Nigerian Food Journal
Official Journal of Nigerian Institute of Food Science and Technology www.nifst.org
NIFOJ Vol. 32 No. 1, pages 73 – 79, 2014

Fungal Profile and Aflatoxin Contamination in Poultry Feeds Sold in Abeokuta, Ogun State, Nigeria

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ABSTRACT

Aflatoxin contamination of animal feeds is common and widely spread, especially in the tropics, due to the ubiquity of the producing fungi. The detection of aflatoxin in five samples of animal feed was carried out; using enzyme linked immunosorbent assay (ELISA). Samples were taken from five different areas in Abeokuta. The aflatoxin level was observed to be the highest in the poultry feed from Lafenwa with the value 93.1 μg/kg; and lowest in the feed from Idi-Aba with the value 13.5 μg/kg. Fungal counts are between 4 **x** 10^3 and 42 **x** 10^3 cfu/g, with highest count occurring in the feed from Lafenwa and lowest in Idi-Aba. The fungal growth was on potato dextrose agar (PDA), and *Aspergillus flavus*, *A. oryzae*, *Rhizopus oryzae* and *Penicillum notatum* were isolated and identified, with *Aspergillus flavus* predominating. Comparison statistical analysis using ANOVA showed a significant mean difference 95% confidence interval. In conclusion, aflatoxin was present in all the samples, which even at low concentration is of great concern to human and animal health. Maize was the main ingredient in all the contaminated feed.

Keywords: Aflatoxin, contamination, animal feed, moulds, concentration.

Introduction

Naturally occurring toxicants produced by microorganisms such as bacteria and fungi (moulds) contaminate foods and feeds and these food borne hazards pose a serious health risk to mammals, fish and poultry. Some fungi produce toxins, viz mycotoxins, and the diseases caused by the ingestion of mycotoxins are called mycotoxicoses. The toxin production can take place in either pre-harvest or post-harvest stage of the crop. Many people died in Russia during World War II owing to alimentary toxic aleukia (ATA), a mycotoxicosis caused by T-2 toxin, sequiterpenoid mycotoxin (Joffe, 1978).

The resurgence of interest in mycotoxin research is directly related to the discovery of the aflatoxins during the 1960s, a group of structurally related hepatocarcinogens, produced on nuts and cereals by *Aspergillus flavus*, *A. parasitus* and *A. nomius* and their role in the aetiology of primary liver cancer in humans (Van Rensburg, 1986; Bressac *et. al.* 1991; and Groopman *et. al.*, 1992). Aflatoxins are one of the most potent toxic substances that occur naturally. They are naturally occurring toxic metabolites and closely related mycotoxins produced by fungi *Aspergillus flavus* and *A. parasiticus*. These are moulds found on food products such as corn and peanuts, peanut butter. Aflatoxins have been associated with various diseases such as aflatoxicosis in livestock, domestic animals and humans throughout the world. Aflatoxicosis is poisoning that result from the ingestion of aflatoxins in contaminated food or

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feed. It acts as a potent liver carcinogen in rodents (and presumably, humans). Aflatoxin poisoning is reported from all parts of the world in almost all domestic and non domestic animals like cattle, horses, rabbits, and other non human primates (Bommakanti and Waliyar, 2004). Aflatoxins were initially isolated and identified as the causative toxins in Turkey X disease (necrosis of the liver) in 1960 when over 100,000 turkeys died in England (Asao *et. al.*, 1963). It was soon found that the disease was not limited to turkeys. Ducklings and young pheasants were also affected and heavy mortality was experienced. There are four generally recognized dietary aflatoxins, designated B1, B2, G1, G2 (AFB1, AFB2, AFG1, AFG2 respectively). The metabolites, M1 and M2, are also found in milk. The order of toxicity is B1 greater than G1 greater than G2, greater than B2 (IARC, 1976). Aflatoxin M1 (AFM1) has been identified in the milk of dairy cows consuming AFB1-contaminated feeds. The occurrence of aflatoxicosis is influenced by the weather (temperature and humidity – warm and wet is worst!); so the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of the peanuts to fungus before they are harvested, and during storage and or processing periods (D'Mello, 2001). The aflatoxigenic Aspergilli are generally regarded as storage fungi proliferating under condition of relatively high humidity and temperature. Aflatoxin contamination, therefore, almost exclusively confined to tropical feeds such as oilseed by-product derived from groundnuts, cottonseed and palm kernel (D'Mello, 2001). Aflatoxin contamination of maize is also an important problem in warm humid regions where *A. flavus* may infect the crop prior to harvest and remain viable during storage (D'Mello, 2000). Thus, aflatoxins have received greater attention than any other mycotoxins because they clearly have a potent carcinogenic effect in laboratory rats and their acute poisonous effects in humans (Hussein *et. al.*, 2001). The study investigated the aflatoxin levels and fungal load of animal feed samples through the use of enzyme-linked immunosorbent assay (ELISA) and microscopy respectively.

Materials and Methods

Collection of samples

Two hundred grams (200 g) each of poultry feed (layers mash) was collected from five different areas of Abeokuta: Lafenwa, Obantoko, Idi-Aba, Kuto, Omida. Fungal count and aflatoxin determination was carried out on each to determine the total fungal population and aflatoxin level.

Serial dilution

For each poultry feed sample, three dilutions, 10^{-1}, 10^{-2}, 10^{-3} were made. From the 10^{-3} dilutions, 1 ml of dilutions was aseptically pipette into sterile petri dishes. The inoculi were covered with the prepared sterile potato dextrose agar (PDA) containing 0.04 g/l of streptomycin, in pour plate method, swirled on the work table and allowed to set. The plates were incubated in an inverted position for 5 – 7 days; after which colonies on the plates were counted and recorded. All colonies were counted and expressed in colony forming unit per gram (cfu/g) of the sample. Colony forming units were determined using the formula:

$$\frac{\text{No of colonies}}{\text{Volume used}} \times \text{dilution factor}$$

Subculture of fungal isolates from primary plates

Fungal isolates on primary plates were subcultured with the help of an inoculating needle and plated on freshly prepared potato dextrose agar (Oxoid). The plates were duly labelled based on source of sample. Incubation at room temperature was carried out for 5 – 7 days.

Identification of fungal isolates

Microscopy was done with the use of cotton blue-in-lactophenol as stain and viewing under the x40 objective lens, for morphology.

Aflatoxin extraction and quantification

In aflatoxin analysis, direct competitive enzyme-linked imnmunosorbent assay is used. The enzyme-