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DETERMINATION OF AFLATOXIN LEVELS IN COMMERCIAL POULTRY FEEDS SOLD IN SOME PARTS OF SOUTHWESTERN NIGERIA

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ABSTRACT

Aflatoxin contamination of animal feeds is common and widely spread especially in the tropics due to ubiquity of the producing fungi. This study was carried out to identify and quantify common moulds and aflatoxin levels in commercial poultry feeds sold in four states of South Western, Nigeria. Twenty samples of poultry feeds, 5 from each state (Lagos, Ogun, Osun and Oyo) were bought from retailers. The feed samples were analysed microbiologically for moulds and total aflatoxins using Enzyme-linked Immunosorbent Assay (ELISA). Mould count ranged between 1 x 10^4 and 8 x 10^4 cfu/g. Common moulds isolated were Aspergillus flavus, A. *parasiticus,* A. t*erreus*, A. *niger,* A. *oryzae*, *Rhizopus* oryzae, and *Penicillium notatum.* Total aflatoxin levels ranged between 13.5 and 270 ug/kg. Maize was the main ingredient in all feeds examined. This study highlights the need for routine surveillance. Feed millers are encouraged to add fungal growth inhibitors or toxin binders to their feeds.

Key words: Aflatoxins, ELISA, moulds, Poultry feeds, South Western Nigeria.

INTRODUCTION

Mycotoxin contamination of foods and feeds has recently gained much attention worldwide due to its adverse effects on human and animal health and consequent national economic implication (Baht and Vashanti, 1999). Reports from various countries have highlighted the co-occurrence of aflatoxin with other mycotoxins such as fumonisin. Mycotoxin contamination has had a considerable economic impact on the food grain and livestock industry (Boudra and Morgavi, 2005).

`Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes (Franzolin *et al*., 2004). Animal feeds also may contain endogenous toxins arising principally from specific primary and secondary substances produced by fodder plants (Cheeke, 1995). Thus, feed toxins include compounds of both plant and microbial origin. Although these toxins are often considered separately, because of their different origins, they share several underlying features. Thus particular compounds within both plant and microbial toxins may exert anti-nutritional effect or reduce reproductive performance in farm animals.

Aflatoxins can cause disease indirectly through their effects on essential nutrients in the diet (Rauber *et al.,* 2007). Fat soluble antioxidants, such as vitamin A, and water soluble antioxidants and vitamins such as vitamin C (necessary for immune function) and thiamin (necessary for metabolic and nervous function), in feeds can be destroyed by toxins. Hence, it is said that aflatoxins depress the immune system, making the organism more susceptible to bacterial, viral, or parasitic diseases (Rauber *et al*., 2007).

Feed contaminants and toxins occur on a global scale but there are distinct geographical differences in the relative impact of individual compounds. In tropical and subtropical conditions, this is further increased due to storage under humid and hot conditions. There are consistent reports of worldwide contamination of feeds with fungi and their spores. In the tropics, *Aspergillus spp.* is the predominant genus in diary and other feeds (Dhand, 1998; Dhand and Jand, 2004). Other species include those of *Penicillium, Fusarium and Alternaria*, which are also important contaminants of cereal grain (D'Mello, 1993).

Fungal contamination is undesirable because of the potential for mycotoxin production. Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity (Marquardt, 1996; D'Mello and MacDonald, 1997).

Mycotoxin contamination of forage and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes (Hohenboken *et al.,* 2000). Contamination may occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi. Moisture content and ambient temperature are key determinants of fungal colonization and mycotoxin production (Arrus *et al*., 2005).

Aflatoxins have been found to be very toxic to some animals, while causing liver damage in others (Wittenberg *et al.,* 1996). Apart from being carcinogenic, aflatoxins can cause mutation and can act as teratogens (Coker *et al.,* 1984).

At low cumulative doses in animals, aflatoxins cause poor feed conversion efficiency and growth rates and subsequent economic losses to the farmer. In the 1960s, more than 100,000 young turkeys on poultry farms in England died in the course of a few months from an apparently new disease that was termed "Turkey X disease now known as aflatoxicosis.

