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Assessment of the mycoflora of commercial poultry feeds sold in the humid tropical environment of Imo State, Nigeria

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ABSTRACT: This study was carried out to identify the common moulds growing in selected commercial poultry feed sold in Owerri, Imo State, Nigeria. Forty-eight bulk samples derived from192 bags of feeds were collected from broiler starter (BS), broiler finishers (BF), grower mash (GM) and layer mash (LM) across 4 different brands of commercial poultry feeds, which included Livestock (LF), Top (TF) Guinea (GF) and Vital (VF) feeds. The feed samples were collected during the rainy season months of June, July and August. The common moulds isolated from these feeds were *Aspergillus sp*., *Penicillium sp*., *Mucor sp*., *Yeast sp*., *Rhizopus sp*., *Epicoecum sp*., *Gymnoaseus sp*., *Cladosporium sp*., *Mortierella sp.* as well as Bacteria. Generally, more organisms were isolated in June than the other months with *Mortierella* sp. being the only one not isolated in that month. Vital feed with 8 different isolates had the highest diversity of fungal species while the others had between 4 and 5 species. Prevalence rates across the feed types, generally ranged from 18.76% in layer mash to 30.03% in broiler finisher. The four *Aspergillus* sp. isolated came from GM and BF. This study highlights the need for constant monitoring of moulds in commercial feedstuff produced in the humid tropical environments such as Imo state, Nigeria. There is also the need to routinely include fungal growth inhibitors in commercial feeds since moulds are capable of reducing the nutritional values of feedstuff as well as elaborating pathogenic toxins.

Key words: *Mycoflora, moulds, mycotoxin, poultry, commercial feeds, Nigeria*

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INTRODUCTION

Feeds supply livestock with nutrients necessary for their body functions and are therefore indispensable for livestock production. Feed quality has been specified on the basis of nutritional value of every individual feed component. However, any given natural feed material also contains various non-nutritional contaminants that may reduce its nutritional value or even exert adverse health effects in animals (Fink-Gremmels, 2004). Many parasitic and saprophytic fungi infect growing crops and may continue to develop through post-harvest, processing and formulation of finished feeds (Vieira, 2003; Mabbett, 2003). Fungal growths cause direct losses in volume and quality of feed ingredients and subsequently feeds made from them, leaving behind some poisonous mycotoxins, which contaminate feed raw materials and finished feeds. Moulds like other micro-organisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrient in the feed (Wilcox, 1988). Such contamination is widespread, especially in tropical countries where poultry production and processing are expanding rapidly (Van den Berghe *et al*., 1990; Mabbett, 2004). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins, trichothecenes such as DON and T-2, ochratoxin and some fusariotoxins (Devegowda *et al*., 1998; Mabbett, 2004). Most mycotoxicoses of poultry are caused by an intake of low concentration of contaminants over a long period of time with the typical chronic symptoms of poor growth, poor feed efficiency and sub-optimal production. Ingestion of higher concentration however leads to acute clinical symptoms associated with specific vital organs, the immune system and other aspects of avian physiology as well as mortality (Mabbett, 2004). According to Richardson (1995), quality control in the feed industry is usually the responsibility of management. This includes documentation and policing of various procedures and processes necessary to guarantee the basic quality of feedstuff and feeds. Commercial feeds manufacturers in developing countries like Nigeria may however not conform to this (Omede, 2003). Generally, mixed feeds by self-composition, especially under favorable conditions, such as high moisture and increased temperature represents excellent substrate for growth and reproduction of fungi. Growth of fungi can occur in feed having as low as 12 % moisture and can result in heat and moisture production. The moisture can cause chains to develop where increased moisture means increased mould production. Currently, mycotoxin binders such as clay minerals, aluminosilicates, esterified glucomannan and modified mannoligosacchande have received ample scientific and industrial attention as effective tools for elimination of mycotoxins from feeds. Available literature reveals that many of the studies carried out on moulds in Nigeria have focused on its effects on human populations (Bankole and Kpodo, 2005; Fandohan *et al*., 2005; Atanda and Akpan, 2005). Fungal and mycotoxin contamination of feedstuff and mycotoxicoses farm animals however, remain neglected in livestock production research issues in Nigeria (Okoli, 2005). There is therefore an urgent need to understand the impact of mycotoxin in animal production in Nigeria. Strategies for reduction of mycotoxin contamination in animal production in Nigeria should however begin with a clear understanding of fungal organisms involved and the type of toxins they produce (Okoli, 2005, Opara and Okoli, 2005). Such information could be utilized in selecting appropriate mould inhibitors and mycotoxin binders for the feed industry in Nigeria. The objective of this study is to identify the common moulds growing in selected commercial poultry feeds sold in Owerri, Imo State, Nigeria. The study was carried out in Owerri, Imo State, Southeastern Nigeria during the rainy season months of June to August 2005.

