A SURVEY OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS IN POULTRY FEED

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

Dedicated to Kinuthia Gathumbi. My beloved grandfather for his interest in what I do at school.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABST.....Abstract
ADMD.....Aspergillus differential medium (dichloran).
A.O.A.C.....Association of official analytical chemists.
CFU.....Colony forming units.
DRYES.....Dicloran rose bengal yeast extract sucrose agar.
ELISA.....Enzyme-linked immunosorbent assay
FAO.....Food and Agriculture Organisation.

g.....gramme(s).

GLC.....Gas Liquid Chromatography.

HPLC.....High Pressure Liquid Chromatography.

HPTLC.....High Performance Thin Layer Chromatography.

Mg.....Milligramme(s).

ML.....Millilitre(s).

MPN.....Most probable number.

MSA.....Malt salt agar.

nM.....Nanometre(s).

PDA.....Potato dextrose agar

ppb.....Parts per billion.

ppm.....Parts per million.

PSA.....Potato sucrose agar

RIA.....Radioimmunoassay

spp.....Species

TLC.....Thin Layer Chromatography

µL.....Microlitre(s).

vol.....volume

wt.....weight

ABSTRACT

The occurrence of fungi and their toxins in poultry feed lowers the hygienic quality of feed and presents several hazards to poultry and sometimes to man. To evaluate the magnitude of these hazards and generally the hygienic quality of feed presented to poultry in Nairobi, Kenya, several experiments were carried out. A total of 90 samples of poultry feeds were randomly sampled from farmers in peri-urban areas Nairobi province and in Kikuyu division of Kiambu district. Thirty samples were collected in January, 30 in March, and 30 in May 1993.

All the samples were subjected to moisture determination, mycological examination and mycotoxin analysis. Enumeration of total mould counts, *Aspergillus* spp. counts, *Penicillium* spp. counts, *Fusarium* spp. counts as well as identification of isolates of species belonging to these three genera was done. The aflatoxin producing ability of the *Aspergillus flavus* group isolates was tested on a synthetic medium. All feed samples were also analysed for aflatoxin B1, B2, G1, G2, ochratoxin A, zearalenone and sterigmatocystin.

The total mould counts recorded were very high compared to the findings from other parts of the World such as Norway and Germany. They varied from 1×10^4 - 1.31×10^6 CFU/g. On the basis of these mould counts, the hygienic quality of 36% of all the samples examined were classified as unacceptable. *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. were the most commonly isolated fungi sometimes accounting for all the fungi identified.

Aspergillus spp. counts varied from 1.0×10^3 - 8×10^5. *Penicillium* spp. counts varied from 2.0×10^2 - 3.0×10^5 while *Fusarium* spp. counts varied from 0 - 3.1×10^5. A total of fifteen species of the genus *Aspergillus*, 19 of *Penicillium* and 3 of *Fusarium* were identified. Overall, *Aspergillus flavus* was the most commonly isolated species being demonstrated in 73.3% of all samples. When the aflatoxin producing ability of these A. *flavus group* isolates was tested on yeast extract sucrose agar, only 19.01% of all the isolates produced aflatoxins.

Analysis of mycotoxins by thin layer chromatography revealed that 35.6% of all the samples were contaminated with at least one of the seven mycotoxins analysed for, with aflatoxins being the most abundant. They were found in 24.4% of the samples at levels of between trace and 50ppb. Ochratoxin A was detected in 10% of all samples, zearalenone in 5.6% and sterigmatocystin in 2.2% of all samples examined. Occurrence of several mycotoxins in one sample was recorded in 13.3% of all the samples. The moisture content varied from 6.79 % to 11.46 %.

Statistical analysis showed no significant differences in the levels of total mould counts, moisture content, mycotoxin contamination and most fungal groups' counts in feed samples from different manufacturers or of different feed formulations. However, almost all these variables showed significant differences when feed sampled at different times was compared.

Several conclusions are drawn from this study. (1) The level of fungal contamination of poultry feeds used in Nairobi is very

high making most of the poultry feed unacceptable. (2) The majority of these contaminants belong to the genera *Aspergillus, Penicillium* and *Fusarium* and include many well known mycotoxigenic fungi species. (3) Aflatoxins are present in some of the feed used in Nairobi and surrounding areas, but at levels insufficient to cause clinical aflatoxicosis. (4) Mycotoxin other than aflatoxins, such as ochratoxin A, zearalenone and sterigmatocystin also occur in poultry feeds used in Kenya, sometimes as co-contaminants to the aflatoxins. (5) The frequency of contamination of poultry feed with mycotoxins vary with the month of the year. (6) Because of the low moisture content of feed recorded in this study, the mycotoxin contamination in mixed poultry feed is most likely as a result of use of contaminated raw materials and not due to fungal growth and toxin production in the mixed feed. For this reason, it is recommended that further studies be done to identify the most susceptible raw material and also have a continuous surveillance for mycotoxins in these raw materials.

1 INTRODUCTION

The poultry industry started in the early ages as a home enterprise whose products were geared to meeting the home consumption alone. With the increase in human population and the corresponding increase in the demand for poultry products, the industry has changed to become a highly competitive commercial enterprise. Extensive systems of poultry husbandry have now been replaced by very intensive systems of poultry production that utilise high energy feeds. In Kenya, these intensive forms of poultry keeping are mainly found in the peri-urban areas and rely on commercially mixed poultry feeds. These feeds as well as other high energy feeds are unfortunately, also highly preferred substrates for many micro-organisms such as bacteria and fungi including yeasts.

The study of micro-organisms in poultry feed has for a long time been a neglected area of poultry science. This attitude is however rapidly changing due to the realization of the important role poultry feed can play as a source of pathogenic organisms and toxins to poultry (Tabib *et al.*, 1981). Specifically, the establishment of poultry feed as a link in the cycle of poultry salmonellosis (Mackenzie and Bains, 1976) and recognition of the filamentous fungal toxins (mycotoxins) as a widespread economic threat to profitable poultry husbandry have led to increased interest in the microbiology of poultry feed (Tabib *et al.*, 1981).

Most poultry feeds are prepared mainly from grain. Since fungi are the major contaminants in these raw materials, they also tend

to dominate the microflora of mixed poultry feeds. Fungal contamination and growth in poultry feed presents several problems to man and poultry. These include lowered hygienic quality of feed, nutrient losses and causation of certain diseases such as aspergillosis. Too many fungal spores in feed may also cause allergy in man handling the feed (Lacey *et al.*, 1980). Certain fungal species can also produce toxic secondary metabolites called mycotoxins. Ingestion of these mycotoxins results in diseases called mycotoxicoses. Some of the most notorious mycotoxin producing fungi species belong to the genera *Aspergillus*, *Penicillium* and *Fusarium* (Moreau, 1979). Demonstration of fungi belonging to these genera and especially the identification of species that are known to be toxigenic, in feed, is an indication of a potential mycotoxin hazard.

There exists a high positive correlation between the incidence of toxigenic fungi and that of mycotoxins (Pitt, 1979). However, the demonstration of toxigenic fungal species in feed does not mean that the feed is toxic. Neither does the absence of toxigenic species in feed indicate freedom from mycotoxin contamination. The only conclusive evidence of the presence of a mycotoxin is the demonstration of the specific mycotoxin itself. This has been done for the aflatoxins during several past outbreaks of acute aflatoxicosis in human beings, dogs and poultry in Kenya (Price and Heinonen, 1978; Muraguri *et al.*, 1981; Mbugua and Etale, 1987). Information on the level of aflatoxin exposure to animals and poultry when no avert aflatoxicosis is evident, however, is

missing. Such information is important in the assessment of the economic losses due to the subacute and subclinical aflatoxicosis. These forms of aflatoxicosis are thought to cause much greater economic loss than the more dramatic acute aflatoxicosis (Pier *et al.*, 1980)

The extent of contamination of Kenyan food and feed with mycotoxins other than aflatoxins is for the large part unknown. This is because almost all the research work on mycotoxins that has been done in Kenya has dwelt on aflatoxins. This narrow scope of work has persisted inspite of accumulated information on the adverse effects and natural occurrence of other mycotoxins in other parts of the World and the recognition of the interactive nature of different mycotoxins in multimycotoxin exposures (Huff *et al.*, 1988b). There was, therefore, need to investigate the occurrence of mycotoxins other than aflatoxins in Kenyan foods and feeds so as to determine the mycotoxins that are relevant to the Kenyan situation.

The purpose of this investigation was to study the fungal microflora and mycotoxins in poultry feeds used in peri-urban areas of Nairobi.

The specific objectives of this study were:-

(1) To determine the level of fungal contamination in poultry feed as a measure of the hygienic quality.

(2) To isolate, enumerate and identify fungal isolates belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*.

(3) To determine the aflatoxin producing ability of *Aspergillus flavus* group isolates.

(4) To analyse poultry feeds for aflatoxins, ochratoxin A, zearalenone and sterigmatocystin.

2 REVIEW OF LITERATURE

2.1 *ASPERGILLUS* Micheli ex Fries

Aspergillus is a latin word for a mop used to distribute holy water. The genus *Aspergillus* was given this name by Micheli in 1729 (Raper and Thom, 1945) in reference to its characteristic sporing structure.

2.1.1 MORPHOLOGY AND IDENTIFICATION

Colonies on Czapek dox agar have varied textures and colours. Textures are either coarse, rough, wooly or cottony. Colours vary from white to pink, orange, yellow, green, blue green, gray brown, with a white to deep red or lavender reverse (Campbell and Stewart, 1980).

The vegetative mycelia consist of colourless, pale or brightly coloured, septate, profusely branching hyphae composed of multinucleated cells (Alexopoulos and Mims, 1979). The conidial apparatus develop as conidiophores that arise from, and more or less perpendicular to specialized thickwalled hyphal cells referred to as foot cells. The conidiophores are septate or non-septate, smooth or rough frequently enlarging to the apex and terminating in a swelling called the vesicle. The vesicle may be globose, subglobose, clubshaped, hemispherical or a mere thickening of the conidiophore. A vesicle may have one or two layers of conidiogenous phialides depending on the species. When two layers of phialides are formed, the uppermost layer is the one from which

conidia arise. Continuously elongating tips of phialides successively cut off to form unbranched chains of conidia (Mehrotra and Aneja, 1990). The conidia vary greatly in size and shape depending on the species. Cleistothecia and sclerotia are occasionally formed. Sexual stage structures, where known, belong to the Ascomycetes genera: *Eurotium, Sartorya, Emericella, Hemicarpenteles* and *Dichlaena* (Smith , 1969 ; Alexopoulos and Mims, 1979).

The genus has been a subject to many taxonomic treatments. These include those of Raper and Thom (1945) and Raper and Fennell (1965) (cited by Smith, 1969). The colour and rate of growth of colonies, the colour and shape of the sporing heads at low magnification (**X** 25-75) and the characteristics of the sporing structure at high magnification are important in identification of *Aspergillus* species. Presence of sclerotia, hulle cells, perithecia and other sexual stage structures are also utilised in identification.

2.1.2 ECONOMIC IMPORTANCE

The genus *Aspergillus* is the most widespread genus throughout the World. It is present in the air, soil and many other substrates (Alexopoulos and Mims, 1979). Some species cause aspergillosis in man and animals (Campbell and Stewart, 1980). *Aspergillus spp.* are also commonly involved in spoilage of food and feeds, sometimes with production of mycotoxins. Some important mycotoxin producers include A. *flavus* which produces aflatoxins, A. *versicolor* which

produces sterigmatocystin, A. *ochraceus* which produces ochratoxins, A. *fumigatus* which produces fumitremogens and A. *clavatus* which produces patulin. Other mycotoxins produced by *Aspergillus* species include some versicolorins, cytochalasins, tremogens, toxic lactones, malformins, roquefortines and secalonic acids (Cole and Cox, 1981).

2.2 *PENICILLIUM* Link ex Fries

Penicillus is a latin word for small artist's brush. The genus *Penicillium* acquired this name due to its characteristic brush or broom-like fruiting structure.

2.2.1 MORPHOLOGY AND IDENTIFICATION

Most species have flat, fast growing, powdery and heavy sporing grey blue to grey green colonies (Pitt, 1976). The vegetative mycelium is colourless, pale or brightly coloured. In most species the mycelium is predominantly submerged or partly submerged. The hyphae are highly branched, septate and made up of unicellate cells. In some species, hyphae intertwine with each other to form ropes. Sclerotia and perithecia are formed in a few species. The perfect (sexual), stages where known, belong to the genera *Eupenicillium* and *Talaromyces* (Mehrotra and Aneja, 1990)

The characteristic conidial apparatus, the penicillus, is composed of a stripe (conidiophore) bearing a tuft of conidiogenous cells (Phialides) and other elements such as rami and metulae. The conidiophores may stand alone or may be aggregated in clusters,

fascicles or definite coremia. Each conidiophore gives rise to a single whorl of phialides or may be twice or severally vertically branched, the final branches being phialides (Smith, 1969; Gilman, 1957; Onions, 1982). Phialides produce unbranched chains of conidia by abscission. The conidia may be ovoid, elliptical or pyriform, smooth or rough and resemble glass beads under a microscope (Alexopoulos and Mims, 1979).

Taxonomy in *Penicillium* has been done by among others, Raper and Thom (1949) (cited by Gilman, 1957) and Pitt (1979) (cited by Onions, 1982). In the latest classification, Pitt (1979) described 150 species of *Penicillium* and its teleomorphic states, *Eupenicillium* and *Talaromyces* (Onions, 1982). The colour and size of the colony, the colour of mycelia and fruiting structures, the manner of branching of the penicillus and other precise characteristics of the sporing structure are some of the important characteristics used to differentiate *Penicillium* species.

2.2.2 ECONOMIC IMPORTANCE

The genus *Penicillium* is cosmopolitan in distribution although it is more prevalent in temperate regions of the World (Mehrotra and Aneja, 1990). Many species cause decomposition of stored products while others cause plant and animal diseases. Some species are also important mycotoxin producers. These include P. *citreonigrum* which produces citreoviridin and citrinin, P. *citrinum* which produces citrinin, P. *islandicum* which produces emodin, luteoskyrin, islanditoxin and rugulosin and P. *rugulosum* which

produces rugulosin. Others are P. *purpurogenum* which produces rubratoxin A and B, P. *cyclopium* which produces ochratoxins, P. *griseofulvum* which produces patulin and griseofulvin and P. *expansum* which produces patulin and citrinin. Other mycotoxins produced by *Penicillium* spp. include tremogens, toxic lactones, roquefortines, epipolythiopiperazine-3,6-diones and secalonic acids (Cole and Cox, 1981).

2.3 *FUSARIUM* Link ex Fries

The genus *Fusarium* was created by Link in 1809 for species with fusiform, nonseptate spores borne on a stroma. Later Fries (1821) validated this genus in terms of the International Botanical code and included it in his order Tuberculariae (Booth, 1971). The presence of fusoid hyaline macroconidia with a footcell shaped into some kind of a heel is the most definite characteristic of the genus.

