ABSTRACT

Poultry farming contributes significantly to poverty alleviation by providing employment opportunities to Nigerians. Feed quality is the most prominent challenge of the poultry sector. This study was aimed at assessing the fungal and proximate qualities of poultry feeds sold in Benin City, Nigeria. Poultry feed samples (starter, grower and finisher) were obtained from five stores in Benin City. Enumeration and isolation of fungi was carried out using the spread plate method on potato dextrose agar and Aspergillus flavus and parasiticus agar (AFPA). Identification of fungal isolates was based on cultural and morphological characteristics. Proximate composition was determined by standard methods. The total fungal counts of starter, grower and finisher feeds ranged from $0.10 - 8.50 \times 10^5$ sfu/g. Fungal species isolated were Aspergillus flavus, Aspergillus niger, Penicillium spp., Saccharomyces spp., Trichoderma spp., Rhizopus spp. and Mucor spp. The results from proximate analysis were as follows: 36.50±0.92 - 70.92±0.51% (protein), 3.53±0.04 - 8.45±0.16% (fibre), 11.61±0.72 - 13.91±0.27% (fat), 2.70 - 8.60% (moisture content) and 3.65 - 37.83% (carbohydrate). The temperature and relative humidity of storage ranged from 33.90°C - 35.20°C and 69% - 75% respectively. Results showed that poultry feeds sampled have diverse fungi present in them. Control measures should be adhered to in poultry industries and stores to prevent microbial contamination.

Keywords: aflatoxigenic fungi, feed storage fungal contamination, poultry feeds, proximate quality
INTRODUCTION
Poultry farming contributes significantly to poverty alleviation by providing employment opportunities to Nigerians. Feed quality is the most prominent challenge of the poultry sector (Dewa and Tikau, 2019). The safety and quality of feed are currently of major concern in developed countries, as safety of feed is a fundamental requirement for all birds. Unsafe feed can lead to great economic losses (Danbappa et al., 2018). Feed is the most important variable cost component, accounting for about 65-70% of production cost (Dewa and Tikau, 2019). Therefore, for efficiency and high productivity, birds must be fed with quality feed to meet their nutritional requirements, hence the need for qualitative feed cannot be over emphasized (FAO, 2013). Healthy poultry require adequate amounts of carbohydrates, lipids and proteins along with necessary vitamins and dietary minerals. These nutritional components are important for the development and growth of farm animals (Dmello and MacDonald, 1998). There are different types of feeds given to poultry. They include starters, growers, layers and finisher feeds (Ubiebi, 2017). The raw materials used for the formulation of feeds are sourced from different origin including animal and plant, most are mainly agro-wastes. Most of these feed additives have been implicated as the primary sources of microorganisms of public health concern in poultry feeds (Afolayan and Afolayan, 2008; Matthew et al., 2017).

Several microorganisms can contaminate poultry feeds. Species of *Aspergillus* and *Penicillium* are frequently isolated as contaminants from feeds (Mehrolia et al., 2015). Feed materials may become contaminated with microorganisms during growing, harvesting, processing, storage and dispersal of the feed (Ubiebi, 2017). Contamination can arise from air, equipment, feed ingredients, farm workers, handling and raw materials. A number of poultry diseases of various pathology and origins including viral, bacterial and fungal have been attributed to poultry feeds. Examples are Avian influenza, New Castle disease, salmonellosis, aspergillosis and candidiasis. The consumption of mycotoxins can result in immunosuppression (Macrowioski et al., 2006; Danbappa et al., 2018). The components of feed especially corn and corn by-products are the most susceptible to aflatoxigenic fungi attack and therefore aflatoxin contamination. Aflatoxins are mould-synthesized, secondary metabolites that are capable of causing disease and death in humans and animals (Granadas-Chinchilla, 2017). The contamination of forages and cereals by mycotoxigenic fungi often occur in the field or during processing, transportation and storage when conditions for growth such as temperature and relative humidity are suitable (Mgabeahuruike et al., 2020).

The presence of fungi affects the organoleptic attributes and nutritional quality of feeds. Moulds will assimilate and utilise the readily available nutrients in the feed thereby leading to loss of nutrients in the feed and spoilage (Danbappa et al., 2018). The genera of most concern globally in poultry feeds are *Aspergillus*, *Penicillium* and *Fusarium* (Ghaemmaghami et al., 2018). The identification of contaminating fungi is essential because it is a helpful indicator to determine feed quality and also provides data on the potential production of mycotoxins. This study was aimed at assessing the fungal and proximate qualities of poultry feeds sold in Benin City, Nigeria.
METHODOLOGY
SAMPLE COLLECTION
Three forms of poultry feeds; starter, grower and finisher from were sampled in five (5) feed stores in Benin City, Edo State, Nigeria. The samples were collected aseptically using sterile spatula in sterile polyethylene bag and labelled accordingly.

