesh *et al.,* 2005).

The extreme sensitivity of poultry species to AF is associated with their livers converting efficiently AF to the metabolically active *exo*-AFB1-8, 9-epoxyde (AFBO).

AF acts as an inhibitor of protein synthesis and, subsequently, dividing cells and tissues with a high protein turnover such as that found in the liver, immune system or gut epithelium, which is most susceptible to the toxic effects of AF. In this respect, exposure to AF has been demonstrated to suppress the immune response in poultry. AF can repress the development of the thymus gland or influence the relative weight of the bursa of Fabricius, which may result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system (Hussain *et al.*, 2010).

There are three reasons for having standards for the maximum amount of aflatoxin in feeds:

(1) To protect human health from possible harmful metabolites in animal products.
(2) To protect livestock from potential negative health and production impacts of aflatoxin.
(3) To protect the environment from contamination.

II. MATERIALS AND METHOD

2.1 Materials: AgraQuant® Total Aflatoxin Assay 4/40 Kit, Order #: COKAQ1048 purchased from ROMER Labs Singapore Pte. Ltd was used for the aflatoxin analysis. It contained:48 antibodies coated microwells (6 eight-well strips) in a microwell holder,48 non-coated dilution microwells (6 eight-well strips marked with blue/green at base),5 vials of 0.75mL of each aflatoxin standard (0, 4, 10, 20 and 40 ppb) ,1 bottle of 12.5mL of aflatoxin conjugate (green-capped bottle),1 bottle of 7.5mL of substrate solution (blue-capped bottle), 1 bottle of 0.75mL of stop solution (red-capped bottle).ELISA reader TECAN infinite F50 (Grödig, Austria),Ohaus® Explorer® Weighing balance,Graduated cylinder: 100mL.70% methanol (ACS grade) Distilled water84% acetonitrile, Whatman No.1 filter paper, Filter funnel,8-channel and single channel pipettes, Wash bottle, Absorbent paper towels, 3 reagent boats for 8-channel pipettes, Porcelain mortar and pestle glass rod., NaOH (Grade:99%Laboratory Reagent;Company: Molychem), Test Tube, Petri dish, Crucibles, 4% Boric Acid, 0.1N HCl, H_2SO_4 (Grade: 98%Laboratory reagent; Company: Loba Chemie), $CuSO_4$ (Grade:99.0%Analytical Reagent; Company:Guangdong Guanghua Sci-Tech Company Ltd), Macro

Kjeldahl digestion and distillation units, Kjeldahl flask (100cm^3 capacity), Conical flask (250 cm^3 capacity), Heating mantle, K_2SO_4 (99.0% Analytical grade ; Company: Loba Chemie PVT LTD), n-hexane (Grade: Laboratory reagent, Company:Molychem), Petroleum ether: (Grade: Laboratory Reagent , Company: Loba Chemie), Trichloroacetic acid, Glacial acetic acid, Nitric acid, Extraction thimbles, Round bottom flask, Soxhlet extraction apparatus, Regulated water bath, Dessicator, Glass Funnel, Drying Oven, Furnace.

2.2 Sample Collection and Treatment

2.3 Sample Collection

Samples of maize, soya bean cake, groundnut cake and the compounded poultry feeds were collected randomly at feed manufacturing plants in Jos, Plateau State and Kaduna in Kaduna State. Samples of raw materials and poultry feeds collected in manufacturing plant in Jos was designated as FM 1 while that collected from manufacturing plant in Kaduna was designated as FM 2.All samples were grounded to powder using electric blender and sieve through a mesh of size 20 prior to analysis. Eighty (80g) of each sample of raw materials were collected from mixture of 50g from 100 bags each of respective raw materials used in poultry feed production while 80g of poultry feed sample were collected from mixture of 50g from 100 bags each of poultry feed.
A total of eight (8) samples: one sample of maize, one sample of soya bean cake, one sample of groundnut cake and one sample of poultry feed from FM 1 and FM 2 respectively now formed composite samples for analysis.

2.4 Determination of pH of Maize, Soya bean cake, Groundnut cake and Feeds from Jos and Kaduna

The pH of the samples was determined using highly sensitive digital pH meter (Montini 095, Romania). Five grams (5 g) of each sample was weighed and transferred to a clean beaker and 50 ml of distilled water was added to form a slurry. A standard buffer solution (pH 6.0) was prepared and was used to standardize the pH meter. The electrode of the digital pH meter was dipped in the slurry at a temperature of about 25°C. The pH readings were recorded.