2.3.1 MORPHOLOGY AND IDENTIFICATION

Fusarium spp. can grow on most standard mycological agar media such as potato sucrose agar, potato dextrose agar and water agar (Booth 1971). Selective media such as Nasu-synder medium, modified Czapek dox and Komada's media have also been developed for isolation of *Fusarium* spp. from fresh samples. Growth on most media is cottony, white, turning rose, lavender or other colours with age (Campbell and Stewart, 1980). Most species are difficult to maintain in a state of stability in artificial culture (Smith,

1969). Members of the genus *Fusarium* produce two types of conidia termed macroconidia or microconidia because of their relative sizes (Alexopoulos and Mims, 1979). Macroconidia are colourless or pale coloured, sickle-shaped with pointed ends usually with several cross-septa. Microconidia are small, ovate, elongate, pyriform or comma shaped usually nonseptate but can rarely be 1-3 septate. Some species produce chlamydospores that may be terminal, intercalary, or both, in the mycelia or in macroconidia. Sclerotia are sometimes formed and are often brightly coloured (Mehrotra and Aneja, 1990). The perfect stages, where known, belong to the genera *Typomyces, Gibberella, Nectria and Colanectria* (Smith, 1969; Booth, 1971).

There are several taxonomic treatments of the genus *Fusarium*. These include that of Synder and Hansen's system of 1945, Toussoun and Nelson of 1968, Gerlach of 1970 and 1978 (cited by Mehrota and Aneja, 1990) and Booth (1971, 1977). Toxicologists mainly use the Booth (1971, 1977) system while plant pathologists use the Synder and Hansen's system (1945). According to Booth (1971), the genus has about 50 species. These are differentiated on the basis of cultural characteristics on Potato dextrose agar (PDA) or Potato sucrose agar (PSA), morphological characteristics of conidia, conidiophores and chlamydospores, and the presence or absence of the perfect stage and its characteristics. The most recent methods in *Fusarium* classification utilise conidial ontogeny characteristics to differentiate species (Hughes, 1953; Alexopoulos and Mims, 1979).

3.3.2 ECONOMIC IMPORTANCE

Fusarium spp. are well distributed in soil and organic matter. They have been isolated from the permafrost in artic and also from sand of the Sahara. Several species are involved in diseases of plants, animals and humans. Others cause major storage rots, often producing mycotoxins (Booth, 1971). Mycotoxins produced include zearalenone and many toxic trichothecenes such as T-2 toxin, HF-2 toxin, neosolaniol, diacetoxyscirpenol, nivalenol, fusarenon-x, diacetylnivalenol, deoxynivalenol and 3-acetyldeoxynivalenol (Cole and Cox 1981).

2.4 METHODS OF DETECTION AND ENUMERATION OF FUNGI

Detection and enumeration of fungi may be achieved by use of a variety of cultural methods, direct microscopic methods and indirect methods (Jarvis, 1978).

2.4.1 CULTURAL METHODS

These methods involve cultivation of fungi in suitable culture media. These include direct plating of samples on solid media, mould propagule (dilution) counts on solid media, membrane filtration techniques and most probable number techniques (Jarvis *et al*, 1983).

2.4.1.1 CULTURE MEDIA

General purpose, selective and differential cultural media have been developed for isolation of fungi. General purpose media allow growth of most fungi and bacteria. They include natural media

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such as pea agar, wort agar and other media based on malt or yeast extract fortified with glucose or other carbohydrates and solidified with 1.5 - 2.0% agar (wt/vol.). The most commonly used general purpose media include potato dextrose agar, Sabouraud dextrose agar, malt extract agar and Czapek solution agar (Jarvis, 1978). Czapek solution agar and its modification are frequently used in the identification of fungal isolates, especially those belonging to the genera *Aspergillus* and *Penicillium* (Pitt, 1976).

In addition to nutrients that support fungal growth, selective media also contain certain selective mold inhibitors such as salts, antibiotics, acids, rose bengal, botran (2,6-dichloro-4-nitroaniline) and oxgall. These inhibitors prevent the spread of fast growing species of fungi and are very important in enumeration of fungi (Frisvad, 1983).

Only a few differential media are used in fungal isolation. A differential medium containing ferric citrate or ferric chloride permits rapid presumptive identification of *Aspergillus flavus* and *Aspergillus parasiticus*, all of which produce an intense orange-yellow reverse colouration on this media. Incorporation of botran (dichloran) to this media has lead to a differential and selective media, ADMB (*Aspergillus* differential media (Dichloran)) for the same species (Hamsa and Ayres, 1977).

2.4.1.2 DIRECT PLATING METHOD

A portion of the sample of interest is placed directly onto the surface of suitable media. Incubation permits development of

moulds *in situ*. The sampling method is non-destructive as mycelia clumps and sporing heads are not fragmented by homogenization procedures that usually precede other cultural methods. The method however, cannot be used to estimate obligate xerophilic fungi in animal feeds such as hay because of problems of equilibration and growth of other contaminants (Jarvis, 1978; Jarvis *et al.*, 1983)

2.4.1.3 MOULD PROPAGULES (DILUTION) COUNTS

This method involves preparation of a sample homogenenate followed by serial dilution in appropriate diluents and plating on to (by surface spread method) or into (by pour plate method) suitable culture media. Diluents used are mostly of physiological osmolarity and incorporate a 'wetting agent ' such as tween 80 (Jarvis et al., 1983). Inoculated agar media plates are incubated at 17 - 27 ^{0}C for 5 - 7 days and the number of colony forming units (CFU) counted (Jarvis, 1978). A major limitation of this method is its heavy bias in favour of organisms profligate in the production of spores and its marked variability associated with different agar media techniques of enumeration (Tabib *et al.*, 1984).

2.4.1.4 MEMBRANE FILTRATION TECHNIQUES

In these methods, liquid samples or supernatant liquid of sample homogenates are filtered through a membrane and the membrane then incubated on a pad soaked with culture media. Counting of fungal colonies is done after a short incubation period using a

hand lens or a stereoscopic microscope. This method is useful in detecting low-level fungal contamination (Jarvis, 1978).

2.4.1.5 MOST PROBABLE NUMBER TECHNIQUES (MPN)

These involve multiple inoculations of samples in liquid media. The methods are rarely used for estimation of fungal contamination although they have been shown to consistently give higher counts than do any fungal propagule count method on solid media (Koburger and Norden, 1975). The precision of MPN techniques are however considerably influenced by the homogeneity of the sample dilutions (Jarvis, 1978).

2.4.2 DIRECT MICROSCOPY METHODS

These include the Howard mould counts method, fluorescent microscopy techniques and the machinery mould counts. In the Howard mould count method, which is most commonly used, examination of a number of standardised microscopic fields provides an estimate of the extent of mould contamination. However, the method lacks precision and has a high coefficient of variation (Jarvis, 1978).

In fluorescent microscopy techniques, the fungal mycelium is detected microscopically following application of fluorescent brightners or fluorescent antibodies. The disadvantage of this technique is that the autofluorescence of food materials may disguise the presence of fluorescent mycelia (Jarvis *et al.*, 1983).

The machinery mould count is a method of quantification of *Geotrichum candidum* and is based on differential staining of fungal

mycelial clumps. Other fungi may however interfere with *Geotrichum candidum* counts (Jarvis, 1978; Jarvis *et al*, 1983).

2.4.3 INDIRECT METHODS

These are rarely used. They utilise estimation of cellwall chitin, metabolic activity, electrical impedance measurements, microbial ATP and particle counting as measures of fungal contamination and growth (Jarvis, 1978).

2.5 REQUREMENTS FOR FUNGAL GROWTH AND MYCOTOXIN PRODUCTION

Fungi require suitable substrates and environmental conditions for growth and mycotoxin production to occur. These requirements vary with the particular fungi or mycotoxin and are not necessarily the same for growth and mycotoxin production (Moreau, 1979).

2.5.1 SUBSTRATE

Fungi need sources of carbon such as carbohydrates and proteins, sources of nitrogen such as proteins and inorganic nitrogen compounds, certain vitamins and minerals for growth. Almost all foodstuffs contain the above nutrients and so may serve as substrates for most fungi (FAO, 1990). The exact nutritional requirements for growth and mycotoxin production however differs for every fungal species (Moreau, 1979). For example, aflatoxin production by *Aspergillus flavus* is enhanced by zinc and inhibited by barium (Venkitasubramanian, 1977). The presence of additives and preservatives in a substrate may also influence mould growth and

mycotoxin production. Other factors include the initial level of mould contamination in a substrate, physical damage of substrates such as grain and genetic properties of some substrates such as maize and peanuts (Mislivec, 1977; Sauer, 1987).

2.5.2 ENVIRONMENTAL CONDITIONS

A number of interrelated factors influence fungal growth and mycotoxin production. Of these factors, temperature, water activity (humidity), $_pH$ and oxygen are the most important. Other factors are light, carbon dioxide, microbial interactions within a substrate, insect activity and time duration when all conditions are suitable (Moore-Landecker, 1972; Mislivec, 1977).

2.5.2.1 TEMPERATURE

Temperature plays an important role in the growth of mycelium and in the formation and germination of spores (Moreau, 1979). The minimum, optimum and maximum temperatures for growth differ widely for the various species of fungi (FAO, 1990). The majority of fungi grow at 20^0C - 25^0C. However, some such as *Cladosporium herbarum* are capable of slow growth at -6^0C while others such as *Humicola lanuginosa* can grow at 60^0C (Moreau, 1979). The optimum temperature for growth is 25^0 - 30^0C for most *Penicillium* spp., 30^0 - 40^0C for most *Aspergillus* spp. and 8^0 - 15^0C for most *Fusarium* spp. (FAO, 1990). The optimum temperatures for mycotoxin production differ for different fungi and are not necessarily the same as those most suitable for growth. For example, *Penicillium chrysogenum* has an

optimum temperature of 30^{0}C for growth and 20^{0}C for mycotoxin production (Moreau, 1979). Some fungi such as *Fusarium sporotrichioides* produce their toxins best at 0^{0}C whereas others such as *Aspergillus flavus* have an optimum temperature of 25^{0}C for toxin production (Pier, 1981). Temperature may also influence the quantity and type of mycotoxin produced by a fungus. For example, *Fusarium tricinctum* produces diacetoxyscirpenol and T-2 toxin at 8^{0}C and HT-2 toxin at 25^{0}C (Ueno, 1977).

2.5.2.2 WATER ACTIVITY

Water activity expresses the availability of water in a substrate for growth of micro-organisms. Water is important for the growth of mycelium, production of fruiting bodies and germination of spores. Fungi can grow more rapidly at low water activity than bacteria. Xerophilic fungi such as *Aspergillus glaucus* can grow at water activity less than 0.7, mesophilic fungi such as *Penicillium cyclopium* at water activity of between 0.8 - 0.9 while hydrophilic fungi such as *Trichothecium roseum* will only grow at water activity of above 0.9 (Moreau, 1979). The range of water activity that sustain growth is however not definite but is considerably narrowed by the composition of food, acidity, temperature and concentration of oxygen (Sauer, 1987).

2.5.2.3 OXYGEN

Oxygen is vital for cellular respiration that provides energy. Moulds, unlike yeasts require oxygen for growth and cannot

proliferate in anaerobic conditions. Thus deoxygenators, gas replacememt methods and vacuum packing are effective in reducing mould growth and mycotoxin production. Reducing atmospheric oxygen below 5 - 1 % for example, reduces sporulation and aflatoxin production by *Aspergillus flavus* (Diener and Davis, 1969). In contrast, very high oxygen pressure may somewhat reduce fungal growth or change the mycelial habit but do not usually have a lethal effect on fungi.

2.5.2.4 PH

Fungi have a comparatively broad PH range over which they grow. Most fungi grow at PH range of between 4 - 8 although some are capable of growth in very alkaline or very acidic conditions. The PH over which a fungus grows may sometimes influence the production of fungal metabolites such as mycotoxins. For example, PH variation between 2.0 and 7.2 has been found to result in marked variations in the amounts and types of aflatoxin metabolites produced by *Aspergillus flavus* (Venkitasubramanian, 1977).

2.6 MYCOTOXINS

The term mycotoxin is derived from two greek words "mykes" (fungus) and "toksikon" (poison) (FAO 1990). It refers to a group of secondary metabolites of filamentous fungi which when ingested by animals or man may cause disease (Jarvis, 1989; Robb, 1993).

The toxicity of certain fungal metabolites has been known since the early ages. However, it was not until the outbreak of

"Turkey X" disease in England in 1960 that interest into the investigation of mycotoxins started (Pitt, 1976). Today, over 800 different mycotoxins showing a large variety of chemical structures are known. These are produced by over 150 species of fungi, most of which belong to the class, fungi imperfecti (Jarvis, 1989). Almost all these mycotoxins are relatively heat-stable and are low molecular weight chemical compounds that are nonantigenic and hence do not provoke an immune response. They are however capable of interfering with vital body metabolic functions at very minute doses, resulting in many different adverse biological effects (Pitt, 1976). These include acute toxic, mutagenic, carcinogenic, teratogenic, hallucinogenic, emetic and oestrogenic effects (FAO, 1990). Human and animals are exposed to mycotoxins through ingestion of contaminated food or feed, inhalation or skin contact.

Animals vary in their susceptibility to mycotoxins according to the species, the age and the specific toxin involved. In general, exposure to large doses of a mycotoxin usually results in acute mycotoxicosis. This form of mycotoxicosis has overt clinical manifestation and is usually easy to recognise. Lower doses of mycotoxins result in less severe mycotoxicosis often characterised by lowered animal productivity with or without mild clinical signs. Low doses may also interfere with the native immunity and immunologic responses in animals and so predispose them to infections (Pier *et al.*, 1980). Although many mycotoxins have shown adverse effects on domestic animals either in natural outbreaks or in experimental intoxications, the mycotoxins that are important in

causation of natural outbreaks in domestic animals are few. These include aflatoxins, toxic trichothecenes, ergot alkaloids, ochratoxins and zearalenone (Pier *et al*, 1980; Pier, 1981).

2.6.1 AFLATOXINS

Aflatoxins are a group of 18 highly flourescent, highly oxygenated, closely related derivatives of difuranocoumarin. Some of these derivatives such as B1, B2, G1, and G2 are produced naturally by *Aspergillus flavus* and the closely related *A. parasiticus*. Other derivatives such as M1, M2, P1, Q1 and aflatoxicol are metabolic products of microbial and animal systems while others such as B29, G29 and D1 are formed spontaneously in response to certain chemical environments (Cole and Cox, 1981). The four major aflatoxins: B1, B2, G1 and G2 are named according to their fluorescent colours when their thin layer chromatographic preparations are viewed under longwave (365nm) ultraviolet light. Aflatoxin B1 and B2 fluoresce bright blue while aflatoxin G1 and G2 fluoresce green (Pier, 1981).

The story of aflatoxins began with the death of 100,000 Turkey poults in England in 1960, from a disease refered to as "Turkey X", whose etiology was unknown (Blount, 1961). The disease was associated with feeding poultry on imported Brazilian groundnut meal. Investigation of this toxic groundnut meal finally led to the discovery that an isolate of common mould *Aspergillus flavus* Link ex Fries was the responsible agent (Sergeant *et al.*, 1961). In view of the fungus of origin, these toxic isolates were named aflatoxin

and the toxicosis induced aflatoxicosis.

