Fungal Contaminants of Feed, Litter and Faecal Dropping Samples of Poultry Birds, their Occurrence and Distribution in Selected Farms in Anambra State, Nigeria

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Abstract: In Nigeria, fungi have been causing high mortality and production loses in the poultry industry. This study aims at quantifying the fungal organisms in feed, litter and birds droppings, and their occurrence and distribution in selected poultry farms in Anambra State, Nigeria. The samples were collected from 10 poultry farms in six local government areas in Anambra State, Nigeria. One gram (1 g) of each sample was serially diluted ten-fold and 0.1 ml of 10^{-4} dilution spread inoculated on Sabouraud dextrose agar (SDA) plates supplemented with 0.05 mg/ml chloramphenicol. After incubation for 3-6 days at 25^0C, fungal load was determined and the isolates characterized using macroscopic, microscopic and genetic features. The range of fungal load of the samples in the farm with floor system was 8.50×10^3 to 2.40×10^5 sfu/g, while that of cage system was 6.0×10^3 to 2.10×10^5 sfu/g. The fungi recovered during the rainy season included *Aspergillus* (40.0%), *Leichthemia* (10.0%), *Paecillomyces* (11.49%), *Penicillum* (10.61%), *Acremonium* (3.89%), *Fusarium* (1.61%), *Chrysonilia* (0.60%) and yeast (21.08%). Those of the dry season were *Aspergillus* (19.16%), yeast (30.75%), *Curvularia* (12.28%), *Penicillum* (9.48%), *Fusarium* (3.75%), *Cunninghamella* (3.33%), *Trichoderma* (4.96%), *Nathrasia* (4.34%), *Syncephalis* (4.16%), *Aureobasidium* (4.59%), *Scopulariopsis* (2.98%). No seasonal effect on fungal loads of the samples from various farms, but significant difference ($p<0.05$) was observed in the occurrence and distribution of the isolates. Fungal quantification of the samples and species identification are essential in the evaluation of potential health risk of workers in the poultry farms.

Key word: Poultry farms, birds, fungal isolates, dry season, rainy season.

INTRODUCTION

Over the last few years, the consumption of animal-source food has led to the intensification of livestock production systems (Rushton and Bruce, 2017; Gomes *et al*., 2022) and globally, poultry production became an important aspect of the animal agriculture (Dei, 2021). In addition, their meat and eggs are cherished worldwide (Dei, 2021) and because of their popularity, irrespective of culture and religion, poultry and poultry products are demanded and consumed in various forms. Poultry products are very important in human nutrition and health, serving as source of proteins, providing essential micronutrients such as vitamin A, B12, riboflavin, calcium, iron and zinc (Dei, 2021). Poultry farming contributes significantly to poverty alleviation by providing employment opportunities to Nigerians (Ogbebor *et al*., 2021) and according to Ajala *et al*. (2012), poultry keeping stands as resource that can generate employment for millions of Nigerian youths in rural and peri-urban areas as entrepreneurs, skilled and unskilled workers. Consumers' perspectives on the quality and safety of animal products are a continuous issue for the poultry industry and its strategic future (Hafez and Attia, 2020). Mycotic diseases have continued to cause havoc in the poultry industry and some of them cause direct harm to human health due to their zoonotic implication (Dhama *et al*., 2013). Fungi in the genus *Aspergillus, Curvularia, Fusarium, Cunninghamella, Leichthemia, Paecillomyces, Penicillium, Acremonium, Syncephalis, Chrysonilia, Alternaria, Trichoderma, Nathrasia, Syncephalis, Aureobasidium, Scopulariopsis* and *Stachybotrys* among others, have been implicated in a number of diseases in poultry causing onychomycosis, keratitis, endophthalmitis (Ferrer *et al*., 2005), mycetoma (Fleming *et al*., 2002; Potekhina

et al., 2023), gastroenteritis, mucormycosis, dermatitis and pulmonary infection such as aspergillosis. These organisms are ubiquitous and are naturally present in the environment. They are saprophytic and parasitic in nature and multiply mainly in feed, litter and droppings, producing spores that act as source of infection and bioaccumulation. The mixture of these bedding materials (litter), chicken excrement (droppings), and feathers seems to play an important role in pathogen development, which may contribute to potential risk of zoonosis and spreading of the disease through the food chain (Gomes *et al*., 2022).

