Mycological and chemical screening of maize at open markets in Osun State, Nigeria

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Abstract

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The aim of the present study was to screen maize at the open markets in Osun state, Nigeria to determine species of fungi, total aflatoxin content and changes in proximate, energy and some vitamins content of maize grains. A total of forty five mouldy maize samples were collected from the six different open markets within the region which were later bulked into six representative samples (20-50Kg) for each market while Aflasafe maize, obtained from the International Institute of Tropical Agriculture (IITA) was used as a control. The representative samples from the bulk were taken for proximate, selected vitamins and total aflatoxin contents determination. 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 of fungi profile were further expressed as fungal isolation frequency (IF) and relative density (RD). Twelve (12) species and seven fungi genera were isolated in this study. The total fungi count ranged from 1.50×10^5 to 2.1×10^7 CFU/g in the six different locations. *Aspergillus flavus*, *Rhizopus stolonifer* and *Aspergillus glaucus* were the most prevalent species *Aspergillus* and *Rhizopus* species occurred at 100% and 87% (IF) respectively. The toxigenic species of *Aspergillus*, *Fusarium* and *Penicillium* had 85.7%, 14.3% and 14.3% RD respectively. The proximate contents of the mouldy maize reduced by 11.54% for crude protein, 12.72% for ether extract and 1.12% for ash while about 5% caloric loss was observed. Total Aflatoxin in mouldy maize rose from near zero in aflasafe maize to 267.41µg/Kg with an average value of 142.0µg/Kg in mouldy maize while retinol and tocopherol contents were depleted by 48.7% and 37.6% respectively. This study indicated substantial loss of nutrients and reduced feed value in maize due to mould contamination and the need for continuous assessment of feed ingredients particularly maize for mycological and nutritive status in order to feed animals for optimal performance.

Keywords: Aflatoxin, maize, mycobiota, proximate content

Introduction

The rank of mould is second after insects as the cause of deterioration and loss of maize (Uzma and Shahida, 2007). Moulds are ubiquitously in many feedstuffs and pose unique challenge in livestock industry (Whithlow and Hagler, 2004). The quality of grains particularly maize used in feeding poultry has become a limiting factor for production performance because these products are ideal substrate for mould growth and mycotoxin when conditions are favourable (Rodrigues *et al.*, 2011)). According to Richard *et al.* (2014) risks

refer primarily to the effects of mould *per se* without regard to possible mycotoxin content and that depressed digestibility, feed intake and performance may occur from high mould content without mycotoxins present. Fandohan *et al.* (2007) also submitted that storage fungi contribute to loss of more than 50% of maize grain in the tropical countries. Mouldy or musty feed will not always contain dangerous mould and mycotoxins, but the presence of considerable mould in itself may adversely affect production and health. Reductions in production performance and increases in

health problems from mouldy feed are often moderate if mycotoxins are not present. For example, a 5% to 10% drop in performance may be typical with mould infestation, whereas mycotoxin contamination leads to greater losses in production, even when mould is not readily apparent. Mycotoxins attract worldwide attention because of significant economic losses associated with their impact on animal productivity and trade (Wagacha and Muthomi, 2008). Mould spores are sometimes counted on mouldy feeds to obtain an indication of the extent of moulding and relative risks in feeding them. Spore counts of less than 10,000 colony forming units per gram (cfu/g) are commonly found on grain. Mould becomes visible at approximately 1,000,000 cfu/g. When conditions are optimal for moulds to colonize grains on the field or in storage, their first effect is utilization of nutrients for their metabolism and propagation. This results in decreased nutritional value of feeds. Energy, crude protein and crude fat values of mouldy maize were reportedly decreased by 5, 7 and 63%, respectively (Tindall, 1983). Dietary fat is affected more extensively than proteins or carbohydrates. Dietary fat decreased 37 to 40% after 25 day or 52 to 57% after 50 day of storage (Bartov, 1985) while digestibility of the ration may be decreased sufficiently to reduce energy content by 5%.