Investigation of other commodities that caused mould toxicosis in domestic animals and poultry in the period just after the discovery of aflatoxins showed that it was not only Brazilian groundnuts that could contain aflatoxins, but also many other commodities from different parts of the World. Today numerous publications of the occurrences of aflatoxins have appeared and virtually no food or feed can be said to be free of possible contamination (FAO, 1990). However, some commodities have been shown to be especially prone to aflatoxin contamination. These include groundnuts, cotton seed, maize, treenuts and other oil seeds (Robb, 1993). Incidence and levels reported so far vary markedly from one geographical area to another or even within regions. In general, the aflatoxin problem appears to be mainly a problem of the tropical and subtropical areas (Moreau, 1979).

In Kenya, the occurrence of aflatoxins was first reported in 1960 by Haig (quoted by Moreau 1979). During this outbreak of aflatoxicosis, 14,000 ducklings were reported to have died in one farm after being fed on a toxic groundnut meal from Uganda. Another outbreak was reported in 1978 when a large number of dogs and poultry from many parts of the country died (Price and Heinonen, 1978). Immediately after this outbreak, a comprehensive survey was carried out and 34.8% of 316 samples of animal feed were found to be contaminated with aflatoxin at levels between 15ppb and 3000ppb (Muraguri *et al.*, 1981). An outbreak of acute hepatitis due to aflatoxins killed 12 people in the Eastern district of Machakos in

1981. Aflatoxins at 12000mg/kg were demonstrated in maize meant for food in one of the affected homes (Nagindu *et al.*, 1982). Reports of aflatoxicosis in poultry (Mbugua and Etale, 1987) and dogs (Mitema, 1986) then gained prominence in the drought years of 1984 and 1985. In this outbreak, imported maize was found to be the source of aflatoxins (Manwiller, 1987). A study carried out at about the time of the last outbreak found 12.6% of all persons in various parts of the country to be positive of aflatoxin exposure (Autrup *et al.*, 1987). In the most recent survey, Muriuki (1992) found aflatoxins in 8.9% of 214 samples of maize and maize products collected from Nairobi city.

2.6.1.1 TOXIC EFFECTS

Many of the biological effects of the aflatoxin derivatives relate to their ability to interfere with protein synthesis by suppressing the synthesis of messenger-RNA and by inhibition of precursor incorporation into RNA (Buck *at al.*, 1976). The biological effects of the different derivatives are almost similar although they differ considerably in potency. Aflatoxin B1 is the most potent of the known compounds. In general, aflatoxins are known to cause acute and chronic liver damage, reduce growth rates in animals, impair immunologic and native defence mechanisms and exert carcinogenic, teratogenic and mutagenic effects in animals exposed to them (Pier *et al.*, 1980).

The toxicity due to aflatoxins varies considerably among species and with regard to sex, age and nutrition. However, the

liver is the primary target organ in most animal species (Cole and Cox 1981). High doses of aflatoxins cause acute hepatic necrosis, coagulopathy and death with extensive haemorrhaging. Chronic ingestion of low doses often leads to poor weight gain and poor productivity in animals although there appears to be a marked individual animal variation in these effects (Pier, 1981). Definitive hepatic lesions such as centrilobular necrosis, periportal fibrosis, enlarged gall bladders, bile duct hyperplasia and hepatic tumours are usually seen (Pier, 1981).

Subacute doses of aflatoxins, especially aflatoxin B1 and M1 have been shown to cause thymic aplasia in a variety of animals. This results in marked suppression of cell mediated immunity. Aflatoxins also impair natural defence mechanisms including phagocytosis by macrophages and production of complement. Antibody titres may be reduced at high (2 - 40ppb) aflatoxin levels (Pier, 1987). As a result of these immunosuppressive effects, there is enhanced susceptibility to infections and suppression of the development of acquired immunity after immunization. Dietary aflatoxin B1 levels of between 0.2 - 10ppm have been shown to significantly predispose chicken to coccidiosis (Edds *et al.*, 1973), candidiasis, salmonellosis (Pier, 1981) and to increase the severity of signs in infectious bursal disease infection (Chang and Hamilton, 1982). Aflatoxicosis also increases the susceptibility of turkeys to candidiasis, salmonellosis and pasteurellosis (Pier, 1981). The efficacy of turkey herpesvirus vaccination against Marek's disease in chicken has been shown to be significantly

lowered by concurrent dietary aflatoxin B1 at 0.5ppm (Batra *et al.*, 1991). Similary, 1.0ppm dietary aflatoxin B1 level can significantly interfere with the vaccination against Newcastle disease (Rao *et al.*, 1988).

Aflatoxins have been found to be carcinogenic to rats, ducks, mice, trout and to subhuman primates (Sinnhuber *et al.*, 1977). This has led to the presumption that aflatoxins, are also human carcinogens. To investigate this possibility, retrospective epidemiological studies were done in Kenya, Indonesia, Mozambique, South Africa, Swaziland, Thailand and Uganda and a high positive correlation between the incidence of human liver and aflatoxin consumption was found. However, these human populations also had a high incidence of hepatitis B viral infection that can also be a cause of liver tumours. Both factors are thought to play a role in the etiology of liver cancer (Jarvis, 1989) although recent studies indicate that aflatoxins may not be human carcinogens. Stoloff (1989), in an *in vitro* experiment with human liver tissues came to a conclusion that the human liver metabolises aflatoxins in a fashion similar to species refractory to aflatoxin carcinogenesis. In animals, carcinogenesis due to aflatoxins is of little concern because livestock take a shorter time to reach market weight than cancer takes to appear (Pier, 1981). However, aflatoxin carcinogenesis may cause losses in the duck industry and in trout hatcheries.

Teratogenic effects such as anencephaly, exencephaly microencephaly, ectopia cordic have been reported after

intraperitoneal aflatoxin administration in hamsters (Pier, 1981) and mice (Arora *et al.*, 1981). Mutagenic effects have been demonstrated in bacteria (*Salmonella typhimurium*) and in plant (*Vicia fabia*) tissues (Pier, 1981).

2.6.1.2 CLINICAL SIGNS AND PATHOLOGICAL LESIONS IN POULTRY

Among poultry, ducklings are the most susceptible to aflatoxins. Turkeys are less susceptible while chickens are comparatively resistant (Allcroft and Carnaghan, 1963). Acute poisoning in ducks manifests as anorexia, poor growth rate, feather pulling, purple discolouration of legs and feet, convulsions, ataxia and death. At postmortem, there is swelling and increased firmness in texture, petechial haemorhages and fatty infiltration of the liver, proliferation of small bile ductiles, portal fibrosis and hepatic cell necrosis (Asplin and Carnaghan, 1961; Edds, 1973).

In turkeys, the signs include listlessness, reduced feed consumption, reduced growth rate, nervous signs and death. The postmortem lesions include engorgement and congestion of kidneys, enteritis, haemorrhage and necrotic foci in the liver, haemorrhages in pancreas, white flecks in air sacs and generalised oedema (Stevens *et al.*, 1960; Clarke *et al.*,1982). Nodular regeneration of the liver and biliary hyperplasia may be seen (Edds, 1973).

In chicken, the clinical signs include anorexia, listleness, reduced egg production, reduced growth rate, cyanotic combs, lameness and high mortality in some cases (Choudary and Rao, 1982; Chen *et al.*, 1985; Choudary, 1986). At postmortem, there are

hepatic changes such as necrosis, haemorrhages, fatty degeneration, hepatomegaly, vacuolation of hepatic cells, fibrosis and bile duct proliferation. Hydropericardium, ascites and ruptured livers are sometimes seen (Choudary and Rao, 1982; Chen *et al.*, 1985; Choudary, 1986).

Aflatoxins may also cause decreased tissue integrity, reduced egg size, increased susceptibility to extreme temperatures (Hamilton, 1987) and impaired hemostatic mechanisms (Witlock and Wyatt, 1981) in poultry. They also exhibit additive synergistic effects with other mycotoxins such as ochratoxin A (Huff and Doerr, 1981) and T-2 toxin (Huff *et al.*, 1988a).

2.6.1.3 ANALYTICAL METHODS

Analysis of aflatoxins may be carried out using biological, chemical and immunochemical methods (FAO, 1990). Biological assays are based on the toxic characteristics of aflatoxins to living organisms, organs or tissue cultures. Micro-organisms such as *Bacillus megaterium* (Clements, 1969) and *Bacillus stearothermophilus* (Reiss, 1975), aquatic animals such as the brine shrimp *(Artemia salina)* and certain fishes (eg. trout and zebra fish), terrestrial animals such as ducklings and chicken embryos (Verrett *et al.*, 1964) have been used as bioassays for aflatoxins. Biossays based on aflatoxin cytotoxicity and cytogenecity to kidney and muscle cell cultures (FAO, 1990) and aflatoxin phytotoxicity to higher plants (Schoental and White, 1965) have also been developed. In general, these biossays are useful for screening known and

unknown mycotoxins but lack specifity, reproducibility and rapidity (FAO, 1990).

Chemical methods of analysis are the most commonly used. These include thin layer chromatography (TLC) methods that were developed in early sixties (Delongh *et al.*, 1964), minicollumn methods (Romer, 1975), the highly sensitive high performance thin layer chromatography (HPTLC) methods, high pressure liquid chromatography (HPLC) methods that are very precise, selective and sensitive and gas liquid chromatography (GLC) methods. Flourotoxinometry, differential pulse polarography and fluorimetry of the iodine derivative of aflatoxin B are sometimes applied in aflatoxin analysis (Coker, 1984; FAO, 1990).

Immunochemical methods that are simple, sensitive and highly specific have also been developed. These include radioimmunoassays (RIA) (Chu, 1984) and enzyme-linked immunosorbent assays (ELISA) (Itoh *et al.*, 1987; Chu *et al.*, 1988; Hongyo *et al.*, 1992).

2.6.2 OCHRATOXINS

Ochratoxins are a group of seven closely related colourless crystalline fungal metabolites produced by several species of *Aspergillus* and *Penicillium* (Krogh, 1977). These metabolites are ochratoxin A and its methyl and ethyl esters, ochratoxin B and its methyl and ethyl esters and 4 hydroxyochratoxin A. Ochratoxin A and its methyl and ethyl esters are the toxic members of the group (Cole and Cox, 1981) but it is only ochratoxin A that is widespread as a natural contaminant (Krogh, 1977).

2.6.2.1 OCHRATOXIN A

This is an isocoumarin derivative first isolated by Van der Marwe and collaborators in 1965 during a laboratory screening programme for toxigenic fungi (Van der Marwe *et al.*, 1965). It was isolated from a culture of *Aspergillus ochraceus* Wilhem from which the name ochratoxin is derived. Ochratoxin A is also produced by A. *sulfureus* (Fres.) Thom and Church, A. *melleus* Yukawa, P. *verrucosum* Dierickx, P. *cyclopium* Westling and P. *vindicatum* Westling (Bauer and Gareis, 1991).

Ochratoxin A, like other mycotoxins, appears to have a considerable regionality in its occurrence (Pier, 1981). Most reports of occurrence are mainly from Europe and North America involving chiefly small grains such as wheat, barley, oats (Roschenthaller *et al.*, 1984). Ochratoxin A has also been reported to occur in swine and poultry tissues in Denmark (Krogh, 1977), in green coffee beans, mixed feeds, peanuts and occasionally hay in many parts of the World (Harwig, 1974; Pier, 1981). In Kenya, a survey of maize and maize products found ochratoxin A In 55% of the 214 samples examined (Muriuki, 1992).

2.6.2.1.1 TOXIC EFFECTS

Ochratoxin A competitively inhibits protein synthesis by inhibiting the enzyme phenylalanyl-t RNA synthetase (Roschenthaler *et al.*, 1984). It is primarily a nephrotoxin and has induced pathological changes in kidneys of all species of animals so far tested (Bauer and Gareis, 1991). Natural cases of the nephropathy

associated with ochratoxin A occur in pigs and in poultry. The incidence of such cases of nephropathy in pigs in Denmark have been found to have a pronounced association to feedborne ochratoxin A. Due to this nephrotoxic activity, ochratoxin A is thought to be one of the causes of endemic (Balkan) nephropathy, a renal disease of man observed in Balkan countries (Krogh, 1977). Experimental studies in a variety of animals have shown that ochratoxin A is also hepatotoxic, teratogenic, immunosuppressive and carcinogenic (Arora *et al.*, 1981; Bauer and Gareis, 1991).

2.6.2.1.2 CLINICAL SIGNS AND POSTMORTEM LESIONS IN POULTRY

Birds dying from acute ochratoxicosis show listlessness, huddling, occasional diarrhoea, ataxia, prostration and death. At postmortem, the kidneys are pale and enlarged. Microspically, there is degeneration of tubular epithelium, dilation of distal tubules and formation of interstitial fibrous tissue (Krogh, 1976; Krogh, 1977). Subacute doses can also cause reduced egg production and reduced weight gain in chicken (Prior *et al.*, 1981) and reduced weight gain in turkey poults (Chang *et al.*, 1981). Ochratoxin A has also been shown to cause anaemia (Huff *et al.*, 1979), impaired phagocytosis by heterophils, a type X glycogen storage disease and intestinal fragility (Manning and Wyatt, 1984). Ochratoxin A residues remain in tissues of exposed birds for a short time (Bauer and Gareis, 1991).

2.6.2.1.3 ANALYTICAL METHODS

Thin layer chromatography methods such as the multiple

mycotoxin method of Eppley (1968) and the official methods of analysis of the A.O.A.C. (A.O.A.C., 1984) are most commonly used for analysis of ochratoxin A. Ochratoxin A fluoresces yellow green under longwave ultraviolet light. Other chemical methods include GLC, HPLC and HPTLC (Coker, 1984; Itoh *et al.*, 1986). Immunochemical methods such as RIA and ELISA have also been developed (Chu, 1984; Itoh et al., 1986).

2.6.3. TRICHOTHECENES

Trichothecenes are a group of fungal metabolites characterised by basic tetracyclic 12,13-epoxytrichothec-9-ene skeleton. They were first discovered in 1948 during a screening program designed to pick up antifungal agents (Freeman and Morrison, 1948). Well over 10 genera of fungi are known to produce trichothecenes. The most important ones are *Fusarium, Trichordema, Tricothecium, Myrothecium, Stachybotrys* and *Cephalosporium* (Cole and Cox, 1981). Trichothecenes are among the most hazardous mycotoxins found in small grains such as wheat, barley, oats and rye in temperate countries (Gareis *et al.*, 1986; Robb, 1993). Over 100 trichothecenes and related biosynthetic metabolites have been reported to be naturally occurring. Some of these trichothecenes such as T-2 toxin, deoxynivalenol, sastra toxin, diacetoxyscirpenol, neosolaniol are known to naturally cause disease in man and animals (Jarvis, 1989).