Chickens and poultry may also become infected during hatching as a result of inhalation of large amount of spores, which may be present in heavily contaminated hatching machines or from environmental litters. In older birds infection can be caused by inhalation of spore from contaminated litter, feed, dried droppings or dust range area (Asfaw and Dawit, 2017). It is also possible that persistent exposure of the farm workers to these fungal agents and direct contact with infected birds increases the risk of other infections like dermatomycoses, otomyosis and onychomycoses (Mba, *et al.*, 2020).

Feed contamination by these fungi can occur during processing of the raw materials, transportation, storage or even within the poultry house. The chance of fungal contamination is also increased with increase in environmental factors such as moisture and temperature (Mgbeahuruike *et al*., 2023). Therefore, fungi presence in feed, destroy the organoleptic and nutritive values of the feed which become unavailable for the birds (Chattopadhyay, 2014; Mgbeahuruike *et al*., 2023). This may lead to decline in birds' health, meat and egg production.

Litter has also been reported as the major contributory factor to fungal contamination in poultry farms (Viegas *et al*., 2012). Studies have shown that spreading of litter is one of the tasks that exposes poultry workers and birds to high level of dust (Whyte, 2010) and fungi and their metabolites, including volatile organic compounds (VOC) and mycotoxins (Viegas *et al*., 2012). Fungal dissemination through litter has also been reported as one of the major problems in the industry as it favours fungal growth (Arne *et al*., 2011; Ostovic *et al*., 2021), therefore, needs to be monitored continuously, in order to control fungal contamination.

Hasan and Al-Temimay (2016), studies on droppings and fungal contamination, carried out in Baghdad, Iraq, stated that the most serious health risks on human comes either from direct contact or inhalation of the fungal spores of the infectious organisms, which grow in the nutrient-rich accumulations of bird droppings. Easy dispersal of the spores by wind makes the fungi airborne, causing diseases in humans. The association between dried droppings and the isolation of fungi was first described by Soltani *et al.* (2013). Birds and their droppings can carry over 60 other disease causing fungal agents; many of which are airborne and can be transferred to humans just by being around them (Zarrin *et al*., 2000). *Rhizomucor variabilis* isolated from bird's droppings have been implicated in serious health issues. It has been reported as a rare mycotic agent in humans causing progressive destruction of the nasal septum and soft and hard palate, leading to collapse of the nose bridge and an ulcerative gaping hole (Hemashettar *et al.,* 2011). *Aspergillus* species can affect any part of the body causing different diseases and symptoms. They mainly affect the respiratory tract, due to their sizes, causing aspergillosis which can manifest as allergic bronchopulmonary aspergillosis, aspergilloma (fungus ball), chronic pulmonary aspergillosis and invasive aspergillosis (Harman and Soo Hoo, 2021).

Most fungal diseases of poultry occur sporadically but sometimes may occur in the form of an outbreak (Asfaw and Dawit, 2017). Seasonal variation plays important role in the spread of their infections. Predominance of infection in closed housing system during summer and the presence of

fungi in the poultry litter material during autumn make the eradication difficult (Solima *et al*., 2012; Asfaw and Dawit, 2017). The aim of this research is to identify the fungal contaminants in feed, litter and dropping samples of poultry birds and their occurrence and distribution in selected farms in Anambra State, Nigeria.

MATERIALS AND METHODS

Description of Study Area: A large scale study was carried out on poultry feeds, litters and droppings, in 10 selected poultry farms located in six Local Government Area of Anambra State, Southeast, Nigeria (Latitude 6º 20'N and Longitude 7º 00'E). These include Apkaka farm in Umuoji, Jospan Farm in Nkpor, Osakwe farm in Ogidi, Cyroby farm in Ogidi , Volant farm in Ogidi, all in Idemili L. G. A.; Agroventures farm in Nnewi North L. G. A,; Government farm in Onitsha South L. G. A.; Takilita farm in Awka South L. G. A.; EM farm in Oyi L. G. A. and Eagle farm in Aguata L.G.A, all in Anambra State, Nigeria. The study was from October, 2014 to September, 2015. The farms housing approximately 227,200 laying birds were examined. Six out of the 10 farms practiced open (floor) housing system, while the other four practiced closed (cage) housing system.

