ISOLATION AND IDENTIFICATION OF FUNGI FROM POULTRY FEEDS SOLD IN BENIN CITY

BY

Oisaseime Rehoboth OHIORENOYA (Miss)
Lsc1605496

UNIVERSITY OF BENIN
BENIN CTY

JULY, 2021

ABSTRACT

Poultry products is one of the most consumed animal products worldwide. Poultry products such as meat and eggs is a rich source of protein, fats and minerals. Poultry feed refers to the feedstuff consumed by poultry birds. This study was aimed at isolating fungi from poultry feeds sold in stores in Benin City. Three (3) forms of poultry feeds (mash, starter and finisher) were sampled in five (5) stores in Benin City. The samples were collected aseptically using in sterile polyethylene bags with sterile spatula and labeled accordingly. Serial dilution was made using water as diluent. Enumeration and isolation of fungi was carried out using potato dextrose agar (PDA) and *Aspergillus flavus* and *parasiticus* agar (AFPA). The pour plate method was employed and were incubated at 28+2°C for 7 days. Temperature and relative humidity of poultry stores were recorded using ThermoPro model (TP49-W-2) thermometer and hygrometer. The total fungal count range was of 0.10x105 on potato dextrose agar from s

CHAPTER ONE

1.0 INTRODUCTION

Poultry is defined as any domestic bird raised specifically for meat and egg consumption. This definition can include chickens, turkey, ducks, quails and others. Poultry is the second most consumed meat in the world next to pork (Danbappa *et al.,* 2018). Poultry feed is food for farm poultry. Feed is a source of organic and inorganic material used for growth of livestock (Sukmawati *et al.,* 2018). However, the principles of nutritional management for chickens are generally applicable to other poultry species (Ravindran, 2013).

Before the twentieth century, poultry were mostly kept on general farms, and foraged for much of their feed, eating insects, grain spilled by cattle and horses and plants around the farm. This was often supplemented by grain, household scraps, calcium supplements such as oyster shell, and garden waste (Eruvbetine, 2009). As farming became more specialized, many farms kept flocks too large to be fed in this way, and nutritionally complete poultry feed was developed. Modern feeds for poultry consists largely of grain, protein supplements such as soybean oil meal, mineral supplements, and vitamin supplements. The quantity and nutritional requirements of the feed, depends on the weight and age of the poultry, growth rate, rate of egg production, weather and the amount of nutrition the poultry obtain from foraging. This results in a wide variety of feed formulation (Ravindran, 2013).

Poultry feeds are formulated in other to meet the complex nutrient requirements of birds. Due to the simple digestive tract of birds and the intestinal flora making little contribution towards food digestion, it is necessary that poultry feed is complete in nutrients necessary for proper growth and egg production) and easily digestible (Matthew *et al.*, 2017). The most commonly used

agricultural product in the production of animal feed includes maize, ground nut, soyabean, sorghum, wheat and barley (Sivakumar *et al.,* 2014).

Healthy poultry require a sufficient amount of protein and carbohydrates, along with the necessary vitamins, dietary minerals, and an adequate supply of water. Lacto-fermentation of feed can aid in supplying vitamins and minerals to poultry. Certain diets also require the use of grit, tiny rocks such as pieces of granite, in the feed. Grit aids in digestion by grinding food as it passes through the gizzard. Grit is not needed if commercial feed is used. The feed must remain clean and dry; contaminated feed can infect poultry (Svihus, 2014).

Poultry production is an important part of national economy and it plays a significant role in providing the high quality food for human beings. Various stresses like low quality feed, naturally occurring toxic contamination in feed stuff, poor management, diseases, climatic extremes and other constraints are ever present threats that can adversely affect performance and health of poultry birds (Gherbawy *et al.,* 2019).

Some of the contaminating fungi such as *Aspergillus niger, Penicillium* spp., *Fusarium* spp*., Rhizopus* spp., *Mucor* spp. and *Cladosporium* spp. grow in the ripening crops in the fields and are termed as field fungi while others propagate in the agricultural commodities during storage conditions and are called storage fungi. The growth of mycoflora on crops is highly dependent upon climatic conditions, for example, rainfall and temperature (Ghaemmaghami *et al*., 2018). Contamination of agricultural commodities by fungi results not only in reduction of feed quality, but toxigenic fungi also develop a health hazard for human, livestock and poultry birds (Uwaezuoke *et al.,* 2008). Agricultural raw materials when contaminated with fungi and used

in the manufacture of poultry feeds may have adverse effects on animal health and productivity when consumed (Mehrolia *et al.*, 2015).

Poultry feeds are subject to contamination from diverse sources, which may have serious consequences on the safety of foods of animal origin (Osho *et al.,* 2007). Public concerns over food safety have heightened in recent years, because of problems such as bovine spongiform encephalopathy (BSE), melamine and dioxin contaminations, outbreaks of food-borne bacterial infections, and potential microbial resistance to antibiotics. Given the direct links between feed safety and the safety of foods of animal origin, it is essential that feed production and manufacture procedures meet stringent safety requirements (Ravindran, 2013). Regular monitoring of toxigenic mycoflora of the agricultural based feeds and foods is an essential pre-requisite for development of strategies to control or prevent mycotoxins exposure of feed animal and human population. Study of prevalence of toxigenic mycobiota of animal/poultry feeds is regularly and frequently reported from many countries (Saleemi *et al*., 2010).

The widely used processing steps in feed manufacturing plants are: receiving the raw materials, grinding or particle size reduction; proportioning or batching, mixing, heating or thermal treatment (or pellet shaping); packaging; warehousing and loading. These steps can influence the feed quality and adversely affect the birds health. Pellet feed is a kind of feedstuff made of raw material, vitamins, mineral and flavoring agents. It is estimated that over 80% of the feeds for poultry and pigs in the U.S. are pelleted (Madmullah *et al.,* 2015). Pellet is a form of mash feed that is compacted by heating, pressing and moisturizing processes. Pelleting reduces microbial contamination and improves the digestibility, palatability and organoleptic characteristics of feeds (Ghaemmagham*i et al.*, 2018). The most critical point for microbial contamination at the feed mills is the post-processing heat treatment. The heating process is required to pellet the feed

and usually kills most of the pathogens but despite this heat treatment, some fungi that are capable of sporulation can survive and propagate after heat stress. Inadequate operating temperatures for the pelleting equipment and feed conditioner are the major risk factors. Contamination of feeds before and after the heating process is common and can be attributed to many factors related to the feed mill factory. Unhygienic feed production provides proper ground for fungal growth in the final product due to insufficient heat temperature in the initial mixture and not following the hygienic standards and recommended guidelines for feed production processes (Dalcero *et al.*, 1997).

1.1 AIM AND OBJECTIVES

The aim of this study was to isolate fungi from poultry feeds sold in stores in Benin City. The specific objectives were to:

1. enumerate, isolate and identify fungi from poultry feeds.

