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## **Survey of Mycobiota of Poultry Feeds Retailed in Ogun State, Nigeria.**

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### **Abstract**

Microscopic fungi occurrences and growth on poultry feeds is one of the major threats to poultry economy and health. This study investigated the microscopic fungi contaminating poultry feeds in Ogun State, Nigeria. It was carried out in the month of May to October and November to April 2011 representing two seasons. Twenty five feed samples of Broilers mash poultry feed were collected from different Feedmills in five towns in Ogun State at these two seasons. Result shows that twenty five Microscopic fungi were isolated from these feed samples collected. *Aspergillus niger* was the most frequent mould floral in the feed samples in the two seasons. The presence of these pathogenic moulds in the poultry feeds reveals that feed produced by these Feedmills are contaminated. Therefore, the commercial poultry Feedmills should be periodically examined for Biosafety so as to prevent the risk of cross contamination of poultry and poultry products.

**Keywords:** Feedmills, Mycotoxin, Contamination, Ogun State

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## Introduction

Microscopic fungi occurrence and growth on poultry feeds is one of the major threats to poultry economy and health. Besides their negative impacts on nutritional and organoleptic properties, microscopic fungi can also synthesize different mycotoxins. Microscopic fungi are generously endowed with extracellular proteolytic or lipolytic enzymes and so can cause softening of product Fun (2006). Microscopic fungi growth also causes off flavour or odours in food. In addition changes in appearance of food and feeds have been related to microscopic fungi growth. Microscopic fungi are capable of producing highly toxic substances during their growth on substrates such as foods or feeds. These toxic metabolites are designated "mycotoxin" which is a collective term for the entire group of toxic substances produced by various moulds (Adegoke, 2004). The diseases that mycotoxin causes are collectively called mycotoxicoses. Mycotoxins are toxic secondary metabolites of about 6900 fungal species, (Nafeesa et al., 2005). The presence of mycotoxin in foods and feeds is potentially hazardous to the health of humans and animals. An example of a well known mycotoxin is aflatoxin. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* and often cause liver damage and cancer, decreased milk production and immune suppression. Usually in developing countries, the best quality grains or cereals are reserved for human consumption and the crops with poorer quality are utilized for the animal feed (Bankole and Kpodo 2005).

The poultry industries rely on the supply of ready-to-use feed from feed mills for handling, unloading, grinding of grains, mixing and usually pelleting of the mixed ration (Uwaezuoke and Ogbulie 2008). These packaged feeds from feed mills constitute the main source of feeds for poultry farmers. Poultry feed component of plants and animal origin are commonly contaminated with microorganisms, mostly bacteria and fungi and/or insects. However, the number and types of microorganisms and insects vary depending on the function of materials, location of its origin, climatic conditions encountered, harvesting, processing, storage transport technologies employed and packaging materials (Uwaezuoke and Ogbulie 2008). Some beneficial poultry feed contaminants such as lactic acid bacteria have been reported (Uwaezuoke and Ogbulie 2008). The importance of LAB in poultry feeds and growth performance in farm animals have equally been documented (Czerwiecki *et al.*, 2002). Other microorganisms that have been implicated as contaminants of poultry feeds include *Escherichia coli*, *Erwinia herbicola*, *Salmonella* spp., *Listeria* sp., *Enterococcus faecalis*, *Aspergillus flavus*, *A. parasiticus*, *Penicillium* spp. and *Fusarium* spp. (Pardo *et al.*, 2004). These microscopic fungi include members of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* which could contaminate many agricultural commodities used in the formulation of poultry finished feed samples like corn, wheat, soybean, barley and others commodities. These microscopic fungi are of great importance because of potential mycotoxin production (Bray and Ryan, 2006).