Collection of Environmental Samples: A total of 300 samples, made up of 100 samples each of feed, litter and droppings were randomly and aseptically collected from ten poultry farms, located in the six Local Government Areas in Anambra state, Nigeria during the dry and rainy season. All samples labeled with the appropriate information (name and location of farm, source of sample, collection time and date) and placed in sampling packets, were transferred to Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. These samples were collected twice a week and the seasonal variations of the fungal loads were determined using standard methods.

Enumeration and Isolation of Fungal Organisms in the Environmental Samples: A 10-fold serial dilution of the environmental sample was prepared in sterile test tubes. One milliliter (1 ml) of 10^{-4} dilution of the sample was inoculated on Sabouraud dextrose agar (SDA) plates, supplemented with 0.05 mg/ml of chloramphenicol. After 3-6 days incubation at 25ºC, the plates were observed for fungal growth (Krnjaja *et al*., 2014; Parvan and Manjunath, 2014). The fungal isolates were counted and pure isolates sub-cultured on SDA slant and stored at 4ºC.

Identification of Fungal Isolates: The fungal isolates were identified based on detailed studies of their macroscopic, microscopic and genetic features (De Hoog, 2000; Diba *et al*., 2007; Nadia *et al*., 2017). Some of the isolates were sent to Macrogen, Europe, Meibergdreef Amsterdam Netherlands, for proper identification and confirmation

Frequency of the Fungal Isolates from Environmental Samples: The percentage frequency of the fungal isolates from the environmental samples was determined. This was calculated using the formula:

$$\text{Percentage frequency (\%)} = \frac{\text{Frequency of occurrence}}{\text{Total number of occurrence}} \times 100$$

Statistical Analysis*:* The results and data obtained from the questionnaires distributed to the poultry farm workers were statistically analyzed using One-way Analysis of Variance (ANOVA) using SPSS version of 21 Software. Values were considered significant if $p < 0.05$.

Consent: Sample collection and filling of questionnaires were voluntarily done on the site of the farm by the workers, with the permission of the management of the farms.

RESULTS

Identification of Fungal Isolates from the Environmental Samples during the Rainy and Dry Seasons: A total of 15 genera of fungal isolates were recovered from feed, lither and droppings from the poultry farms during the rainy and dry seasons. Table 1, shows the distribution of fungal genera

isolated during the rainy and dry seasons. As observed in Table 1, *Aspergillus* spp., yeasts, *Penicillium* sp. and *Fusarium* sp. were isolated during rainy and dry seasons. Others were isolated at different seasons. *Aspergillus* spp. and yeast were the most abundant isolates in all the samples examined.

Table 1: Fungal genera isolated from feed, lither and droppings during rainy and dry seasons

Fungal Genera	Rainy season	Dry season
Aspergillus spp.	+	+
Yeast	+	+
Penicillium sp.	+	+
Fusarium sp.	+	+
Lichtheimia sp.	+	-
Paecelomyces sp.	+	-
Acremonium sp.	+	-
Chrysonilia sp.	+	-
Curvularia sp.	-	+
Cunnighamella sp.	-	+
Syncephalis sp.	-	+
Aureobasidium sp.	-	+
Nathrasia sp.	-	+
Trichoderma sp.	-	+
Scopulariopsis sp.	-	+