MATERIALS AND METHODS

A preliminary field survey was carried out to identify reputable commercial poultry feed sellers in Owerri. These sellers were informed of the nature and purpose of the research and based on the preliminary survey, the 4 most popular commercial feed brands that included Guinea feed (GF), Livestock feed (LF), Top feed (TF) and Vita feed (VF) were purposively selected for the study. Feed types such as broiler starter (BS), broiler finisher (BF), grower mash (GM and Layer mash (LM), were sampled at random across the selected brands using the method described by Okoli (2003). These brands were sampled at four different sales outlets representing the most popular feed collection points for farmers in the State.

Sample collection: A total of 48 bulked samples were collected from the list of 4 selected commercial feed brands. Each selected feed outlet was visited 3 times for samples collection during the rainy season months of June, July and August 2005. Altogether, the 48 bulked samples were obtained by sampling 192 feed bags of the different feed types across the selected brands (Table 1). A standard commercial feedbag in the state weighs 25 kg. Each feed brand was sampled by carefully opening 4 randomly selected bags of the same feed type and collecting about 3 g of feed using a sterile universal bottle. These were thereafter homogenized to obtain a representative bulk sample of about 12 g of the feed type. The samples were transported to the laboratory for analysis within 2 hours of collection.

Fungal cultivation and isolation: The two growth media used for the study were sabouraud's agar and potato dextrose agar. The media were prepared according to standard procedure and thereafter sterilized by autoclaving at a temperature of 121 °C for 15 minutes at 15 PSI. They were then allowed to cool to 45 °C on the workbench before plating out into petri dishes. The dishes were inoculated with feed samples and incubated at room temperature for 5 days at the end of which they were examined for fungal growth. Growths were further sub-cultured onto fresh media for another 5 days to obtain pure cultures. After isolation, individual species were identified on the basis of their macro- and micro-morphology (light microscope) in accordance with the interpretative picture keys to some common genera of common moulds (Buchana and Gibbons, 1974; Samson *et al*., 1995; Pitt and Hocking, 1997).

Statistical analysis: The raw data genereated were analyzed to obtain the prevalence of mould.

RESULTS

Table 2 showed the overall frequency of isolation of different fungal species from commercial poultry feeds sold in the state. Fifty-eight isolates made up of 10 fungal species were isolated from the feed samples with

Table 1: Distribution of commercial feeds sample types collected for isolation of fungal organisms in Imo State, Nigeria

		Feed type in bags				
VISIT	Feed Brand	GM	LM	BS	BF	Total Bags
June	LF	3	3	3	3	12
	VF	3	3	3	3	12
	TF	3	3	3	3	12
	GF	3	3	3	3	12
	Total Bulk	4	4	4	4	48 bags
		=16 Bulk samples				
July	LF	3	3	3	3	12
	VF	3	3	3	3	12
	TF	3	3	3	3	12
	GF	3	3	3	3	12
	Total Bulk	4	4	4	4	48 bags
		= 16 bulk samples				
August	LF	3	3	3	3	12
	VF	3	3	3	3	12
	TF	3	3	3	3	12
	GF	3	3	3	3	12
	Total Bulk	4	4	4	4	48 bags
		= 16 bulk samples				
Grand Total = 48 bulk sample made up of 192 bags of feed						