Despite the great attention that has been paid to the study of toxigenic microscopic fungi and their mycotoxins in various foods and feeds, little is known about fungal and mycotoxin contamination of poultry feed. Furthermore, it is well established that contamination of poultry feeds with mycotoxins may induce sanitary disturbances and mortality among the birds and secondary contamination of the human consumer via eggs, poultry meat and giblets (Kamalavenkatesh *et al.*, 2005). Variety of complex and diverse clinical signs of potential mycotoxicosis have been observed in different broiler, broiler breeder and layer farms with potential mycotoxicosis. Affected flocks showed one or more of the following symptoms; decreased weight gain; anorexia; reduced feed conversion efficiency; decreased egg production; poor egg shell quality; increased egg blood spots; spiking mortalities; immunosuppression and failure of vaccination programs; increased susceptibility to diseases especially E-coli infection; reduced fertility and hatchability; visceral hemorrhages; leg weakness and high percentages of leg deformities; pale bird syndrome; fatty liver with pale, muddy to yellowish discoloration; increased bruising; enlarged pale kidneys; wet litter; urate deposition in the body cavities; increased incidence of viral diseases like Newcastle disease, infectious bursal disease and inclusion body hepatitis; oral lesions; tibial dyschondroplasia; gizzard erosions; paralysis; extension of leg and neck (Kamalavenkatesh *et al.*, 2005).

The survey of mycobiota of poultry bird feeds became imperative in view of the recent birds infections and diseases outbreak in Nigeria. The outbreak resulted in massive destruction of birds championed by the Federal Government of Nigeria. In addition, many poultry farmers have not recovered from the shock and huge financial losses created by the scenario. Therefore, the main objective of this study was to initiate a study on the mould contaminating poultry feed in Ogun State, Nigeria with the aim of ascertaining the safe quality of the feeds.

## Materials and Methods

Poultry finished broilers mash feeds were collected in a sterile paper bag from five towns in Ogun State, Nigeria. The towns are: Abeokuta, Ijebu Ode, Shagamu, Ijebu Remo and Wahasimi which were selected in state representing the cardinal points. Samples of poultry feeds (broilers mash) were collected in May- October 2008 (raining season of the year) and November 2008-April 2009 (dry season of the year) from different Feedmills in the State. Feeds were sampled at random across the selected brands using the method described by Okoli (2003). These brands were sampled at different feed mill outlets representing the most popular feed collection points for farmers in Ogun States, Nigeria. The samples were transported to the laboratory for analysis within 24 hours of collection.

The growth media used for the study were potato dextrose agar (PDA), Potato dextrose salt agar (PDAS 7.5g of NaCl in 100ml of medium), malt extract agar (MEA) and media formulated from feed ingredients (Onifade *et al.*, 2010). 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 pouring into Petri dishes. The dishes were inoculated with feed samples by pour plate technique and incubated at 28±2°C for 1-2 weeks at the end of which they were examined for mould growth. Growths were further sub-cultured onto fresh media to obtain pure cultures. After isolation, individual species were identified on the basis of their macro- and micro-morphology under

compound microscope in accordance with the interpretative picture keys to some common genera of common microscopic fungi (David 2009).

Dilution plate and direct isolation techniques were used for the isolation of microscopic fungi from the feed samples. To prevent bacterial growth 100 ppm oxytetracycline was added (Omede, 2004). Feed samples were aseptically milled and the samples were diluted in sterile distilled water with 0.05% Tween 80 at a ratio of 1/10 (v/w). One gram from a sample lot from each location were assayed for mould using pour plate serial dilution technique. Afterwards petridishes with visible colonies were selected and the mould contamination levels for 1g samples were determined. The samples were examined with the naked eye and with a stereomicroscope. The colonies with different morphological characters were repeatedly inoculated on appropriate media for purification. Identification of the mould isolates was made according to David (2009).

### Results and Discussions

The result of microscopic fungi isolated from broilers poultry feeds in the two seasons of the year were; *F. oxysporum*, *F. solani*, *P. expansum*, *A. niger*, *R. oligosporus*, *A. flavus*, *F. verticillioides*, *A. versicolour*, *A. terreus*, *P. implicatum*, *P. italicum*, *P. lapidosum*, *P. digitatum*, *A. wentii*, *F. gramineum*, *P. rubrum*, *P. graminearium*, *A. oryzae*, *A. fumigates*, *P. notatum*, *A. parasiticus*, *P. spinulosum*, *P. viridicatum*, *A. japonicas* and *A. ochraceus*. The commonest mould isolated in feed samples during the dry and rainy season within state were; *A. parasiticus*, *F. solani*, *A. niger*, *A. flavus*, *F. verticillioides*, *P. italicum*, *P. digitatum* and *A. wentii*. While *A. niger* was the most frequent mycobiota of the feed samples in the two seasons (Table 1-5). During the raining season (May-October) *P. spinulosum* ( $8.0 \times 10^4$  cfu/g) had the highest count followed by *A. niger* ( $1.7 \times 10^4$  cfu/g) while *A. parasiticus* ( $8.3 \times 10^4$  cfu/g) had the highest count and the least count were *F. verticillioides* and *F. solani* ( $3 \times 10^3$  cfu/g) during the dry season (November- April).