Key + = Positive – Present, - = Negative –Absent

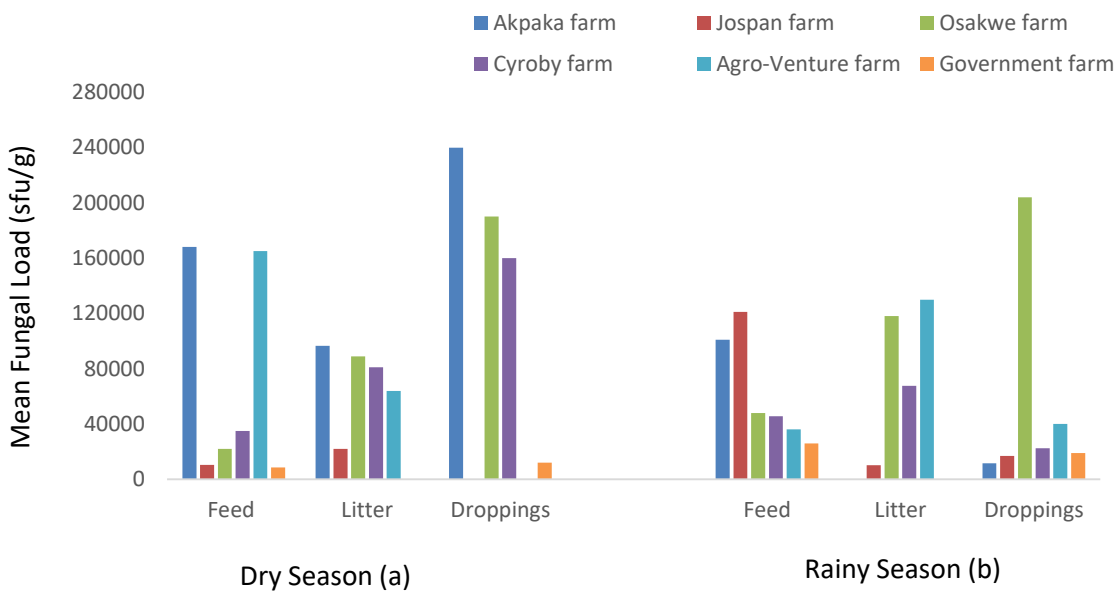

Figure 1: Mean fungal load of environmental samples from six poultry farms with floor/open system during (a) dry season (b) rainy season

Enumeration and Isolation of Fungal Organisms in the Environmental Samples: The mean fungal loads obtained from the different environmental samples during dry and rainy season in the farms that practiced floor/open system and caged/ close system are shown in Figures 1a and 1b respectively. In the six farms from three local governments that practiced floor/open system during the dry season, the fungal load in feed samples showed a mycological counts ranging from 8.50 x 10^3 – 1.68 x 10^5 sfu/g, while the litter sample has a range from 2.20 x 10^4 sfu/g - 9.65 x 10^4 sfu/g . The fungal load for the droppings samples range from 1.20 x 10^4 sfu/g – numbers too numerous to count (TNTC) (Figure 1a). During the rainy season, the mean fungal

load for the feed range from 2.60 x 10^4 – 1.21 x 10^5 sfu/g, while that of litter showed a range of 1.10 x 10^4 sfu/g – number too numerous to count (TNTC). For the droppings, the range observed was 1.15 x 10^4 – 2.04 x 10^5 sfu/g (Figure1a).

For the four farms in four local governments that practiced the cage/closed system during the dry season, the mean fungal load in feed samples range from 9.50 x 10^3 – 2.10 x10^5 sfu/g, while that of droppings was from 1.10 x 10^4 – 1.70 x 10^4 sfu/g (Figure 1b). The fungal load for feed samples in farms that have the caged / close system, during the rainy season, range from 9.50 x 10^3 - 2.10 x 10^5 sfu/g. The droppings samples have a range of 1.50 x 10^4 - 4.50 x 10^4 sfu/g.

