

Full Length Research Paper

Mycological quality of commercially prepared and self compounded poultry feeds in Sokoto Metropolis, Sokoto, Nigeria

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Evidences arising from epidemiological studies as well as from detailed experimental investigations have indicated that there is strong relationship between certain feed ingredients and incidence of fungal infections. Over a 12 month period, a total of two hundred and thirty nine poultry feed samples comprising of two hundred and four commercially prepared and thirty five self compounded feeds were collected from seventy six identified poultry farms in Sokoto metropolis were assessed for their mycological quality. A total of 198 (82.85%) of the samples yielded positive growth for at least one fungal organism which comprises of 163 (79.90%) commercially prepared and 35 (100%) self compounded feeds. The fungi comprises of *Aspergillus* sp. 136 (49.10%), *Penicillium* sp. 51 (18.41%), *Rhizopus* sp. 37 (13.36%), *Fusarium* sp. 21 (7.58%) and *Mucor* sp. 32 (11.55%). The frequency of isolation was observed to be highest in rainy season in which out of total 239, 148 (63.52%) fungal isolates were yielded. The presence of the aforementioned fungi in all the feed samples calls for attention in the storage methods employed by the poultry farmers, livestock feed manufacturers, distributors and the retailers.

Key words: Poultry feeds, mycological quality, fungi, Sokoto.

INTRODUCTION

In Nigeria, chickens are the most important of the poultry species in terms of number and development. The exotic breeds are managed intensively using either battery cages or deep litter system of management, while the local breeds are managed extensively and are allowed to scavenge food for survival. The major constraints in raising these chickens include shortage and cost of feeds and the substantial economic losses due to different diseases of which viral infections account for the highest percentage of the mortality in chickens because of their contagious nature (Adeboyega, 1999).

Animal feed may serve as a carrier for a wide variety of microorganisms including pathogenic fungal species (Beuchat, 1978). Some of these microorganisms are adapted to the desiccated and relatively nutrient-deficient conditions of the soil and survive in similar niches on growing crops (Beuchat, 1978). Gastrointestinal pathogens can be introduced into the food chain by animals defecating in the farm environment or by fertilization of crops with manures; also microorganisms are introduced during storage (Maciorowski et al., 2006). In general, the amount of available water in the feed matrix determines whether a microorganism will grow or survive. Some microorganisms, primarily moulds, are adapted to the low amount of available moisture and grow actively within stored seeds and grains. Others will produce spores or enter survival state until the moisture

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is high enough for bacterial action (Maciorowski et al., 2006).

There are numerous ways by which microorganisms can affect feed quality negatively including reducing dry matter and nutrients, causing musty or sour odours, causing caking of the feed and most importantly producing mycotoxins. Feed can also act as a carrier for animal and human pathogens. The type of feed, processing treatments and storage conditions can all be factors that influence the population levels and types of microorganisms present (Maciorowski et al., 2006).

Regular monitoring of toxigenic mycoflora of the agricultural based feeds is a pre-requisite for designing strategies to control and prevent exposure of animal and humans to mycotoxins. Study of prevalence of toxigenic mycobiota of poultry feeds is regularly and frequently reported from many countries (Dalcero et al., 1997, Accensi et al., 2004 and Krnjaja et al., 2008, 2010). In Nigeria However, Osho et al. (2007) reported the isolation of *Rhizopus* and *Aspergillus Niger* in poultry feed in south western region of Nigeria. He reported that *Rhizopus* sp. has the highest frequency of isolation (44%) and *Aspergillus Niger* the lowest (38%). In another survey of fungal contamination in commercial poultry feed in southern state of Nigeria by Oyediji (1986), 57 to 62% of the chick and broilers starter and finishers feed ratios were found to be contaminated with Aflatoxin. Other reported studies on isolation of pathogenic fungi genera in poultry feed samples in Nigeria include that of Uwaezuoke and Ogbule (2008) in Owerri, Imo state and Obi and Ozugbo (2007) in Umuahia, Abia state.