Key: BS-Broiler starter, LM- Layer mash, GM-Grower mash, BF-Broiler finisher, LF-Livestock feed, VF-Vital feed, TF-Top feed, GF-Guinea feed

Table 2: Overall frequency of isolation of different fungal species from commercial poultry feeds sold in Imo State, Nigeria

Fungal specie	Frequency	Percentage
1. *Mucor sp.*	33	56.90
2. *Aspergillus sp.*	4	6.90
3. *Penicillium sp.*	1	1.72
4. *Epicoecum sp.*	1	1.72
5. *Yeast sp.*	5	8.62
6. *Rhizopus sp.*	6	10.34
7. *Cladosporium sp.*	1	1.72
8. *Gymnoaseus sp.*	3	5.17
9. *Mortierella sp.*	1	1.72
10. *Bacteria sp.*	3	5.17
Total	58	100.00

Table 3: Frequency of isolation of fungal species during three sampling periods (June, July and August) of commercial poultry feeds sold in Imo State, Nigeria

Fungal specie	June	July	August
Mucor sp.	11(42.31)	12(63.16)	10(76.92)
Aspergillus sp.	4(15.38)	0(0.00)	0(0.00)
Penicillium sp.	1(3.85)	0(0.00)	0(0.00)
Epicoecum sp.	1(3.85)	0(0.00)	0(0.00)
Yeast sp.	3(11.54)	1(5.26)	1(7.69)
Rhizopus sp.	2(7.70)	3(15.79)	1(7.69)
Cladosporium sp.	1(3.85)	0(0.00)	0(0.00)
Gymnoaseus sp.	1(3.85)	0(0.00)	0(0.00)
Mortierella sp.	0(0.00)	0(0.00)	1(1(7.79)
Bacteria sp.	2(7.70)	1(5.26)	0(0.00)
Total	26(44.83)	19(32.76)	13(22.84)

Mucor sp. being the most prevalent. Table 3 highlighted the variations in the prevalence of the isolated species across the months of study. Generally, more organisms were isolated in June than the other months with *Mortierella* sp. being the only one not isolated in that month, while 4 fungal species each were isolated in July and August respectively. Although Bacteria species was included in the present result, they are not fungal organisms but are known to occur in mix cultures with moulds.

Tables 4 and 5 show frequency of isolation of the fungal species from four different of commercial poultry feed brands and feed types within brands respectively. Vital feed (VF) with 8 different isolates had the highest diversity of fungal species while the others had between 4 and 5. Percentage isolation across the feed brand was however similar at 24.14 for LF and VF, and 25.86 for TF and GF. Four *Aspergillus* sp. were isolated from TF, GF and VF. Across the feed types (Table 5), prevalence rates generally ranged from 18.76% in layer mash (LM) to 30.03% in broiler finisher (BF). Diversity of fungal species among the feed types was also similar, however the 4 *Aspergillus* sp. isolated came from GM and BF.

Table 4: Frequency of isolation of fungal species from four different of commercial poultry feed brands sold in Imo State, Nigeria

Fungal specie	No isolated	LF	TF	GF	VF
Mucor sp.	33	9 (27.27)	9 (27.27)	9 (27.27)	6 (18.18)
Aspergillus sp.	4	0 (0.00)	1 (25.00)	1 (25.00)	2 (50.00)
Penicillium sp.	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)
Epicoecum sp.	1	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Yeast sp.	5	2 (40.00)	0 (0.00)	2 (40.00)	1 (20.00)
Rhizopus sp.	6	2 (33.33)	3 (50.00)	0 (0.00)	1 (16.67)
Cladosporium sp.	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)
Gymnoaseus sp.	3	0 (0.00)	1 (33.33)	1 (33.33)	1 (33.33)
Mortierella sp.	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)
Bacteria sp.	3	0 (0.00)	1 (33.33)	2 (66.67)	0 (0.00)
Total	58	14 (24.14)	15 (25.86)	15 (25.86)	14 (24.14)

