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Survey of Mycotoxigenic Fungi in Concentrated Poultry Feed in Niger State, Nigeria

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Abstract

In Nigeria, concentrated poultry feed is a vital component of animal production and health but there was no reliable mycogram in Niger state, Nigeria. As a result this study determined the incidence of mycotoxigenic fungi in both commercial and privately milled concentrated poultry feeds. A total of 100 poultry feed samples consisting of 52 privately milled poultry feed and 48 commercial feed samples were collected. Mycoflora in the feed was determined. Nine fungi genera were isolated. The most frequently isolated fungi genera in both privately milled and commercial feed was *Aspergillus spp* which was about 40% of mould isolate. *Penicillium spp* is 20% in private feed and 13% in commercial feed. A total of 874 fungi were isolated consisting of 458 fungi species in privately milled feed and 416 fungi species found in commercial feed. Mycotoxigenic fungi genera, *Aspergillus, Penicillium, Fusarium and Alternaria* were isolated. *Aspergillus flavus* is the commonest isolated fungi species.

Keywords: poultry feed, fungi, mycological survey, commercial

1. Introduction

Worldwide fungi contamination of food and feedstuff has become a source of concern. Fungi are well adapted for growth and development over surfaces and through solid materials (Moss, 1992). The hyphae of some fungi are capable of penetrating hard surfaces (Gow et al., 2002). This group of microorganisms are ubiquitous in nature (Park et al., 2005), and are mostly saprophytes that breakdown and transport the available nutrients to the actively growing hyphae (Smith & Moss, 1985). Or they may be parasites (Moss, 1992) by invading and exhibiting their pathogenic potentials on both plant and animal species (Parker & Gilbert, 2004). Forages and cereals are often contaminated by fungi in the field, or during processing, transportation and storage when conditions such as temperature and relative humidity are favourable. Temperature and relative humidity of above 30°C and 80-100% respectively are favourable for fungal growth (Blaha et al., 1990). Other conditions include nutrient availability (Njobeh, 2003) and oxygen supply (Filtenborg et al., 2000). Most of the fungi invade only a minor fraction of feed particles with appropriate condition for their growth.

The presence of visible mould spores may not likely serve as a reliable guide to mycotoxin content (Smith & Seddon, 1998) but must not be disregarded when assessing the occurrence of mycotoxins. This is because it provides a foundation for evaluation of risk of mycotoxin contamination. Observations of fungi in food and animal feed are likely indications of the presence of mycotoxins and it is only through the cultivation of fungi under laboratory conditions when the presence or absence of fungi can be appreciated.

Pathogenic micro organisms and their secondary metabolites (mycotoxins) in general chain of nutrition represent the most important potential risk to animal and human health. Because of the harmful effect of mycotoxins in chain of nutrition of humans and animals, in this paper, presence of potentially mycotoxigenic fungi in samples of poultry feed was determined.

2. Materials and Methods

2.1 Collection of Samples

100 poultry feed samples were collected between February and March 2011 from different poultry farms in Minna, Niger state, which is a state in the North western part of Nigeria and the largest state in the country, a state with favourable climate (average annual temperature of 31.7°C and average humidity of 51.6%) and it is hot and humid for most part of the year especially between May and October (29.5°C and 73.1%) which is conducive for fungal growth and mycotoxin production on food. The microclimatic environment is dry at the time of sample collection. The annual rainfall in Niger state is between 1000-1200 mm.

52 samples from privately milled poultry feed were collected from six different farms and 48 samples from six commercial poultry feed brands were also taken from different farms using commercial feed. These samples were taken before the feed was served to birds. The samples were labeled based on the manufacturer as commercial feeds or privately milled feeds. The commercial feeds were labeled according to their different brands and the private feed labeled with the farms' name. About 200 grams of the feed were taken and packaged in well labeled paper bags and immediately taken to the laboratory for fungi isolation and identification.

2.2 Isolation and Identification of Fungi

Fungi were isolated and cultured according to the method described by Pitt and Hocking (1997). About 1 g of the ground grain was diluted in 9 ml of sterile distilled water which was followed by five other serial dilutions. One millitre of the extract was placed at random in each of the Petri-dishes containing potato dextrose agar (PDA) and chloramphenicol (500 mg per litre). The pure culture was used for identification between five and seven days of incubation. Determination of each species of fungi was done using the keys of Klich and Pitt (1988) and Klich (2002) for *Aspergillus spp*. Nelson et al. (1983) for *Fusarium spp.* and Pitt and Hocking (1997) for *Penicillium* and other genera. This is done by observing both microscopic characteristics of the colonies on various media used as well as the microscopic morphology and measurement of the candidiophores (after staining mycelia with 0.1% fucshin dissolved in lactic acid) under microscope. The pure culture of different isolate (identified fungi) were aseptically sub-cultured in potatoes dextrose agar slant and incubated.