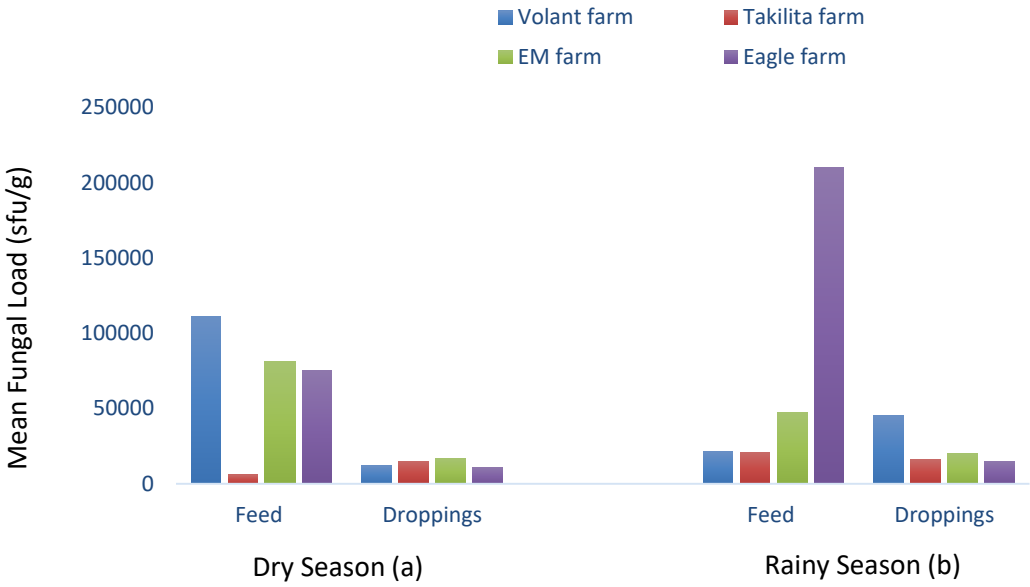

Figure 1b: Mean fungal load of environmental samples from four poultry farms with caged/closed system during (a) dry season (b) rainy season

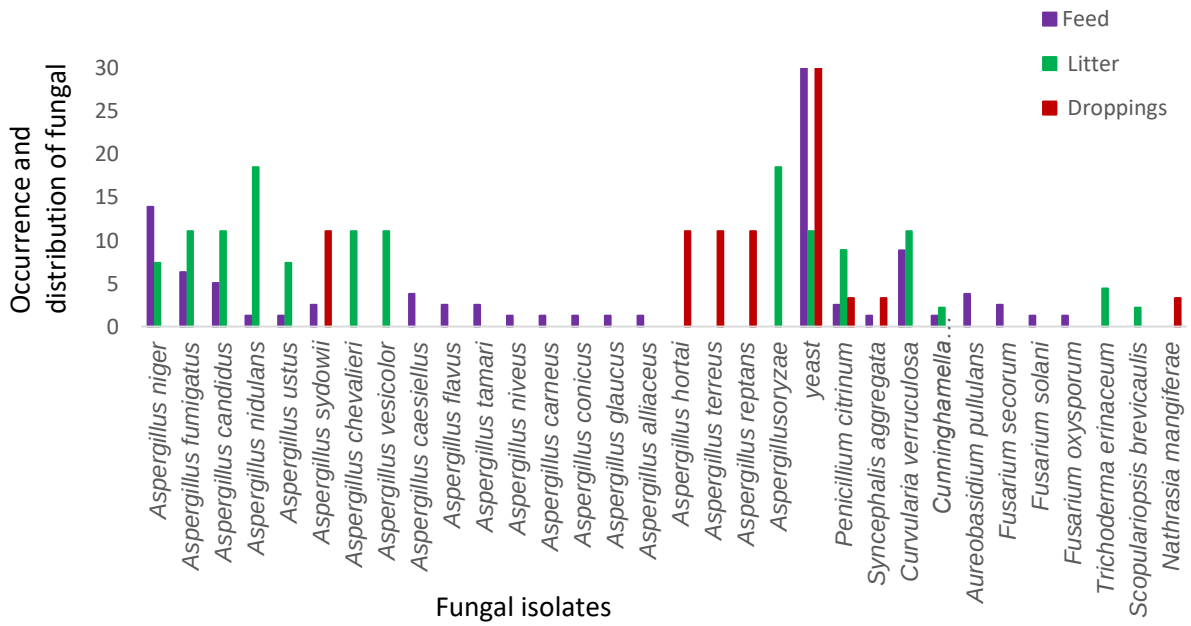

Figure 2a: Occurrence and distribution of fungal isolates in environmental samples during dry season

