THE EFFECT OF MOISTURE, FEED ADDITIVES AND BIOLOGICAL AGENTS ON PERFORMANCE, NUTRIENT RETENTION, BLOOD PARAMETERS AND AFLATOXIN IN BROILERS.

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A THESIS SUMITTED TO THE DEPARTMENT OF ANIMAL PRODUCTION, FACULTY OF AGRICULTURE, UNIVERSITY OF ILORIN, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.) IN ANIMAL PRODUCTION

MARCH, 2018

CERTIFICATION PAGE

We, the undersigned Examiners hereby certify that Salako, Abiola Olayemi (06/10AC358) has satisfactorily effected all the necessary correction pointed out to her during the Oral Examination of his thesis entitled "Effects of moisture, feed additives and Aflatoxin on performance, nutrient retention and blood parameters of broilers." held _____ and recommended that he be awarded the degree of Doctor of Philosophy (Ph.D.) in Animal Production.

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DECLARATION

I, **SALAKO, ABIOLA OLAYEMI** hereby declare that this thesis entitled **"**Effects of moisture, feed additives and Aflatoxin on performance, nutrient retention and blood parameters of broilers.**"** is a record of my research work. It has neither been presented nor accepted in any previous application for a higher degree. All sources of information have been specifically acknowledged.

In addition, the research work has been ethically approved by the University Ethical Review Committee.

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SALAKO, Abiola Olayemi Date

DEDICATION

To Jesus, the same yesterday, today and forever.

And to my loving Wife (Mrs Bukola Folasade Salako) and Daughter (Sophia Oluwamurewa Salako): Thank you for your sacrifice.

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I would like to praise and thank Almighty God for the countless blessings and privileges that I am constantly immersed in, for the effort I put forth in this work is merely an attempt to follow His most excellent prophecies for my destiny.

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I am confident that the skills acquired throughout my doctoral training, along with the positive influences of aforementioned individuals, have positioned me well for greater advantages in the academics and research.

Salako, Abiola Olayemi

July, 2018.

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Table 7.7b: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Creatinine. **Error! Bookmark not defined.**
Table 7.7c: Details interactions between types of maize treated with atoxigenic organisms and turmeric on ALT. **Error! Bookmark not defined.**
Table 7.7d: Details interactions between types of maize treated with atoxigenic organisms and turmeric on ALP. **Error! Bookmark not defined.**

ABSTRACT

Aflatoxin, a secondary metabolic product of *Aspergillus* spp is economically important in animal health due to its biological effects, manifested in reduced performance. Eradicating aflatoxin induced disease in poultry is an essential strategy for improving productivity. The objectives of this study were to determine: (i) effects of moisture and temperature on the growth of *Aspergillus paraciticus* and aflatoxin concentration; (ii) *in vitro* binding ability of xylanase and herbal extracts to attenuate the growth of *Aspergillus paraciticus* and (iii) effects of the screened additives and atoxigenic *Aspergillus paraciticus* on performance and blood parameters of broilers fed aflatoxin containing diet and high, medium and low moisture maize (HMM, MMM and LMM).

Five experiments were conducted. Experiment 1 investigated the *in vitro* effects of temperature (15^{o}C, 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C) and moisture (4%, 8%, 12%, 16% and 20%) on *Aspergillus paraciticus* count and aflatoxin concentration in feed inoculated with *Aspergillus paraciticus* in a 6×5 factorial design. Experiment 2 examined the effects of xylanase, herbal extracts of ginger, turmeric and garlic and their concentrations (0, 10, 20, 30, 40 and 50 mg/ml) on inhibition of *Aspergillus paraciticus* in a 4x6 factorial design. Experiment 3 evaluated the effects of dietary aflatoxin concentration (0 and 500 ppb) and herbal extracts on performance, digestibility and blood parameters of broilers in a 2x4 factorial design. Experiment 4 studied the response of broilers to dietary aflatoxin concentration (0 and 500 ppb) with or without atoxigenic *Aspergillus flavus* in a 2x2 factorial design. Experiment 5 studied the response of broilers to types of maize (HMM, MMM, LMM) and types of binder (0, turmeric, atoxigenic *Aspergillus flavus*) in a 3x3 factorial design. Data obtained were subjected to analysis of variance ($p<0.05$) and means were separated using Duncan multiple range test.