2.5 Determination of the Moisture Content of Maize, Soya Bean Cake, Groundnut Cake and Feeds from Jos and Kaduna

As recommended by Association of Official Analytical Chemist (AOAC, 2005), 5g
each of the samples of maize, soya bean cake and groundnut cake were weighed up into a petri dish of a known weight and then dried up in the oven at 105.5 ± 1.5^{o}C for four (4) hours. The
samples were allowed to cool in a desiccator and weighed. The percent moisture composition of the samples was calculated as follows:

$$\text{Moisture}(\%) = \frac{(W_2-W_3)}{W_2-W_1} \times \frac{100}{1}$$

Where W_1= weight of the empty petri dish; W_2=weight of the dish and sample before drying; W_3=weight of the dish and sample after drying to a constant weight.

2.6 Determination of Crude protein content of Maize, Soya bean cake, Groundnut cake and Feeds from Jos and Kaduna by Kjeldahl method

The Kjeldahl method was performedusing standard methods prescribed by Association of official analytical chemists (AOAC,2000). Exactly 0.5 g (for cake and Maize) and 1g (for feed) of sample was weighed in different crucibles ,0.8g $CuSO_4$ salt was added to each, and the contents were transfer into a digestion tube, where 12cm^3 of concentrated H_2SO_4 was added into the digestion tube, the digestion tube was then transferred into a heating mantle and heated for about 45 minutes at 420°C until a clear digest was obtained. The tube was removed from the heating mantle and kept in a tube rack to cool. 75 cm^3 of distilled water was added to the solution digested and allowed to stand for 5 minutes before taking it to the distillation unit. 25cm^3 of 4% Boric acid was measured into a conical flask, the receiving tube was inserted inside the flask containing the Boric at the receiving end of the distillation unit, the distillation was then initiated by discharging NaOH and steam. The flask was removed when the distillation stopped for titration, 0.10 N HCl was titrated against the solution and the titre value was recorded.

$$\text{Protein}(\%) = \frac{(A-B)\times N\times 1.4007\times 6.25}{W}$$

Where A =volume(ml)of0.1NHClusedsampletitration

B =volume(ml)of0.1NHClusedinblanktitration

N =NormalityofHCl

W =weight(g)ofsample

14.00 =atomicweightofnitrogen

6.25 =theprotein-nitrogenconversationfactor.

2.7 Determination of Ash content of Maize, Soya bean cake, Groundnut cake and Feeds from Jos and Kaduna

The crucible and lid were placed in the furnace at 550°C overnight to ensure that impurities of the surface of the crucible are burned off, the crucible was then cooled in the desiccator for 30 minutes, the weight of the crucible and lid was taken to 3 decimal places using Ohaus® Explorer® Weighing balance. Exactly 5g of sample Maize, Soya bean cake, Groundnut cake and Feeds was weighed into the crucible and heated over low Bunsen flame with lid half covered. When fumes were no longer produced, the crucible and lid was placed in the furnace and heated at 550°C overnight for 7 hours and then cooled in the desiccator after heating. The ash with the crucible and lid was weighed when the sample turned into gray

$$\text{Ash}(\%) = \frac{\text{Weightofash}}{\text{Weight of sample}} \times 100$$

2.8 Sample Extraction for total aflatoxin content of Maize, Soya bean cake, Groundnut cake and Feeds from Jos and Kaduna

Sample extraction was done as described by (Hussain *et al.*, 2010). Briefly, samples were individedly ground using Christy and Norris laboratory mill so that 75% would pass through a 20-mesh screen. One hundred ml of methanol/water (70/30) was added to 20g of each ground sample and was shaken for 30 minutes. Sample was allowed to settle and the supernatant was filtered through a whatchman no. 1 filter paper. The filtrate was collected for further analysis'