2. enumerate, isolate and identify aflatoxigenic fungi from poultry feeds.

3. determine the temperature and relative humidity of storage of the poultry feeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 POULTRY FEED AVAILABILITY

Worldwide, production of poultry meat and eggs has increased consistently over the years, and this trend is expected to continue. It is predicted that most increases in poultry production during the next two decades will occur in developing countries, where rapid economic growth, urbanization and higher household incomes will increase the demand for animal proteins. Several factors have contributed to the consistent growth in world poultry production, including: genetic progress in poultry strains for meat and egg production; better understanding of the fundamentals of nutrition; and disease control (Ravindran, 2013).

For example, the age for a meat chicken to reach the market weight of 2kg has steadily decreased from 63 days in 1976 to 35 days in 2009, and the efficiency of converting feed into poultry products also continues to improve. This growth in poultry production is having a profound effect on the demand for feed and raw materials. Feed is the most important input for poultry production in terms of cost, and the availability of low-priced, high-quality feeds is critical if poultry production is to remain competitive and continue to grow to meet the demand for animal protein (Afolayan, 2008).

2.1 PRODUCTION SYSTEMS AND FEEDING

Historically, the poultry sector has evolved through three phases: traditional systems, which include family poultry consisting of scavenging birds and backyard raising; small-scale semi-commercial systems; and large-scale commercial systems. Each of these systems is based on a

unique set of technologies. They differ markedly in investment, type of birds used, husbandry level and inputs such as feeds. The feed resources, feeding and feed requirements required to raise poultry also vary widely, depending on the system used. The traditional system is the most common type of poultry production in most developing countries. Possible feed resources for the local birds raised in this system include: household wastes; materials from the environment (insects, worms, snails, greens, seeds); crop residues, fodders and water plants; and by-products from local small industrial units (cereal by-products) (Ravindran, 2013).

The survival and growth of extensive poultry systems are determined by the competition for feed resources in villages. This system works well where biomass is abundant, but in areas with scarce natural resources and low rainfall, the competition for natural resources with other animals can be extreme. Between the two extremes of traditional and commercial production systems is the semi-commercial system, which is characterized by small to medium-sized flocks (50 to 500 birds) of local, crossbred or improved genotype stock, and the purchase of at least part of their feed from commercial compounders (Nelder, 2012). Several feeding strategies may be used in this system: on-farm mixing of complete rations, using purchased and locally available feed ingredients; dilution of purchased commercial feeds with local ingredients; and blending of a purchased concentrate mixture with local ingredients or whole grains (Applegate and Angel, 2014).

Large-scale commercial system is the dominant production system in developed countries, and this sector has also recently expanded in many developing countries. Commercial systems are characterized by large vertically integrated production units and the use of more productive modern strains of birds. In these systems, feed is the most important variable cost component, accounting for 65 to 70% of production costs. High productivity and efficiency depend on

feeding nutritionally balanced feeds that are formulated to meet the birds' nutritional requirements (Ravindran, 2013).

2.2 POULTRY FEED NUTRITION

For maximum growth and good health, intensively reared poultry need a balanced array of nutrients in their diet. The nutrients required by birds vary according to species, age and the purpose of production – whether the birds are kept for meat or egg production. In order to meet these specific needs, different classes of poultry have to be fed different types of diets (Ravindran, 2013).

Poultry require nutrients to maintain their current state (maintenance) and to enable body growth (weight gain) or for egg production (Afolayan, 2004). Birds need a steady supply of energy, protein, essential amino acids, essential fatty acids, minerals, vitamins and, most important, water. Poultry obtain energy and required nutrients through the digestion of natural feedstuffs, but minerals, vitamins and some key essential amino acids (lysine, methionine, threonine and tryptophan) are often offered as synthetic supplements (Araujo, 2004). The common ingredients used in typical poultry feed formulations are: energy sources (which includes cereals (mainly maize), cereal by-products, animal fats and vegetable oils; plant protein sources (soybean meal); animal protein sources (fishmeal, meat and bone meal) and mineral supplements (Ravindran, 2013).

2.2.1 ENERGY

Poultry can derive energy from simple carbohydrates, fat and protein. They cannot digest and utilize some complex carbohydrates, such as fibre, so feed formulation should use a system based on utilizable energy (van der Klis and Vinyeta, 2008). Birds eat primarily to satisfy their

energy needs, provided that the diet is adequate in all other essential nutrients. The energy level in the diet is therefore a major determinant of poultry's feed intake. When the dietary energy level changes, the feed intake will change, and the specifications for other nutrients must be modified to maintain the required intake. For this reason, the dietary energy level is often used as the starting point in the formulation of practical diets for poultry (Arauju *et al*., 2004).

The protein requirement of growing chicken includes the amount of protein needed for maintenance plus the amount needed for tissue growth with an allowance for the losses in the digestion and metabolism. Different classes of poultry need different amounts of energy for metabolic purposes, and a deficiency will affect productive performance. In order to sustain high productivity, modern poultry strains are typically fed relatively high-energy diets. The dietary energy levels used in a given situation are largely dictated by the availability and cost of energy-rich feedstuffs. Due to the high cost of cereals, particularly maize, the use of low-energy diets for poultry feeding is not uncommon in many developing countries (Ravindran, 2013). Although all the usual ingredients of typical diets for poultry, except salt, limestone and oystershell, supply some energy, the chief sources of energy are the cereal grains and the grain sorghums. The latter, however, are not used so extensively in the feeding of poultry as are the former (Ajauro *et al.*, 2004).

2.2.2 PROTEIN AND AMINO ACIDS

The function of dietary protein is to supply amino acids for maintenance, muscle growth and synthesis of egg protein. The synthesis of muscle and egg proteins requires a supply of 20 amino acids, all of which are physiological requirements. An appropriate level of protein shall be

provided in the diet to ensure that the range and concentration of amino acids available are adequate to satisfy the nutritional needs of the poultry flock (Afolayan, 2008).

Ten of these are either not synthesized at all or are synthesized too slowly to meet the metabolic requirements, and are designated as essential elements of the diet. These need to be supplied in the diet. The balance can be synthesised from other amino acids; these are referred to as dietary non-essential elements and need not be considered in feed formulations. From a physiological point of view, however, all 20 amino acids are essential for the synthesis of various proteins in the body. The essential amino acids for poultry are lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, valine, phenylalanine and arginine. In addition, some consider glycine to be essential for young birds. Cysteine and tyrosine are considered semi-essential amino acids, because they can be synthesized from methionine and phenylalanine, respectively (Applegate and Angel, 2014). Lysine, methionine and threonine are the most limiting in most practical poultry diets of the ten essential amino acids (Araujo *et al.,* 2004).

