MYCOLOGICAL AND CHEMICAL SCREENING OF MOULDY CORN USED IN LAYERS FEED IN WESTERN PART OF NIGERIA

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Abstract
The aim of the present study was to determine species of the fungal genera, the total aflatoxin contamination and changes in nutritional profile of mouldy maize grain intended for poultry farm in western part of Nigeria. A total of six representative mouldy corn samples (20-50Kg) per sample were collected from six different open markets in Ile-Ife and its environs. The quantitative enumeration of fungi as colony-forming units per gram of the grains (CFU/g) was performed using the surface spread method in different culture media. The results were expressed as fungal isolation frequency and relative density. Fungal total counts ranged from $<1\times10^2$ to 4.7×10^4 CFU/g. Seven mould genera were recovered, 3 of which are known to be mycotoxigenic. More than 12 species were determined. Among toxigenic genera, Aspergillus (83%) was the most prevalent, followed by Fusarium (16.7%) and Penicillium species (16.7%). Aspergillus flavus, Aspergillus niger and Aspergilus parasiticus were the most prevalent species. The proximate contents of the mouldy maize fall by 11.54% crude protein, 12.72% ether extract and 1.12% ash. Total Aflatoxin in mouldy maize rose from near zero in aflasafémaize to 267.41 µg/Kg in mouldy maize. This study indicates the need for continuous assessment of the mycological status of animal feed production, in order to feed animals for optimal performance.

Key words: Aflasafe safe, mouldy maize, Mycological profile,aflatoxin, proximate content

Introduction

Feed cost and feed contamination are the major problems affecting the poultry industry in Nigeria. Feed mouldiness poses unique challenge in livestock industry. At present, fungal contaminations appear to have a higher health risk, in terms of exposure and severity of chronic lesions, anthropogenic contaminants, some phytotoxins, and imbalanced diets (Kuiper-Goodman, 1998). There are consistent report of worldwide contamination of feeds with fungi and its metabolites. Mycotoxins attract worldwide attention because of significant economic losses associated with their impact on animal productivity and trade (Wagacha and Muthomi, 2008). In recent years, data on mycotoxins of maize in Africa have begun to raise concern on feed safety (Probst *et al.*, 2007).

Moulds are ubiquitously in many feedstuffs including roughages and concentrates (Whithlow and Hagler, 2004). The quality of products used in feeding poultry has become a limiting factor for performance because these products are ideal substrate for mould growth which has potential to produce mycotoxin when conditions are favourable. Reports of Akande *et al.* (2006) has indicated that many feed mill industries in Nigeria exposed both human and animal to numerous health risks due to uncontrolled use of mouldy grains in compounding animal feeds. Fandohan *et al.* (2007) also submitted that storage fungi contribute to loss of more than 50% of maize grain in tropical countries. Poor storage system and facilities in this part of the world and weak feed regulation have exacerbated the problem of mould contamination.

Most toxic species that have been identified belong to the genera *Aspergillus, Penicillium, Fusarium,* and *Alternaria* (Pittand andHocking, 2009).Since maize constitutes over 50% of diet of commercial poultry, the nutrient requirement and performance traits of the poultry may be seriously compromised as earlier indicated by Hernadez *et al.*(2012) that infection of maize by storage fungi results in discoloration, dry matter losses, chemical and nutritional changes and overall reduction of maize grain quality. **The** ever-presence of mould contamination in hot and humid tropics like the western part of Nigeria, specifically Ile-Ife townand its environs in Osun State and the associated low performance of poultry industry call for concern. This study attempts to evaluate the mycological profile and chemical constituents of maize grains commonly used in formulating diets for poultry within Ile Ife and its environs.

Materials and Methods

Sample collection:A total of six representative mouldy corn samples (20-50Kg) per sample were collected from six (6) different markets in Ile-Ife and its environment (Table 1). Details of samplesare as presented in Table 2. All the samples were homogenized in order to obtain 500gm each per sample for analysis. Each sample collected were ground with ceramic mortar and