ENUMERATION AND ISOLATION OF FUNGI
The fungi were isolated by serial dilution and the spread plate method. Poultry samples were serially diluted and 0.10 ml (dilution $10^{-1}$) of samples was inoculated on potato dextrose agar (PDA) and *Aspergillus flavus* and *parasiticus agar* (AFPA). The inoculated plates were incubated at 28 ±2°C for 3-5 days. Colonies that developed were counted and recorded as spore-forming units per gram (sfu/g). Pure cultures were obtained by sub-culturing and identification was done by cultural and microscopic characterisation. One drop of lactophenol cotton blue stain was placed on a clean glass slide. A sterilized inoculating pin was used to pick suspected fungal colony placed in the stain on the slide. This was then teased out and covered with glass cover slip. The slide was observed under ×40 magnification of a light microscope. Microscopic characteristics of fungi such as hyphae, conidial heads and arrangements of conidia were observed (Barnet and Hunter, 1972).

PROXIMATE ANALYSIS
The crude protein, fibre, ash, fat, total carbohydrate and moisture contents of the poultry feeds were analysed according to standard methods given in Association of Official Analytical Chemists (AOAC, 2016). Relative humidity and temperature of the feed stores were determined using a thermometer humidity meter (ThermoPro Model TP49-W2) by methods described by Nwabueze and Nwabueze (2011).

RESULTS AND DISCUSSION
The evaluation of fungal contamination in poultry feeds is one of the important steps taken to control feed quality and hygiene. The total fungal counts of starter, grower and finisher feeds ranged from $0.10 \times 10^5$ – $8.50 \times 10^5$ cfu/g. The fungal count of the poultry feeds exceeded the accepted European standard for finished poultry feed (1 x $10^5$ cfu/g) (Ghaemmaghami et al., 2018). The high fungal counts depict the level at which feed ingredients used in feed formulation is contaminated. The condition of feed storage and handling is a source of contamination. There is a build-up of microbial contamination when feeds are not hygienically handled and stored (Ubiebi, 2017). The fungal species isolated from poultry feeds sampled in this study include *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp., *Saccharomyces* spp., *Trichoderma* spp., *Rhizopus* spp. and *Mucor* spp. Similar fungi including *Aspergillus niger*, *Mucor* spp., *Rhizopus* spp., *Fusarium* spp., *Penicillium* spp., and *Cladosporium* spp. were also isolated and identified from poultry feeds sampled by Danbappa et al. (2018). Ghaemmaghami et al. (2018) also reported that *Fusarium* species, yeast and *Aspergillus* species were the most frequently recovered genera from mashed and pelleted feeds. It is reported that some species of *Rhizopus* are also mycotoxigenic (Matthew et al., 2017). The feed ingredients and nutritional quality of feed can influence the capability of fungi to inoculate and make use of their genetic machinery to produce aflatoxins within the substrate. Feed is especially susceptible to aflatoxin...
contamination (Granadas-Chinchilla, 2017). The isolation of aflatoxigenic fungi; *Aspergillus flavus* from poultry samples in this study is of great public health concern. Agricultural raw materials that are contaminated with fungi and used for the manufacture of poultry feeds may have adverse effects on the animal’s health and productivity (Mehrolia *et al.*, 2007).

Vakili *et al.* (2015) reported that the major nutrients of poultry feed that are considered for formulating diets are crude protein, moisture, crude fibre, calcium and phosphorus. Contamination of feeds by moulds may decrease nutritional value and affects animal health (Obiajuru *et al.*, 2015). The results from the proximate composition of the poultry feeds (starter, grower and finisher) are shown in Table 3. The protein content of the poultry feed samples ranged from 36.50±0.92 - 70.92±0.51%. Crude protein is one of the most important nutrients to quantify in a prospective feed, due to the fact that a deficiency of protein has a drastic effect on growth and production. Starter rations are generally higher in protein whereas grower and finisher feeds usually contain lesser protein, because old birds need less (Vakili *et al.*, 2015). The moisture content ranged from 2.70 - 8.60%. According to Vakili *et al.* (2015), the standard moisture content for poultry feeds ranges from 10.30-11.00%. A high moisture content can affect the shelf life of feeds, thereby making them unsuitable for long storage. However, the moisture content of the feeds analysed in this study were low, this could be the reason for the high level of fungi obtained in this study (Matthew *et al.*, 2017). The fat content ranged from 11.61±0.72 - 13.91±0.27%, which is greater than the recommended requirements by NRC (1994). A high fat content indicates a good source of energy to poultry. The carbohydrate contents of the sampled poultry feeds were high except for starter feeds. A high carbohydrate content indicates availability of energy in the feeds.