2.6.3.1 TOXIC EFFECTS

Trichothecenes inhibit protein synthesis in eucaryotic cells by binding to the 60S subunit of ribosomes and interfere with peptidyltransferase enzyme (Jarvis, 1989). They exhibit a broad range of toxicity in animals. Toxicity may be seen as vomiting, dirrhoea, anorexia, gastroenteritis, nerve degeneration, hemorrhages in cardiac muscle and lesions in lymphnodes, testis and the thymus. Topical application leads to dermal inflammation and necrosis. Trichothecenes also show antibacterial, antiviral, antifungal, phytotoxic, cytostatic and insecticidal activity (Cole and Cox, 1981). Under natural conditions, trichothecenes have been implicated in the causation of alimentary toxic aleukia, akakabi-Byo, stachybotryotoxicosis, dendrochiotoxicosis, T-2 toxicosis and several other conditions in man and animals (Smalley and Strong, 1974).

2.6.3.2 CLINICAL SIGNS AND PATHOLOGICAL LESIONS

The clinical signs of trichothecene toxicosis vary widely but usually include prominent signs in the alimentary tract vascular and coagulatory systems (Buck *et al.*, 1976).

Several natural occurring clinical syndromes have been attributed to trichothecenes. These include staggering grain toxicosis, stachybotryotoxicosis, bean-hull toxicosis, mouldy corn toxicosis and red-mold toxicosis. In staggering grain toxicosis in man and animals, there is headache, vertigo, shivering chills, nausea, vomiting and visual disturbance. In stachybotryotoxicosis

of swine, horses, calves and poultry, there is stomatitis, dermal necrosis, haemarrhages, thrombocytopenia, leukopenia, nervous disorders and shock. Bean-hull toxicosis of horses presents as convulsions and cyclic movements. Emesis, haemorrhages and feed refusal are seen in pigs and cattle suffering from mouldy corn toxicosis. Red-mold toxicosis in man, horses pigs and cattle is characterised by vomiting, diarrhoea, emesis and abortion (Ueno, 1977)

T-2 toxin, which is one of the most important naturally occurring trichothecenes, has been shown to cause reduced weight gain, reduced egg production (Shlosbera *et al.*, 1984) necrosis of beak and oral cavity and gastroenteritis in poultry (Pier, 1981). Neurological signs and coagulopathy have also been demonstrated in poultry on T-2 toxin contaminated diets (Chi *et al.*, 1981).

2.6.3.3 ANALYTICAL METHODS

Biological, chemical and immunochemical methods have been developed. Biological methods are based on the antibiotic, phytotoxic, cytotoxic and dermatitic qualities of trichothecenes. These include assay methods utilising fungistatic activity against *Rhadotorula rubra*, phytotoxic activity against germinating pea seed, inhibition of protein synthesis in rabbit reticulocytes and dermatitic activity on rabbit skin. These biological assays have a good sensitivity but little selectivity for the detection of different trichothecenes (Pathre and Mirocha, 1977; Watson and Lindsay, 1982).

Thin layer chromatography is the chemical method most commonly used for trichothecenes although it suffers from the limitation of high detection limits and the non-fluorescence of some trichothecenes. Other methods are GLC, HPLC and liquid chromatography (Scott, 1982). Radioimmunoasssays (RIA) and ELISA tests have been developed for some trichothecenes such as T-2 (Scott, 1982; Itoh *et al.*, 1986).

2.6.4 ZEARALENONE

Zearalenone (6-(10-hydroxy-6-0x0-trans-1-undecenyl)-B-resorcyclic acid lactone) is an oestrogenic, white crystalline fungal metabolite produced by *Fusarium* spp. It was first isolated from a culture of F. *roseum "graminearum"* (Stob et al., 1962). This fungus had earlier been associated with signs of hyperestrogenism in pigs in USA (McErlean, 1952). Other *Fusarium* species such as F. *oxyporum*, F. *lateritium*, F. *culmorum*, F. *moniliforme*, F. *equiseti*, F. *gibbosum*, F. *avernaceum* and *F. nivale* also produce this toxin (Cole and Cox, 1981).

The highest incidence and levels of zearalenone in nature are seen in corn, but barley, wheat, oats, sorghum, sesame, hay, corn and mixed animal feed may also contain the toxin (Mirocha and Christensen, 1974; Pier, 1981). It is commonly found occurring together with toxic trichothecenes produced by *Fusarium* spp. especially deoxynivalenol and T-2 toxin (Mirocha *et al.*, 1977; Pier, 1981; Gareis *et al.*, 1986).

2.6.4.1 TOXIC EFFECTS

Zearalenone exerts oestrogenic effects mainly on the female reproductive organs. Although it is not steroidal in nature but a resorcyclic acid lactone and of mycological origin, zearalenone can produce true heat as well as other anabolic effects associated with steroidal oestrogens in animals (Mirocha and Christensen, 1974). Swine is the most sensitive and most commonly affected. Rats, mice, guinea pigs, monkeys and lambs are experimentally affected (Mirocha *et al.*, 1977).

2.6.4.2 CLINICAL SIGNS AND PATHOLOGICAL LESIONS IN POULTRY

In poultry, zearalenone causes enlargement of the cloaca, bursa and oviduct, and cystic development of the ovary. Swelling of vents and eversion of the cloaca have also been reported in turkeys. The anabolic effects of zearalenone in chicken are seen as high body weight gains, increase in ovarian length and increase in comb weight. In mature hens, zearalenone has little influence on the various performance, reproductive and physiological parameters (Mirocha and Christensen, 1974). Depending on the concentration of zearalenone, fertility of ganders and turkey cocks have been demonstrated to be decreased or completely inhibited (Mirocha *et al.*, 1977). This does not seem to occur in males of other animal species.

2.6.4.3 ANALYTICAL METHODS

A rat uterotrophic biossay with the sensivity of 0.3 - 1.0mg

total zearalenone has been developed (Pier, 1981). However, thin layer chromatography is the most widely used method. Zearalenone emits a greenish blue fluorescence under short wave (256nm) ultraviolet light. Other methods are HPLC, HPTLC, ultraviolet spectrophotometry and gas chromatography (Shotwell, 1977; Coker, 1984). Immunoassays have also been developed (Chu, 1984).

2.6.5. STERIGMATOCYSTIN

This is a pale yellow xanthone derivative first isolated by Hatsuda and co-workers (cited by Kamasaki and Hatsuda, 1977) in 1954 from a culture of *Aspergillus versicolor* (Vuill) Tiraboschi during a systematic survey of fungal metabolites. It belongs to a group of related fungal metabolites characterised by fused xanthone and difurano or tetrahydrodifurano moieties referred to as sterigmatocystins. These are produced by a variety of species belonging to the genera *Aspergillus*, *Bipolaris* and *Penicillium* (Cole and Cox, 1981).

Sterigmatocystin is mainly produced during storage of feedstuffs. It has been found in rice, wheat, barley and other food samples from Japan, United Kingdom, Canada, Czechoslovakia, Poland and Mozambique. It has also been demonstrated in the soil and in green coffee (Van der Watt, 1974; Robb, 1993).

2.6.5.1 TOXIC EFFECTS

The toxicological effects of sterigmatocystins are similar but not identical to those of aflatoxins. This is probably because

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sterigmatocystins are biosynthetic precursors of the aflatoxins (Cole and Cox, 1981). Sterigmatocystin has been demonstrated to cause acute and chronic hepatic and renal toxicity in rats and subhuman primates. It is also carcinogenic and causes hepatic tumours in rats and monkeys and neoplastic skin lesions on topical application in rats (Van der Watt, 1974). Mutagenic effects have been demonstrated in several experiments with *salmonella typhimurium* and with cultured chinese hamster cells (Van Egmond, 1984).

2.6.5.2 ANALYTICAL METHODS

Sterigmatocystin is detected on TLC preparations, that have been sprayed by aluminium chloride and heated, by its yellow fluorescence under short wave ultraviolet light (Van Egmond, 1984). High performance thin layer chromatography method has also been developed (Coker, 1984).

2.6.6 CONTROL OF MYCOTOXIN CONTAMINATION

The most effective strategy for the control of mycotoxins is to reduce or eliminate the initial toxin production process (Lillehoj and Wall, 1987). This may be achieved through several approaches such as development of crop hybrids that are less susceptible to fungal growth and mycotoxin production, control of insect crop pest, reducing crop stress during growth, reducing physical damage to crops at harvest, drying crops below 0.68 water activity, use of fungal inhibitors, anaerobic storage and use of

suitable and clean containers for storage (Cerutti and Vecchio, 1988; Robb, 1993). These preventive approaches may only be partially successful since some unanticipated environmental conditions such as excessive drought, rainfall etc., often result in widespread occurrence of mycotoxins (Lillehoj and Wall, 1987). A more practical strategy in the control of mycotoxins is that of decontamination (Gonzalez, 1987).

Methods of decontamination of mycotoxins can be physical, chemical or biological (Dollear, 1969; Robb, 1993). The physical methods include use of heat, gamma radiation, ultraviolet radiation, visible light, hand or machine sorting, washing, PH changes and autodegradation (Lillehoj and Wall, 1987; Robb, 1993)

Biological methods explore the possibility of using micro-organisms or their enzymes to transform or degrade a mycotoxin to a less toxic or non-toxic metabolite. For example a bacterium, *Flavobacterium aurantiacum* has been found to remove aflatoxins from solutions or preparations of peanut milk (Ciegler et al., 1966).

Chemical methods are most promising. They include the use of acids, sodium hypochlorite, alkaline hydrogen peroxide, bisulphite, formaldehyde, methylamine, ammonia, urea and hydrated sodium calcium aluminosilicates (Gonzalez, 1987; Lillehoj and Wall, 1987; Connaughton, 1989; Robb, 1993). Of the chemical methods, the use of ammonia and hydrated sodium calcium aluminosilicate are the most promising in decontamination of aflatoxins. Aqueous or gaseous ammonia is applied to contaminated feed at high pressure or temperature and monocalcium phosphate added to remove the residue

ammonia. This method is cheap, efficient and has been used commercially to detoxify peanuts at industrial scale in Senegal, France and India (Robb, 1993). Hydrated sodium calcium aluminosilicate is an anticaking agent used in preparation of mixed feeds. It works by breaking down aflatoxins and binding them to prevent absorption in the intestines (Connaughton, 1989).

3 MATERIALS AND METHODS

3.1 SAMPLING

A total of 90 samples of commercially compounded poultry feed were randomly sampled from poultry farmers in Nairobi province and Kikuyu division of Kiambu district. Thirty samples were taken in January, thirty in March and thirty in May 1993. In picking the areas of sampling, 10 administrative sublocations, out of a total of 85, were randomly selected. From each of these selected sublocations, three farms were randomly sampled. The samples consisted of a half to one kilogramme of poultry feed. A 30cm probe was used to obtain feed from different parts of a bag of feed the farmer was about to give to birds.

3.1.1 SUBSAMPLING AND SAMPLE PROCESSING

Samples were transported to the laboratory within three hours of collection. In the laboratory, every feed sample was thoroughly mixed and the following subsamples taken:

(1) About 20g feed for the determination of the moisture content. This was put in air tight bottles.

(2) About 100g feed for mycological examination. This was stored at 4 °C before being plated onto suitable media 1-2 days after collection.

(3) The rest of the feed was put in sealed polythene bags and stored in a deep freezer for mycotoxin analysis.

3.2 DETERMINATION OF MOISTURE CONTENT

This was done within 2 days of sample collection, by the oven method. Two grams of feed in a dish was placed in an oven at 105°C overnight. The feed was then weighed and percentage moisture was calculated as the percentage weight loss.

$$\% \text{ moisture} = \frac{Mo - M1}{Mo} \times 100$$

where Mo = initial weight in grammes

M1 = final weight after drying in grammes

This was done in duplicate for every sample and the average calculated

3.3 ENUMERATION OF TOTAL MOULD COUNTS

Ten grams of feed was weighed and diluted in 90ml of a sterile solution of 0.9% NaCl and 0.2% tween80 in water. This mixture was further diluted to concentrations of 0.01g/ml and 0.001g/ml feed. From each of these dilutions, aliquots of 0.1ml were taken and spread on to the surface of each of the following media: Malt salt agar (MSA) (Christensen, 1946), *Aspergillus* differential media (Dichloran)(ADMB)((Hamsa and Ayres, 1977) and Dichloran rose bengal yeast extract sucrose agar (DRYES) (King et al., 1979). The composition of these media is given in appendix 1. All plates were incubated at room temperature for 4 to 5 days.

The total number of colony forming units (CFU) were counted in

al. plates where mould growth was not too dense for reliable counting. The total CFU/gram for every sample was calculated from the media yielding the highest number of colonies at the highest dilution of feed.

Total CFU/gram = Total number of colony forming units **X** dilution factor of feed

3.4 ENUMERATION, ISOLATION AND IDENTIFICATION OF *ASPERGILLUS* SPP., *PENICILLIUM* SPP. AND *FUSARIUM* SPP.

Colonies of *Aspergillus* spp. and *Penicillium* spp. were counted on MSA and DRYES plates. *Fusarium* spp. were counted on MSA and ADMB plates. *Aspergillus flavus* were counted on the ADMB plates. Counts were multiplied by the dilution factor to obtain the number of the colony forming units per gram of feed (CFU/gram)

Aspergillus spp. and *Penicillium* spp. were isolated from MSA and DRYES media and subcultured on Czapek solution agar. *Aspergillus flavus* group isolates were isolated from ADMB agar media and subcultured on Czapek solution agar. *Aspergillus* spp. were identified according to Raper and Thom (1945). *Penicillium* spp. were identified according to the classification of Raper and Thom (1949)(as adapted by Gilman, 1957 and Smith, 1969) and according to Pitt (1979) (as adapted by Onions, 1982).

Fusarium spp. were isolated from MSA and ADMB media and subcultured on potato dextrose agar (Oxoid). Single spore isolation

techniques were used to obtain cultures suitable for identification. *Fusarium* spp. were identified according to Booth (1971) and Booth (1977).

3.5 SCREENING *ASPERGILLUS FLAVUS* GROUP ISOLATES FOR AFLATOXIN PRODUCTION

Isolates of A. *flavus* were obtained from ADMB agar media. They were subcultured on Czapek solution agar and stored at 4° C for 2 to 16 weeks. Aflatoxin production test was performed using the agar plug screening method for extracellular mycotoxins described by Filtenborg and Frisvad (1980) (cited by Stenwig, 1988). All isolates were grown on yeast extract sucrose agar for two weeks at room temperature. A sterile end of a pasteur pippette with an inner diameter of about 4mm was used to cut out the colonies and agar substrates. These plugs were placed directly on silica gel GHR TLC plates that had previously been activated by heating at 110 ° for 1 hour. These plugs were wetted with chloroform and the liquid allowed to ooze onto the TLC plates. Three plugs of each isolate were placed on one spot to increase the amount of aflatoxin to be detected. Aflatoxin B1, B2, G1 and G2 standard solutions were also spotted alongside the agar plugs. All spots were allowed to dry before the TLC plates were developed in chloroform:acetone (88:12) under saturated conditions. Aflatoxins were detected by their blue and green fluorescence under longwave (365 nm) U.V. light.