### Conclusion and Recommendations

This study revealed high microscopic fungi counts in the poultry feeds investigated. This tends to reflect the level of Biosafety and hygienic practices in the production, handling and storing of poultry feeds by Feedmills. Incorporation of feed additives into poultry feeds that would prevent mould contamination should be encouraged. These findings emphasize the need for constant quality assessment of these commercial Feedmills and feeds on sale in order to prevent the risk of cross contamination of poultry and poultry products.

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## Tables

**Table 1:** Total number of microscopic fungi (cfu/g) Isolated from poultry feeds in Abeokuta Ogun State Nigeria within the two season of the year

| Mould Isolates            | Counts of mould (cfu/g) May-Oct | Counts Counts of mould (cfu/g) Nov-April |
|---------------------------|---------------------------------|--|
| <i>F. oxysporum</i>       | 6x10 <sup>3</sup>               | 0  |
| <i>F. solani</i>          | 7 x10 <sup>3</sup>              | 3 x10 <sup>3</sup>                       |
| <i>P. expansum</i>        | 0                               | 7 x10 <sup>3</sup>                       |
| <i>A. niger</i>           | 1.1 x10 <sup>4</sup>            | 1.7 x10 <sup>4</sup>                     |
| <i>R. oligosporus</i>     | 0                               | 8 x10 <sup>3</sup>                       |
| <i>A. flavus</i>          | 0                               | 5 x10 <sup>3</sup>                       |
| <i>F. verticillioides</i> | 0                               | 3 x10 <sup>3</sup>                       |

**Table 2:** Total number of microscopic fungi (cfu/g) Isolated from poultry feeds in Wahsimi, Ogun State Nigeria within the two season of the year

| Mould Isolates            | Counts of mould (cfu/g) May-Oct 2008 | Counts Counts of mould (cfu/g) Nov2008-April2009 |
|---------------------------|--------------------------------------|--|
| <i>A. versicolour</i>     | 7 x10 <sup>3</sup>                   | 0  |
| <i>A. terreus</i>         | 3 x10 <sup>3</sup>                   | 0  |
| <i>A. niger</i>           | 5 x10 <sup>3</sup>                   | 5 x10 <sup>3</sup>                               |
| <i>P. implicatum</i>      | 2 x10 <sup>3</sup>                   | 0  |
| <i>A. flavus</i>          | 5 x10 <sup>3</sup>                   | 7 x10 <sup>3</sup>                               |
| <i>P. italicum</i>        | 3 x10 <sup>3</sup>                   | 6 x10 <sup>3</sup>                               |
| <i>F. verticillioides</i> | 6 x10 <sup>3</sup>                   | 3 x10 <sup>3</sup>                               |
| <i>P. lapidosum</i>       | 0                                    | 2 x10 <sup>3</sup>                               |

**Table 3:** Total number of microscopic fungi (cfu/g) Isolated from poultry feeds in Ijebu Remo, Ogun State Nigeria within the two season of the year

| Mould Isolates           | Counts of mould (cfu/g) May-Oct 2008 | Counts Counts of mould (cfu/g) Nov2008-April2009 |
|--------------------------|--------------------------------------|--|
| <i>A. terreus</i>        | 0                                    | $7.1 \times 10^4$                                |
| <i>A. niger</i>          | $5 \times 10^3$                      | 0  |
| <i>P. implicatum</i>     | 0                                    | $8 \times 10^3$                                  |
| <i>P. digitatum</i>      | $2 \times 10^3$                      | $6 \times 10^3$                                  |
| <i>A. wentii</i>         | $9 \times 10^3$                      | $7 \times 10^3$                                  |
| <i>F. verticilliodes</i> | 0                                    | $1.0 \times 10^4$                                |
| <i>F. graminearum</i>    | 0                                    | $3 \times 10^3$                                  |
| <i>R. oligosporus</i>    | 0                                    | $2 \times 10^3$                                  |
| <i>A. flavus</i>         | 0                                    | $7 \times 10^3$                                  |