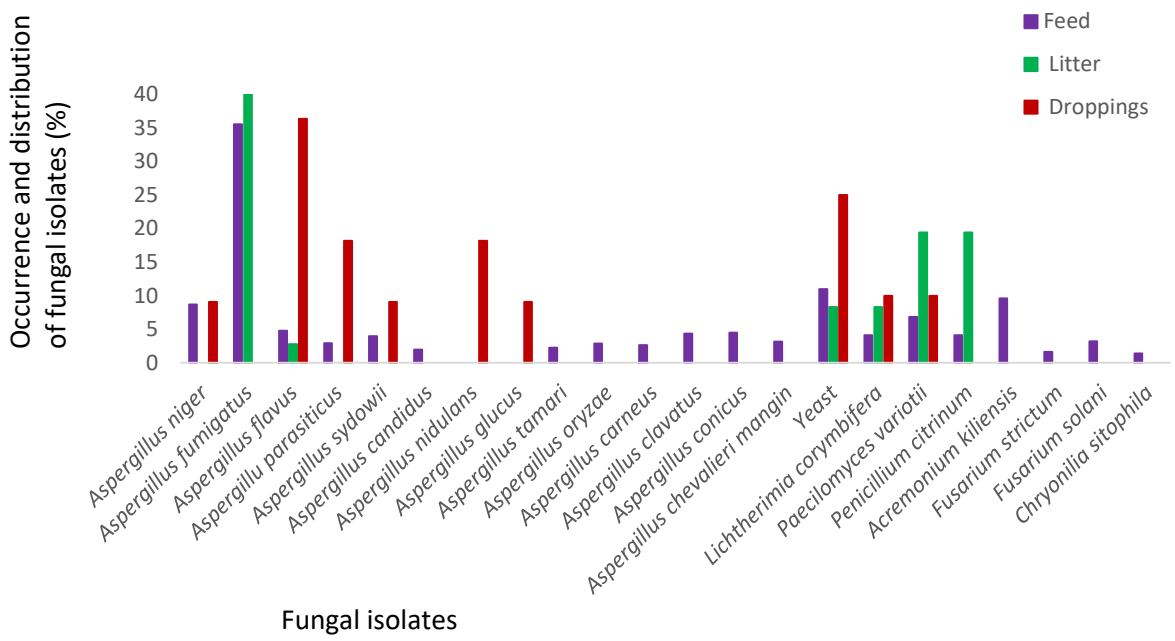

Figure 2b: Occurrence and distribution of fungal isolates in environmental samples during rainy season

Occurrence and Distribution of Fungal Isolates in Environmental Samples during Dry and Rainy Season: The occurrence and distribution of fungal isolates in feed, litter and faecal droppings from poultry farms is presented in Figure 2a and Figure 2b. During the dry season, the most prevalent organism was yeast (Figure 2a), while *Aspergillus* species was the most prevalent during the rainy season (Figure 2b).

DISCUSSION

In this study, it was observed that all the environmental samples were overly contaminated by fungi, although to a variable extent**.** Fungal counts is an indicator of the quality of feed. The fungal counts of feed above 30,000 sfu/g for young animals or 50,000 sfu/g for older ones, do not meet the hygienic quality of feed, therefore, not good for animal consumption (Krinjaja *et al*., 2014). Based on this, it was observed that only 4 feed samples out of 6 studied, were good for animal consumption in the floor house/open system farms, during the dry and rainy seasons. Similar observation was recorded in the cage house/closed system but only 3 out of the studied feed samples were good for animal consumption during the dry season and 4 out of the feed samples during the rainy season.

From the records of International Organization for Standardization in 2008, total fungal counts of food and animal feed should be below 1.0 x 10^4 sfu/g (Parviz *et al*., 2014). In line with this, only one feed sample (8.5 x 10^3 sfu/g) from the farm that practiced floor housing system (Figure 1a) and one feed sample (6 x 10^3 sfu/g) from farm that practiced cage housing system (Figure 1b), had acceptable fungal load during rainy season. On the contrary and based on the European accepted standard for finished poultry feed of 1 x10^3 sfu/g (Paterson, 2017, Anifowose and Bakre, 2021), the fungal load in the feed samples studied, exceeded the standard limit, and therefore, cannot be accepted for use in the poultry farms. The presence of these fungi could affect the taste, reduce the nutrient absorption and feed quality which could affect the performance of the birds.