The involvement of poultry feeds in the transmission of fungal agents of Aflotoxicosis which is the most prevalent and economically significant mycotoxin is of great health concern to the poultry farmers and the extended consumers respectively.

The production of poultry feeds for local and commercial utilization in the developing countries including Nigeria requires microbiological methods that will reduce microbial contamination of the product (Obi and Ozugbo, 2007). Thus the present study is to investigate the mycological contamination of poultry feeds used in poultry establishments in Sokoto metropolis.

MATERIALS AND METHODS

Study area

The study area is Sokoto metropolis in Sokoto state. Sokoto is the capital of Sokoto State, located at latitude 13°N and between longitude 30°E and 90°E in the North Western part of Nigeria the extreme Northwest of Nigeria. It lies roughly between longitude 30°E and 15°E of Greenwich and between 4°N and 14°N of the equator. It covers approximately an area of 56,000 km².

The state shares border with Niger Republic to the north, Kebbi State to the south and Zamfara State to the east (Anon, 2001).

Based on the 2006 population census, It has a projected population of about 4,244,399 as at 2009 (NPC Nigeria, 2006). Sokoto State is endowed with livestock resources; indeed the state is placed second with regard to livestock population which has a mean livestock population for Cattle (3 million), Goat (4 million), Sheep (3.85 million), Camels (0.8 million) and 1 million Poultry (SSIPC, 2008).

Sample collection

During sampling visits of the 76 participating farms, a total of 239 poultry feed samples comprising of commercially prepared feed ($n=204$) and self-compounded feed ($n=35$) were collected. Samples were collected during the rainy season ($n=102$), the cold-dry season ($n=64$) and during the hot-dry season ($n=73$). From each visited farm, feed present in the farm were sampled by selecting 20 to 25% of the bags present in the farm by simple random sampling. For each sample, 5 to 10 g of feed was collected in a polyethylene bag and then collectively transported to the Veterinary Microbiology laboratory for immediate processing. In some cases the feed samples were stored at room temperature (22 to 25°C) for a maximum of 24 h prior to inoculation onto culture media.

Isolation and Identification of fungi

For each feed sample obtained, a ten-fold serial dilution of 1g of feed was carried out using sterile distilled water and 0.1 ml of the dilution was cultured by spread plate technique into Sabouraud dextrose agar (SDA) supplemented with chloramphenicol at 40 µg/ml and Gentamycin at 500 µg/ml (Davis et al., 1980). The inoculated plates were incubated at room temperature for 5 to 14 days. Pure culture of the different colonies (based on morphology) was obtained by sub-culturing of the isolates on nutrient agar and SDA plates respectively. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics of the isolates obtained from pure cultures. Such characteristics include; rapidity of growth, colour of obverse and reverse site of the culture plate, shape, texture and consistency of the growth, Septation of hyphae, shape, size, texture and arrangement of the conidia, etc. according to methods of Carter and Cole (1991), and Cheesbrough (2002). The fungal isolates were also sub-cultured on SDA slants, incubated at 27°C for 10 days and stored in refrigerator for future studies (Saleemi et al., 2010).

Data analysis

The data generated in the study were presented as frequency distribution in the form of tables and figures using frequency distribution. Chi-square formula (Araoye, 2003) was used to test if there is any statistical association between seasons of the year, feed sample type and the rate of isolation of fungi.

RESULTS

Isolation and identification

Of the total 239 feed samples analysed for the presence of fungal agents, 198 (82.8%) were found positive for one or more fungal specie. Fungal isolates were found among 163 (79.9%) of the 204 commercially prepared feed and

Table 1. Percentage culture positive feed samples.

Sample feed types	Number tested	Number positive for fungi	Positive (%)
Commercially prepared	204	163	79.9
Compounded by self	35	35	100.0
Total	239	198	82.8

Chi² = 0.3091, p = 0.74, p<0.05

Table 2. Monthly distribution of fungi contaminated feed samples.