2.9 Total Aflatoxin Determination of Maize, Soya bean cake, Groundnut cake and Feeds from Jos and Kaduna

Detection of total aflatoxins in the samples was done by Enzyme Link Immunosorbent Assay (ELISA) method as described by Murshed *et al.,* (2019). Two hundred μL of enzyme conjugate w1as dispensed into each green-bordered dilution well and 100 μl of each standard (0, 4, 10, 20 and 40 ppb)/ sample (in duplicate) were added into the appropriate dilution well containing the 200 μl of conjugate. Each well was carefully mixed by pipetting it up and down three times and 100 μl of the contents from the dilution well was transferred into the antibody-coated well to initiate the reaction. This was then incubated for 15 min at room temperature (20–25°C) for reaction to take place. After incubation for 15 min at room temperature (20–25°C), the contents of the wells were discarded and the wells were washed four times to remove any unbound toxin. One hundred microliters of substrate (Chromogen) were added to each well and mixed gently by shaking the plate manually. Following 5 min incubation at room temperature in the dark, the reaction was stopped by adding 100 μl of stop solution into each well, and the colour changes from blue to yellow.

Finally, absorbance was measured at 450nm by the ELISA reader within 30 min after the addition of stop solution was added.

III. RESULTS AND DISCUSSION

PROXIMATE COMPOSITION OF FEED MATERIALS

Table 1: shows the proximate composition of feed ingredients and super starter feed. There were significant ($P<0.05$) variations in the moisture content, crude protein and crude fibre of feed ingredients. The lipids, ash, NFE and pH were similar ($P>0.05$) across the dietary groups. Soyabean cake significantly ($P<0.05$) had the highest crude fibre content followed by GNC, super starter while maize recorded the least. The C.P content of SBC and SS were significantly highest, followed by GNC while the least was maize.

Table 1:

Proximate and pH composition of feed ingredients and super starter feed

Parameters (%)	GNC	Maize	SBC	SS	P values
Moisture	6.1 ± 0.14^{c}	8.6 ± 0.21^{a}	2.2 ± 0.24^{d}	7.1 ± 0.04^{b}	0.001*
Crude Protein	28.7 ± 15.7^{b}	7.9 ± 0.4^{b}	30.2 ± 14.1^{a}	30.7 ± 18.6^{a}	0.04*

Crude fibre	13.9±2.3[b]	2.9±1.9[d]	16.6±0.9[a]	9.8±0.6[c]	0.004*
Lipids	12.4±8.2	4.5±0.5	10.6±1.6	7.1±0.2	0.35
Ash	5.8±3.1	7.5±3.1	6.4±3.1	9.6±3.1	0.83
NFE	33±21.2	68.6±6.5	33.9±15.5	35.7±17.6	0.22
pH	6.4±0.08	6.4±0.08	6.3±0.08	6.4±0.08	0.20

NFE-Nitrogen free extract; GNC-Groundnut cake; SBC-Soyabeancake; SS-Super starter; [abcd]means differs significantly across the column. P<0.05-significant different.

TOTAL AFLATOXIN AND CONTAMINATION FACTOR OF FEED MATERIALS

The total aflatoxin and contamination factor in groundnut, maize, SBC and super starter samples are shown in Table 2. SBC had significantly (p<0.05) highest concentration of total aflatoxin (37.3 µg/kg), followed by GNC (33.8 µg/kg), SS (20.3 µg/kg) and maize (3.46 µg/kg). SBC had the highest contamination factor (9.32) while SS had the least (4.06). The range of total aflatoxin concentration was highest in SBC (30.57-43.96 µg/kg), GNC (33.78-33.90 µg/kg), SS (20.33-20.35µg/kg) and maize (3.19-3.73).

Table 2:

Total aflatoxin and contamination factor in groundnut cake, maize, SBC and super starter samples.

	Total aflatoxin(µg/kg)	Samples above Regulatory Limit of 20 µg/kg for S.S, Gnc, Sbc and 4µg/kg for maize		Contamination factor
Feed materials	Range	Mean±S.D		Mean
GNC	33.78-33.9	33.8±0.17b	2	8.46b
Maize	3.19-3.73	3.46±0.38d	0	5.08c
SBC	30.57-43.96	37.3±9.47a	2	9.32a
SS	20.33-20.35	20.3±0.01c	2	4.06d

GNC-Groundnut cake; SBC-Soyabeancake; SS-Super starter; [abcd]means differs significantly across the column. P<0.05-significant different; S.D-Standard deviation.