**Table 4:** Total number of microscopic fungi (cfu/g) Isolated from poultry feeds in Shagamu, Ogun State Nigeria within the two season of the year

| Mould Isolates           | Counts of mould (cfu/g) May-Oct 2008 | Counts Counts of mould (cfu/g) Nov2008-April2009 |
|--------------------------|--------------------------------------|--|
| <i>P. rubrum</i>         | $7 \times 10^3$                      | 0  |
| <i>F. graminearum</i>    | $6.1 \times 10^3$                    | 0  |
| <i>A. oryzae</i>         | $2.5 \times 10^4$                    | 0  |
| <i>A. niger</i>          | $2.1 \times 10^4$                    | 0  |
| <i>A. digitatum</i>      | 0                                    | $5 \times 10^3$                                  |
| <i>A. fumigatus</i>      | 0                                    | $7.7 \times 10^4$                                |
| <i>P. notatum</i>        | 0                                    | $8 \times 10^3$                                  |
| <i>A. parasiticus</i>    | 0                                    | $2.6 \times 10^4$                                |
| <i>F. verticilliodes</i> | 0                                    | $9 \times 10^3$                                  |

**Table 5:** Total number of microscopic fungi (cfu/g) Isolated from poultry feeds in Ijebu Ode, Ogun State Nigeria within the two season of the year

| Mould Isolates           | Counts of mould (cfu/g) May-Oct 2008 | Counts Counts of mould (cfu/g) Nov2008-April2009 |
|--------------------------|--------------------------------------|--|
| <i>F. verticilliodes</i> | $6.2 \times 10^4$                    | 0  |
| <i>P. spinulosum</i>     | $8.0 \times 10^4$                    | 0  |
| <i>A. fumigatus</i>      | $5.2 \times 10^4$                    | 0  |
| <i>A. parasiticus</i>    | $7 \times 10^3$                      | $8.3 \times 10^4$                                |
| <i>P. viridicatum</i>    | NP                                   | $4 \times 10^3$                                  |
| <i>A. japonicas</i>      | 0                                    | $2.1 \times 10^4$                                |
| <i>F. solani</i>         | 0                                    | $5.1 \times 10^4$                                |
| <i>A. niger</i>          | 0                                    | $7 \times 10^3$                                  |
| <i>A. ochraceus</i>      | 0                                    | $6.5 \times 10^4$                                |

**Table 6:** Percentage Occurrence of poultry feeds collected from Abeokuta Ogun State Nigeria within the two season of the year

| Isolate                       | No of occurrence in May-Oct | No of occurrence in Nov-April | % occurrence in May-Oct | % occurrence in Nov-April |
|-------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| <i>F. oxysporum</i>           | 4                           | 0                             | 33.33                   | 0.00                      |
| <i>F. solani</i>              | 3                           | 3                             | 25.00                   | 13.04                     |
| <i>P. expansum</i>            | 0                           | 5                             | 0.00                    | 21.74                     |
| <i>A. niger</i>               | 5                           | 5                             | 41.66                   | 21.74                     |
| <i>R. oligosporus</i>         | 0                           | 3                             | 0.00                    | 13.04                     |
| <i>A. flavus</i>              | 0                           | 4                             | 0.00                    | 17.39                     |
| <i>F. verticilliodes</i>      | 0                           | 2                             | 0.00                    | 8.70                      |
| <b>Total No of occurrence</b> | <b>12</b>                   | <b>23</b>                     |                         |                           |

**Table 7:** Percentage Occurrence of poultry feeds collected from Wahsimi, Ogun State Nigeria within the two season of the year

| Isolate                       | No of occurrence in May-Oct | No of occurrence in Nov-April | % occurrence in May-Oct | % occurrence in Nov-April |
|-------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| <i>A. versicolour</i>         | 3                           | 0                             | 16.66                   | 0.00                      |
| <i>A. terreus</i>             | 2                           | 0                             | 11.11                   | 0.00                      |
| <i>A. niger</i>               | 4                           | 3                             | 22.22                   | 20.00                     |
| <i>P. implicatum</i>          | 1                           | 0                             | 5.56                    | 0.00                      |
| <i>A. flavus</i>              | 4                           | 5                             | 22.22                   | 33.33                     |
| <i>P. italicum</i>            | 1                           | 3                             | 5.56                    | 20.00                     |
| <i>F. verticilliodes</i>      | 3                           | 2                             | 16.66                   | 13.33                     |
| <i>P. lapidosum</i>           | 0                           | 2                             | 0                       | 13.33                     |
| <b>Total no of occurrence</b> | <b>18</b>                   | <b>15</b>                     |                         |                           |