Poultry do not have a requirement for protein per se. However, an adequate dietary supply of nitrogen from protein is essential to synthesize non-essential amino acids. This ensures that the essential amino acids are not used to supply the nitrogen for the synthesis of non-essential amino acids (van der Klis and Vinyeta, 2008). Satisfying the recommended requirements for both protein and essential amino acids therefore ensures the provision of all amino acids to meet the birds' physiological needs. The amino acid requirements of poultry are influenced by several factors, including production level, genotype, sex, physiological status, environment and health status. For example, high levels of lean meat deposition require relatively high levels of lysine. High levels of egg output or feather growth require relatively high levels of methionine. However, most changes in amino acid requirements do not lead to changes in the relative

proportions of the different amino acids. There is therefore an ideal balance of dietary amino acids for poultry, and changes in amino acid requirements are normally expressed in relation to a balanced protein or ideal protein (Ravindran, 2013).

2.2.3 FATS AND FATTY ACIDS

Due to the greater energy density of fat compared with carbohydrates and protein, poultry diets usually include fats to achieve the needed dietary energy concentration. Fat accounts for about 3 to no more than 5% of most practical diets. Other benefits of using fats include better dust control in feed mills and poultry houses, and improved palatability of diets (Araujo et al., 2004). Poultry do not have a specific requirement for fats as a source of energy, but a requirement for linoleic acid has been demonstrated. Linoleic acid is the only essential fatty acid needed by poultry and its deficiency has rarely been observed in birds fed practical diets. Linoleic acid's main effect in laying birds is on egg size (Ravindran, 2013).

2.2.4 MINERALS

Minerals are needed for formation of the skeletal system, for general health, as components of general metabolic activity, and for maintenance of the body's acid-base balance. Calcium and phosphorus are the most abundant mineral elements in the body, and are classified as macro-minerals, along with sodium, potassium, chloride, sulphur and magnesium. Macro-minerals are elements required in the diet at concentrations of more than 100 mg/kg. Calcium and phosphorus are necessary for the formation and maintenance of the skeletal structure and for good egg-shell quality. In general, 60 to 80 percent of total phosphorus present in plant-derived ingredients is in the form of phytate-phosphorus (Afolayan, 2008).

Under normal dietary conditions, phytate phosphorus is poorly utilized by poultry owing to the lack of endogenous phytase in their digestive enzymes. It is generally assumed that about one-third of the phosphorus in plant feedstuffs is non-phytate and is biologically available to poultry, so the phosphorus requirement for poultry is expressed as non-phytate phosphorus, rather than total phosphorus. A ratio of 2:1 must be maintained between calcium and non-phytate phosphorus in growing birds' diets, to optimize the absorption of these two minerals. The ratio in laying birds' diets is 13:1, because of the very high requirement for calcium for good shell quality (Ravindran, 2017).

2.2.5 VITAMINS

Vitamins are classified as fat-soluble (vitamins A, D, E and K) and water-soluble (vitamin B complex and vitamin C). All vitamins, except for vitamin C, must be provided in the diet. Vitamin C is not generally classified as a dietary essential as it can be synthesized by the bird. However, under adverse circumstances such as heat stress, dietary supplementation of vitamin C may be beneficial. The metabolic roles of the vitamins are more complex than those of other nutrients. Vitamins are not simple body building units or energy sources, but are mediators of or participants in all biochemical pathways in the body (Ravindran, 2013).

2.2.6 WATER

Water is the most important, but most neglected nutrient in poultry nutrition. Water has an impact on virtually every physiological function of the bird. A constant supply of water is important to: the digestion of feed; the absorption of nutrients; the excretion of waste products; and the regulation of body temperature (Araujo *et a*l., 2004).

Unlike other animals, poultry eat and drink all the time. If they are deprived of water for even a short time, production and growth are irreversibly affected. Water must therefore be made available at all times. Both feed intake and growth rate are highly correlated with water intake. Precise requirements for water are difficult to state, and are influenced by several factors, including ambient conditions, and the age and physiological status of the birds. Under most conditions, water intake is assumed to be twice the amount of feed intake. Drinking-water temperatures should be between 10-25 °C (Afolayan, 2008).

2.3 STORAGE OF POULTRY FEED.

Long storage of cereals during post-harvest periods and improper storage conditions are known to favour fungal growth, resulting in aflatoxin production in feed. Most feed mills suffer huge losses due to diseases caused by poor processing of feeds and feed contamination with microbial metabolites. The poultry industry in Nigeria has an annual growth rate of 2.17%. Livestock feed quality may however be affected by various microorganisms such as bacteria and fungi growing in different parts of the world (Sivakumar *et al.,* 2014). Most fungal contaminants in stored feed materials usually arise from infestations that began in the field, although some can directly infest storage grains as well when conditions are right. Moulds require about 12% moisture, more than 7°C temperature, oxygen and energy for their growth (Ravindran, 2013).

Fungal growth causes direct loss in volume and quality of feed raw materials and subsequently feed made from them leaving behind some poisonous mycotoxin, which contaminate feed raw materials and finished feeds. Feed spoilage by fungi also results in heating and dustiness (Adeniran *et al.,* 2013). The three most important genera of toxigenic fungi in the tropics are

Aspergillus, Fusarium and *Penicillium.* In Nigeria, much of the studies carried out on moulds focused on the agronomic dimensions of the problem (Okoli *et al.,* 2007).

As high-level contamination of food crops used as raw materials for the manufacture of poultry feeds by mycotoxigenic fungi occurs during storage, probably the point at which it would be most effective to intervene is during pre-harvest because contamination of crops in the field will determine the rate at which they will deteriorate in storage, particularly under conditions of high temperatures and humidity (Ghaemmaghami *et al.,* 2018). It is advisable to destroy waste parts of maize that already harbor *Aspergillus flavus*, otherwise they remain in the soil and could contaminate the next season's crop. It may be possible to reduce fungal contamination through the use of fertilizer, irrigation when necessary and weeding at appropriate times to reduce stress to the crops. The use of non-toxigenic bacterial or fungal species that out-compete toxigenic fungi in the field and safe storage may be an effective biological control (Aliyu *et al.,* 2016). In an experiment where biological control was tested on groundnut and cotton, aflatoxins were reduced by more than 70% and 68% respectively (Bankole and Kpodo, 2005). Growing varieties of crops resistant to fungal growth will reduce the chances of occurrence of mycotoxins. Delayed harvesting of crops makes them likely to suffer fungal infection. However, rapid drying of the harvested crop, physical separation of apparently contaminated grain, improved storage structures, using effective traditional methods, like smoking and local plant products, may reduce the chances of fungal contamination. Synthetic chemicals can also be used in coating cereals to reduce contamination with the moulds (Ravindran, 2013). Chemoprevention is the use of natural or synthetic agents to block, retard or reverse the effects of toxigenic fungi. Use of selective high affinity hydrated sodium calcium aluminosilicate (HSCAS) to bind aflatoxin in animal feeds can protect the animals against mycotoxins. Detoxification through ammonization is also effective

but residual ammonia can affect the health of the animals that feed on such detoxified material. Detoxification does not work efficiently for all types of mycotoxins (Bankole and Kpodo, 2005).