pestle in the laboratory and preserve in polythene bags for subsequent analysis

Mycobiota determinations: Plate count was carried out to analyze the six maize samples microbiologically in respect of the total heterotrophic fungi (THF). One gram of sample was suspended in 100ml of sterile distilled water in a conical flask in which some glasses chips were inserted (1/100 w/v) in the first instance. Hundred folds serial dilution of the suspension was carried out five times in a set of test tubes, each containing 9.9ml sterile distilled water. 1.0ml of each dilution was then plated out respectively in duplicates, employing the use of malt extract agar (sterile) kept in molten form. Pour plate techniques was adopted. The culture plates were labeled appropriately.The culture plates, having allowed the agar medium to set, were incubated aerobically at 30°c for 3-5days (until the plates grew). The plates were observed daily for growth and selected for count after the expiration of the incubation period. The culture plate in which the number of colonies was less than 300 and it's duplicate for each sample was selected. The viable count was obtained by multiplying the number of colonies counted with the dilution factor at that dilution and expressed as colony forming unit (CFU) per gram of the original sample.

The fungal isolates from each of the samples were gotten from the previous culture plates. As each colony matures, a portion of it was cut and transferred unto a fresh culture agar medium and labelled appropriately. The isolates were observed and their growth characteristics (i.e texture, pigmentation, form, spore, formations etc) studied microscopically. Sub culturing was repeated until a pure isolate was obtained. The pure isolate was then preserved on agar slant for further study. The pure isolate were then identified morphologically through a staining technique using Lactophenol-in-cotton blue and the prepared slides examined under the microscope with magnification X40 objective.Drawings of the various structures (i.e sporangium, sporangiophores, celumellae, myccluim,sporesetc) of each isolates were made and compared with the drawing of described commonly encountered moulds. The isolation frequency (Fr) and relative density (RD) of genus/species were calculated (Tables 2 and 3) according to (Pacinl *et al.*, 2003 and Saleemi *et al.*2010) as follows:

$$\text{Frequency (\%)} = \frac{\text{number of samples with a genus or species}}{\text{total number of samples}} \times 100,$$

Total number of sample

$$\text{Relative Density (\%)} = \frac{\text{number of isolates of a genus or species}}{\text{total number of fungi isolated}} \times 100$$

Total number of sample

Proximate analysis of test sample: samples were analyzedfor moisture content, crude protein (CP), ether extra (EE), crude fibre (CF), and ash according to procedure of A.O.A.C. (2000).

Mycotoxin Analysis: feed samples were subjected to quantitative analysis using ELISA-based analytical test kits for aflatoxin. In brief, 5g of ground sample was extracted with 25mL of 70% methanol for aflatoxins.

Results and Discussion

Mycological isolation and identification: Theresults were expressed as fungal isolation frequency and relative density. Fungal total counts ranged from <1 × 102 to 4.7 × 104 CFU/g. seven mould genera were recovered, 3 of them known to be mycotoxigenic. Twelve species were determined. Among toxigenic genera, Aspergillus(83%) was the most prevalent, followed by *Fusarium* and *Penicillium* species (16.7%) each. ***Aspergillus flavus***, *Aspergillus niger* and *Aspergilus parasiticus* were the most prevalent species. In all the samples collected, Aspergillus species continued to reoccur and became more notable and predominant and particularl, A. flavus had higher relative density among all the species identified. This is an indication that aflatoxin is likely to be the major mycotoxin present in the mouldedmaize in western part of Nigeria. This findings is similar to report of Fandohan *et al.* (2005); Shephard *et al.* (2002);Omemu ***et al,*** (2005). In other words, this study has shown that, in hot humid region of Nigeria, the likely fungi species that may contaminate maize are *Aspergillus flavus* ,***Aspergillus glacus***, *Rhizopus stolonifer*, *Scopulariopsis brevicaulis* and that production of aflatoxin is likely when conditions favour growth of these fungi.

Proximate analysis of both infected and un-infected maize

There was difference in the moisture content of the mouldy maize with about 30% increase over the aflasafe maize. This difference was necessary as it creates a favourable condition for mouldiness. This was in line with reports of Omniski *et al* (1994). This implies that higher moisture in feed grains increases possibility of infection by fungi (Chunk-Hernandez *et al.*, 2012) because it create

conducive environment for fungal growth. Hence, low moisture content of maize probably not more than 10% should be considered for storage.