In recent years, data on mould and mycotoxins of maize in Africa have begun to raise concern on feed safety (Probst *et al.*, 2007). More recently, Rodrigues *et al.* (2011) conducted a survey of the occurrence of mycotoxins in feedstuffs and finished feeds in the Middle East and Africa, which included numerous samples from Western and Central Africa including Nigeria, Sudan, Egypt, Algeria, Kenya, Ghana, South Africa, Israel, Jordan,

Lebanon, Syria and Yemen. The authors found that 98% of the ingredients used in animal feed formulation are positive for aflatoxin B1. It was further shown that maize was a preferred substrate for fungal growth and mycotoxin production in comparison with soybean and wheat. Information on mycological and chemical profile of maize grains in the western Nigeria is however scanty. In this part of the country, mouldy grains may end up as animal feed because of their availability in open markets and involvement of many unskilled individuals in feed mill business who prioritize monetary benefits at the expense of quality of products. 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 indiscriminate use of mouldy grains in compounding animal feeds. Poor storage system and facilities in this part of the world coupled with weak regulation of feed industry have exacerbated the problem of mould contamination. Since maize constitutes about 50% of diet of commercial poultry, the nutrient requirement and performance traits of the poultry could be compromised with mouldy maize diets as earlier indicated by Hernadez *et al.* (2012). The ever-presence of mould in hot and humid tropics like the western Nigeria and the associated low performance of poultry industry calls for a deep concern. This study thus attempts to evaluate the mycological profile and chemical constituents of maize grains commonly found in open markets in western Nigeria.

Materials and methods

Sample collection

Survey was carried in six major open markets in Osun State of Nigeria. The maize grains were carefully selected because there were three substandard maize grains available in open markets: the mouldy

maize, the weevil infested maize and mouldy and weevil infested maize. About 50kg mouldy maize grains were collected from each market between 5 and 10 different points totaling forty five samples from the six markets. The grains from different points for each market were bulked. Details of samples are as presented in Table 1. Since mycotoxins are present in

such small quantities and may only occur in isolated spots, representative samples were taken according to procedure of Richard *et al.* (2014). 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.

Table 1: Sample collection in six different open markets in Osun State

Market	Quantity (Kg)	Number of samples per market	Sample characteristics
1	45	6	Grains with cobb
2	20	7	Grain
3	47	8	Grain
4	40	5	Grain
5	55	10	Grain
6	50	9	Grains with cobb

Mycobiota determinations

Plate count was carried out to analyze the six representative maize samples from the six different study locations for the total heterotrophic fungi (THF). One gram of sample was suspended in 100ml of sterile distilled water in a conical flask in which some glass 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 of 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 labeled appropriately. The isolates were observed and their growth characteristics (i.e texture, pigmentation, form, spore, formations) 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, spores etc) 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) using the formula stated below:

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

Total number of sample

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 analyzed for moisture content, crude protein (CP), ether extra (EE), crude fibre (CF), and ash according to procedure of AOAC (2000).

Aflatoxin analysis

A representative sample of 1 kg of maize grains was finely ground for each sample and then a 50 g sub- sample of flour was blended with 4 g of sodium chloride (NaCl) and 250 ml of 60% methanol. The extract was diluted with 250 ml of distilled water and then filtered using Whatman filter paper No. 4. Twenty five to fifty millilitres of the filtrate was collected, 10 ml of the filtrate was then passed through the immunoaffinity column so that the antibodies absorb the aflatoxins. The column was then washed twice with distilled water. Pure methanol was passed in the column to release the aflatoxins after which it was collected in a test tube. Water and chloroform were added (1 ml each) to the aflatoxin eluted and shaken gently then left to stand. The bottom chloroform layer was pipette out and then passed through a florisil tip. The florisil tip was then viewed under 366nm UV light and the fluorescence

of the toxins was compared with a standard comparator card (a component of the aflascan) with semi- quantitative values to obtain the range of total aflatoxin contamination in an extract according procedure of Bullerman (1979).

Results and discussion

Mycobiota determinations

The result of fungi count is shown in Table 2. Fungal total counts ranged from 1.50×10^5 to 2.1×10^7 CFU/g. The results were further expressed as fungal isolation frequency and relative density (Tables 3 and 4) to compare the various genera and species identified. Seven mould genera were isolated, 3 of them known to be toxigenic. Twelve species were determined among toxigenic genera as *Aspergillus*, with 85.7% relative density was the most prevalent, followed by *Fusarium* and *Penicillium* species at 14.3% relative density each (Table 4). This report aligned with assertion of Jones and Toal (2003) that fungi from five genera (*Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Calviceps*) are responsible for the production of the great majority of the mycotoxins that are of agricultural relevance. Similarly, Binyam Tsedaley (2016) concluded in his study in Ethiopia that *Aspergillus*, *Fusarium*, *Penicillium* were predominant storage fungi found in maize. In this study, *Aspergillus flavus*, *Aspergillus niger* and *Aspergilus parasiticus* were the most prevalent species (Table 3). This was in consonance with earlier report of Reddy and Salley (2011) on co-occurrence of moulds in maize. It then becomes imperative that in feed quality control, identification of the contaminating organisms should be obligatory to determine feed hygiene and the potential for mycotoxins production (Khosravet al., 2008).