3.6 MYCOTOXIN ANALYSIS

This was perfomed using the method described by Stahr *et al.*, (1977).

3.6.1 PREPARATION OF THE SAMPLE EXTRACT

A 50-gram feed subsample was drawn from every sample according to the official methods of the Association of the Official Analytical Chemists (A.O.A.C) (14th edition, 26.003C). This was put into a waring blender (model 32 BL 79). 200ml of acetonitrile-4% KCl (90 + 10) was added and the mixture blended at a high speed for two minutes. 100ml of the resultant supernatant was filtered into a separatory funnel.

The sample extract was defatted twice with 50ml petroleum ether and then decolorised using a gel prepared from a solution of ferric chloride. Mycotoxins were extracted twice from the decolourised sample extract using two 50 ml portions of chloroform. The chloroform extract was then evaporated to near dryness on a water bath before being quantitatively transfered into a 10ml centrifuge tube and evaporated to dryness. The residue was re-dissolved in 200 μL of benzene:acetonitrile (98+2).

3.6.2 THIN LAYER CHROMATOGRAPHY

3.6.2.1 PREPARATION OF THIN LAYER CHROMATOGRAPHY PLATES

These were prepared according to the method of the A.O.A.C. (14th edition, 26.031). Silica gel-GHR (MW-kieselgel G-HR) powder

was used to coat 20cm X 20cm glass plates to 0.3mm thickness using an automatic TLC plate coater (Camag). The plates were activated by placing them in an oven at 110 degrees centigrade for an hour. They were stored in a cabinet with an active silica gel desiccant until just before use.

3.6.2.2 CHROMATOGRAHY

Twenty microlitre aliquots of sample extracts dissolved in benzene + acetonitrile (98+2) were spotted on the activated TLC plates along with appropriate standards using microtitre syringes (Hamilton). The spots were allowed to dry and the plates developed in suitable solvents.

3.6.2.3. DETECTION OF AFLATOXINS, ZEARALENONE AND STERIGMATOCYSTIN

TLC plates spotted with sample extracts and aflatoxin B1, B2, G1, G2, zearalenone and sterigmatocystin standard solutions were developed in toluene-ethylacetate-acetone (3+2+1), in a dark room, until the solvent front reached 12cm from the bottom. The plates were dried and observed under long wave (365nm) U.V. light. Aflatoxins were detected by their blue or green flourescence and sterigmatocystin by its red flourescence. The wave-length was then changed to shortwave (256nm) U.V. light to detect zearalenone that emits a greenish-blue flourescence. yConfirmation of the identity of aflatoxins was done by spraying flourescent spots with 50% (V/V)

sulphuric acid solution. Flourescence due to aflatoxins changed from blue or green to yellow.

3.6.2.4 QUANTIFICATION OF AFLATOXINS

This was done by visual estimation. 20µl of sample extract was spotted alongside an increasing dilution of the appropriate standard aflatoxin solution. The quantity of aflatoxin in the sample extract was estimated by comparing the intensity of flourescence of the sample extract spot to those of the standard aflatoxin solution spots. The concentration of aflatoxins in a sample was calculated using the formula:-

$$\text{Aflatoxin B1 or G1 or B2 in ug/kg} = \frac{S \; Y \; V}{X \; W}$$

Where:

Y = Concentration of aflatoxin standard solution in µg/ml

V = Volume of the final solution of the sample extract in ml

X = Volume of the sample extract in ml giving spot intensity equal to S

W = Mass of the sample representative of the final extract in grammes.

S = Volume of aflatoxin standard giving an intensity of flourescence equal to the sample extract.

3.7.2.5 DETECTION OF OCHRATOXIN A (OTA)

Twenty microlitre aliquots of sample extracts and 20µl of standard OTA solution (20µg/ml) were spotted on TLC plates and the plates developed in the toluene+ethylacetate+acetone (3+2+1)+1% formic acid solvent. The plates were viewed under longwave (365nm) U.V.light. Blue flourescence was observed for standard OTA solution spots and positive sample extract spots.

3.7 STATISTICAL ANALYSIS

Total mould counts, *Aspergillus* spp. counts, *Penicillium* spp. counts and *Fusarium* spp. counts were expressed on the logarithmic scale (base 10). One way analysis of variance was used to test for differences between different manufacturers, feed formulations and the times of sampling in the means of total mould counts, *Penicillium* counts, *Aspergillus* counts, *Fusarium* spp. counts and moisture content. The 0.05 level of significance was used. Where necessary, Tukey's test was used to further compare the means.

Overall correlations among the continous variables (moisture, total mould counts, *Aspergillus* spp. counts, *Penicillium* spp. counts and *Fusarium* spp. counts) were calculated using Pearsons correlations co-efficient (r^2).

Mycotoxin contamination was expressed as either positive or negative. Chi-square test was used to examine for any differences in the frequencies of mycotoxin contamination amongst the various feed manufacturers, feed formulation and times of sampling.

4 RESULTS

4.1. MOISTURE CONTENT

The moisture content varied from 6.79% to 11.56%. The average moisture content was 8.66% while the median was 8.56%. The moisture content for individual samples is shown in Appendix 2. There were no statistically significant ($P>0.05$) differences in moisture content of feeds from different manufacturers (Appendix 7.) and of different feed formulations (Appendix 6). However feed sampled in January had a significantly higher moisture content than that sampled in March and in May (Appendix 8).

4.2. ENUMERATION OF TOTAL MOULD COUNTS

Fungi were isolated in all feed samples examined. The total mould counts obtained varied from 1.0×10^4 to 1.31×10^6 with an average of 1.115×10^4 and a median of 1.8×10^5 CFU/gram of feed. Classification of these mould counts according to the hygienic classification of Flatscher and Willinger (1981) (cited by Stenwig and Liven, 1988) showed that only 41% of all the feed samples examined had a good to acceptable level of mould contamination. Twenty three percent and 36% of the samples were of inferior to unacceptable level of mould contamination respectively (Table 1). Feed samples from different feed manufacturers had no significantly ($P>0.05$) different total mould counts (Appendix 7). Broiler feed had significantly ($P<0.05$) lower total mould counts than other feed

formulations (Appendix 6). Feed sampled in May had significantly higher total mould counts than that sampled in January and March ($P<0.05$) (Appendix 8).

TABLE 1. Classification of total mould counts according to Flatscher and Willinger, (1981) (cited by Stenwig and Liven, 1988)

Ranges (CFU/g)	Category	Number of samples	%
0 - 1.0 X 10^5	Good to acceptable	37	41
1.0 X 10^5 - 2.0 X 10^5	Inferior	21	23
Over 2.0 X 10^5	Unacceptable	32	36

4.3 ENUMERATION AND IDENTIFICATION OF *ASPERGILLUS* SPP., *PENICILLIUM* SPP. AND *FUSARIUM* SPP.

Aspergillus spp. and *Penicillium* spp. were isolated in all samples examined. *Fusarium* spp. were isolated in 76.7% of the samples. A summary of the estimated counts (CFU/g) of these genera are shown in Table 2 and Appendix 3. Pearsons ($r2$) correlations between the counts of different fungal genera, total mould counts and moisture content are shown in Table 3. Pearsons r^2 was high and positive for comparisons between most fungal counts but low and

sometimes negative for comparisons between fungal counts and moisture content.

4.3.1 *ASPERGILLUS* SPP.

Aspergillus spp. counts varied from 1.0×10^3 - 8.0×10^5. They were significantly ($P<0.05$) higher in feed sampled in May than that sampled in January and March (Appendix 8) and significantly ($P<0.05$) lower in broiler feed than in feed of other formulations (Appendix 6). Feed samples from "Belfast" manufacturer had significantly higher ($P<0.05$), and that from "Muus" significantly lower counts than feed from other feed manufacturers (Appendix 7).

A total of 15 different *Aspergillus* species were identified. (Appendix 4). The most commonly isolated species were A. *flavus* Link (75.6%), A. *Wentii* Wehmer (73.3.%), A. *chevalieri* Thom and Church (36.7%) and A. *candidus* Link (27.8%). Other species identified were A. *ruber* (Brem.), A. *niger* Van Tieghem, A. *tamarii* Kita, A. *chevalieri intermedius* Thom and Raper, A. *fumigatus* Fresenius, A. versicolor (Vaill) Tiraboschi, A. *sydowi* (Bain. and Sart.), A. *ochraceus* Wilhelm, A. *ustus* (Bainier), A. *parasiticus* Speare and A. *terreus* Thom. Some morphological characteristics of some of the most commonly isolated species are shown in plates 1-5.

4.3.2 *PENICILLIUM* SPP.

Counts of *Penicillium* spp. ranged from 2.0×10^2 to 3.0×10^5. The counts were significantly ($P<0.05$) lower in broiler feed than in other feed formulations (Appendix 6). They were also

significantly ($P<0.05$) lower in feed sampled in January than in that sampled in March and May (Appendix 8). Variations in *Penicillium* spp. counts amongst feed samples from different manufacturers was not statistically significant ($P>0.05$) (Appendix 7).

A total of 19 *Penicillium* spp. were identified (Appendix 5). The most commonly isolated species were P. *cyclopium* Westling (60%), P. *expansum* Thom (27%) and P. *vindicatum* Westling (17.7%). Other *Penicillium* species identified were P. *crustosum* Thom, P. *martensii* Biourge, P. *implicatum* Biourge, P. *purpurogenum* Stoll, P. *oxalicum* Carrie and Thom, P. *rugulosum* Thom, P. *digitatum*, Saccardo, P. *brevicompactum* Dierckx, P. *granulatum* Bainier, P. *diversum* Raper and Fennel, P. *italicum* Wehmer, P. *duclauxi* Delacroix, P. *funiculosum* Thom, P. *chrysogenum* Thom, P. *puberulum* Bainier, and P. *roqueforti* Thom. Morphological characteristics of some of the species isolated are shown in plates 6 - 9.

4.3.3 *FUSARIUM* SPP.

Fusarium spp. counts varied from 0 to 3.1×10^5. The counts were not significantly different in feed of different formulation (Appendix 6). However, they were significantly lower in feed sampled in March than that sampled in January and May (Appendix 8). *Fusarium spp* counts were also lower in feed from 'Milling' and 'Memake' than in feed from "Unga", "Muus" and "other" manufacturers. Three species of the genus *Fusarium* were identified. These were F. *moniliforme* Sheldon (38.8%), F. *oxysporum*

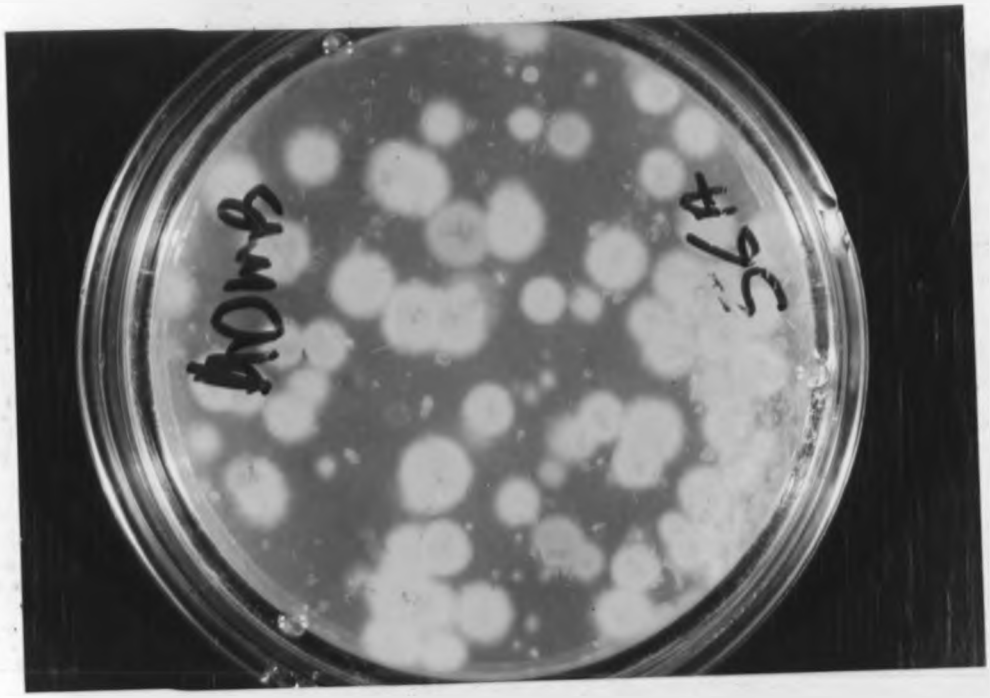
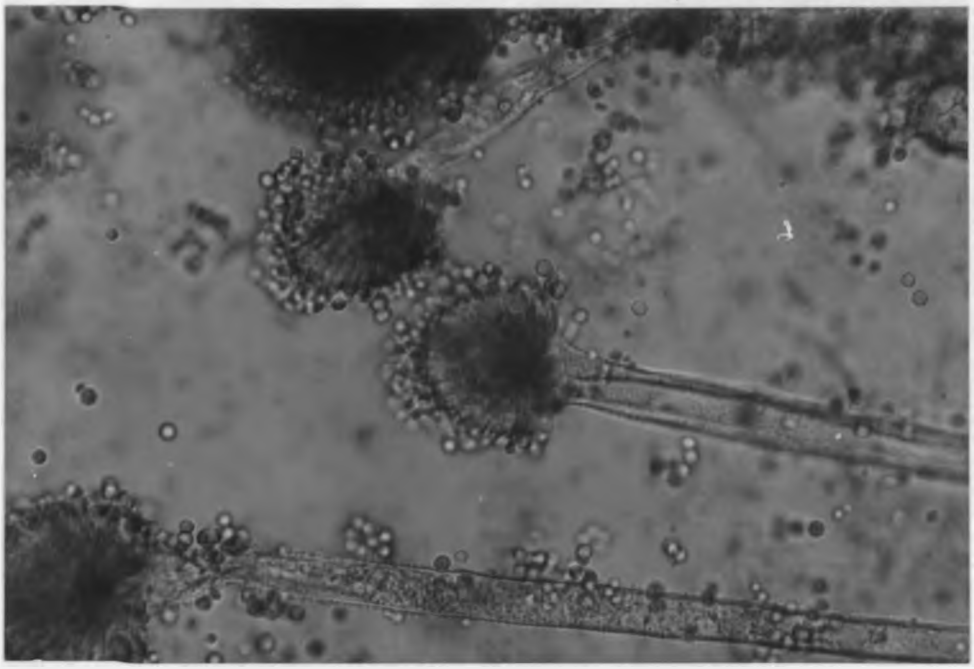


Plate 1. (above). Reverse of ADMB agar media plate showing yellowish-orange reverse colour of *Aspergillus flavus* group colonies.