Months	Number of feed sample tested	Number positive for growth (%)
June	23	21 (91.3)
July	15	13 (86.7)
August	16	16 (100.0)
September	16	16 (100.0)
October	15	15 (100.0)
November	18	14 (77.8)
December	22	9 (40.9)
January	24	18 (75.0)
February	20	17 (85.0)
March	26	21(80.7)
April	27	22 (81.5)
May	17	16 (94.1)
Total	239	198 (82.8)

35 (100%) of 35 self compounded feed (Table 1).

The rate of isolation of fungi in feed samples were found to be highest (100%) in the months of August, September and October, followed by May (94.1%) and the least rate (40.9%) was in the month of December (Table 2).

Seasonally, the rate of isolation of fungi in feed samples was found to be highest (95.3%) in rainy season, followed by hot-dry season (84.4%) and the least (64.1%) was cold-dry season (Table 3).

Of the fungal agents isolated from commercially prepared feeds, *Aspergillus* sp. 112 (48.3%) has the highest frequency of occurrence, followed by *Penicillium* sp. 43(18.5%) and the least is *Fusarium* sp. 18 (7.8%). Similarly, in case of self-compounded feeds, *Aspergillus* sp. 24(53.3%) and *Fusarium* sp. 3 (6.7%) has the highest and the lowest rate of occurrence in feeds respectively (Table 4).

Of the 136 *Aspergillus* sp. isolates, 79 (58.09%) was *Aspergillus fumigatus*, and 57 (41.9%) was *Aspergillus flavus*.

DISCUSSION

In the study area, fungal contamination was present in a high proportion of the feed samples (82.85%) (Table 1)

which is in line with the reports from parts of the country Osho et al., 2007; Obi and Ozugbo, 2007; Uwaezuoke and Ogbulie, 2008). Similar findings were also reported from many parts of the world (Dalcero et al., 1997; Oliviera et al., 2006; Rossa et al., 2006; Krnjaja et al., 2008; 2010; Saleemi et al., 2010).

Different genera of contaminating fungi in the present study ranked according to their isolation frequency were *Aspergillus* sp. (49.1%) [Which constitute *A. fumigatus* (58.09%) and *A. flavus* (41.91%)] appeared to be the most prevalent, followed by *Penicillium* sp. (18.41%), *Rhizopus* sp. (13.36%) *Mucor* sp. (11.55%) and the least the *Fusarium* sp. (7.58%) (Table 4). This finding is in agreement with that of Pacin et al. (2003) in Ecuador, Abdul-wahab (1996) in Saudi Arabia, Dalcero et al. (1997) in Argentina and Shareef (2010) in Iraq but not consistent with that of Glender et al. (2006) in Rio de Janeiro.

It may be stated that *Aspergillus*, *Penicillium* and *Fusarium* are the typical fungal genera inhabiting poultry feed mixtures in this area of study. In fact, they are very important contaminants being renowned for their ability to form a huge number of various types of toxic extrolites-mycotoxins (Leistner, 1984).

In the study area, high temperature and humidity might be responsible for higher frequency of isolation of *Aspergillus* sp. especially *A. fumigates* in poultry feeds

Table 3. Seasonal distribution of fungi contaminated feed samples.

Season	Number of feed sample tested	Number positive for growth (%)
Rainy season	85	81 (95.3)
Cold-dry season	64	41 (64.1)
Hot-dry season	90	76 (84.4)
Total	239	198 (91.6)

Chi² (Tabulated) = 5.991, Chi² (calculated) = 3.02, p<0.05.

Table 4. Percentage distribution of fungal isolates in poultry feeds.