Table 5: Frequency of isolation of fungal species from four different feed types of the commercial poultry feeds sold in Imo State, Nigeria

Fungal specie	No isolated	GM	BS	BF	LM
Mucor sp.	33	11 (33.33)	4 (12.12)	11 (33.33)	7 (21.21)
Aspergillus sp.	4	2 (50.00)	0 (0.00)	2 (50.00)	0 (0.00)
Penicillium sp.	1	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Epicoecum sp.	1	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)
Yeast sp.	5	0 (0.00)	2 (40.00)	3 (60.00)	0 (0.00)
Rhizopus sp.	6	0 (0.00)	5 (83.33)	0 (0.00)	1 (16.67)
Cladosporium sp.	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)
Gymnoaseus sp.	3	1 (33.33)	0 (0.00)	2 (66.67)	0 (0.00)
Mortierella sp.	1	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)
Bacteria sp.	3	1 (33.33)	0 (0.00)	0 (0.00)	2 (66.67)
Total	58	16 (27.59)	13 (22.41	18 (31.03)	11 (18.97)

DISCUSSION AND CONCLUSION

The present results show that the common moulds growing in commercial poultry feeds sold in Imo State, Nigeria during the rainy season months of June to August are *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Yeast* sp., *Rhizopus* sp., *Epicoecum* sp., *Gymnoaseus* sp., *Cladosporium* sp. and *Bacteria* sp. Bacteria were identified even though they are not fungi because according to Buchanan and Gibbons (1974), they will always be seen where fungi occur and form wet or slimy colonies often with rather bright colors and unpleasant odors. Maurice (1999) stated that 10 to 20% of the world's estimated annual cereal crop is lost through spoilage by mould and this problem is particularly acute in the humid tropical countries such as southern Nigeria. Some effects of mould growth in feed include caking of feed, darkening of feed and grain, dustiness, poor feed flow out of bins, moldy and mildew smell of the grains as well as production of mycotoxins (Mabbeth, 1998; Maurice 1999; Danike, 2002). Routine monitoring of mould growth in feedstuff could therefore provide useful clues to variations in raw materials quality and of possible contamination during production of feedstuffs (Adam, 1987). The fact that a much higher number of *Mucor* sp. was isolated in the present study is expected since mucor is one of the commonest moulds that thrive under tropical environment. The present study conducted during the rainy months of June to August revealed high presence of moulds in the commercial feeds in June. This result probably reflects the effects of relatively higher temperatures experienced in Imo state during the month of June on mould growth when compared to the other months (Okoli, 2003; Adams, 1987; Danike, 2002). Again the higher diversity of fungal types in Vital feeds samples probably reflects the different climatic conditions of northern Nigeria where the feed is produced before transportation to the south. This climatic effect and the fact that rains usually come much later in the north (Agboola, 1979) may explain the growth of peculiar species such as *Penicillium, Cladosporium* and *Mortierella* in Vital feed samples.

The ability of moulds to produce mycotoxins potentially dangerous or lethal to man and animals is widespread and depends upon several factors such as the organism, the nature and moisture content of the stored commodity, relative humidity and temperature (Awan, 1983). For example it has been shown that *Aspergillus* sp. produces aflatoxins, while *Penicillium* sp. produces citrinin. Again the two species have been implicated in the production of ochratoxin thus highlighting the importance of their isolations in commercial feeds sold in Imo state (Danike, 2002). The data generated from this study highlights the need for constant monitoring of moulds in commercial feedstuff in humid tropical environments such as Imo state and the need to routinely inhibit their growth since some of them are known to elaborate mycotoxins and may also lower feed quality.

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