Crude protein fraction of the maize reduced significantly by 11%% indicating a debilitating effect of fungi in the infected sample. This decrease was in line with the report of Ratcliff (2002) and Akande *et al* (2006) who earlier reported that mould contamination have effects on economic andhealth impact of maize due to decline in nutritive value.There was also a difference in the ether extract content of the mouldy maize with the aflasafe maize by 12.72% resulting from the effect of the mouldiness in grains as a result of microbial activities (Ratcliff 2002). Thiswas also in line with report of Chunk-Hernandez *et al.* (2012) who reported that infection of maize grain by storage fungus results in nutritional changes of the grain.The ash content of the maize reduced slightly as opposed to the previous finding that ash content increases slightly due to activities of fungi in the infected maize (Chunk-Hernandez *et al.*, 2012, Akande et al., 2006).Theresult of crude fibre of the maize content agrees with Ratcliff (2002) who reported increase in crude fibre as a result of the impact formed by the fungus activities.

Fungi and mould growth in feedstuffs is associated with the utilization of nutrients from the host, causing alteration in the nutritional content of the feedstuff. The germ of the grain is the main site for *Aspergillus* spp. development, thus the reduction of energy value due to fat, protein and sugar utilization in contaminated grain should be expected, leading to decrease in nutritive value.The differences observed with proximate composition of the contaminated sample indicate clearly that the use of such maize grains in poultry feed will certainly compromise the requirement of the animal considering the inclusion level of maize about 50% in the diets of poultry. Hence, contaminated maize should be avoided in formulating poultry diet as nutrient requirement may be compromised apart from tendency of exposure to mycotoxicoses.

Total Aflatoxin in mouldy maize rose from near zero in aflasafe maize to 267.41 µg/Kg in mouldy maize. The World Health Organization recommends that the current action level (the concentration above which a commodity is condemned) of total aflatoxin in food as 20 µg/kg (Muzaffer *et al.*, 2003; WHO, 2006). Foodstuffs and livestock feed are deemed contaminated if they contain this concentration. Above these range clinical symptoms such as diseases and mortality can arise.

Conclusion

The study has indicated clearly that feeding of mouldy maize compromised nutrient requirement of animals, it depressed performance and expose both man animal to various health risks. Due to preponderance of mould infestation of maize and animal feed, there is the need for continuous assessment of the mycological status of animal feed production, in order to feed animals for optimal performance.

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Table 1: sample collection in Ile-Ife market and its environment

Market	Quantity (Kg)	Number of samples/market	Sample characteristics
Oja Tun-Tun (Ile-Ife)	20	3	Grains with cobb
Ita Akogun (Ile-Ife)	50	5	Grain
Better life (Modakeke)	30	4	Grain
Akinola (Ipetu)	40	3	Grain
Olode (Oarage Olode)	25	3	Grain
Moro (Moro town)	30	2	Grains with cobb

Table 2. : Fungal Species identified in mouldy corn in Ile-Ife area of Osun state Nigeria

s/no	species	Number of isolates	Fr (%)	RD (%)
1	*Aspergillusflavus*			
2	*A.* glacus			
3	*A.* fumigatus			
4	*A.* niger			
5	*A.* parasiticus			
6	*Botrytis spp*			
7	*Fusarium proliferatum*			
8	*F. oxysporum*			
9	*Penicillium spp*			
10	*Rhyzopus stolonifer*			
11	*Scopulariopsis brevicaulis*			
12	*Trichoderma spp*			

Fr: isolation frequency, RD: isolation Relative Density

Table 3: Fungal genus identified in mouldy corn in Ile-Ife area of Osun state Nigeria

Genus	No. of isolates	Fr (%)*	RD (%)**
Aspergillus	6	100	85.7
Botrytis	2	33.3	28.6
Fusarium	1	16.7	14.3
Penicillium	1	16.7	14.3
Rhyzopus	5	83.3	71.4
Trychoderma	2	33.3	28.6
scopulariopsis	1	16.7	14.3

Table 4; Proximate and aflatoxin content of aflasafe and mouldy maize

Components	Mouldy Maize	Aflasafe Maize	Percentage loss
Proximate (%)			
Crude Protein	8.05	9.10	11.54
Crude fibre	5.40	4.10	-31.7
Ether extract	3.50	4.01	12.72
Ash Content	10.01	10.13	1.18
Dry matter	87.86	90.37	-0.54
Nitrogen free extractives	76.04	72.66	-0.52
Aflatoxin and vitamins			
Total Aflatoxin(µg/Kg)	267.41	0.23	-99.91
*ME (Kcal/kg)	3174.27	3240.94	2.05