Identification of isolates was carried out at the Microbiology laboratory of Federal University of Technology, Minna and Microbiology Department, Faculty of Veterinary Medicine, University of Abuja.

3. Result and Discussion

In this survey nine fungi general were isolated as the natural contaminant of both commercial and private feeds milled in Minna Niger state. The fungi genera includes *Aspergillus*, *Penicillium Fusarium*, *Mucor*, *Rhizopus*, *Cladosporum*, *Alternaria*, and *Torula*. This result is similar to those published by (Makun et al., 2010; Atehnkeng et al., 2008; Kpodo et al., 2010) as the contaminant of cereal crops which are the ingredient used in poultry feed production.

Table 1 showed the frequency of isolated fungi genera in both private and commercial poultry feed samples, where a significant difference was obtained ($X^2 = 199.818$; $P < 0.05$ and $X^2 = 237.053$; $P < 0.05$) respectively, being the genus *Aspergillus* and *Penicillium* the most frequently isolated. This result is similar to the ones obtained by Rosa et al. (2006), Oliveira et al. (2006) and Figueroa et al. (2009), who found that *Aspergillus* and *Penicillium* were the most frequently isolated genera in the poultry feed samples analyzed. *Aspergillus* has been shown to be predominant in cereals and other ingredients used in producing poultry feeds in the tropics as in the case of Nigeria (Pitt & Hocking, 1997; Makun et al., 2009). A total of 456 species of fungi were isolated in private milled feed and 416 fungi commercial poultry feeds. Table 2 shows that *Aspergillus spp* has the highest frequency of occurrence and *Torula spp* is the least fungi isolated. *Aspergillus* and *penicillium* are frequently isolated are the commonest and widespread in nature, and have been shown as fungal contamination of African foods and feeds (Atehnkeng et al., 2008; Essono et al., 2009; Njobeh et al., 2009; Makun et al., 2010).

Table 1. Occurrence and frequency of fungi genera isolated from privately milled and commercial poultry feed

	Private feed		Commercial feed	
Genera	Incidence	Frequency (%)	Incidence	Frequency (%)
Alternaria	11	2	8	2
Aspergillius	189	41	167	40
Cladosporum	19	4	20	5
Fusarium	31	7	47	11
Mucor	54	12	65	16
Penicillum	91	20	54	13
Rhizopus	51	11	45	11
Torula	1	0.2	3	1
Yeast	9	2	7	2

Private feed: *** X2= 199.818 Highly significant at P<0.05;

Commercial feed: *** X2= 273.053 highly significant at P<0.05.

Table 2. Occurrence and frequency of fungi species isolated from privately milled and commercial poultry feed

	Private Milled Feeds		Commercial Feeds	
Fungi Spp	Incidence	Frequency (%)	Incidence	Frequency (%)
Alternaria spp	6	1	5	1
Aspergillius flavus	43	9	47	11
A.fumigatus	29	6	21	5
A.malleus	11	2	8	1
A. nidulans	4	1	5	1
A. niger	41	9	33	7
A.ochraceus	19	4	6	1
A. parasiticus	32	7	34	8
A. flaviceps	10	2	13	3
Cladosporum spp	11	2	14	3
Curvularia spp	8	1	6	1
Fusarium spp	19	4	29	6
F. oxysporum	5	1	7	2
F.semitectum	2	0.4	5	1
F.solani	5	1	6	1
Mucor spp	54	11	65	15
Penicillium spp	46	10	33	7
P. citrinum	7	1	5	1
P. notatum	8	1	2	0.4
P. rubrum	8	1	6	1
P.verrucosum	22	4	8	1
Rhizopus spp	15	11	45	10
Torula spp	1	0.2	3	1
Yeast	9	1	7	1