It was observed that high fungal loads occurred in feed samples of some farms in dry and rainy seasons for both floor and

caged housing systems in this study. Contrary to the observation of high fungal load in both seasons, Aliyu *et al*. (2012), reported that fungal load in feed samples usually occur during rainy season. Similarly, WHO (2004), also reported high level of fungal load during rainy season (80 – 90%) than during dry season (50 – 70%). Ghaemmaghani *et al.* (2016), suggested that these fungal contaminants may have come from production activities within the farms, quality of raw materials, hygienic processes, lack of proper drying, transportation and inappropriate storage silage and climate conditions. Parviz *et al*. (2014), reported that feed contamination can result from different facilities and failure to comply with health codes, However, Kabak *et al*. (2006) and Murphy *et al.* (2006), noted that these fungal contaminations may be retained at various points along the production line, contaminating successive batches of meal as they are processed.

Litter fungal quantification and species identification have important implications in the evaluation of potential adverse health risk to exposed workers and animals (Viegas *et al*., 2012a). The fungal counts of the litter samples in this study range from 2.20×10^4 – 1.30×10^5 sfu/g in both rainy and dry seasons. This observation supports the work of Ostovic *et al*. (2021), who reported that litter fungi concentration range from 10^2 – 10^5 sfu/g. The presence of different level of fungi contaminants observed in this study also supported the work of Heshmatollah *et al*. (2009). The authors reported that various litter materials used in poultry farms can have diverged fungi species and counts. Litters were not sampled in farms that practiced cage housing system as the birds are kept in cages. Studies have shown that biodiversity and fungi load of litter depends on material choice, litter aging and handling techniques (Arne *et al*., 2011).

Mycological examination of bird droppings shows a fungal load range 1.10×10^4 – 2.40×10^5 sfu/g for both floor and cage housing system and during rainy and dry seasons. A similar trend was observed in the report of Adegunloye and Adejumo (2014), who detected high fungal loads which ranged from 12.38×10^5 – 28.05×10^5 sfu/g and 10.60×10^5 – 34.09×10^5 sfu/g for turkey and duck feaces respectively. The high fungal load in poultry droppings observed in this study, may have given rise to the fungal load in litter samples. This finding supports the work of Adegunloye and Adejumo (2014), who also reported possible cross contamination of air, water, feed and the environment from the bird droppings.

The following fungal genera were isolated from feed, litters and droppings: *Aspergillus* spp., yeast, *Penicillium* sp., *Fusarium* spp., *Lichthermia* sp., *Paecelomyces* spp., *Acremonium* sp., *Chrysonilia* sp., *Curvularia* sp., *Cunninghamella* sp., *Syncephalis* sp., *Aureobasidium* sp., *Nathrasia* sp., *Trichoderma* sp. and *Scopulariopsis* sp. The fungal genera isolated from feed samples in both rainy and dry seasons include *Aspergillus* spp., yeast, *Lichthermia corymbifera, Paecelomyces variotii, Penicillium citrinum, Acremonium kiliensis, Fusarium strictum, Fusarium solani, Chrysonilia sitophila, Syncephalis aggregate, Curvularia verruculosa, Cunninghamella bertholletiae, Aureobasidium pullulans, Fusarium secorum and Fusarium oxysporum.*

Ghaemmaghani *et al.* (2016), showed that the organisms commonly isolated from poultry feeds are *Aspergillus* species, *Fusarium* species, *Penicillium* species, *Mucor, Scopulariopsis* species and yeast. Most of the isolates except *Scopulariopsis*, *Mucor* and *Rhizopus* were recovered in this study. Saliyo *et al*. (2014), isolated different genera of fungi from poultry feed with *Aspergillus* species, *Penicillium* species and *Fusarium* species, having the highest occurrence.