Filamentous fungi are able to grow on all types of food especially on a wide variety of agricultural products worldwide. Contamination of feed stuff can occur during handling, storage, processing and transport. About 19 genera of fungal species notably *Fusarium* spp., *Aspergillus* spp., *Penicillum* spp. and *Stenocarpella* spp. affect maize (a major feed ingredient) (Onyeze *et al.,* 2013). Development of toxicogenic molds in the animal feed is favoured by factors such as condensation, heating, leakage of rain water and insect infection. Such toxicogenic species include *Aspergillus fumigates, Aspergillus parasiticus, Aspergillus flavus, Fusarium verticillioides, Monascus rubes, Penicillium roqueforti* and *Trichoderma viride* (Shareef, 2010). Under certain environmental conditions, such as high temperature and high rainfall, maize grain is infected by ear rot fungi which produce mycotoxins (Saleemi *et al.,* 2010). Moldy growth on agricultural products results in the change in the texture, smell and taste of the infected foodstuff, this is due to the excretion of enzymes and volatile compounds by the fungus (Sivakumar *et al*., 2014).

Various animal feed raw materials are however derived from the same sources as human food, thus any fungal problem in an environment would equally manifest in the health of animals and may serve as early warning sign of an impending outbreak in human populations (Nafeesa *et al.,* 2005). Mould contamination is wide spread in tropical countries where poultry production and processing are expanding rapidly (Ibrahim *et al.,* 2017). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins, trichothecenes, ochratoxins and fusariotoxins. Numerous grain and root tuber-based raw materials are used in compounding poultry feeds. Usually one or more of these may be infested with mycotoxigenic fungi. It is therefore necessary to understand

the fungal population of these different materials since they are usually sourced from wide geographical areas and may therefore harbor diverse microbial populations (Mgbeahuruieke *et al.,* 2020).

Although much work has been done on fungal contamination of animal feeds in the temperate region, and the application of anti-oxidants and mould inhibitors have become routine for feed manufacturers, these products are rarely used in developing countries like Nigeria. There is an urgent need to understand the impact of fungi and their mycotoxin products on animal production in Nigeria. Strategies for reduction of mycotoxin contamination in animal production in Nigeria should however be based on a clear understanding of the fungal organisms involved and the type of toxins they produce (Okoli *et al*., 2007). Additionally, there is a limited information on the major fungal species causing aflatoxin contamination of poultry feeds in Nigerian feed mills (Mehrolia *et al*., 2015).

2.4 MYCOTOXIN CONTAMINATION OF POULTRY FEEDS

The term "mycotoxin" refers to all toxins produced by various types of fungus when they grow on agricultural products before or after harvest or during transportation or storage (Rodrigo *et al.,* 2016). The most commonly affected feedstuffs are cereals, oilseeds and oilseed meals. These toxins have the capacity not only to impair bird performance, but also to affect humans through toxin residues that can be deposited in animal tissues (Astoreca *et al.,* 2011). Many mycotoxins with different chemical structures and biological activities have been identified. Mycotoxin contamination of grains may start in the field when environmental conditions are conducive to fungal growth and can also take place during processing and storage of harvested products (Bankole and Kpodo, 2005). The moisture content of the harvested product and the ambient

temperature are principal determinants of fungal contamination and mycotoxin production. Some fungi, such as *Fusarium* spp., normally infest grains before harvest; others, such as *Penicillium* spp., invade after harvest, while *Aspergillus* spp. can grow both before and after harvest (Binder *et al.,* 2007). However, the presence of fungi does not necessarily indicate contamination with mycotoxins (Hassan, 2020).

Different mycotoxins affect animals in different ways. Some are cancer-causing toxins (for example, aflatoxin B1, ochratoxin A, fumonisin B1) and some are oestrogenic (zearalenones). Some affect the nervous system (fumonisin B1), while others affect the kidneys (ochratoxins) or suppress the immune system (aflatoxin B1, ochratoxin A, and T-2 toxin). Depending on the degree of contamination, these effects will eventually have negative impacts on performance (Jeff-Agboola *et al.,* 2012). It is often difficult to diagnose the effects of a mycotoxin because they are not necessarily unique to a given mycotoxin, but may be shared by others or magnified by interactions with others. Many fungal species are also capable of producing several mycotoxins (Mahmudullah *et al.,* 2015). Recent evidence has highlighted the co-contamination of feed samples with multiple mycotoxins, which has serious consequences for both feed safety and animal performance. The hazards induced by the simultaneous presence of several mycotoxins are not clearly understood (Nwiyi *et al.,* 2019).

In addition, depending on the degree of contamination, mycotoxins or their metabolites can be deposited in meat, visceral organs and eggs (Krnjaja *et al.,* 2008). Their concentration in animal products is considerably lower than the levels present in the feed consumed by the animals, and will not cause acute toxicity in humans, but residues of carcinogenic mycotoxins, such as aflatoxins and ochratoxin A, can affect human health. In most cases, however, the principal

source of mycotoxins for humans is contaminated cereals and legumes rather than animal products (Queiroz *et al.,* 2013).

2.4.1 AFLATOXIN

Aflatoxins are secondary metabolites produced by the aflatoxigenic fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations (Faparusi and Alagamba, 2018). Aflatoxicosis is poisoning resulting from ingestion of moderate to high levels of aflatoxins in contaminated food or feed (Habib *et al.,* 2015). Toxicological studies demonstrate that ducklings, hamsters, rats, trout, rabbits, and a number of other vertebrates are all susceptible to aflatoxin poisoning. Acute aflatoxicosis results in rapid progressive jaundice, edema of the limbs, pain, vomiting, necrosis, cirrhosis, or in severe cases, acute liver failure and death (Queiroz *et al.,* 2013).

Aspergilli, the fungi producing aflatoxins, proliferate under conditions of relatively high humidity and temperature, and are generally regarded as storage fungi (Oliveira *et al.,* 2006). Aflatoxin contamination is therefore almost exclusively confined to hot climates. Aflatoxin levels in certain types of feeds (cereals and oilseed meals) are a major problem in tropical countries, and require careful monitoring and appropriate treatment. All poultry species are susceptible to aflatoxin, especially young ducks (Ravindran, 2017).

Fungi are continuous threat to livestock feeds of economic importance such as compound feeds. They may affect feed either directly by causing mechanical damage throughout feeding, or indirectly by secreting and spreading mycotoxins such as aflatoxins in the case of aflatoxin producing fungi (Salisu *et al.,* 2020). Mycotoxins affect feed quality by reducing the nutritive value and producing unpleasant smell. Aflatoxin-contamination of poultry feeds results in

increased mortality of birds, decreased blood cell count, lower egg production, lower feed consumption rate, impaired resistance to infectious diseases, reduced vaccination efficiency and induced pathological damage to the liver and other organs (Mgbeahuruike *et al*., 2020).