The important factors for optimal feed storage are suitable temperature and humidity, hygiene, a place to store feed away from pesticides, direct sunlight, water and damp (Sukmawati *et al.*, 2018). The temperature and relative humidity of storage ranged from 33.90°C - 35.20°C and 69% - 75% respectively. Mehrolia *et al.* (2007) reported that temperature and humidity above 30°C and 80-100% respectively are favourable for fungal growth. Ghaemmaghami *et al.* (2018) noted that many studies have suggested that the presence of *Aspergillus* might potentially lead to mycotoxin production when processes of storage and transportation are not appropriate.

**Table 1:** Fungal Count of Poultry Feeds Sold in Benin City

| Sample      | Fungal Count (× 10^5 cfu/g) |
|-------------|-----------------------------|
| A (Finisher E) | 0.10 ± 0.00                 |
| B (Grower F)   | 2.00 ± 0.00                 |
| C (Finisher F) | 0.60 ± 0.00                 |
| D (Starter F)  | 0.10 ± 0.00                 |
| E (Grower E)   | 8.50 ± 1.00                 |
| F (Starter E)  | 3.00 ± 2.00                 |
| G (Starter D)  | 1.00 ± 0.50                 |
| H (Starter C)  | 0.50 ± 0.00                 |
| I (Finisher C) | 0.20± 0.00                  |
| J (Finisher B) | 1.55 ± 0.50                 |
| K (Grower B)   | 1.55 ± 0.50                 |
| L (Grower C)   | 1.05 ± 0.50                 |
| M (Grower A)   | 0.75 ± 0.20                 |
| N (Finisher A) | 3.15± 1.50                  |
| O (Starter A)  | 0.65 ± 0.04                 |
Table 2: Percentage Occurrence of Fungal Isolates from Poultry Feed Samples.

| Organism             | Frequency | Percentage (%) |
|----------------------|-----------|----------------|
| *Mucor sp.*          | 3         | 12.50          |
| *Penicillium sp.*    | 1         | 4.17           |
| *Aspergillus niger*  | 2         | 8.33           |
| *Aspergillus flavus* | 4         | 16.66          |
| *Saccharomyces cerevisiae* | 12 | 50.00     |
| *Trichoderma sp.*    | 1         | 4.17           |
| *Rhizopus sp.*       | 1         | 4.17           |
| Total                | 24        | 100            |

Table 3: Proximate Quality of the Poultry Feeds Samples

| Sample | Ash (%) | Fat (%)  | Fibre (%) | Protein (%) | Moisture (%) | Carbohydrate (%) |
|--------|---------|----------|-----------|-------------|--------------|------------------|
| Starter| 3.69±0.32| 13.91±0.27| 3.53±0.04 | 70.92±0.51  | 4.30         | 3.65             |
| Grower | 4.70±0.33| 12.50±0.07| 8.45±0.16 | 41.00±0.61  | 8.60         | 27.71            |
| Finisher| 4.99±0.33| 11.16±0.72| 6.82±0.41 | 36.50±0.92  | 2.70         | 37.83            |

Table 4: Temperature and Relative Humidity of the Poultry Feed Stores

| Stores | Temperature (°C) | Relative Humidity (%) |
|--------|------------------|-----------------------|
| A      | 33.90            | 71                    |
| B      | 34.80            | 75                    |
| C      | 35.10            | 72                    |
| D      | 34.10            | 66                    |
| E      | 35.20            | 65                    |

CONCLUSION

Most of the fungi isolated from this study are potentially toxigenic, making it necessary to establish quality control measures that will be adhered to during formulation, packaging, transport, storage and use. There is need to store feeds properly to avoid moisture uptake and damage by heat. Feeds should be stored suitably where it cannot be attacked by microorganisms, insects and rodents. Good manufacturing practice, handling and retailing methods should be improved to enhance the microbial quality of these products. It is highly recommended that government and public health organizations should raise awareness on poultry diseases that could be contracted from the consumption of contaminated feeds.
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