The study was conducted in order to asses the levels of aflatoxins in commercial poultry feeds sold in some parts of South Western Nigeria.

MATERIALS AND METHODS

Twenty samples of poultry feeds were bought from four states in South Western Nigeria. The states were Lagos, Ogun, Osun and Oyo. Five samples were randomly bought from different locations in each state. The feeds were transported in sterile containers to the laboratory and stored at 4^{o}C and analysed within 24 hours of collection.

Microbiological analysis

Serial dilutions of each sample were made and dilution 10^3 and 10^4 were plated on Saboraud Dextrose Agar. The plates were incubated at 28^{o}C for five days. Each dilu-

tion was replicated 5 times. All colonies were counted and expressed in colony forming unit per gram (cfu/g) of the sample.

Isolates were identified using morphological and cultural characteristics according to the schemes of Klich (2002).

Aflatoxin extraction and quantification

The Agra Quant total aflatoxin assay which is a direct competitive enzyme-linked immunosorbent assay (ELISA) was used. Twenty grams of each sample was weighed into a conical flask containing 1000 ml of 70% methanol. The suspension was shaken in a rotary shaker at 80 rpm. for 3 minutes. This was filtered using Whatman No.1 filter paper. One hundred microlitres of filtrate of each sample was added into micro strip dilution wells containing 200µl of conjugate solution. The contents of the wells were vortexed for 1 minute and 100 µ of the mixture was transferred into a corresponding antibody coated microwell and incubated at room temperature for 15 minutes. The contents of the wells were emptied and washed with deionised water 4 times. One hundred microlitres of stop solution was added to each well. Optical density of each microwell was measured using a microwell reader with an absorbance filter of 450 nm. The optical densities of the samples were compared with optical densities of standards and an interpretative result was determined.

Statistical analysis

Data obtained from fungal counts and aflatoxin levels were analysed using one way Analysis of variance and Duncan multiple range test.

RESULTS AND DISCUSSION

Results from the study showed fungal counts and organisms isolated from the samples using the identification manual of Klich (2002). The fungal counts ranged from 10^3 to 10^4 cfu/g. The mould spore counts which were low may underestimate the amount of mold present and be a poor indicator of the potential risk. However, the International Standard Organization minimal count for pathogenic organisms should be as low as 10^2 (Deilo, 1996).

Moldy feeds have reduced digestibility and energy content may need to be adjusted by 5%. Molds grow and propagate deriving energy from the feed's protein, fat and carbohydrate. Dietary fat in particular is reduced in mold infected feeds. Arumolzhi *et al*.(2002) had observed that aflatoxin at molecular level may interfere with energy production. All samples had *A. parasiticus, A. flavus, A. terreus, A. niger*, with exception of one sample made of wheat from Osun state from which *Candida albicans* was isolated.

Khan and Smith (1994) and Vlachou *et al*. (1994) assessed animal feeds from Greece and recorded mould counts as high as 10^5 cfu/g. Abarca *et al*.(1994) examined animal feeds and recorded fungal counts of 10^2 -10^8 cfu/g but noted that the moulds isolated were aflatoxigenic. Khan and Smith (1994) reported 26 x 10^4 cfu/g in mouldy feed and 7 x 10^3 in non-mouldy feed. All results obtained in this study fell within these values. The presence of these pathogenic organisms in feeds pose serious health hazard. Total aflatoxins levels in feeds ranged from 5 to 270 µg/kg. Three samples (one sample from Ogun State and two samples from Osun State) only fell below the 20 µg/kg regulatory limit set by Food and Drug Administration. Most of the animal feeds were formulated

Table 1: **Fungal counts and probable moulds from poultry feeds sold in South Western Nigeria**

States	Fungal count(cfu/g)	Probable isolates
Lagos	4×10^4	*A. flavus, A. niger, A. terreus,*
	9×10^4	*A.parasiticus, Rhizopus sp*
	4×10^4	
	4×10^4	
	1×10^4	
Ogun	29×10^3	*Aspergillus flavus, A. parasiticus, A. terreus, A. niger*
	4×10^3	
	26×10^3	
	42×10^3	
	5×10^3	
Osun	10×10^4	*Aspergillus flavus, A. parasiticus, A. niger, Candida albicans*
	8×10^4	
	8×10^4	
	3×10^4	
	8×10^4	
Oyo	2×10^4	*Aspergillus flavus, A. terreus, A. parasiticus, A. niger, Rhizopus sp*
	7×10^4	
	4×10^4	
	2×10^4	
	5×10^4	