2.4.2 OCHRATOXINS

Ochratoxins are produced by *Aspergillus* spp. and two *Penicillium* spp. Both are storage species, but *Aspergillus* sp. thrives in hot, humid conditions, whereas *Penicillium* spp. are essentially temperate (Saleemi *et al.,* 2010). Ochratoxins are therefore problems in both tropical and temperate regions. Ochratoxin A and B are two forms that occur naturally as contaminants, with ochratoxin A being more ubiquitous and occurring predominantly in cereal grains and the tissues of animals fed with contaminated feedstuffs (Queiroz *et al.,* 2013).

2.4.3 *Fusarium* MYCOTOXINS

Fusarium are "field moulds", as arable conditions (high moisture) favour their survival and growth. *Fusarium* are ubiquitous, cereal grains and animal feed are contaminated with *Fusarium* mycotoxins all over the world (Cegielska-Radziejewsk *et al.,* 2013). Fumonisins (FBs), produced by *Fusarium verticillioid*es and *Fusarium proliferatum,* occur worldwide and are predominantly found in maize and in maize-based animal feeds. Fumonisin B1 (FB1) is the most common and the most thoroughly studied, causing toxicities in animals such as equine leukoencephalomalacia and porcine pulmonaryoedema, diseases long associated with the consumption of mouldy feed by horses and pigs, respectively (Queiroz *et al*.,2013).

The majority of *Fusarium* sp. have the ability to produce toxins. Trichothecenes, zearalenone (ZEN) and the fumonisins are of particular importance (Danbappa *et al.,* 2018). The

trichothecenes include T-2 toxin and deoxynivalenol (DON; also known as vomitoxin). In addition, a given species can produce several different toxins, and grain crops are often contaminated by several *Fusarium* species at the same time. Thus, several toxins may be present simultaneously in contaminated feeds (Ravindran, 2013).

2.5 METHODS OF CONTROLLING OR DECONTAMINATING MYCOTOXINS.

Prevention of the contamination of agricultural commodities by fungi and their mycotoxins can be divided into the following three levels.

2.5.1 PRIMARY PREVENTION

The best pre- or post-harvest strategy to use in a particular year depends on the climatic conditions of that year. Unfortunately, avoiding weather that favours fungal infection is beyond human control (Turner *et al.,* 2005). Nonetheless, understanding the environmental factors that promote infection, growth and toxin production is the first step in minimizing mycotoxins in feeds (Okoli *et al.,* 2007). Several practices may help to maintain conditions that are unfavourable for fungal growth: development of fungal-resistant crop varieties; control of onfield infection with fungicides; scheduling of harvests in the period suitable for the region; and lowering the moisture content of the feedstuff after harvest and during storage (Ravindran, 2013).

2.5.2 SECONDARY PREVENTION

This level of prevention is required when the fungi are already in the feedstuff. The fungi should be eliminated or their growth stopped to prevent further deterioration and mycotoxin contamination. The following measures may be useful: protecting stored products from conditions that favour continuing fungal growth; using mould inhibitors (such as organic acids)

against fungal growth; storing commodity at low temperatures, where economically possible; stopping the growth of infested fungi by re-drying the products; and removing contaminated material (Ravindran, 2013).

2.5.3 TERTIARY PREVENTION

When the product is heavily infested by toxic fungi, primary and secondary prevention are no longer feasible. If the mycotoxin levels are known, it may be possible to dilute the contaminated material and produce a final blended feed that contains less than the critical level of the specific mycotoxin (Danike, 2002). Such blending of feeds to reduce mycotoxin concentrations is officially permitted, with restrictions in several countries. A number of additives are available for use in practical diets to remove or detoxify mycotoxins and reduce their negative effects on animals (Torres *et al.,* 2003). These additives fall into two categories: mycotoxin binders, which bind and adsorb the mycotoxins and prevent their absorption in the gut; and mycotoxin deactivators, which deactivate specific mycotoxins (Mehrolia *et al.,* 2015). The effects of some mycotoxins (aflatoxin, ochratoxin and fumonisin) can be effectively reduced by the inclusion of appropriate adsorbent-type binders, while others (trichothecenes and zearaleone) can be removed only by deactivation. Common mycotoxin binders include hydrated sodium calcium aluminosilicate, esterified yeast-wall polysaccharides, and clays such as zeolites and bentonites. Different sorbents have differing affinities for specific mycotoxins. However, there is a risk that non-specific adsorbing agents may prevent the uptake of micronutrients in the gut (Ravindran, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sample Collection

Three (3) forms of poultry feeds (mash, starter and finisher) were sampled in five (5) stores in Benin City. The samples were collected aseptically using sterile spatula in sterile spatula in sterile polyethylene bags and labeled accordingly.

3.2 Determination of Temperature of Storage

A thermometer {ThermoPro(TP4a-w-2)} was used to take the temperature readings from the different stores.

3.3. Determination of Relative Humidity

A hygrometer {ThermoPro(TP4a-w-2)} was used to take the relative humidity readings of the different stores.

3.4. Sterilization of Glasswares and Working Surface

Glasswares used for this research were washed with detergent and rinsed with water. They were sterilized by wrapping them in aluminum foil paper and placed in hot air oven at 160°C for 1hr.

3.5. Preparation of Media

3.5.1. Potato Dextrose Agar

Weighed 39g of Potato Dextrose Agar (PDA) was poured in a conical flask and 1000ml of distilled water measured with measuring cylinder was poured into the conical flask was tightly

covered with cotton wool wrapped in foil paper, after which it was sterilized by autoclaving for 15 min at 121°C. The medium was cooled to 45°C and then dispensed aseptically into sterile Petridishes. The poured culture plates with PDA were allowed solidify.

3.5.2 *Aspergillus flavus* and *parasiticus* Agar (AFPA)

Weighed 22.75g of *Aspergillus flavus* and *parasiticus* agar (AFPA) and it was poured in a conical flask. 500ml of distilled water measured with measuring cylinder was poured into the conical flask and was stirred thoroughly with a clean stirrer and the mouth of the conical flask was tightly covered with cotton wool wrapped in foil paper, after which it was sterilized by autoclaving for 15 min at 121°C. The medium was cooled to 45°C and then dispensed aseptically into sterile Petri-dishes. The poured culture plates with AFPA were allowed solidify.

3.6 Serial Dilution

Five-fold serial dilution was carried out on each of the samples by transferring 1.0ml of the stock into a sterile test tube containing 9ml of distilled water and mixed thoroughly. This was labelled 10^{-1}(test tube 1). From tube 1, 1ml was pipette into 10^{-2} (tube 2). Subsequent dilution were carried to the last test tube (tube 5). This procedure was done for all the samples from 5 different poultry feeds stores.