CORRELATION BETWEEN TOTAL AFLATOXIN AND PROXIMATE COMPOSITION OF FEED MATERIALS

Table 3 shows the correlation between total aflatoxin and proximate composition of feed materials. Total aflatoxin had positive, high and significant relationship crude protein (r=0.48), crude fibre (r=0.925) and lipids (r=0.631). Total aflatoxin had negative, high and significant relationship with moisture, NFE and pH. The highest correlation was between total aflatoxin and crude fibre (r=0.925) while the highest negative relationship was between crude protein and NFE (r=-0.967).

Parameters	Total aflatoxin	Moisture	Crude Protein	Crude fibre	Lipids	Ash	NFE	pH
Total aflatoxin	1.0000							
Moisture	-0.8330	1.0000						
Crude Protein	0.4888	-0.4263	1.0000					
Crude fibre	0.9250	-0.8601	0.5282	1.0000				
Lipids	0.6314	-0.4713	0.6782	0.5379	1.0000			
Ash	-0.1618	0.1543	-0.0810	-0.3025	-0.2139	1.0000		
NFE	-0.6268	0.5064	-0.9675	-0.6265	-0.7772	-0.0088	1.0000	
pH	-0.7425	0.6226	-0.1587	-0.8141	-0.1786	-0.0339	0.3130	1.0000

Table 4 shows the correlation between contamination factor and proximate composition of feed materials. Contamination factor had positive, high and significant relationship with crude fibre (r=0.825) and lipids (r=0.623). Contamination factor had negative, high and significant relationship with moisture, ash, NFE and pH. The highest correlation was between contamination factor and crude fibre (r=0.825) while the highest negative relationship was between crude protein and NFE (r=-0.967).

Table 4: Correlation between Contamination factor and proximate composition in groundnut, maize, SBC and super starter

Parameters	Contamination factor	Moisture	Crude Protein	Crude fibre	Lipids	Ash	NFE	pH
Contamination factor	1.0000							
Moisture	-0.8188	1.0000						
Crude Protein	0.3274	-0.4263	1.0000					
Crude fibre	0.8258	-0.8601	0.5282	1.0000				
Lipids	0.6239	-0.4713	0.6782	0.5379	1.0000			
Ash	-0.4603	0.1543	-0.0810	-0.3025	-0.2139	1.0000		
NFE	-0.4261	0.5064	-0.9675	-0.6265	-0.7772	-0.0088	1.0000	
pH	-0.4774	0.6226	-0.1587	-0.8141	-0.1786	-0.0339	0.3130	1.0000

IV. DISCUSSION

Determination of proximate compositions of energy and protein source will go a long way in providing substantive nutritional information on livestock diets, for effective guide on animal dietetics and optimization of animal health. Ash contents represent the presence of total amount of minerals of a specified material (Olagunju *et al.,* 2013). Minerals are inorganic substances which are required to maintain the physicochemical characteristics of living beings. Although, they do not produce energy but are important in the performance of many processes within the body (Soetan *et al.,* 2010).

Protein plays a crucial role in feed formulation. The high protein value in SBC and GNC is required to meet nutrients requirement of chicken of different categories (Bhatti *et al.,* 2002). The low CP value of maize is connected to the fact that maize is an energy source. Dietary crude protein (CP) requirement is based on the amino acid content of the protein. Amino acids are used as the building blocks of structural proteins (skin, ligaments and muscles), enzymes, metabolic proteins and precursors of several body component. Fat is thought to be an economical and practical source of energy in poultry feed. The adding fat to diets not only supply energy but also increases the absorption of fat-soluble vitamins, improves the palatability of the feed, lowers the pulverulence and improves the efficacy of the consumed energy. The lipid content in raw ingredients and super starter feed are within the reported range in the literatures (Soetan *et al.,* 2010).