Table 2: The identification and enumeration of the fungi in the mouldy maize at 30°C

Sample	organism identified	cfu/gram at 30°C
Sample 1	<i>Rhizopus stolonifer</i>	3.00 X 10 ⁵
	<i>Aspergillus glaucus</i>	1.30 X10 ⁷
	<i>Trichoderma spp</i>	7.20 X10 ⁵
	<i>Botrytis spp</i>	2.10 X 10 ⁶
	<i>Aspergillus flavus</i>	3.05 X 10 ⁶
Sample 2	<i>Rhizopus stolonifer</i>	3.00 X10 ⁶
	<i>Aspergillus glaucus</i>	2.1 X10 ⁶
	<i>Trichoderma spp</i>	4.00 X 10 ⁶
	<i>Botrytis spp</i>	9.01 X10 ⁵
	<i>Aspergillus flavus</i>	2.05 X10 ⁶
Sample 3	<i>Rhizopus stolonifer</i>	2.77 X10 ⁶
	<i>Aspergillus glaucus</i>	1.50X10 ⁵
	<i>Aspergillus niger</i>	3.72X10 ⁶
	<i>Fusarium proliferatum</i>	1.75X10 ⁶
Sample 4	<i>Rhizopus stolonifer</i>	2.20X10 ⁵
	<i>Aspergillus glaucus</i>	2.11X10 ⁶
	<i>Aspergillus flavus</i>	2.90X10 ⁶
	<i>Scopulariopsis brevicaulis</i>	ND
Sample 5	<i>Aspergillus flavus</i>	3.01 X10 ⁵
	<i>Aspergillus niger</i>	5.71X10 ⁵
	<i>Penicillium spp</i>	6.42X10 ⁶
Sample 6	<i>Rhizopus stolonifer</i>	5.82X10 ⁵
	<i>Aspergillus parasiticus</i>	4.30X10 ⁶
	<i>Aspergillus fumigatus</i>	ND
	<i>Fusarium oxysporum</i>	5.33X10 ⁶

ND-not determined

Table 3: Fungi Species identified in mouldy maize in Osun State, Nigeria

s/no	Species	No of isolates	Isolation Frequency (%)	Relative Density (%)
1	<i>Aspergillus flavus</i>	5	83.3	41.7
2	<i>A. Glaucus</i>	4	66.7	33.3
3	<i>A. Fumigatus</i>	1	16.7	8.33
4	<i>A. Niger</i>	2	33.3	16.7
5	<i>A. Parasiticus</i>	1	16.7	8.33
6	<i>Botrytis spp</i>	2	33.3	16.7
7	<i>Fusarium proliferatum</i>	1	16.7	8.33
8	<i>F. oxysporum</i>	1	16.7	8.33
9	<i>Penicillium spp</i>	1	16.7	8.33
10	<i>Rhyzopusstolonifer</i>	5	83.3	41.7
11	<i>Scopulariopsis brevicaulis</i>	1	16.7	8.33
12	<i>Trichoderma spp</i>	2	33.3	16.7

Table 4: Fungi genus identified in mouldy maize **maize in Osun State, Nigeria**

Genus	No of isolates	Isolation Frequency (%)	Relative Density (%)
Aspergillus	6	100	85.7
Botrytis	2	33.3	28.6
Fusarium	1	16.7	14.3
Penicillium	1	16.7	14.3
Rhizopus	5	83.3	71.4
Trichoderma	2	33.3	28.6
scopulariopsis	1	16.7	14.3