3.7. Enumeration and Isolation of Fungi

Aliquot of 1ml each from dilution 10^{-3} and 10^{-5} respectively in duplicates accordingly to their corresponding label were inoculated into culture plates of the prepared medium aseptically using spread plate method.

3.9 Identification of Fungi

Colonies were examined macroscopically for both sides of the plates to investigate their cultural characteristics, viz: size, shape, pigmentation and elevation. For the microscopic characterization, a loopful of the fungal mycelium was teased out on a glass slide, and then stained with lactophenol cotton blue (LPCB). A coverslip was placed on the stained preparation and then examined under X40 objective lens of a microscope, for investigating the microscopic features.

CHAPTER FOUR

4.0 RESULTS

Table 1 shows the fungal count of poultry feed samples obtained from feed stores A, B, C, E and F. The fungal count ranged from 0.1×10^5 to 8.5×10^5 (CFU/g) for 10^{-3} dilution factor and 0.1×10^5 to 1.05×10^5 (CFU/g) for 10^{-5} dilution factor in the PDA (Potato Dextrose Agar).

Table 2 shows the aflatoxigenic fungal count of poultry feeds sampled from poultry stores A, B, C, E and F. The count ranged from 2.00×10^4 to 4.50×10^4 (cfu/g) for 10^{-3} dilution factor. *Aspergillus flavus* displayed a yellowish colony. Also, there was not growth for *Aspergillus parasiticus* in all poultry feeds sampled.

The results of isolation of fungi in poultry feed revealed the presence of different species of fungi which included *Aspergillus flavus, Aspergillus niger, Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and *Trichoderma* spp. is shown in table 4.

The frequency of occurrence of fungal isolates from poultry feed samples ranged from 4.16-50% are shown in table 5.

Table 6 reveals the values of temperature (°C) and relative humidity(%) obtained from five different poultry feed stores in Benin City.

Table 1: Fungal Count of Poultry Feeds Sold in Benin City

Sample	10^5(cfu/g)
A (finisher E)	0.10 ± 0.00
B	2.00 ± 0.00
(grower F)	
C (finisher F)	0.60 ± 0.00
D (starter F)	0.10 ± 0.00
E (grower E)	8.50± 1.00
F (starter E)	3.00± 2.00
G (starter D)	1.00± 2.00
H (starter C)	0.50 ± 0.00
I (finisher C)	0.20 ± 0.00
J (finisher B)	1.55 ± 0.50
K (grower B)	1.55 ± 0.50
L (grower C)	1.05 ± 1.50
M (grower A)	0.75± 1.50
N (finisher A)	3.15 ± 1.50
O (starter A)	0.65 1.50

Mean ± SD

- = No growth

Table 2: Aflatoxigenic Fungal Count of Poultry Feeds Sold in Benin City.

.

Sample.	10^4(CFU/g)
A. (finisher E)	-
B. (grower F)	2.00± 0.00
C. (finisher F)	-
D. (starter F)	-
E. (grower E)	-
F. (starter E)	1.50 ± 1. 19
G. (starter D)	-
H. (starter C)	3.00 ± 0.00
I. (finisher C)	-
J. (finisher B)	-
K. (grower B)	-
L. (grower C)	-
M. (grower A)	-
N. (finisher A)	4.50 ± 0.00
O. (starter A)	-

]

Mean ± SD, - = No growth

Table 3: Cultural and Morphological Characteristics of the Fungal Isolates

Name of colony	Nature of hyphae	Spore type	Organism
Cream-coloured colony with entire margin	Pseudohyphae	Chlamydospore	*Saccharomyces cerevisae*
Dark grey	Non-septate	Sporangiospore	*Trichoderma* spp.
Flat, smooth and thick colony with dirty white reverse coloration (greeen spores).	Septate	Conidiospore	*Penicillium* spp.
Fluffy colonies with reverse side yellow (black spores).	Septate	Conidiospore	*Aspergillus niger*
Fluffy colonies with reverse side yellow (yellow spores).	Septate	Conidiospore	*Aspergillus flavus*
White to dark grey	Septate	Sporangiospore	*Rhizopus* spp.
White to dark grey colony	Septate	Sporangiospore	*Mucor* spp.
Woody white to pink colony	Septate	Chlamydospore	*Fusarium* spp.

Table 4: Distribution of Organisms on Fungal Isolates

The results of isolation of fungi in poultry feed revealed the presence of different species of fungi which included *Aspergillus flavus, Aspergillus niger, Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and *Trichoderma* spp. shown in Table 5.

Sample	*Mucor* spp.	*Penicillium* spp.	*A. niger* spp.	*A flavus* spp.	*Saccharomyces cerevisae*	*Trichoderma* spp.	*Rhizopus* spp.
A(finisher)	-	-	-	-	+	-	-
B(grower)	-	-	-	+	+	-	-
C(finisher)	-	-	-	-	+	-	-
D(starter)	-	-	-	-	+	-	-
E(grower)	-	-	-	-	+	-	+
F(starter)	-	-	-	+	+	-	-
G(starter)	-	-	-	-	+	-	-
H(starter)	-	+	-	+		+	-
I (finisher)	-	-	-	-	+	-	-

J(finisher)	-	-	-	-	+		-	-
K(grower)	+	-	-	-	-		-	-
L(grower)	-	-	-	-	+		-	-
M(grower)	+	-	+	-	-		-	-
N(finisher)	-	-	+	+	+		-	-
O(starter)	+	-	-	-	+		-	-

- = Absent

Table 5: Percentage Occurrence of Fungal lsolates from Poultry Feed Samples.

Organism	Frequency	Occurrence (%)
Mucor spp.	3	12.50
Penicillium spp.	1	4.17
Aspergillus niger	2	8.33
Aspergillus flavus	4	16.66
Saccharomyces cerevisae	12	50.00
Trichoderma spp.	1	4.16
Rhizopus spp.	1	4.16
Total	24	100

Table 6: Temperature and Relative Humidity of the Poultry Feed Stores.

Stores	Temperature (°C)	Relative humidity (%)
A	33.90	71
B	34.80	75
C	35.10	72
E	34.10	66
F	35.20	65

CHAPTER FIVE

Discussion

The assessment of fungal contamination of poultry feeds is one of the important steps taken to control the feeds quality and hygiene. In this study, the total fungal count of the poultry feed samples ranged from 0.10×105 to 8.50×105 cfu/g which is similar to a research conducted on 130 samples from two feed mills in Argentina which demonstrated a range of 6.60×103 to 6.30×105 cfu/g feed fungal contamination (Dalcero et al., 1998) and a study carried out on the analysis of the total fungal load of finished feed samples carried out in Abia State, Nigeria, which were about 1.90x106 cfu/g (Nwiyi *et al.,* 2019). The fungal count from these feed samples exceeds the accepted European standard for finished poultry feed of 1×103 cfu/g (Ghaemmaghami *et al.,* 2018).