The moisture content of maize in the current study is slightly lower than the earlier research on maize/maize products. Typically, Samir *et al.* (1998) reported moisture content of maize as 9 %. The slight variation in maize (8.6%) in this study may be attributed to the maize variety used, environmental factors and agronomic practices. These lend credence to the assumption that lower moisture content is important as it enables long storage by minimizing fungal contamination and spoilage of the maize/maize products. Maize bran is an important source of protein supplement and energy for ruminant (Ghol, 1981). The percentage ash content (5.8-9.6%) was within the range reported in the literature by some researchers. Samir *et al.* (1998 reported ash content of maize in the range of 1.4 – 3.3% which was lower than 7.5% reported for maize in this study. May *et al.* (2005) reported ash content of maize/bran as 5.1%. The percentage crude protein of maize (7.9%) in the current study was found closely related to those reported on different maize varieties in Nigeria. Notably, Ujabadenyi and Adebolu (2005) reported protein of three maize varieties growth in Nigeria within the range of 10.67 –11.25. The protein content of maize can be improved through technological processes by moving gene responsible for protein synthesis from the ribosomal DNA of high protein plant. The percentage fat obtained for maize in this study was consistent and in agreement with other researchers (Matida *et al.,* 1993; Ikenie *et al.,* 2002) but slightly differs from the findings of Ujabadenyi and Adebolu, (2005) that found higher fat content of 5.0%.

Animal feed is at the beginning of the food chain, and any in-feed contaminants may reach the final consumer through food matrixes, such as eggs or meat products. However, the worldwide occurrence of aflatoxins in agricultural products is well documented, with the major contamination occurring in countries with high temperature and humidity. While it is generally recognized globally that there is no safe level of aflatoxin exposure, the regulatory bodies, including the Standards Organization of Nigeria (SON) sets standards on many food commodities, taking into account global standards as well as national production and target export markets. Animal feed derived from aflatoxin prone crops are also susceptible to aflatoxin contamination. In this study, all the samples were contaminated at levels ranging from 3.46-33.8 µg/kg. In consonance with our study, reported an aflatoMatida *et al.,*(1993)in contamination of South African animal feeds at the levels of >20µg/kg. These high levels of aflatoxin might be due to the grains and other ingredients contents that may harbour these toxins with high levels. Nigeria has no regulatory standards for maximum aflatoxin concentrations for animal feed

(Hussaini *et al.,* 2012). But the level of >20.0µg/kg is within the EEC maximum permitted level of aflatoxin for poultry feed of 30-40µg/kg which corroborates the findings in our study.

The mean contamination level of groundnut cake is 33.8 µg/kg, although the level of aflatoxin in groundnut was within the range reported elsewhere (Matida *et al.,* 1993) in Nigeria who reported an aflatoxin levels in Homemade and unrefined groundnut oil ranging between 20-2000µg/kg and (Matida *et al.,* 1993), who reported an aflatoxin contamination in Nigerian groundnut cake at levels ranging between 20-455µg/kg. The contamination in our study generally exceeded the national mean of 16.4 ppb in groundnut from Plateau (in Mid- Altitude zone), Katsina (in Southern Sudan Savannah) and Sokoto (in Sahel Savannah zone). Aflatoxin levels groundnut cake samples were above the EU limit of 4 µg/kg and US limit of 20 µg/kg. The difference between results obtained in our study and that of the previous investigations might be due the difference in geographical location, seasonal variations, weather conditions of the study areas, and also the method or technique used. The reason why the incidence of aflatoxin is more frequent in groundnut than in other agricultural commodities is not fully understood. Soya bean cake serves as animal feeds and its oil is used as a substitute of groundnut oil due to its scarcity and expensiveness. The mean aflatoxin level of soya bean samples is 37.3 µg/kg in this study which was were above the EU limit of 4 µg/kg and US limit of 20 µg/kg.

The significant positive high correlation between protein source and total aflatoxin in the feedstuff implies that protein synthesis is affected by aflatoxins in food grains by inhibiting the inclusion of amino acids into protein and resulting in nongermination of embryo. This also implies that as the concentration of total aflatoxin is increased there is a corresponding increase in the concentration of crude protein. Aboloma *et al.* (2012) postulated that this increase in protein contents might be due to the proliferation of microorganisms which assimilate the protein in the synthesis of new protoplasm thereby reducing its protein contents. However, Adaku and Chinyerum (2012) reported that significant increase in protein contents of *Dialium guineense* occured by the inoculation with *A. flavus*, though this was similar with the pattern obtained for maize in this study. Our results of positive association between total aflatoxin and fat content contradict the previous study of Ali *et al.* (2009) who reported that the almond samples inoculated by *A. flavus* produced the aflatoxins and significantly caused reduction of crude fat.