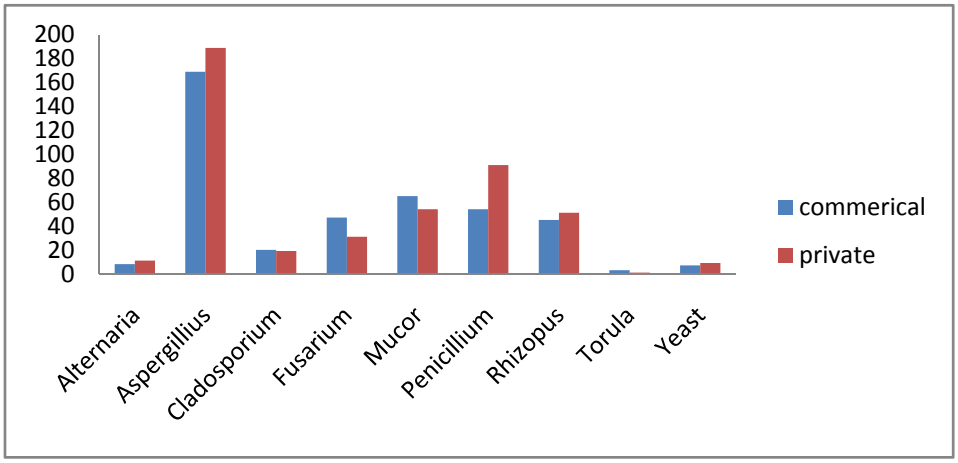

Figure 1. Frequency of fungi general isolated from private and commercial feed

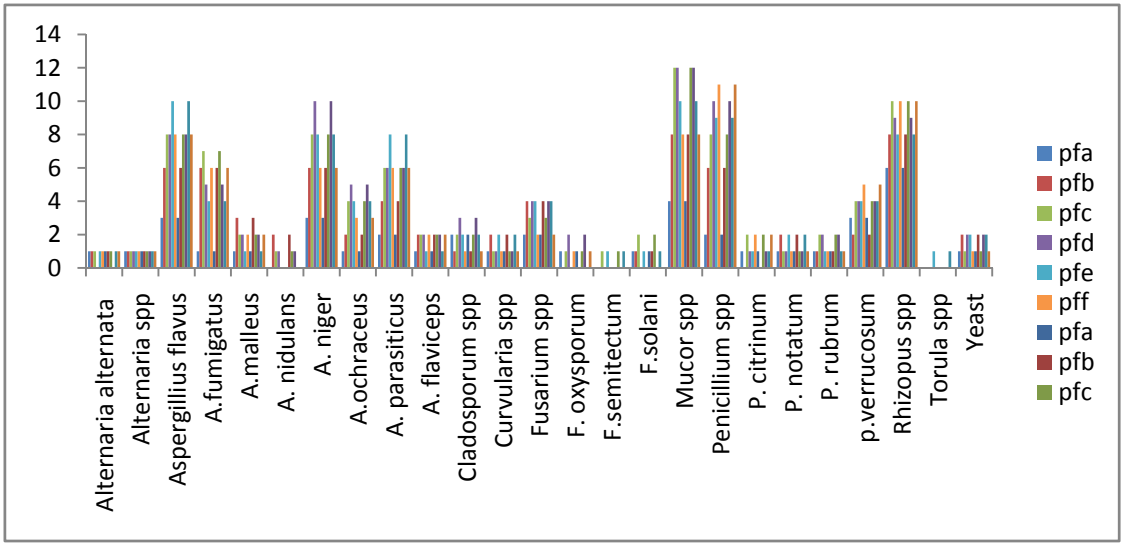

Figure 2. Frequency of fungi species isolated from private feed

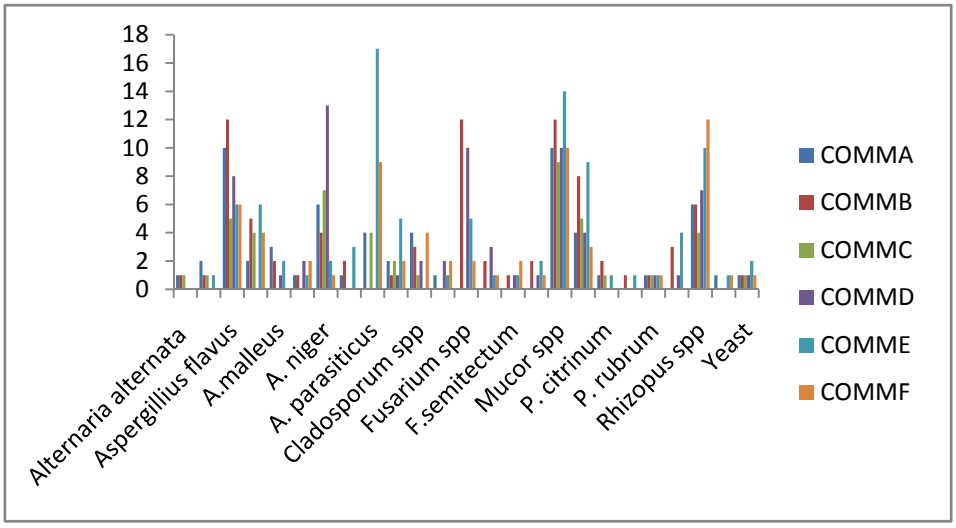

Figure 3. Frequency of fungi species isolated from commercial feed

Aspergillus flavus is the most isolated species in this work both in private and commercial poultry feed. *Aspergillus flavus* was the most isolated species in poultry feeds in different countries (Rosa et al., 2006; Figueroa et al., 2009). *A. flavus* produces mycotoxin like aflatoxin, kojic acid, aspergillic acid and cyclopiazonoid acid (FAO, 2003). Aflatoxin B1 is carcinogenic to human (IARC, 1993), in chickens, aflatoxin increased susceptibility to, or severity of ceacal coccidiosis, Marek's disease (Edds, 1976), salmonellosis (Smith et al., 1969; Wyatt & Hamilton, 1975), inclusion body hepatitis (Singh et al., 1996), and infectious bursal disease virus (Chang & Hamilton, 1982; Somvanshi & Mohanty, 1991). Vaccination failures were linked to aflatoxicosis in chickens (Anjum, 1994; Pruthi et al., 1992), and impaired response to vaccination responses was demonstrated for Newcastle disease, infectious bronchitis, infectious bursal disease, and fowl cholera (Azzam & Gabal, 1997; Azzam & Gabal, 1998; Bunaciu et al., 1998).

A. niger is higher in private milled poultry feed than the commercial feed because the commercial feed manufacturer uses cotton seed as their ingredient compared to private feed which used maize or corn sources from local market as their ingredient. *A. niger* and *A. ochraceus* has a great capacity to produce Ochratoxin A (OTA) (Dalcero et al., 1998; Rosa et al., 2006). *A. ochraceus* is capable of producing high amount of secondary toxic metabolites such as Ochratoxin A, penicillin acid, Xantomegnin, Viomellein and Vioxantin. Effect of OTA in poultry include delay of sexual maturity in pullets, reduction in mean body weight, decrease in egg weight and reduced egg production, immunosupression, impaired vaccination response (Kozaczynski, 1994; Politis et al., 2005). OTA also has sensitive effect on the kidney and liver. Rosa et al. (2006) was able to demonstrate that 9 strain out of the 74 strains found of *A. ochraceus* in bird feeds in Brazil were capable of producing OTA in concentration of between 53 and 116 ug/kg.