Fungal genera	(% Rate of occurrence)		Total	(%)
	Com. prepared (%)	Self comp. (%)		
<i>Aspergillus sp.</i>	112 (48.3)	24 (53.3)	136	49.1
<i>Fusarium sp.</i>	18 (7.8)	3 (6.7)	21	7.6
<i>Mucor sp.</i>	27 (11.6)	5 (11.1)	32	11.6
<i>Penicillium sp.</i>	43 (18.5)	8 (17.8)	51	18.4
<i>Rhizopus sp.</i>	32 (13.8)	5 (11.1)	37	13.4
Total	232 (83.8)	45 (16.2)	277	(100.0)

compared with other species of *Aspergillus* which might be due to their high temperature tolerance character (Battilani et al., 2003). Fungal contamination frequency was higher in self compounded poultry feeds (100%) as compared with commercial poultry feeds (79.99%). A possible reason for low fungal contamination frequency in commercially prepared feeds might be inclusion of antifungal agents to prevent fungal growth during prolonged and varied storage conditions at farms whereas in self compounded poultry feeds such inclusions might be skipped due to short duration of storage. Another reason might be that commercial poultry feeds are usually in pellets or crumbs form which are prepared at high temperature thus reducing its fungal loads (Chelkowsky 1991; Dalcero et al., 2002) whereas self compounded feeds is invariably in mash form, not exposed to high temperatures and might have high microbial count.

Among the *Aspergilli* isolated from feed samples, *A. fumigatus* were the predominant species followed by *A. flavus*; These results differ from some reports describing *A. niger* as the most predominant followed by *A. flavus* (Saleemi et al., 2010); also *A. flavus* as the predominant species followed by *A. niger* aggregates (Accensi et al., 2004; Somashekar et al., 2004 and Rosa et al., 2006).

Statistical analysis of the isolates revealed no significant association between Feed types and the isolation rate of each of the fungal species (Chi² tabulated at df 1 = 0.3841, p<0.05); this implies that the rate of isolation of each of the five fungal species does not depend on whether the feed is commercially prepared or

self compounded poultry feeds. Similarly, there is no significant association between the seasons of the year and proportional rate of isolation of fungi in feeds where *p* values of each statistically analyzed variables are greater than 5% (p<0.05). This implies that seasonal variation has no influence on the rate of isolation of fungi. Therefore, the rate of occurrence of fungi is by chance, and does not depend on seasonal variation. The rate gotten may be due to pre and post-harvest contamination of the feed ingredient, bad manufacturing process, contamination of the feed by the handlers in the farm, bad feeds storage facilities in the farm among others.

Conclusion

The high fungal recovery in this study may indicate a potential hazard to both animals, and humans. The high occurrence of fungal species of public health concern may indicate obvious health hazard in terms of direct consumption of fungal contaminated feed or their toxins by farmed animal and subsequent public health problem. Due to this fact, regular microbiological but also mycotoxicological analysis should be necessary methods for determination of quality and safety of poultry feed.