Our results further contradicted the work of Akande *et al.,* (2006) who reported that the fat contents showed reduction by 62.5% in stored maize when the good corns were compared with moldy corns. As lipases are present in *A. flavus* which can breakdown the fat for the uptake of nutrients (Tripathi and Mishra, 2009). also found appreciable. Aboloma *et al.* (2012) change in the lipids and free fatty acids of groundnut cake as contaminated with *A. flavus* and *A. parasiticus*. The increase in fat content of raw ingredients for poultry feed in our study might be due to the lipolytic activity of the fungi as reported by Aboloma *et al.* (2012) Favorable temperature and water activity which is an intrinsic parameter for the moisture content are crucial for mycotoxigenic fungi and mycotoxin production.The climatic conditions in tropical region are favorable for fungal development with high relative humidity, high temperature and moisture content and little aeration, all conditions that accelerate fungal and mycotoxin development. The significant and negative correlations between aflatoxin and moisture content implies that moisture content are crucial for mycotoxigenic fungi and mycotoxin production which negatively affected the quality of the feed ingredient and animal feed.

V. CONCLUSION

Aflatoxins are the major class of mycotoxins which are toxic secondary metabolic products made by microorganisms of the fungus kingdom, generally known as molds. Aflatoxins are seen in grains before, during, and after harvest. The aflatoxins levels associated with the grain were within the When improperly processed, consumption of these feed ingredient and feeds may expose the poultry birds as well as individuals to the risk of aflatoxicosis, liver cancer, urinary tract cancer, and kidney damage.

Results showed that the presence of aflatoxins have effects on the nutritional properties of samples at various levels of significance.

The total aflatoxin level in GNC, soyabean and super starter is higher than 20µg/kg FDA acceptable level for poultry.

There was a significant high negative correlation between total aflatoxin and moisture, NFE, pH and ash in feed material, thus suggest that these candidate markers could promote the growth of total aflatoxin in animal feed raw materials.

permissible limit while that of sbc, gnc and super starter were above.

REFERENCES

[1]. Aboloma, R. I., Onifade, A. K. and Adetuyi, F. C. (2012). Effect of deterioration on the proximate composition of some fruits of the family Cucurbitaceae. Journal of Microbiology and Biotechnology Research. 2(1): 240-243.

[2]. Adaku, N. I. and Chinyerum, I. N. (2012). Biochemical changes induced by the effect of six pathogenic fungi on Dialium Guineense: Black velvet edible fruit. Journal of Pharmacy and Biological Sciences. 2(4): 20-24.

[3]. Akande, K. E., Abubakar, M. M. Adegbola, T. A. and Bogoro, S. E. (2006). Nutritional and health implications of mycotoxins in animal feeds: a review; Pakistan Journal of Nutrition. 5(5): 398-403.

[4]. Ali, E., Saleemullah, F. Anjum, B. Khan, A. and Zubair, A. (2009). Aflatoxin contamination and mineral profile of almond seeds. Mycopathology. 7(1): 39-44

[5]. Andretta, I., Kipper, M., Lehnen, C. R., Hauschild, L., Vale, M. M. & Lovatto, P. A. (2011). Meta-analytical study of productive and nutritional interactions of mycotoxins in broilers. Poultry Science, 90, 1934-1940.

[6]. Ariyo A.l., Anthony, M.H. and Lami, M.H. (2013). Survey of mycotoxigenic fungi in concentrated poultry feed in Niger State, Nigeria. Journal feed in Niger State, Nigeria. Journal of Food Research, 2(2):128-135

[7]. Bhatti, B.M. (2005). Poultry development Centre Rawalpindi., Pakistan

[8]. European Food Safety Authority (EFSA) (2013). Aflatoxins (sum of B1, B2, g1, g2) in cereals and cereals-derived food products. EFSA supporting publications 10(3) DOI:10.2903/sp.efsa.2013.EN-406

[9]. European Food Safety Authority (EFSA). (2007). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. The EFSA Journal, 446, 1-127.

[10]. Fratamico PM, Bhunia KA, and Smith LJ (2008). Foodborne Pathogens: Microbiology and Molecular Biology. Norofolk, UK: Horizon Scientific Press. ISBN 978-1-898486-52-7.