The presence of six genera of filamentous fungi in feeds were indicated namely: *Aspergillus flavus, Aspergillus niger, Mucor* spp., *Penicillium* spp., *Rhizopus* spp., *Trichoderma* spp. and *Saccharomyces cerevisae* in comparism to the fungal isolates mostly found in poultry feeds by other researchers which are *Fusarium* spp., *Aspergillus* spp., *Penicillum* spp. and *Rhizopus* spp . affect maize which is a major raw material for processing poultry feed (Onyeze *et al*., 2013). Also, Astoreca *et al.,* (2017) reported that *Aspergillus, Penicillium* and *Fusarium* were the most common isolated from feed samples in Buenos Aires. Development of toxigenic molds in the animal feed is favored by factors such as condensation, heating, leakage of rain water and insect infestation (Okoli *et al.,* 2007). Inappropriate waste disposal, lack of access to hygienic water

and insufficient heat process on the initial mixture of raw materials are the main causes of exceeding fungal loads and spreading the pathogenic microorganisms to final products (Aliyu *et al.,* 2016).

The important factors for optimal feed storage conditions in a warehouse are suitable temperature and humidity, the need for hygiene, the place to store feed away from pesticides and direct sunlight and the place which protect the feed from water and damp (Sukmawati *et al.,* 2018). Mehrolia *et al.,* (2007) also reported that temperature and relative humidity of above 30°C and 80-100% respectively are favourable for fungal growth.

The aflatoxigenic fungal count of poultry feed samples ranged from 1×104 to 4.5×104 cfu/g, there was no growth for Aspergillus parasiticus in all poultry feeds sampled. In a study on the mycoflora and toxicity of feedstuffs from a production plant in Argentina reported the presence of 15 genera of filamentous fungi in feeds, Fusarium spp. and Penicillium spp. were isolated in 67.5% of the samples and Aspergillus spp. in 57.5% of them (Magnoli et al., 2002). A study carried out on 96 finished feeds from four feed mills in Brazil, showed more than 1×105 cfu/g fungal contamination of poultry feed, in which Aspergillus and Penicillium were the most frequent isolated genera (Rosa et al., 2006). A research conducted on 130 samples from two feed mills in Argentina, demonstrated a range of 6.60×103 to 6.30×105 cfu/g feed fungal contamination, and the most frequent isolates were Aspergillus spp. and Fusarium spp. (Dalcero et al., 1998). A survey on 35 feed samples in Buenos Aires, showed that the contamination loads were 4.00×104 to 1.60×105 cfu/g and Aspergillus, Penicillium and Fusarium were the most common genera (Astoreca et al., 2011). Analysis of mycobiota in commercial poultry feeds in Nigeria has detected common moulds isolated, Aspergillus spp.,

Penicillium spp. and Mucor spp. (Okolo et al., 2007). The total fungal load in the analyzed finishedfeed samples in a study carried out in Abia State, Nigeria, were about 1.90x106 cfu/g (Nwiyi *et al.,* 2019). Development of toxigenic molds in the animal feed is favored by factors such as condensation, heating, leakage of rain water and insect infestation (Okoli *et al.,* 2007). Inappropriate waste disposal, lack of access to hygienic water and insufficient heat process on the initial mixture of raw materials are the main causes of exceeding fungal loads and spreading the pathogenic microorganisms to final products (Aliyu *et al.,* 2016).

Shop E had the highest fungal count of isolates (8.5x105) and the lowest fungal count of 0.1x105 where its temperature was 35.20°C and that of relative humidity 65%. Also, the grower feed form is reported to have the highest count of fungal isolates within the range of 0.75x105 to 8.5x105 cfu/g. The finisher feed form had the lowest count of fungal isolates of 0.10x105 to 1.55x105 cfu/g. The fungal count from these feed samples exceeds the accepted European standard for finished poultry feed of 1×103 cfu/g (Ghaemmaghami *et al.,* 2018). The frequency of toxigenic fungi (4.17%, 16.66% and 8.30%) was lower than the non-toxigenic fungi (12.50%, 50% and 4.16%). The temperature range of the poultry feed stores were from 33.90-35.20°C and that of relative humidity was 65-75%. Storage temperatures between 25°C -30°C and a relative humidity of 97% favor the production of toxins during storage (Saleemi et al., 2010). The temperature range of the poultry feed stores were from 33.90-35.20°C and that of relative humidity was 65-75%. Aspergilli, the fungi producing aflatoxins, proliferate under conditions of relatively high humidity and temperature, and are generally regarded as storage fungi (Oliveira et al., 2006). The moisture content of the harvested product and the ambient temperature are principal determinants of fungal contamination and mycotoxin production. Some fungi, such as

Fusarium spp., normally infest grains before harvest; others, such as Penicillium spp., invade after harvest, while Aspergillus spp. can grow both before and after harvest (Binder et al., 2007). However, the presence of fungi does not necessarily indicate contamination with mycotoxins (Cegielska-Radziejewsk *et al.,* 2013).

In order to minimize the fungal infestation in agricultural commodities at pre-harvest stage, proper planting date of the crop, good irrigation practices and pest management have to be followed and these are effective in the reduction of fungal infestation and subsequent mycotoxin accumulation. Fungal infestation at post harvest stage largely relies on proper storage for future use. Storage of agricultural commodities and animal feed formulations for long periods poses a great threat of fungal infestation and remains as a big challenge in the developed and developing countries (Hussein and Basel, 2001). Climate represents the key agro-economic driving force of fungal colonization and mycotoxin production in this study. The most important parameters in fungal growth and mycotoxin production are moisture and temperature (Saleemi *et al.,* 2010).

Fungal spores are common component of aerosols and they drift by air current leading to dispersion in short and long distances. As these spores come in contact with feeds, they tend to germinate in the presence of moisture (Mgbeahuruieke et al., 2020).

CONCLUSION

The feed mill manufacturing process, should be maintained properly and post-processing contamination should be avoided. On this basis, regular microbiological and mycotoxicological analysis should be performed for maintaining quality and safety of poultry feeds.

REFERENCES

Adeniran A. L., Anthony M. H. and Lami M. H. (2013). Survey of mycotoxigenic fungi in concentrated poultry feed in Niger State, Nigeria. *Journal of Food Research.* **2**(2):128-135.

Afolayan, M. O. and Afolayan, M. (2008). Nigeria Oriented Poultry Feed Formulation Software Requirements. *Journal of Applied Sciences Research.* **4**(11):1596-1602.