Plate 2. (below). *Aspergillus flavus*. Conidiophores, conidial heads and conidia. (X100)



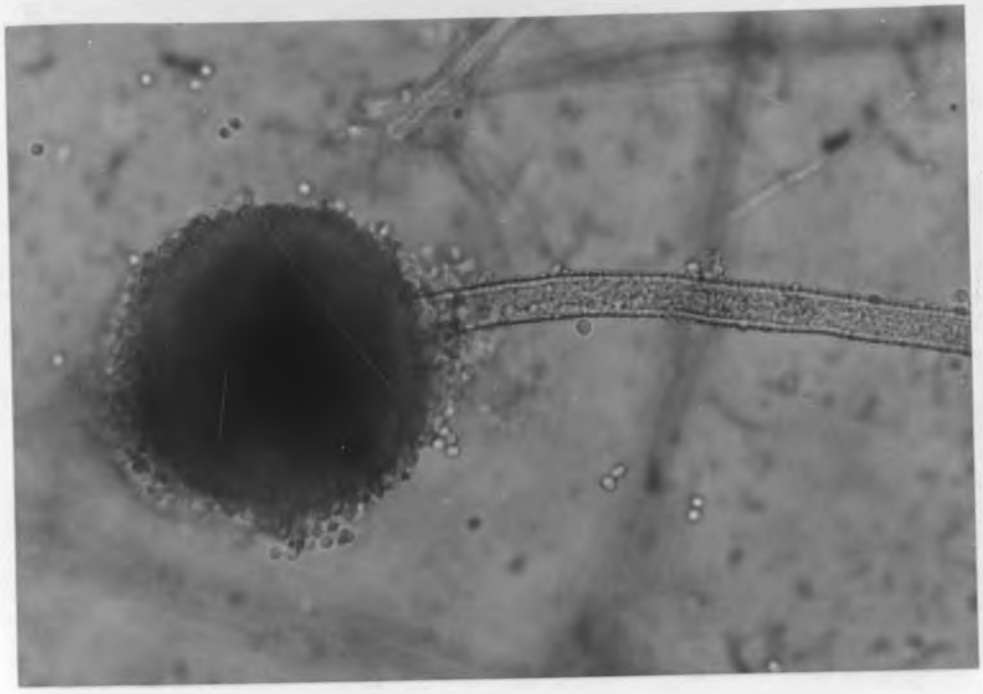


Plate 3. (above). *Aspergillus ochraceus*. Conidiophores, conidial heads and conidia.(X400)

Plate 4. (below). *Aspergillus terreus*. Edge of colony showing collumnar conidial heads. (X35).

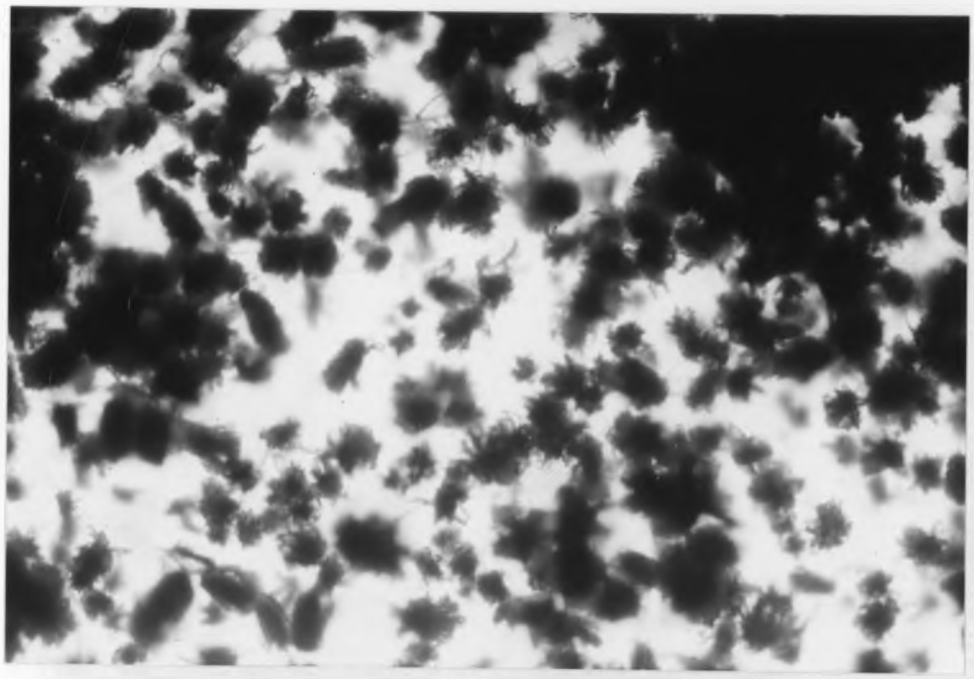
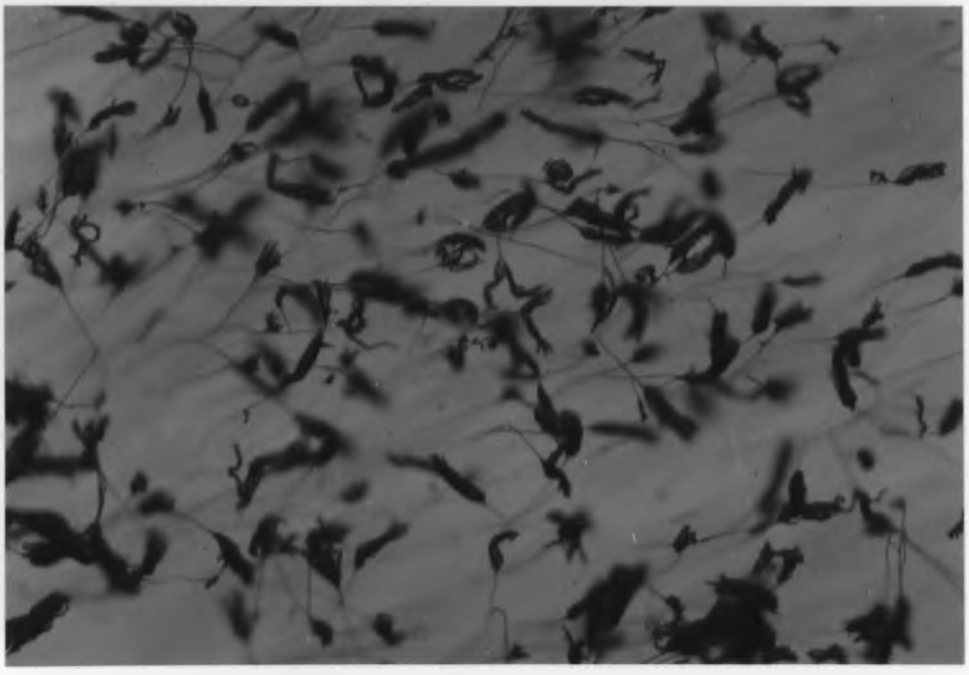


Plate 5. (above). *Aspergillus candidus*. A two-week old colony growing on Czapek solution agar.

Plate 6. (below). *Penicillium cyclopium*. Edge of a colony showing fasciculation (X35).



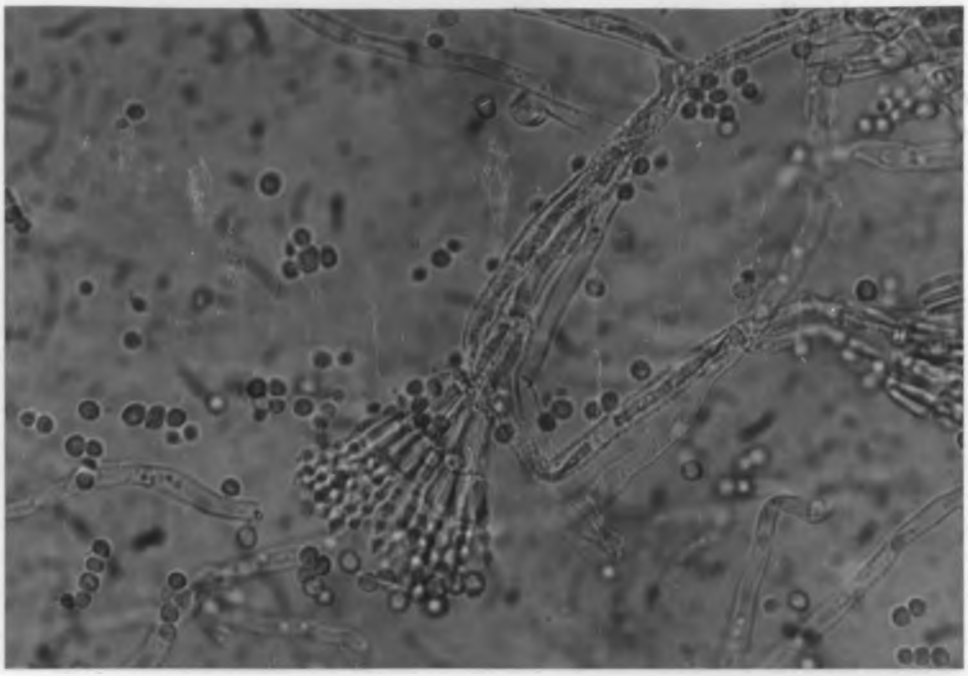


Plate 7. (above). *Penicillium cyclopium*. Conidiophores and conidia. (X400)

Plate 8. (below). *Penicillium expansum*. A 2-week old colony growing on Czapek solution agar.

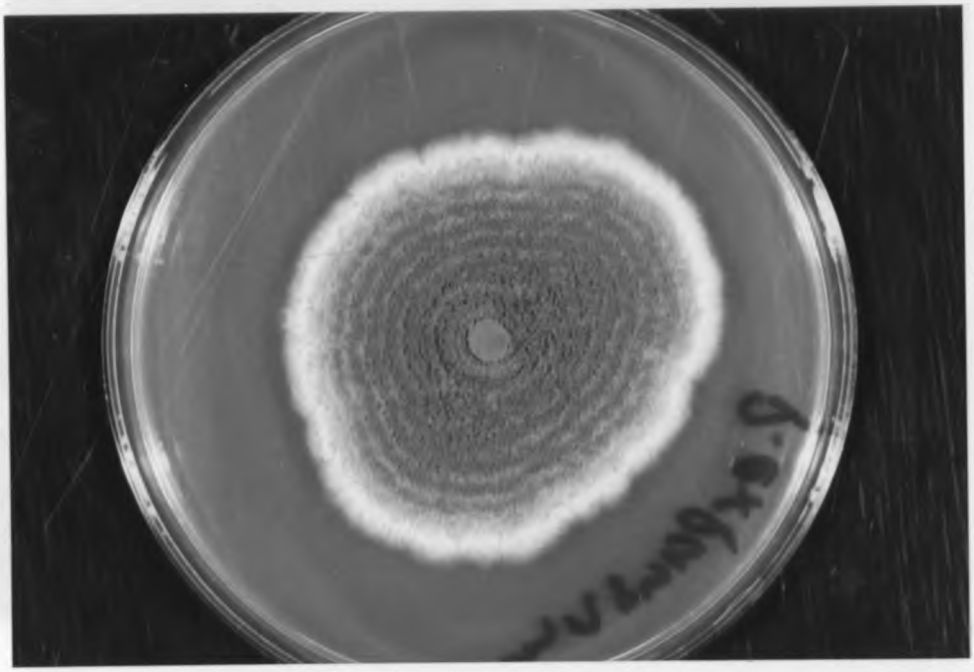
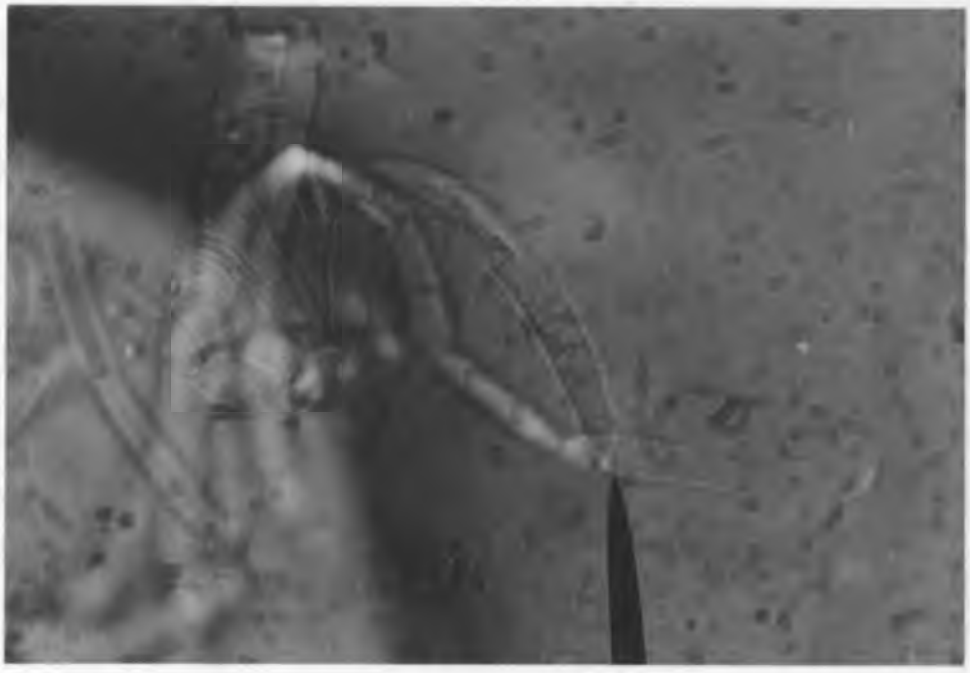


Plate 9. (above). *Penicillium funiculosum*. Conidiophores arising from a rope of hyphae. (X400).

Plate 10. (below). *Fusarium lateritium*. Macroconidia and hyphae. (X400)

Plate 11. (above). *Fusarium moniliforme*. Seven day old colony growing on Potato dextrose agar.

Plate 12. (below). *Fusarium oxysporum*. Macroconidia showing a heel-shaped footcell. (X1000).



4.4 AFLATOXIN PRODUCING ABILITY OF *ASPERGILLUS FLAVUS* GROUP ISOLATES

Thirteen (19.01%) isolates of a total of 68 isolates of A. *flavus* group screened for aflatoxin production on yeast extract sucrose agar produced aflatoxins. Eleven (16.2%) isolates produced aflatoxin B1, 2 (2.9%) produced aflatoxin B2, and 2 produced aflatoxin G1. Two isolates produced both aflatoxin B1 and aflatoxin G1.

4.5 MYCOTOXIN CONTAMINATION

Mycotoxins were found in 32 (35.56%) of the 90 samples examined. Aflatoxins were the most commonly occurring mycotoxins being detected in 22 (24.4%) of the samples at levels of between trace to 50ppb (Appendix 11). Ochratoxin A was detected in 9 samples (10%), zearalenone in 5 samples (5.5%) and sterigmatocystin in two samples (2.2 %) (Table 4, Appendix 9). Five samples were positive for more than one toxin (Appendix 10).

There were no significant ($P>0.05$) differences in aflatoxin and mycotoxin contamination between feeds from different manufacturers, or of different formulations. However, these two variables varied with the time of sampling (Table 5).

