MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Food Science and Technology (ISSN: 2354-2527) Vol. 5(1) pp. 001-005, July, 2020
Available online http://www.meritresearchjournals.org/fst/index.htm

DOI: 10.5281/zenodo.3967805

Original Research Article

Saprotrophs Associated with Poultry Feeds of South - Eastern Nigeria

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Abstract

Poultry feeds sold in South Eastern Nigerian States were screened for the presence of saprotrophs. The aim was to ascertain the mycopopulation in the poultry diet. Samples of Starters, Growers, Layers and Finishers mash sold in South- Eastern Nigeria States of Abia, Anambra, Ebonyi, Enugu and lmo States were used in the study. Standard methods of isolation, characterization and identification, involving cultural media, microscopy and statistical analysis of the occurrence frequencies of the isolates were employed in the evaluation. The results showed the presence of *Aspergillus flavus, A. niger, Penicillium* sp, *Rhizopus* sp and Yeast in the test feed samples. In the starters mash, *A. flavus* occurrence range from 20% in Abia state to 40% in Anambara state. In growers mash, the occurrence range from 20% in Enugu state to 40% in Abia. In layers mash, it ranges between 20% in Ebonyi state and 40% in Enugu. In the finishers mash, the range is between 20% in Anambara and 80% in Abia. *A niger*, starters mash, range from 40% in Imo state to 100% in Abia. Growers mash, 20% in Imo to 80% in Ebonyi, 40% in Anambara to 60% in the rest of the states. Layers mash, 60% in Abia, Enugu and Imo to 100% in Anambara and Ebonyi respectively. *Penicillium* spp, starters mash, 20% in Abia, Anambara and Ebonyi. Growers mash, 20% in Enugu and Imo and 40% in the rest of the states. Layers mash, the range is from 20% in Abia to 80% in Ebonyi. Finishers mash, 20% in Enugu and Ebonyi to 60% in Abia. *Rhizopus* spp in starters mash is 20% in Ebonyi and Enugu. Growers mash, 20% in Anambra and 40% in Ebonyi and Enugu. Layers mash, 20% in Abia and Enugu and 40% in Imo. Finishers mash, it is 20% in Imo and 40% in the rest of the states. Yeast in starters mash range between 60% in Imo to 100% in Abia. Growers mash, 40% in Anambara to 100% in Enugu. Layers, 60% in Enugu and Imo to 100% in Abia, Anambara and Ebonyi. Finishers mash, 60% in Abia, Anambara and Imo state and 100% in Ebonyi and Enugu states. The incidence of these deteriorative fungi in feed products and trophic levels is a serious danger, considering mycotoxicosis and other associated health hazards

Keywords: Feed products, Health hazard, Mycopopulation, Mycotoxicosis, trophic levels

INTRODUCTION

Mycoflora of a microhabitat, usually refer to the population of fungi in a unique environmental unit.

Fungi are usually multicellular eukaryotic organisms that are heterotrophs. In other words, they are saprotrophs that play important role in nutrient recycling in our ecosystem.

Mycotoxigenic fungi are those fungi with genetic potentials to synthesize and exude mycotoxin and other xenobiotics in foods and feed before and after harvest (Bondy and Peska, 2000). Some of the fungi that have

genetic potentials to produce the toxins include: *Aspergillus flavus* and *A.parasiticus.* These toxins are known to cause both acute and chronic toxicity in humans and many other animals.

When lactating mammals ingest fungal contaminated foods or feeds, the toxic metabolites can be found in their milk as mycotoxins such as Aflatoxin M1 (AFM_1) and (AFM_2)' Aflatoxigenic fungi can contaminate wide range of food commodities such as cereals, oil seeds, nuts, dried fruits, black pepper, cheese, dairy products etc.

Prior to Aflatoxin contamination, food must first be infested with aflatoxigenic fungi with inherent capability to produce and deposit the toxins in the food commodities (Adebayo and Ettah, 2010).

A strain of mold may have genetic potentials to produce toxins but the level of production depends on the environmental conditions such as, availability of nutrients, temperature, moisture content etc. (Amadi and Adeniyi, 2009).

Nigerian animals are exposed to mycotoxins by feeding them with mycotoxigenic fungal infected rations or domestic wastes. Humans are in turn exposed to the toxins by feeding on the poisoned meat from such animals like pigs, chickens, rabbit etc. and food products (Njobeh *et al.,* 2009).

Mycotoxin exposure has high health and socio-economic implications. The AFB 1 are naturally strong carcinogens, hence have raised investigations by medical professionals. Infestation of mycotoxigenic fungi can lead to lungs cancer, immune suppression, kwashiorkor and impaired reproduction (Oyelamin and Ogunleshi, 2007).

In view of the fact that these toxins get into human and livestock through ingestion of contaminated rations, this study therefore, aims at generating information about poultry livestock feeds, in relation to the sources and transmissions of mycotoxin through screening of the four major livestock feed types for possible mycoflora including mycotoxigenic fungal species.