Aspergillus fumigatus causes a condition called aspergillosis which is an infectious, non contagious respiratory disease affecting birds characterized by gasping, sleepiness, loss of appetite and sometimes convulsion and death. Occasionally the organism invades the brain, causing paralysis or other forms of nervous symptoms. *Aspergillus, penicillium* and *fusarium* are toxigenic fungi; they produce very important mycotoxins like Ochratoxin, aflatoxin, fumonism and trichothecene.

High level of *Mucor spp* and *Rhizopus spp* was a result of these feed ingredient which are higher in Carbohydrate in Nature. The high incidence of *Mucor spp* and *Rhizopus spp i*s in conformity with the Infeanyi et al. (2007) which showed a high frequency of these fungi in poultry feed ingredients. *Mucor* and *Cladosporun* species may cause mycotic abortion and allergy in animal and human as a result of systemic and respiratory transmission respectively (Rippon, 1988).

The mycotoxigenic fungi general identified in this research were *Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium*.

Controlling mould growth and mycotoxin production is very important to the feed manufacturer and livestock producer. Control of mould growth in feeds can be accomplished by keeping moisture low, feed fresh, and equipment clean and using mould binders and inhibitors. Grains and other dry feedstuffs should be stored at a moisture level of less than 14 percent to prevent mould growth.

Aeration of grain bins is important to reduce moisture migration and keep the feedstuff dry (Jones et al., 1994).

The use of chemical mould inhibitors is a well establish practice in the feed industry. However, mould inhibitors are only one of several tools useful in the complex process of controlling the growth of moulds, and they should not be relied upon exclusively.

4. Conclusion and Recommendation

It is important to note that the impact of mycotoxigenic fungi on animals extend beyond the symptoms. The mycotoxin produced by the fungi poses a serious economic impact worldwide. The economic impact result from lowered productivity, reduced feed conversion efficiency which causes reduced weight gain, less meat and egg production, increased disease incidence because of immunosupression, damage to organs in the body. Since there has not been a mycogram of poultry feed in Niger state, there is need to develop one using this mycological survey as a baseline data for further studies which will involve more local government areas under different microclimatic condition.

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