[11]. Geiser, D. M., Dorner, J. W., Horn, B. W., & Taylor, J. W. (2000). The phylogenetics of mycotoxin and sclerotium production in Aspergillus flavus and Aspergillus oryzae. Fungal genetics and biology. Fungal Genetics and Biology, 31(3), 169–179. https://doi.org/10.1006/fgbi.2000.1215

[12]. Ghol B (1981). Tropical feeds. feed information and nutritive values. FAO, Rome pp

[13]. 529.

[14]. Hussain Z, Khan MZ, Khan A, Javed I, Saleemi MK, Mahmood S, et al. Residues of aflatoxin B1 in broiler meat: effect of age and dietary aflatoxin B1 levels. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc. 2010;48(12):3304–3307

[15]. Hussain, I., J. Anwar, M. A. Munawar and M. R. Asi. (2008). Variation of levels of aflatoxin M1 in raw milk from different localities in the central areas of Punjab, Pakistan. Food Control. 19(12): 1126-1129

[16]. Hussein HS, Brasel JM. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology. 167(2):101–134.

[17]. Hussein, H.S., & Brasel J.M. (2001). Toxicity, metabolism, & impact of mycotoxins on humans & animals. Toxicol. 167 (2): 101–34.

[18]. Ikenie, J.E, Amusan, N.A and Obtaolu, V.O. (2002). Nutrient composition and weight evaluation of some newly developed maize varieties in Nigeria. J. Food Techn. in Africa. Vol. 7; Pp 27-29.

[19]. Kamalavenkatesh, P., Vairamuthu, S., Balachandran, C., Muralimanohar,B. and Dhinakarray, G. (2005). Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. Mycopathologia,159:273-279.

[20]. Matilda Asiedu, Rune Nilsen, Oyvind Lie, Einar L. (1993). Effect of Processing (Sprouting and or Fermentation) on Sorghum and Maize.1: Proximate Composition, Minerals and Fatty Acids. Food chemistry. 46:351-353.

[21]. May PS, Pereka AE, Balthazary ST, Phiri EJ, Hvelplund T, W eisbjerg MR, Madsen J. (2005). The Effect of Maize bran or Maize Bran mixed with sunflower cake on the performance of small holder diary cows in urban and semi-urban area in Morogoro, Tanzania.

[22]. Olagunju, A., Onyike, E. Muhammad, A. Aliyu, S. and Abdullah, A. S. (2013). Effects of fungal (Lachnocladium spp.) pretreatmenton nutrient and antinutrient composition of corn cobs. African Journal of Biochemistry Research. Vol. 7(11), pp. 210-214, December, 2013, DOI: 10.5897/AJBR2013.0715.

[23]. Ortatatli, M., Oguz, H., 2001. Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. Research in Veterinary Science 71, 59–66.

[24]. Rodrigues, I.; Handl, J.; Binder, E.M. Mycotoxin occurrence in commodities, feeds and feed ingredients sourced in Middle East and Africa. Food Addit. Contam. B Surveill. 2011, 4, 168–179.

[25]. Samir, T, Kraszaewsk, A. W, Nelson, S.O. (1998). Non destructive microwave Characterizationfor Determining the bulk density and moisture Contents of Shelled Gru. Sci. Technol.,9:1548-1556.

[26]. Smith JE and Sivewright-Henderson R (1991). Mycotoxins and animal foods. CRC Press. p. 614. ISBN978-0-8493-4904-1.

[27]. Soetan, K.O., Olaiya, C.O. and Oyewole, O.E.(2010). The importance of mineralelements for humans, domestic animalsand plants: A review. African Journal ofFood Science, 4(5): 200-222

[28]. Tripathi, S. and Mishra, H. N. (2009). Nutritional changes in powdered red pepper upon invitro infection of Aspergillus flavus. Brazilian Journal of Microbiology. 40(1): 139144

[29]. Ujabadeniyi, A.O and Adebolu, J.T. (2005). The effect of processingmethod on nutritional properties of ogi produced from three maize varieties.J. Food,Agric and Environment.3:108-109.

[30]. Verma, J., Johri, T. S., Swain, B. K. & Ameena, S. 2004. "Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers." British Poultry Science, 45, 512- 518.

[31]. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D, (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 80: 1106-1122.