Aliyu, R. M., Abubakar, M. B.,Yakubu, Y., Kasarawa, A. B., Lawal, N., Bello, M. B., Fardami A. M., Odera, P.,Muchiri, M., Omanya, N. and Werehire, P. (2016). Prevalence of Potential toxigenic Aspergillus species isolated from poultry feeds in Sokoto metropolis. *Sokoto Journal of Veterinary Sciences.* **14**(1):39-44.

Applegate, T. J. and Angel, R. (2014). Nutrient Requirements of Poultry Publication: History and Need for an Update. *The Journal of Applied Poultry Research.* 23:567–575.

Araújo L. F., Junqueira O. M., Araújo C. S., Faria D. E and Andreotti M. O. (2004). Different Criteria of Feed Formulation for Broilers Aged 43 to 49 Days. *Brazilian Journal of Poultry Science.* **6**(1):61-64.

Binder, E.M, Tan, L.M, Chin, L.J, Handl, J., and Richard, J. (2007) "Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology.* **136**(3-4), 265-282.

Cegielska-Radziejewsk R., Stuper K. and Szablewski T. (2013). Microflora and mycotoxin contaminations in poultry feed mixturesfrom western Poland. *Annals of Agricultureand Environmental Medicine*. **20**(1):30-35.

Dalcero A., Magnoli C., Chiacchiera S., Palacios G. M. and Reynoso (1997). Mycoflora and incidence of aflatoxins B1, zearalenone and deoxinyvalenol in poultry feeds in Argentina. *Mycopathologia.* **137**: 179-184.

Danbappa, R. A., Alhassan, K. A. and Shah M. A. (2018). Isolation and Identification of Microbial Contaminants Associated with Commercial Poultry Feeds. *Journal of Applied and Advanced Research.* **3**(5):142−147.

Danike, S., (2002). Prevention and Control of Mycotoxins and in poultry production. Chain: *A European View, World's Poultry Science Journal.* **58**(4): 457-474.

Ghaemmaghami, S. S, Nowroozi, H. and Tohidi moghadam, M. (2018). Toxigenic Fungal Contamination for Assessment of Poultry Feeds: Mashed vs. Pellet. *Iranian Journal of Toxicology* . (5):5-10

Gherbawy, Y. A., Shebany, Y. M. and Alharthy, H. (2019). Aspergilli and Their Aflatoxins Contamination of Poultry and Animal Feedstuff Samples in Western Region of Saudi Arabia. *Sains Malaysiana.* **48**(4): 765–771.

Ibrahim, M. J., Kabir, J., Kwanashie, C. N., Salawudeen, M. T. and Joshua, Z. (2017). Occurrence of mycotoxigenic fungi in poultry feeds at livebird markets, Zaria, Nigeria. *Sokoto Journal of Veterinary Sciences.* **15**: 53 - 59.

Islam M. T, Hossain M. K, Elahi A. T, Purkayastha M., Rahman M. M. (2014). Isolation and Identification of Common Fungal spp. from Commercial Broiler Feeds Available in Market of Sylhet District, Bangladesh. *International Journal of Natural Sciences.* **4**(2):38-41.

Mahmudullah B., Rupomanjury S., Shafqul I. and Fahmida B. A. (2015). Prevalence of microflora and potentially toxigenic fungi in poultry feed mixtures. *Annals Food Science and Technology* **16**(1): 267-273.

Matthew, O., Chiamaka, R. and Chidinma, O. (2017). Microbial Analysis of Poultry Feeds Produced in Songhai Farms, Rivers State, Nigeria. *Journal of Microbiology & Experimentation.* **4**(2):1-3.

Mehrolia, M. B., Kalkar, S. A. and Bhiwagade, S. D. (2015). Isolation and Identification of Fungi from Poultry Feed. (2015). *International Journal of Researches in Biosciences, Agriculture and Technology.* 1:72-75.

Mgbeahuruike, C. A, Nwoko, E. I. and Idolor, S. O. (2020). A Survey of the Aflatoxin Level and Molecular Identification of Fungal Contaminants in Poultry Feedmills from Different Geopolitical Zones of Nigeria. *African Journal of Biotechnology.* **19**(1):5-9

Nelder, R. (2012). Making Poultry Feed More Sustainable. (2012).*Organic Research Centre.*

Nwiyi, P. O, Nwabuko, C. N., Amaechi, N. and Ozioko C. (2019). Isolation and Identification of Mycotoxigenic Organisms in Poultry Feed from Selected Locations in Abia State, Nigeria. *Asian Journal of Research in Animal and Veterinary Sciences.* **3**(4): 1-9.

Okoli, I. C, Ogbuewu, I. P, Uchegbu, C. M, Opara, M. N, Okorie, J. O, Omede, A. A, and Ibekwe, V. I. (2007).Assessment of the Mycoflora of Poultry Feed Raw Materials in a Humid Tropical Environment. *Journal of American Science.* **3**(1):5-9.

Onyeze R. C., Udeh M. C., Akachi B. O. and Ugwu P. C. (2013).Isolation and Characterization of Fungi Associated with the Spoilage of Corn (Zea mays). *International Journal of Pharmaceutical Medicine and Biological Sciences.* **2**(3):87-91.

Ravindran, V. (2013). Poultry Feed Availability and Nutrition in Developing Countries. *Australian Journal of Agricultural Research*.

Queiroz, B., Pereyra, C. M., Keller, K. M., Almeida, T., Cavaglieri, T. R., Magnoli, C. E. and da Rocha Rosa, C. A. (2013). Fungal Contamination and Determination of Fumonisins and Aflatoxins in Commercial Feeds Intended for Ornamental Birds in Rio de Janeiro, Brazil. *Letters in Applied Microbiology.* **57**:405-411.

Saleemi, K. M, Khan, Z. M, Khan, A and Jareb, I. (2010). Mycoflora of Poultry Feeds and Mycotoxin Producing Potential of *Aspergillus* species. *Pakistan Journal of Botany.* **42**(1):427-434.

Salisu D. B. and Almajir, I. R. (2020). Aflatoxins and aflatoxigenic fungal contamination of common poultry feed products in Katsina State, Nigeria. *Novel Research in Microbiology Journal.* **4**(1): 653-665.

Shareef A.M., 2010. Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis. *Iraqi Journal of Vetinery Science.* **24**: 17-25.

Sivakumar, V. K, Singaravelu, G. and Sivamani, P. (2014). Isolation, Characterization and Growth Optimization of Toxicogenic Molds from Different Animal Feeds in Tamilnadu. *International Journal of Current Microbiology and Applied Sciences.* **3**(9):430-445.

Sukmawati, D., Saidah, N., Handayani, T. and Rahayu, S. (2018). The Characteristics of Fungi Contaminating Chicken Feed in Regal, Bigot, West Java. *Asian Journal of Agriculture and Biology.* **6**(4):472-480.

Uwaezuoke, J. C and Ogbulie, J. N. (2008). Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. *Journal of Applied Science and Environmental Management.* **12**(1):113 - 117.