MATERIALS AND METHODS

Sample collection

Starters, Growers, Finishers and Layers marsh, were collected from the artisan stores of the study area. The study area spans the geopolitical zones of the South-Eastern Nigeria comprising of: Abia, Anambra, Ebonyi, Enugu and Imo states.

Eastern Nigeria is situated between latitude 4.0S'N and longitude 7.30^{o}E within the tropical rain forest guinea savannah zones of Nigeria. The samples were collected in sterile polythene bags and taken to the laboratory at Research and Development microbiology unit, Umuahia, Abia state where the assay was conducted.

Media Preparation and Sterilization

The media used in the culture were Sabouraud Dextrose Agar (SDA). The medium was prepared according to the manufacturers' instructions. The melted and autoclaved media were allowed to cool down to about 45^{0}C and 20ml were poured into sterile petri dishes and allowed to solidify.

Sample Preparation and Inoculation

The feed sample was homogenized by grinding into powder using ceramic pestle and mortar. One gram (1 g) of the pulverized sample was mixed with 10ml of sterile distilled water (stock solution) followed by serial dilution up to 10-^{6}. Aseptically, 0.lml sample was collected from each tube and plated out by spread and streak plate methods respectively (Chesbrough, 2005). The triplicate of each tube sample were labeled and incubated at room temperature for 7 to 14 days. The plates were examined daily for growth and plates with growth were sub-cultured.

Identification characterization of fungal isolates

The methods described by the International Commission of Microbiological Specification of Food (ICMSF, 2002), was used. The pure culture obtained from each sub-culture was used for characterization and subsequent identifications. The nomenclatural determination of the organisms were based on the cultural characteristics and micro morphological features of the isolates, using standard manuals (ICMSF, 2002)

Microscopic Examination

Wet preparation of ground and serially diluted sample was made on a clean grease free slide by putting one or two drops of sterile distilled water on the slide and emulsified with a loopful of the suspension and viewed under the microscope. Slide preparation for the plated culture was done by first observing the colonial characteristics. A drop of the mounting medium, (Lacto phenol cotton blue) was put on the slide. Using flamed or sterile teasing needle, small portion of the colony was removed from the margin together with a very thin layer of agar surface, if the colony is not thick and woolly. The portion of the colony was emulsified with the mountants. With another sterile needle, the filaments were spread on the slide. Coverslip was lowered into the slide and viewed under the microscope with x40 objective. Spores and hyphae were observed.

Table 1. Percentage (%) Occurrence of Fungal Isolates in Starter Mash

Location	Fungal Isolates				
	Yeast	***A. Flavus***	***A. Niger***	***Penicillium***	***Rhizopus***
Abia	100	20	100	20	0
Anambra	100	40	80	20	0
Ebonyi	80	20	60	20	20
Enugu	60	0	40	0	20
Imo	60	0	40	0	0
X%	80%	16%	64%	12%	8%

Table 2. Percentage (%) Occurrence of Fungal Isolates in theGrowers Mash

Location	Fungal Isolates				
	Yeast	***A. Flavus***	***A. Niger***	***Penicillium***	***Rhizopus***
Abia	80	40	60	40	0
Anambra	40	0	40	40	20
Ebonyi	60	0	80	40	40
Enugu	100	20	60	20	40
Imo	60	0	20	20	0
X%	68%	12%	52%	32%	8%

Table 3. Percentage (%) Occurrence of Fungal Isolates in the Layers Mash

Location	Fungal Isolates				
	Yeast	***A. Flavus***	***A. Niger***	***Penicillium***	***Rhizopus***
Abia	100	0	60	20	20
Anambra	100	0	40	20	0
Ebonyi	100	20	60	80	0
Enugu	60	40	60	40	20
Imo	60	20	60	40	40
X%	85%	16%	56%	40%	16%

Determination of fungal loads in the test samples

The analysis was carried out in accordance with method described by Fawole and Oso, (2005). From the established growth, the number of fungi colonies in each of the three plates was counted using microbial colony counter. The formula below was used to calculate the fungi loads. It was expressed as the total viable count showing the number of colony forming unit per feed in solid form or liquid form per gram of test samples. Discrete colonies were sub-cultured in separate sterile SDA or petri plates. Fungi load was calculated following Fawole and Oso (2005) thus;

Fungi load (cfu/ml/g = 1/s x N x D,

Where

S= amount of sample collected from serially diluted feed sample.

N = number of colonies counted from the plates

D= the dilution factor.

RESULTS AND DISCUSSION

The percentage occurrence of the mycoflora in the test feed samples are shown in Tables: 1, 2, 3, 4

The occurrence of the aflatoxigenic fungi *Aspergillus flavus* within the samples from the feed types in the study area showed that Starters contamination is 16%, Growers 12%, Layers 16% and Finishers 48%. The range showed that the fungal infestation range from the lowest 12% in Growers mash to the highest 48% in the Finishers mash (Tables 2 and 4).