Out of a total of 30 feed samples collected in May, only 2 were positive for mycotoxins (Table 6). This frequency of contamination was significantly lower than that of feed sampled in March ($P=0.001$, CHI-Square=10.76) and lower than that of feed sampled in January ($P=0.00001$, CHI-Square=17.33). The frequencies

of mycotoxin contamination in feed sampled in January and March were not significantly different (P=0.3017, CHI-Square=1.07).

Fifteen out of 30 feed samples collected in January were contaminated with aflatoxins (Table 6). This rate of contamination was significantly higher than that of feed sampled in March (P=0.0006, CHI-Square=7.5) and that sampled in May (P=0.0.0002, CHI-Square=13.87). The frequencies of aflatoxin contamination of feed sampled in March and that sampled in May were not significantly different (P=0.2276, CHI-Square=1.46).

TABLE 4. A summary of mycotoxin contamination

Mycotoxin	Number of samples	% (N=90)
Aflatoxins	22	24.4
Ochratoxin A	9	10.0
Zearalenone	5	5.6
Sterigmatocystin	2	2.2
All mycotoxins	32	35.6

TABLE 5. Chi-square comparison between various feed formulations manufacturers and times of sampling, in mycotoxin and aflatoxi contamination

	MYCOTOXIN		AFLATOXINS	
	Overall Chi-square	P-value	Overall Chi-square	P-value
Different feed formulations	1.37	0.7120	4.21	0.2397
Different feed manufacturers	7.81	0.1621	5.87	0.3187
Different times of sampling	17.55	0.0002	16.72	0.0002

TABLE 6 Frequencies of mycotoxin and aflatoxin contamination at different times of sampling

	MYCOTOXIN		AFLATOXINS	
	samples positive	samples negative	samples positive	samples negative
JANUARY	17	13	15	15
MARCH	13	17	5	25
MAY	2	28	2	28

5.0 DISCUSSION AND CONCLUSIONS

One of the aims of this study was to assess the level of fungal contamination in feed presented to poultry in Nairobi and surrounding areas as a measure of the hygienic quality. Using the classification of Flatscher and Willinger (1981) (cited by Stenwig and Liven, 1988), that is one of the most widely accepted classification of the hygienic quality of feed on the basis of the level of fungal contamination, it will be seen from the results (Table 1) that 41% of all the feed samples were of good to acceptable hygienic quality. Twenty three percent were of inferior quality while 36% were of unacceptable hygienic quality. The proportion of feed classified as inferior or unacceptable in the present study is very high compared to the findings of workers in other parts of the World. Stenwig and Liven (1988) for example, found only 8.5% of poultry and swine feed used in Norway to have more than 1 X 10^5 Cfu/gram. Routine examination of more than 23,000 samples in Germany showed that only 4.5% of samples contained more than 1 X 10^5 Cfu/gram (Kohler *et al.*, 1984 cited by Stenwig and Liven, 1988). The high level of mould counts reported here represent an immediate health hazard to poultry keepers. Indeed, two farmers encountered during the survey were reported to have developed pneumonic allergy from poultry houses. Moulding also decreases the nutritive value of feed (Lacey *et al.*, 1980). For this reason, nutrient losses due to moulding should be allowed for in calculations of poultry rations in Kenya. Pelleting of feed has

been shown to reduce the mould counts in chicken feed by a factor of between 100 - 10,000 times depending on the type of feed (Tabib *et al.*, 1984). This should be recommended to poultry feed manufacturers in Kenya.

Aspergillus spp., *Penicillium* spp. and *Fusarium* spp. were the most commonly isolated fungi, sometimes accounting for all the fungi isolated (Table 1). This is in agreement with the findings of Gatumbi *et al.*, (1989) who surveyed microfungi of stored cereals, legumes and oil seeds from various parts of Kenya. *Aspergillus* spp. and *Penicillium* spp. were isolated in all the samples and *Fusarium* spp. in 76.7% of all samples examined. These frequencies of isolation are high when compared to those reported in other parts of the world. For example, Romo and Fernandez (1985) isolated *Penicillium* spp. in 83.2%, *Aspergillus* spp. in 80.8% and *Fusarium* spp. in 54.4% of the 125 samples of Spanish commercially mixed poultry feeds they examined. Many fungi in these genera are important mycotoxin producers. Others such as *Aspergillus fumigatus*, and some *Penicillium* and *Fusarium* species are opportunistic pathogens of poultry.

Important toxigenic *Aspergillus* spp. isolated in this study were A. *flavus*, A. *candidus*, A. *versicolor*, A. *sydowi*, A. *ochraceus* and A. *terreus* (Appendix 4). *Aspergillus flavus* was the most commonly isolated species being found in 75.6% of all the samples examined. This frequency of isolation is within the range of other workers. Bryden *et al.*, (1975) isolated A. *flavus* in 76.1% of

animal feedstuffs in Australia, Carballo and Miguel (1987) found A. *flavus* in 71% of mixed animal feeds in Spain, while Romo and Fernandez (1986) reported a frequency of isolation of 63% of poultry feeds in Spain. The frequency of isolation of A. *flavus* in this study was however higher than that reported from poultry feeds in Punjab, India (46.3%) (Mangat *et al.*, 1987) but lower than that reported from poultry feed used in Norway (100%) (Stenwig and Liven, 1988).

Only 19.01% of *Aspergillus flavus* group isolates in the present study produced aflatoxins on a synthetic medium. This proportion of toxigenic strains is low considering the fact that in general, isolates from tropical regions are considered to be more toxigenic (46%) compared to those from temperate countries (15%)(Moreau, 1979). It is also low when compared to the frequencies reported for A. *flavus* group isolates from poultry and animal feed in other parts of the World. These include 80% in Australia (Bryden *et al.*, 1975), 64% in Punjab, India (Mangat *et al.*, 1987) and 53% in Bihar, India (Ranjan and Singh, 1991). The thin layer chromatography of plugs of agar cultures employed in the present study is only a simple screening method utilising the ability of A. *flavus* strain to produce toxins in artificial cultures. A higher proportion of strains produce aflatoxins in natural substrates than in synthetic substances. Furthermore, the sampling of cultures in the present study was limited to only one colony per sample. This means that there was a high probability of

leaving out toxigenic colonies in a sample and picking a nontoxigenic one. Thus the frequency of occurrence of aflatoxigenic strains demonstrated in the present study must be considered to be an underestimation and that the actual frequency could be much higher.

The most commonly isolated *Penicillium* spp. in this study were incidentally well known mycotoxin producers. *Penicillium cyclopium* which produces penicillic acid was isolated in 60% of the samples, P. *expansum* which produces patulin and citrin in 27.8% of the samples and P. *vindicatum* which produces ochratoxins in 17.7% of the samples (Appendix 5). Although the toxigenicity of the *Penicillium* isolates in this study was not tested, a high proportion of *Penicillium* spp. commonly occurring in food and feeds are usually toxigenic. For example, Leistner (1984) found 828 (55.9%) of 1481 *Penicillium* isolates from various foods and feeds in Germany to be toxigenic, producing over 20 different mycotoxins. The high frequency of occurrence (100%) and the high levels of contamination (2×10^2 - 3×10^5 Cfu/gram) recorded in this study indicate how highly significant the potential mycotoxin hazard due to *Penicillium* spp. is. Indeed, ochratoxin A and sterigmatocystin which are some of the mycotoxins that can be produced by *Penicillium* spp. were found in 12.2% of all samples examined. A higher proportion of samples would most probably have been found to be contaminated with these mycotoxins if more of such mycotoxins were analysed for.

Fusarium moniliforme was the most commonly isolated *fusaria*, being found in 38.9% of all samples. This fungus is cosmopolitan and ubiquitous on corn and occurs both in the temperate and warmer parts of the world. It is known to produce several mycotoxins such as moniliformin, zearalenone and the trichothecene (Cole and Cox, 1981), but the chemical nature of other mycotoxins produced by this fungi is unknown. *Fusarium moniliforme* cultures or contaminated feeds are associated with certain toxicosis such as esophageal cancer in man and encephalomalacia in equines. In poultry, laboratory studies have shown F. *moniliforme* contaminated diets to be toxic to duckling (Jeschke *et al.*, 1987) and immunosuppressive to chicken (Marijanovic *et al.*, 1991) although isolates of F. *moniliforme* are known to vary greatly in their toxicity to chicken (Bryden *et al.*, 1987). The isolation of F. *moniliforme* in this study is therefore in itself a demonstration of a possible toxic factor in poultry feed. Other *Fusarium* spp. isolated in this study are F. *oxysporum* which is reported to produce zearalenone and F. *lateritium* which produces several toxic trichothecene (Cole and Cox, 1981).

A total of 32 (35.56%) samples out of the 90 samples examined were contaminated with at least one of the seven mycotoxins analysed. Aflatoxins were the most commonly encountered mycotoxins being demonstrated in 24.4% of all the samples examined. This is in agreement with the widely held view that together with trichothecenes, aflatoxins are the most commonly occurring

mycotoxins in nature (Robb, 1993). The frequency of aflatoxin contamination in this study compares well to, though slightly less than the frequencies reported in surveys in other parts of the world. Ranjan and Singh (1991) for example, found aflatoxins to contaminate 36.1% of poultry feed used in Bihar, India. A survey of farms with history of prevalent disease in Punjab, India found 29.1% of poultry feed samples to be contaminated with aflatoxins (Mangat *et. al.*, 1987). Other similar studies have yielded much higher frequencies of contamination. These include 61.9% of poultry feed from farms with history of disease in Punjab, India (Raina and Singh, 1991), 41.8% of molded feeds in Australia (Bryden *et al.*, 1980) and 63.9% poultry feed used in Kuwait (Natour *et al.*, 1985).

Aflatoxins were detected at levels ranging from detection limit (4ppb) to 50ppb. These are quite low and are within the range of regulatory levels of 20 - 50ppb recommended in many countries (Jelinek *et al.*, 1989). These levels would not be expected to have any adverse effects on poultry in Kenya even though the susceptibility of the Kenyan strains of poultry has not been tested. This is because the lowest level of aflatoxin B1 demonstrated to have an adverse effect in poultry is 200ppb. This level significantly predisposed the highly susceptible New Hampshire chicks to *Eimeria tenella* infection (Edds *et al.*, 1973). But the levels of aflatoxins in this study should not be taken to be absolutely safe let alone harmless for the following two reasons. First, the safety limits set up by regulatory authorities

and data derived from experiments designed to demonstrate effects of mycotoxins may have little relationship to the actual minimum effective dose. Epidemiological studies coupled with laboratory experiments and mathematical corrections have yielded an estimated minimum effective dose of below 10ppb aflatoxin B1 in broiler chicken (Hamilton, 1987). On the basis of such a criteria, 10.0% of all samples examined would be considered to have a level higher than the minimum effective dose. Second, the level of a particular mycotoxin that produces an effect under field conditions can be much lower than the level that produces the same effect in the laboratory (Huff *et al.*, 1988b). It would have been important to carry out a feeding trial on young chicks using the contaminated feeds sampled in this study, to see if there would arise any adverse effects attributable to mycotoxins. This was not done due to lack of funds.

The method used to analyse for mycotoxins in this study did not allow for quantification of ochratoxin A, zearalenone and sterigmatocystin. The method could however detect the mycotoxins at levels of or above the detection limits of 40ppb for ochratoxin A, 1000ppb for zearalenone and 200ppb for sterigmatocystin. 5.6% of all samples examined were contaminated with more than one mycotoxin (Appendix 10). The occurrence of several mycotoxins in the same sample is not improbable considering the number of different toxigenic fungi isolated in most feed samples and the frequent likelihood of occurrence of conditions suitable for mycotoxin

production. Mycotoxins such as aflatoxins and ochratoxins have been demonstrated to have synergistic additive effect (Huff and Doerr, 1981). Thus, whereas the levels of aflatoxins in this study were low, the concurrent occurrence of ochratoxin A in the same feed could result in mycotoxicoses. Another consequence of the occurrence of several mycotoxins in feed is that of diagnosis. Most veterinarians rely on presenting clinical signs and pathological lesions that have been determined in the laboratory with pure toxins, for the diagnosis of mycotoxicosis. However, the effects observed during a multiple mycotoxin exposure often differ greatly from those observed in animals exposed to a single toxin. For example, fatty livers that are used as a presumptive diagnostic identification of aflatoxicosis in poultry do no occur in a simultaneous aflatoxin and ochratoxin intoxication (Huff et al., 1988b).

All the samples examined had lower moisture content than the minimum level of about 13% (Pier, 1981) required to support fungal growth. This probably means that mycotoxin contamination in feeds is as a result of the use of contaminated raw materials. Karstad (1987) shares this view. He cites improper storage, bulk importation of raw materials, importation of low quality of feed ingredients, use of grain rejected for human consumption, reluctance of millers to dispose of contaminated raw materials or products, and poor or non-existent quality control of feed constituents as the reasons why Kenyan feeds are prone to

mycotoxicity. Maize and oilseed cake are the feed ingredients most commonly implicated. These and other raw materials prone to mycotoxin contamination should be closely monitored and contaminated batches discarded or detoxified (Jewers, 1988) to prevent the occurrence of mycotoxins in mixed feeds.

Other than through feed, poultry flocks are also exposed to mycotoxin hazards through litter (Kaya *et al.*, 1991) and through deliberate use of antibiotic drugs derived from fungal sources (Linsell, 1982). Mycotoxin risk through these two sources is much less compared to that from feed but not negligible. There is therefore, need to investigate the possible occurrence of mycotoxins in poultry litter from poultry houses in Kenya.

The presence of mycotoxins in animal feed represents the first stage of one of the food chains that can carry mycotoxins to man. Fortunately for chicken products' consumers, chicken have a high clearance rate for both aflatoxins (Wolzak *et al.*,1985) and ochratoxins (Bauer and Gareis, 1991; Ruprich *et al.*, 1991). They also have a very high (2200:1) feed : egg ratio and a high feed : meat ratio (1200:1) for aflatoxins (Rodricks and Stolloff, 1977). Thus the poultry consuming the feed sampled in the present study would not be expected to show any aflatoxins in eggs or tissues. However, since poultry flocks are also exposed to mycotoxins through other routes such as litter, determination of mycotoxins in poultry tissues would have added more information on the extent of mycotoxin exposure to poultry. This was not possible due to

shortage of funds.

Infestation of animal feeds with mycotoxigenic fungi and subsequent mycotoxin formation is likely to continue into the future. What is most required to reduce the extent of mycotoxin exposure to animals and poultry is the enforcement of more stringent regulations on mycotoxin contamination in feed ingredients and mixed feeds by quality control authorities. Availability of cheaper and unsophisticated mycotoxin assay test that can be used by farmers and veterinarians under field conditions and animal feed manufacturers will also help. Information on mycotoxin decontamination should be availed to feed manufacturers and decontamination recommended for mycotoxin contaminated batches of animal feed raw materials.