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REFERENCES

- Abdul-wahab RH (1996). Seed-borne fungi in domestic bird feed in Saudi Arabia. *Iraqi J. Vet. Sci.* 37:223-226.
- Accensi E, Abarca ML, Cabanes FJ (2004). Occurrence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce ochratoxin A. *Food Microb.* 21:623-627.
- Adeboye MA (1999). Serological Evidence of Newcastle Disease and Infectious Bursa Disease Agents in Ducks and Guinea Fowls. Africa Press Ibadan pp. 5-7.
- Anon (2001). Sokoto state – The seat of the Caliphate. In Sokoto State Diary pp.2-8.
- Araoye MO (2003). Research Methodology with Statistics for Health and Social Sciences. Nathadex Publishers, Ilorin p.118.
- Battilani P, Pietri A, Bertuzzi T, Languasco L, Giorni P, Kozakiewicz Z (2003). Occurrence of ochratoxin A producing fungi in grapes grown in Italy. *J. Food Prot.* 66:633-636.
- Beuchat LR (1978). Microbial alterations of grains, legumes, and oilseeds. *Food Technol.* 32:193-198.
- Carter GR, Cole JR (1991). Diagnostic Procedure in Veterinary Bacteriology and Mycology. 5th Ed. Academic Press Inc. pp.372-373.
- Cheesbrough M (2002). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press pp.62-70.
- Chelkowsky J (1991). Mycological quality of mixed feeds and ingredients. In: Cereal grain, mycotoxins, fungi and quality in drying and storage. (Ed.): J. Chelkowsky. Elsevier, Amsterdam pp.217-227
- Dalcerio A, Magnoli C, Chiacchiera S, Palacios G, Reynoso M (1997). Mycoflora and incidence of aflatoxins B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. *Mycopathologia* 137:179-184.
- Davis BD, Dulbecco R, Eisen HN, Ginsberg HS (1980). Microbiology Including Immunology and Molecular Genetics. 3rd Ed. Harper International Edition pp.832.
- Glender R, Oliveira J, Ribeiro M, Marcelo EF, Lilia RC, Gloria MD, Kelly MK, Ana MD, Carlos AR (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia* 162:355-362.
- Krnjaja V, Stojanović Lj, Cmiljanić R, Trenkovski S, Tomašević D (2008). The presence of potentially toxigenic fungi in poultry feed. *Biotechnol. Anim. Husb.* 24(5-6):87-93.
- Krnjaja V, Stojanović Lj, Trenkovski S, Bijelić Z, Tomašević D (2010). The frequency of pathogenic fungi genera in poultry feed. *J. Food Agric. Environ.* 8(3&4):589-591.
- Leistner L (1984). Toxinogenic penicillia occurring in feeds and foods: A review. *Food Technol. Australia* 36:404-413.
- Maciorowski KG, Herrera P, Jones FT, Pillai SD, Ricke SC (2006). Effects on poultry and livestock of feed contamination with bacteria and fungi. Elsevier B.V. pp.109-136.
- National Population Commission (NPC) (2006). Population census of Federal Republic of Nigeria: Preliminary Report. pp.1-2.
- Obi CN, Ozugbo IJ (2007). Microbiological analysis of poultry Feeds sold in Umuahia main market, Abia State, Nigeria. *Res. J. Appl. Sci.* 2(1):22-25.
- Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcerio AM, Rosa CAR (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia* 162:355-362.
- Osho IB, Awoniyi TAM, Adebayo AI (2007). Mycological investigation of compound poultry feeds used in poultry farms in south west Nigeria. *Afr. J. Biotech.* 6:1833-1836.
- Oyediji AN (1986). Prevalence of aflatoxin B1 in commercial poultry rations in Nigeria. *J. Inst. Fur. Tropische Land.* 61:1134-1139.
- Pacin AM, Gonzalez HH, Etcheverry M, Restnik SL, Vivas L, Espin S (2003). Fungi associated with food and feed commodities from Ecuador. *Mycopathologia* 156:87-92.
- Rosa CAR, Riberio JMM, Fraga MJ, Gatti M, Cavaglieri LR, Magnoli CE, Dalcerio AM, Lopes CWG (2006). Mycoflora of poultry feed and ochratoxin- producing ability of isolated *Aspergillus* and *Penicillium* species. *Vet. Microb.* 113:89-96.
- Saleemi MK, Khan MZ, Ahrar K, Javed I (2010). Mycoflora of Poultry Feeds and Mycotoxins Producing Potential of *Aspergillus* Species. *Pak. J. Bot.* 42(1):427-434.
- Shareef AM (2010). Molds and Mycotoxins in Poultry Feeds from Farms of Potential Mycotoxicosis. *Iraqi J. Vet. Sci.* 24(1):17-25.
- Somashekar D, Rati ER, Anad S, Chandrashekar A (2004). Isolation, enumeration and PCR characterization of Aflatoxigenic fungi from food and feed samples in India. *Food Microb.* 21:809-813.
- SSIPC (2008). Beef up Your Profits by Investing in Sokoto State's Livestock Sector pp.1-8.
- Uwaezuoke JC, Ogbulie JN (2008). Microbiological Quality of Commercially Available Poultry Feeds Sold in Parts of Eastern Nigeria. *J. App. Sci. Envi. Manag.* 12(1):113-117.