In the Sabouraud Dextrose Agar culture the isolates identified as *Aspergillus flavus* appeared as greenish-yellow mesh work and progressed to dark-green as the culture gets older. These cultural characteristics concur with the observations of Duru and Anyadoh. (2009) on cultural features of *A. flavus as* a rot pathogenic fungi. The microscopic features of the same organism showed a highly filamentous mycelia with septate hyphae, hyaline and non septate conidiophore. The conidia were ornamented with spines and occur in chains. These micro morphological structures were also akin with the features described by Bankole and Mabekoje (2004) and Duru *et al*. (2012).

The mean fungal loads range from 2.5 to 4.3 in Starters mash, 2.7 to 6.1 in Growers mash, 3.5 to 5.8 in Layers mash and 3.8 to 8.8 in Finishers mash (Table 5).

Table 4. Percentage (%) Occurrence of Fungal Isolates in the Finisher Mash

Location	Fungal Isolates				
	Yeast	*A. Flavus*	*A. Niger*	Penicillium	Rhizopus
Abia	60	80	60	40	40
Anambra	60	20	100	40	40
Ebonyi	100	60	100	40	40
Enugu	100	40	60	40	40
Imo	60	40	60	20	20
X%	76%	48%	36%	36%	36%

Table 5. Statistical Analysis of the Fungi Loads of Feeds Marketed In South East

States	Starter	Grower	layer	Finisher	ANOVA F-ratio
Abia	$3.6\pm O.77^{b}$	$4.3\pm O.84^{b}$	$3.9\pm O.44^{b}$	5.5 ± 1.77^{b}	2.859^{n5}
Anambra	$3.5\pm O.67^{b}$	6.1 ± 1.81^{b}	$3.5\pm O.22^{b}$	4.3 ± 0.96^{b}	***6.208
Ebonyi	3.8 ± 1.33^{b}	4.2 ± 1.09^{b}	4.4 ± 1.04^{b}	6.8 ± 1.97^{b}	***4.600
Enugu	2.5 ± 0.22^{b}	5.4 ± 0.70^{b}	4.3 ± 1.24^{b}	8.8 ± 1.59^{b}	***29.958
Imo	4.3 ± 1.35^{b}	2.7 ± 0.25^{b}	5.8 ± 1.03^{b}	3.8 ± 1.17^{b}	***7.669
Overall	3.6 ± 1.07^{a}	4.5 ± 1.53^{b}	4.4 ± 1.14^{b}	5.8±2.31 b	***5.280

Note: Results from total mean loads of feed samples ± standard deviation of means of five replicates. Superscripts a = not significant, while b shows significant difference at 0.05 level of significance. Ns = not significant, *** = significant at alpha = .0.05

Statistical Table above showed no significant difference in the fungal loads between the feed types in Abia State, while there were significant differences in other states. For the states that had significant differences in their feed types, a multiple comparison test (Tukey Highest Significance Difference) was carried out to identify the particular feed type(s) that were actually significant.

In Anambra State, the mean differences between Starter and Grower, Starter and Layer, and Grower and Layer feed types are significant. However, there are more fungal loads in Growers feed type than in others. There is no significant difference in the fungal loads between Growers and Finishers, Starters and Finishers, Starters and Layers, and Layers and Finishers feed types.

Results showed heavy presence of the saprotrophs in the test feed sample types. This proved that the feed samples served as over wintering incubator for those fungi. The higher fungal loads suggests more mycotoxin production. This agrees with the findings of Amadi and Adeniyi, (2009) that humidity; nutrient and high temperature encourage fungal growth and aflatoxin production. Statistically, there was no significant difference in the fungal loads between the feed types in Abia State, while there were significant differences in other feed types, a multiple comparison test approach (Turkey Highest Significance Difference) should be carried out to identify the particular feed type(s) that were highly significant and potential danger in the trophic levels.

Fungi isolates evaluated from the feed sample types were mostly filamentous fungi that belong to the *Aspergillus* species. The isolates include: Yeast, *Rhizopus*, *Penicillium* species, *A. niger* and *A. flavus*. Looking at the fungi loads and occurrences in Abia state, the entire sample feed types were contaminated but finisher had the highest load (5.5×10^2cfu/ml/g). **In** Anambra state, the whole sample feeds were also contaminated but the Grower mash had the highest load (6.1×10^2cfu/ml/g). Ebonyi state is not left out, all the samples evaluated were contaminated but Finisher had the highest load of (6.8×10^2cuf/ml/g). In Enugu state all samples were contaminated but finisher had the highest load of 8.8×10^2 / cfu / ml / g. Imo state, feed samples were also heavily infested by fungi. The highest was Layers mash (5.8×10^2cfu/ml/g).

The results of the fungal loads and their occurrences shown on the tables are in agreement with the findings of (Fabian *et al*. 2013; Nwaiwu and Imo, 1999; Makun *et al*., 2010; Wagacha and Muthomi, 2008) 1isolated similar organisms from feeds and other infected processed food materials.

CONCLUSION

Within the limit of this study, it was evident that all the feed samples types screened were contaminated with mycotoxigenic saprotrophs. This poses danger to the food safety situation of South Eastern Nigeria, a

geographic unit within the ecological Tropical Rainforest belt of Africa.

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