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7.0 APPENDICES

APPENDIX 1. COMPOSITION OF AGAR MEDIA USED

(1) ADMB medium (Hamsa and Ayres,1977)

Tryptone	15g
Yeast extract	10g
Ferric chloride	0.5g
Botran (2,6 Dichloro-4-nitroaline)	20mg
Agar	15g

Distilled water added to make up to 1 Litre
Final pH = 6.5

(2) Czapek solution agar

Sodium nitrate ($NaNo_3$)	3.0g
Potassium Phosphate (K_2HPO_4)	1.0g
Potassium Chloride (KCl)	0.5g
Magnesium Sulphate ($MgSO_4.7H20$)	0.5g
Ferrous Sulphate ($FeSO_4$)	0.01g
Sucrose	30.0g
Agar	15.0g
Distilled water	1000 ml

(3) DRYES Agar (King et al., 1979)

Yeast extract	20g
Sucrose	150g
Agar	20g
Chloramphenicol	100mg
Botran (2,6 dichloro-4-nitroaniline)	2mg
Rose bengal	25mg

Distilled water added to make upto 1 litre

(4) Malt salt agar (Christensen, 1946)

Malt extract	20g
Sodium chloride	75g
Agar	20g
Streptomycin	250mg

Distilled water to make upto 1 litre
All media were sterilised by autoclaving for 15 minutes at 121 °c

APPENDIX 2. RAW DATA ON MANUFACTURERS, FORMULATIONS, MOISTURE CONTENT AND TOTAL MOULD COUNTS

Sample No.	Manufacturer	Formulation	% moisture	Total mould count (Cfu/g X 10^5)
1	Belfast	Layers	9.45	5
2	Unga	Layers	8.23	2
3	Unga	Layers	8.23	1
4	Unga	Layers	7.18	9
5	Merchant	Layers	9.35	11
6	Ideal	Layers	10.31	1
7	Unga	Growers	9.37	7
8	Unga	Layers	10.40	3
9	Unga	Broilers	10.25	3
10	Ideal	Layers	10.10	35
11	Bora	Layers	8.46	30
12	Unga	Growers	11.56	26
13	Unga	Growers	9.64	10
14	Unga	Growers	10.40	7
15	Unga	Chick mash	10.11	18
16	Unga	Layer	9.01	9
17	Own Made	Layer	9.31	18
18	Muus	Broiler	9.02	13
19	Unga	Grower	10.14	21
20	Unga	Chick mash	9.68	4
21	Unga	Grower	9.44	13
22	Unga	Layer	9.73	60
23	Belfast	Chick mash	9.59	58
24	Belfast	Grower	9.42	44
25	Belfast	Chick mash	9.05	27
26	Belfast	Grower	7.60	10
27	Belfast	Layer	10.02	11
28	Muus	Broiler	9.02	2
29	Muus	Broiler	9.34	4
30	Unga	Layers	8.37	4
31	Unga	Layers	8.29	40
32	Memake	Chick mash	8.32	9
33	Belfast	Layers	7.47	28
34	Memake	Layers	8.13	20
35	Belfast	Chick mash	8.59	60
36	Memake	Growers	7.86	70
37	Milling	Layers	9.06	12
38	Unga	Growers	9.39	7
39	Milling	Growers	7.74	9
40	Unga	Layers	6.85	11
41	Unga	Chick mash	7.70	8
42	Milling	Chick mash	9.10	9
43	Unga	Layers	7.36	21
44	Belfast	Layers	7.74	21

Sample No.	Manufacturer	Formulation	% moisture	Total mould count (Cfu/g X 10^5)
45	Unga	Growers	9.49	4
46	Kibos	Layers	8.23	44
47	Kibos	Growers	8.69	5
48	Ideal	Chick mash	8.46	5
49	Ideal	Layers	8.62	6
50	Unga	Layers	8.05	12
51	Unga	Broilers	9.05	2
52	Unga	Layers	7.47	10
53	Unga	Chick mash	8.94	10
54	Milling	Chick mash	9.65	2
55	Unga	Chick mash	7.78	13
56	Unga	Layers	8.25	49
57	Unga	Growers	8.94	30
58	Unga	Layers	7.59	15
59	Unga	Chick mash	7.82	6
60	Unga	Growers	8.14	5
61	Unga	Layers	7.67	33
62	Unga	Chick mash	7.42	6
63	Belmill	Layers	9.37	90
64	Unga	Chick mash	9.33	9
65	Unga	Growers	8.63	41
66	Unga	Layers	6.93	78
67	Unga	Layers	6.79	70
68	Unga	Chick mash	8.63	7
69	Unga	Growers	8.00	17
70	Unga	Growers	7.46	44
71	Unga	Growers	8.18	12
72	Unga	Chick mash	8.46	17
73	Unga	Growers	8.06	9
74	Unga	Chick mash	7.90	22
75	Unga	Layers	7.32	29
76	Unga	Layers	7.61	13
77	Unga	Chick mash	10.52	36
78	Unga	Growers	10.72	40
79	Belfast	Growers	7.07	131
80	Belfast	Chick mash	8.03	5
81	Belfast	Layers	8.43	22
82	Unga	Chick mash	8.52	49
83	Unga	Layers	8.50	7
84	Unga	Growers	9.35	29
85	Unga	Growers	7.32	18
86	Unga	Layers	8.91	8
87	Unga	Growers	8.81	12
88	Unga	Layers	9.15	33
89	Unga	Layers	8.02	4
90	Unga	Layers	8.44	27

APPENDIX 3. ESTIMATED *ASPERGILLUS* SPP., *PENICILLIUM* SPP. A *FUSARIUM* SPP. COUNTS

Sample No.	*Aspergillus* spp. CFU/g X 10^3	*Penicillium* spp. CFU/g X 10^3	*Fusarium* spp. CFU/g X 10^3
1	160	20	0
2	70	3	2
3	70	3	2
4	60	10	90
5	150	10	0
6	20	10	0
7	10	2	70
8	20	20	0
9	10	1	0.2
10	60	0	310
11	40	3	30
12	10	10	130
13	60	1	70
14	60	2	50
15	90	2	20
16	70	2	10
17	50	3	120
18	20	10	10
19	170	10	5
20	20	2	20
21	20	5	20
22	130	7	80
23	10	10	0
24	10	10	10
25	40	4	0
26	90	50	0
27	60	30	0
28	50	0.2	0
29	10	1	0
30	1	4	10
31	160	130	7
32	70	10	10
33	70	10	10
34	,60	. 6	0.5
35	150	30	10
36	20	10	10
37	10	20	1
38	20	4	10
39	10	30	/2
40	60	10	6
41	40	2	0.1
42	10	30	40
43	60	100	5
44	60	100	40

Sample No.	*Aspergillus* spp. CFU/g X 10^3	*Penicillium* spp. CFU/g X 10^3	*Fusarium* spp. CFU/g X 10^3
45	9	20	2
46	70	120	40
47	50	4	50
48	20	3	0
49	170	2	0
50	20	50	0
51	20	2	0
52	13	2	150
53	10	50	30
54	10	10	10
55	40	30	40
56	90	70	60
57	60	20	10
58	50	80	10
59	10	7	0
60	1	1	110
61	160	100	0
62	10	1.3	4
63	350	300	100
64	50	6	2
65	40	270	20
66	140	10	30
67	50	3	20
68	40	10	10
69	10	100	8
70	140	140	20
71	30	60	0
72	50	70	8
73	20	50	4
74	90	70	30
75	80	100	30
76	40	60	40
77	220	90	20
78	150	100	24
79	800	30	9
80	50	16	0
81	110	60	3
82	160	60	0
83	40	10	11
84	120	90	12
85	30	90	40
86	20	40	2
87	40	40	0
88	40	2.3	10
89	30	0.5	10
90	100	10	90

APPENDIX 4. *ASPERGILLUS* SPECIES ISOLATED

	Species	Number of samples	%(N=90).
1	A. *flavus* Link	68	75.6
2	A. *wentii* Wehmer	66	73.3
3	A. *chevalieri* Thom and Church	33	36.7
4	A. *candidus* Link	25	27.8
5	A. *ruber* (Brem.)	16	17.8
6	A. *niger* van Tieghem	12	13.3
7	A. *tamarii* Kita	8	8.9
8	A. *chevalieri* var. intermedius Thom and Raper	7	7.8
9	A. *fumigatus* Fresenius	7	7.8
10	A. *versicolor* (vaill) Tiraboschi	5	5.6
11	A. *sydowi* (Bain. and Sart.)	5	5.6
12	A. *ochraceus* Wilhelm	4	4.4
13	A. *ustus* (Bainier)	2	2.2
14	A. *Parasiticus* Speare	1	1.1
15	A. *terreus* Thom	1	1.1

APPENDIX 5. *PENICILLIUM* SPECIES ISOLATED.

Species	Number of samples	% (N=90)
1. P. *cyclopium* Westling	54	60.0
2. P. *expansum* (Link) Thom	25	27.8
3. P. *vindicatum* Westling	16	17.7
4 P. *crustosum* Thom	8	8.9
5 P. *martensii* Biourge	8	8.9
6 P. *implicatum* Biourge	6	6.7
7 P. *purpurogenum* Stoll	6	6.7
8 P. *oxalicum* Currie+Thom	6	6.7
9 P. *rugulosum* Thom	6	6.7
10 P. *digitatum* Saccardo	6	6.7
11 P. *brevicompactum* Dierckx	6	6.7
12 P. *granulatum* Bainier	4	4.4
13 P. *diversum* Raper and Fennel	3	3.3
14 P. *italicum* Wehmer	3	3.3
15 P. *duclauxi* Delacroix	3	3.3
16 P. *funiculosum* Thom	2	2.2
17 P. *chrysogenum* Thom	1	1.1
18 P. *puberulum* Bainier	1	1.1
19 P. *roqueforti* Thom	1	1.1
20 *Penicillium* spp. (unidentified)	14	15.6

APPENDIX 6. RESULTS OF ANALYSIS OF VARIANCE TO COMPARE THE MEANS OF VARIOUS VARIABLES AMONGST DIFFERENT FEED FORMULATIONS

	Means for various feed formulation				
Variable	layers	growers	chick mash	broiler	P-Value
Log Moisture content	8.420	8.843	8.7667	9.1417	0.1800
Log total mould count	5.4195	5.5685	5.4514	4.5824*	0.0453
Log *Aspergillus* counts	4.7241	4.5766	4.5040	4.0502*	0.0113
Log *Pennicillium* counts	4.2270	4.3469	4.1655	4.1003*	O.0009
Log *Fusarium* counts	4.0740	4.0405	3.6451	3.1505	0.0940

Key:- * mean significantly lower

APPENDIX 7. RESULTS OF ANALYSIS OF VARIANCE TO COMPARE THE MEANS OF VARIOUS VARIABLES AMONGST DIFFERENT FEED MANUFACTURERS

Means for the various feed manufacturer

Variable	Unga	Belfast Milling	Memake	Muus	Others	P--Value
Log Moisture content	8.6122	8.5383	8.8875	8.0100	9.37	9.0663	0.3337
Log Total mould count	5.5378	5.6071	4.8222	5.1860	4.8903	4.8630	0.0518
Log *Aspergillus* count	4.4762	4.9039[a]	4.000	4.6923	3.8306[b]	4.5387	0.0061
Log *Penicillium* count	4.2192	4.1418	4.2386	4.2143	3.4005	3.7641	0.0952
Log *Fusarium* count	4.0579	3.8113	2.6667[b]	2.805[b]	4.7457[a]	4.1596[a]	0.0002

Key:- a - mean significantly higher.
b - mean significantly lower.

APPENDIX 8. RESULTS OF ANALYSIS OF VARIANCE TO COMPARE THE MEANS OF VARIOUS VARIABLES AMONGST DIFFERENT TIMES OF SAMPLING

	Means for the different times of sampling			
Variable	January	March	May	P-Value
Moisture content	9.3897[a]	8.2760	8.3200	< 0.0001
Log Total mould count	5.8727	6.0462	7.3137[a]	< 0.0001
Log *Aspergillus* count	4.2205	4.4722	4.8029[a]	0.0002
Log *Penicillium* count	3.6847[b]	4.2460	4.5779	< 0.0001
Log *Fusarium* Count	4.3480	3.4139[b]	4.1335	< 0.0001

Key:- a - mean significantly higher.
b - mean significantly lower.

APPENDIX 9. MYCOTOXIN CONTAMINATION

Sample	aflatoxins	ochratoxin A	zearelenone	sterigmatocystin
1	-	-	-	-
2	-	-	-	-
3	+	-	+	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	+	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	+	-	-	-
14	+	-	-	-
15	+	-	-	-
16	+	-	-	-
17	-	-	-	-
18	+	-	-	-
19	+	-	-	-
20	+	-	-	-
21	+	-	-	-
22	+	+	-	-
23	+	+	-	-
24	+	-	-	-
25	+	-	-	-
26	+	-	-	-
27	-	+	-	-
28	-	-	-	-
29	-	-	-	-
30	-	-	-	-
31	+	-	-	-
32	-	-	-	-
33	+	-	-	-
34	-	-	-	-
35	-	-	-	-
36	-	-	-	-
37	-	+	-	-
38	-	-	-	-
39	-	-	+	-
40	-	-	+	-
41	-	+	-	-
42	-	-	-	-
43	-	-	-	-
44	-	+	-	-
45	-	-	-	-
46	-	-	-	-

Sample	aflatoxins	ochratoxin	zearelenone	sterigmatocystin
47	-	-	-	-
48	-	-	-	-
49	-	-	-	-
50	-	-	-	-
51	-	+	-	-
52	-	-	-	-
53	-	-	-	-
54	-	-	-	-
55	-	-	-	-
56	-	-	-	-
57	+	-	-	+
58	-	+	-	-
59	-	-	-	-
60	-	-	-	-
61	-	-	-	-
62	-	-	-	-
63	-	-	-	-
64	-	-	-	-
65	-	-	-	-
66	-	-	-	-
67	-	-	-	-
68	-	-	-	-
69	-	-	-	-
70	+	-	-	-
71	-	-	-	-
72	-	-	-	-
73	+	-	-	+
74	-	-	-	-
75	-	-	-	-
76	-	-	-	-
77	-	-	-	-
78	-	-	-	-
79	-	-	-	-
80	-	-	-	-
81	-	-	-	-
82	-	-	-	-
83	-	-	-	-
84	-	-	-	-
85	-	-	-	-
86	-	-	-	-
87	-	-	-	-
88	-	-	-	-
89	-	-	-	-
90	-	-	-	-
Total	22	10	5	2

KEY: + = Positive for mycotoxin tested.
- = Negative for mycotoxin tested.

APPENDIX 10. A SUMMARY OF MULTIMYCOTOXIN CONTAMINATION

Mycotoxins	Number	% (N=90)
Aflatoxin + Zearalenone	1	1.1
Aflatoxin + Ochratoxin A	2	2.2
Aflatoxin and sterigmatocystin	2	2.2
Total	5	5.6

APPENDIX 11. LEVELS OF AFLATOXINS DETECTED

	Number of samples containing aflatoxins:			
Levels	AFB1	AFB2	AFG1	AFG2
Trace	5	4	0	0
12ppb	2	2	0	0
15ppb	1	0	0	0
18ppb	1	1	0	3
25ppb	4	1	3	1
50ppb	1	0	0	0
Total	14	8	3	1

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