Table 2: Aflatoxin levels in poultry feeds from four states in South Western Nigeria

States	Optical density	Aflatoxin level (ug/g)
Lagos	2.067	86
	2.800	59
	2.601	63
	1.7	100
	2.712	57
Ogun	1.522	43.2
	2.391	13.5
	2.168	24.0
	0.840	95.1
	0.700	100
Osun	0.700	100
	0.252	270
	2.400	77
	2.800	5
	2.700	12
Oyo	3.122	40
	2.067	86
	2.800	59
	3.204	40
	2.601	63

from maize, peanuts, and soybeans, food staples that are notable culprits in crops contaminated with aflatoxins. Okoli *et al*. (2006) reported the presence of moulds in poultry feeds sold in Imo State of Nigeria and reported the presence of *Aspergillus sp., Penicillium sp., Mucor sp., Yeast Rhizopus sp., Epicoecum sp., Gymnoaseus sp., Cladosporium sp.* Following the EU standard, probably only the wheat sample may be accepted. This sample had low aflatoxin level of 5 μg/kg. The reasons might be due to several reasons such as proper drying of grains after harvest, proper storage, packaging and inclusion of mould inhibitors. Aflatoxins are carcinogenic and they expose animals to different health hazards because of their toxic effects (Kabak *et al*., 2006). The levels of aflatoxin quantified in all the feeds are able to stimulate responses in animals. Oluwafemi and Taiwo (2004) simulated very low doses of aflatoxin (690, 1380, 2010 ng/ml) in cockerels and recorded various responses in liver enzymes, leucocytosis, microctic hypochromic anemia while liver sections revealed gross histological lesions. Gupta et al. (2002) reported that 40ug/kg of aflatoxin was responsible for birds having congested lungs, enlarged pale liver and kidneys, distended ureters with chalky white deposits on kidneys, pericardium, lungs, liver and serosal surfaces of gastrointestinal tract and air sacs.

Rauber *et al.(*2007) fed turkey poults with 20, 50, 100, 200, 500, 1000 ug/kg of aflatoxin B1 and at the end of 21 d of experiment, both feed consumption and Body Weight(BW) were significantly affected by the aflatoxins present in the diet. In addition gizzard relative weight, total plasmatic proteins, and cholesterol levels were also affected. At the 42-d evaluation, besides feed consumption and BW, gizzard and liver relative weights and cholesterol levels were also affected by the presence of aflatoxins in the diet. Khan and Smith (1994) fed steers with mouldy wheat straw for 19 days and recorded BW loss of 6kg. They also reported increase in alkaline phosphates.

The presence of aflatoxins in commercial poultry feeds possibly is one of the contributory factors for diseases in chicken. Severe outbreaks of aflatoxicosis result in high mortality and great economic loss to the farmers. In many such diseases outbreaks, feed consumed at the farms, on analysis invariably showed aflatoxin contaminations and the pathological features were similar to those described for aflatoxicosis by earlier investigators.

Although sample size was small in this study due to high cost of importing chemicals, there is nevertheless unequivocal evidence of contamination of poultry feed in South Western Nigeria. The factors which promote elaboration of aflatoxins in the poultry feed ingredient are inadequate pre and post harvest conditions, untimely rains, floods, drought, lack of facilities for quick drying of the harvests, insect attack and poor storage conditions. Besides, poultry feed mill owners use feed ingredients of inferior quality for more profit. It has been suggested that improvement in agricultural practices such as Good Agricultural Practice (GAP), development of traceability systems and establishment of Hazard Analysis Critical Control Point (HACCP) systems will contribute in no small measure in controlling the adverse effects of aflatoxins in poultry feed ingredient are inadequate pre and post harvest conditions, untimely rains, floods, drought, lack of facilities for quick drying of the harvests, insect attack and poor storage conditions. Besides, poultry feed mill owners use feed

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