Chemical constituents of mouldy and Aflasafe maize

The results of chemical composition are shown in Table 5. There was difference in the moisture content of the mouldy maize with about 27.4% increase over the aflasafe maize. This difference obviously created the favorable condition for mouldiness. This was in line with reports of Omniski *et al.* (1994) that implied higher moisture in grains increases possibility of infestation by fungi because it creates conducive environment for fungal growth. Similar assertion were made by Pitt and Hocking (1997) that high temperatures and relative humidity provide ideal conditions for growth and development of moulds with possible production of mycotoxins. Hence, low moisture in maize probably below 15% should be considered for storage as earlier reported by Chunk-Hernandez *et al.* (2012). Protein content in mouldy maize declined by 11.54%, indicating the sapping effect of fungi on the cereal grains. This decrease was in line with the report of Ratcliff (2002) and Akande *et al.* (2006) who earlier reported depletion of organic nutrient of maize contaminated with mould thereby reducing its nutritive value. There was also a reduction in both the ether extract and ash content of the mouldy maize by 12.72% and 1.18% respectively resulting from the microbial activities (Ratcliff 2002). This was also in line with report of Chunk-Hernandez *et al.* (2012) that infection of maize grain by storage fungus results in

nutritional changes of the grain. The result of crude fiber of the maize content agreed with Ratcliff (2002) on increase in crude fiber in grains contaminated with mould. There was reduction in both retinol and tocopherol contents of the mouldy maize by 48% and 37%, respectively. This study corroborated the assertion by Bains (2002) that vitamins are destroyed by mould growth in the feed ingredients.

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* species 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 indicated 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 diets as nutrient requirement may be haggled apart from tendency of exposure to mycotoxins. Richard *et al.* (2014) also indicated that mouldy feed are less palatable and may lower the intake of energy, dry matter, and critical nutrients. The authors further suggested that, in case mouldy feed find their entry into animal feed, digestibility of the

ration may be decreased sufficiently to reduce energy content by 5% and it is best to discount energy values by multiplying them by 0.95. This is was a measure to reduce possible compromise in caloric need of animals fed such feed.

Total Aflatoxin in mouldy maize in the six regions, samples 1 to 6 were: 50.6, 107, 75.1, 267.4, 183 and 169.7 µg/kg respectively. The value rose from near zero in aflasafe maize to 267.41 µg/kg in mouldy maize though with an average value of 142.2 µg/kg. The World Health Organization recommends that the current action level (the concentration above which a commodity is condemned) of total aflatoxin in food is 20 µg/kg (Muzaffer *et al.* 2003; WHO, 2006). Above this range, clinical symptoms such as diseases and mortality could arise. From the six regions surveyed, all had grains whose aflatoxin levels were beyond the recommended level.

It means then, that mould infested grains as indicated in this study in this part of the world should be completely prevented from getting into poultry feed. Similar result was obtained by Mpuchane *et al.* (1997) in a mycological study of stored maize and sorghum grains in Botswana. Aflatoxin contamination of maize has been shown to occur in virtually all maize producing areas of the world and it has been shown that contamination can occur either pre- or post-harvest (Miller, 1995). Pre-harvest contamination is brought about by *Aspergillus flavus* colonising the maize silks and then invading the developing kernels and by boring insects causing further moisture stress in the crop (Payne, 1992). Globally, there has been a high reliance on maize grains as the main energy source in poultry diets, there is a need for legislative framework that will focus directly on feed industry with the major objective of ensuring food safety

Table 5: Some chemical constituents of aflasafe and mouldy maize

Components	Mouldy Maize	Aflasafe Maize	Percentage loss/gain
<i>Proximate (%)</i>			
Crude Protein	8.05	9.10	11.5
Crude fibre	5.40	4.10	31.7
Ether extract	3.50	4.01	12.7
Ash Content	10.0	10.1	1.18
Dry matter	87.9	90.4	2.77
Nitrogen free extracts	60.2	63.1	4.60
<i>Aflatoxin and vitamins</i>			
Total Aflatoxin(µg/Kg)	+142	0.23	617
VIT.A (µg/kg)	7.89	15.4	48.7
VIT.E (µg/kg)	4.23	6.78	37.6
*ME (Kcal/kg)	3177	3244	2.05

*values obtained by formula of Pauzenga 1985; +average values in the six regions surveyed

Conclusion

The study has shown that mould contamination reduced feed value and such feed when fed may compromise nutrient requirement of animals. *Aspergillus flavus* was the preponderant toxigenic fungi isolated in this study and found to constitute a risk factor in maize sourced from open markets in Osun Nigeria. Higher aflatoxin

content in mouldy maize in this part of the world accentuates the need for continuous assessment of both the mycological and mycotoxin status of animal feed for optimal performance.

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