In a study by Khahfa *et al*. (2022), *Aspergillus* species, *Acremonium* species, *Fusarium* species *Paecilomyces* species, *Lichtheimia* species and *Penicillium* species, among other fungal isolates were observed in the sampled feed. This finding is supported by the results obtained in this

study. The isolation of *Aspergillus* species as the most prevalent fungal organism recovered from the feed samples in this study supports the works of Anifowose and Akindele (2021) and Oyekemi *et al*, (2022). The authors separately reported *Aspergillus* species as the most prevalent fungi in feed samples. Ghaemmaghami *et al.* (2016), reported that the prevalence of *Aspergillus* species in feed samples is caused by the widely distributed nature of the spores. Anifowose and Akindele (2021), however, stated that inappropriate disposal of waste, lack of access to hygienic water supply as well as insufficient exposure to heat during production, may likely increase the chances of fungal contamination in finished products. Bedding material is an important requirement of floor based poultry production system to meet the health and welfare requirements (Munir *et al*., 2019). The organisms isolated from the litter samples in both rainy and dry seasons included *Aspergillus* spp., yeast, *Penicillium citrinum, Curvularia verruculosa, Cunninghamella bertholetiae, Trichoderma erinaceum, Scopulariopsis brevicaulis, Lichtheimia corymbifera* and *Paecilomyces variotii*. The finding is in line with the work of Chuwang *et al*. (2013), who observed that poultry litter presents a conducive environment for fungal growth and reported *Mucor* species, *Penicillium notatum* and *Aspergillus* species as the most encountered organisms. However, in this study a different *Penicillium* species was isolated, and *Cunninghamella bertholletiae* (Mucor) was the least isolated. The findings obtained in this study agrees with the report of Dalcero *et al*. (1998) and Arne *et al*. (2011) who isolated *Aspergillus* and *Penicillium* species as the major contaminants in the litter samples.

Regarding fungal assessment in litter samples, Gomes *et al*. (2022) observed that *Penicillium* species was the most dominant, while *Scopulariopsis* species and *Aspergillus* species were equally prevalent. The Viegas *et al*. (2012a), suggested that these findings need to be taken into consideration since some detected fungal species are considered potential agents of infection to both humans and animals.

Microbial analysis of birds droppings showed the presence of *Aspergillus* species, *Penicillium citrinum, Syncephalis aggregate, Nathrasia mangiferae,* yeast during the dry season and *Aspergillus* species, *Lichtheimia corymbifera, Paecilomyces variotii,* yeast during the rainy season. The result obtained in this study is in agreement with the findings of Mendes *et al*. (2014), who isolated *Aspergillus* species, *Penicillium* and yeast from bird droppings. The authors also isolated *Malassezia pachydermatis*, *Trichosporon ashir* and *Geotrichum klebalnii*, which were not observed in this study. The organisms recovered are similar to those isolated by Maryam *et al*. (2013), with *Penicillium* species as the most prevalent isolate but yeast as the most prevalent fungal isolate in this study. Labuda and Tancinova (2006) and Abo-Shama (2015) reported that *Eurotium* spp., *Fusarium* spp. and *Aspergillus flavus* were wide spread throughout the samples of birds droppings. In this study, *Fusarium* spp. was not isolated from birds droppings, and as suggested by Dennis and Gee (1973) and Arne *et al*. (2011), may have resulted from the age of the litter being sampled.

Statistically, no significant difference was observed in the fungal load of the feed samples during rainy and dry season ($p>0.05$). This implies that seasonal variation has no significant influence on the fungal load within the sampled feeds, which may have been due to pre or post –harvest contamination of feed ingredients, bad manufacturing processes, contamination of the feed by the handlers in the farm and bad storage facilities in the farm among others.

In the case of litter samples, there was no significant difference ($p>0.05$) in fungal counts between rainy and dry seasons. This findings corroborates the report of Ostovic *et al*. (2021), who found no significant difference between the summer and winter periods. The authors noted that fungi

concentrations highly correlated with litter moisture and pH, which did not significantly differ between the seasons.

CONCLUSION

Fungal contamination of feed, litter and faecal dropping, showed high fungal loads in all the poultry farms investigated, however, no seasonal effect was observed. The occurrence and distribution of the fungal isolates showed seasonal variations, with *Aspergillus* species and yeast being the most prevalent organisms. It is important to note that most of these fungi have been implicated in fungal infections. There is, therefore, need to evaluate their potential adverse health risk to exposed workers and animals in the poultry farms.

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