**Table 8:** Percentage Occurrence of poultry feeds collected from Ijebu Remo, Ogun State Nigeria within the two season of the year

| Isolate                       | No of occurrence in May-Oct | No of occurrence in Nov-April | % occurrence in May-Oct | % occurrence in Nov-April |
|-------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| <i>A. terreus</i>             | 0                           | 2                             | 0.00                    | 10.00                     |
| <i>A. niger</i>               | 3                           | 0                             | 42.86                   | 0.00                      |
| <i>P. implicatum</i>          | 0                           | 2                             | 0.00                    | 10.00                     |
| <i>P. digitatum</i>           | 1                           | 2                             | 14.29                   | 10.00                     |
| <i>A. wentii</i>              | 3                           | 3                             | 42.86                   | 15.00                     |
| <i>F. verticilliodes</i>      | 0                           | 4                             | 0.00                    | 20.00                     |
| <i>F. graminearum</i>         | 0                           | 1                             | 0.00                    | 5.00                      |
| <i>R. oligosporus</i>         | 0                           | 2                             | 0.00                    | 10.00                     |
| <i>A. flavus</i>              | 0                           | 4                             | 0.00                    | 20.00                     |
| <b>Total no of occurrence</b> | <b>7</b>                    | <b>20</b>                     |                         |                           |

**Table 9:** Percentage Occurrence of poultry feeds collected from Shagamu, Ogun State Nigeria within the two season of the year

| Isolate                       | No of occurrence in May-Oct | No of occurrence in Nov-April | % occurrence in May-Oct | % occurrence in Nov-April |
|-------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| <i>P. rubrum</i>              | 2                           | 0                             | 14.29                   | 0.00                      |
| <i>F. graminearum</i>         | 3                           | 0                             | 21.43                   | 0.00                      |
| <i>A. oryzae</i>              | 4                           | 0                             | 28.57                   | 0.00                      |
| <i>A. niger</i>               | 5                           | 0                             | 35.71                   | 0.00                      |
| <i>A. digitatum</i>           | 0                           | 1                             | 0.00                    | 6.66                      |
| <i>A. fumigates</i>           | 0                           | 4                             | 0.00                    | 26.67                     |
| <i>P. notatum</i>             | 0                           | 3                             | 0.00                    | 20.00                     |
| <i>A. parasiticus</i>         | 0                           | 4                             | 0.00                    | 26.67                     |
| <i>F. verticilliodes</i>      | 0                           | 3                             | 0.00                    | 20.00                     |
| <b>Total no of occurrence</b> | <b>14</b>                   | <b>15</b>                     |                         |                           |

**Table 10:** Percentage Occurrence of poultry feeds collected from Ijebu Ode, Ogun State Nigeria within the two season of the year

| <b>Isolate</b>                | <b>No of occurrence in May-Oct</b> | <b>No of occurrence in Nov-April</b> | <b>% occurrence in May-Oct</b> | <b>% occurrence in Nov-April</b> |
|-------------------------------|------------------------------------|--------------------------------------|--------------------------------|----------------------------------|
| <i>F. verticilliodes</i>      | 5                                  | 0                                    | 27.78                          | 0.00                             |
| <i>P. spinulosum</i>          | 4                                  | 0                                    | 22.22                          | 0.00                             |
| <i>A. fumigates</i>           | 5                                  | 0                                    | 27.78                          | 0.00                             |
| <i>A. parasiticus</i>         | 4                                  | 5                                    | 22.22                          | 21.74                            |
| <i>P. viridicatum</i>         | 0                                  | 4                                    | 0.00                           | 17.39                            |
| <i>A. japonicas</i>           | 0                                  | 3                                    | 0.00                           | 13.04                            |
| <i>F. solani</i>              | 0                                  | 3                                    | 0.00                           | 13.04                            |
| <i>A. niger</i>               | 0                                  | 4                                    | 0.00                           | 17.39                            |
| <i>A. ochraceus</i>           | 0                                  | 4                                    | 0.00                           | 17.39                            |
| <b>Total no of occurrence</b> | <b>18</b>                          | <b>23</b>                            |                                |                                  |