The findings of the study were that:

i. *Aspergillus paraciticus* count and Aflatoxin B1 increased from (1.15-5.52cfu) and (23.07-110.45ng) respectively ($p<0.05$) as moisture level increased. There were significant ($p<0.05$) interactions between moisture and temperature on growth of A*spergillus paraciticus* and aflatoxin concentration;

ii. herbal extracts at 30mg/ml was most effective in controlling growth of *Aspergillus paraciticus* and were in the order turmeric>ginger>garlic ($p<0.05$);

iii. aflatoxin in diet reduced feed intake (30g/bird/day), whereas dietary inclusion garlic, ginger and turmeric increased feed intake (32, 46 and 44g/bird/day respectively). There were significant interactions between dietary aflatoxin concentration and herbs on performance, digestibility and blood parameters of broilers ($p<0.05$);

iv. dietary atoxigenic *Aspergiluus flavus* increased feed intake (50g/bird/day), there were significant interactions between dietary aflatoxin concentration and atoxigenic *Aspergiluus flavus* on performance, digestibility and blood parameters of broilers ($p<0.05$); and

v. dietary HMM increased feed intake (63.23g/bird/day) relative to LMM (57.54g/bird/day) and MMM (57.25g/bird/day), turmeric and atoxigenic *Aspergillus flavus* improved performance relative to control ($p<0.05$).

The study concluded that poultry feed is best stored at 4% moisture and 40^{o}C to control aflatoxin. The use of the herbal extracts and atoxigenic *Aspergillus flavus* are effective in controlling aflatoxicosis. The study recommends that the strategy of incorporating turmeric and atoxigenic *Aspergillus flavus* should be used to control *Aspergillus paraciticus* risk in poultry feed.

CHAPTER ONE

1.0 INTRODUCTION

Food safety is an important prerequisite in ensuring human and animal health among all nations (Kumar *et al*., 2017). It can be compromised by many factors which include handling, storing and processing methods, physicochemical or microbiological toxic and poisonous agents that affect plant, human, animal wellness and productivity (Mohapatra *et al*., 2017). Protein from animal is an essential need in human nutrition and poultry species have been used as source of protein for human consumption across the nations of the world for years. Products of animals especially meat, milk and avians viscerals organs have played an important role in human's nutrition (Neumann *et al*., 2002). These products listed are compromised by factors that make its nutrition use improper due to different reasons (Allameh and Abyaneh, 2001). Human's health can also be impaired when exposed to contaminated foods like meat, milk and other animal products. One of the most potent and implicated microbiological food and feed contaminants are mycotoxins which are of greatest concern in the food chain. The secondary metabolites produced by different types of fungi are Mycotoxins. There are more than two hundred mycotoxins existing in nature and the most common ones are aflatoxins, ochratoxin and fumonsin (Daghir, 1995). Among these, we have aflatoxinswhich are of greatest concern for tropical countries like Nigeria and other African countries. Today, aflatoxin contaminated products are of economic importance to agricultural products and countries have legislated laws for grains and food production, importation and consumption (Allameh and Abyaneh, 2001). In Unites State of America, grains, food and drugs with more than 20ppb total aflatoxin and 15ppb aflatoxin B1 are not suitable for selling and selling which also include exports and imports (Gourama and Bullerman, 1995).

Aspergillus namely *Aspergillus flavus* and *Aspergillus parasiticus* are mostly implicated in Aflatoxicosis. Aflatoxins are field, processing and storage mycotoxins. *Aspergillus spp* thrives favorably under hot and humid conditions. It is for this reason that aflatoxins are limited to tropical climates and are mostly found in grain, legumes and cereals (Hell *et al*., 2000). *A. flavus* and *A. parasiticus* may invade plant species such as maize during growth and may remain on cereal grains even after the maize is harvested. Such infected maize contaminates the whole diet when used to formulate poultry diet, although some ingredients in the diets are also susceptible to Aflatoxin contamination from the source. (Hell *et al*., 2000; Hawkins *et al*., 2005; Turner and Sylla, 2005).

Aflatoxicosis contamination occurs due to interactions between the *Aspergillusspp*, the host animal and the environment. The response to aflatoxins contamination by livestock species also depends on some factors such as level of exposure, environmental condition and the type and susceptibility of the invaded species. Aflatoxin as the cause of health and wellness disorders has been one of the major cause for low life expectancy in the third world (Williams *et al*., 2004). Since its isolation and identification, aflatoxin has become economically important to animal and human health, this is because different biological effects that include carcinogenesis, mutagenesis, teratogenicity, hepatotoxcitiy, nephrotoxicity, dermaltoxicity, immunosuppressive and different biochemical effects including their effects on digestion, assimilation and metabolism, protein and nucleic acid synthesis (Dudhe *et al*. 1998). Higher doses of aflatoxin in diets results in hyperplasia of the bile duct and acute necrosis of the liver resulting in reduced digestibility of protein, fat and fiber in animal diets (Yunus *et al*., 2013). The presence of Aflatoxin may cause reduced feed consumption, feed conversion ratio, digestibility and increased risk of infection (Marquardt, 1996).

Haematological and serum biochemical indices of poultry can be elevated due to aflatoxicosis. Raju and Devegowda, 2000 reported the changes in haematology which include decrease in haematocrite, haemoglobin, red blood cell (RBC) counts and lymphocyte percentage and increased white blood cell count and heterophil percentage. The most important economic effect of poultry ingesting Aflatoxin at higher dosage is the increase of the mortality index (Jain, 2004). Lower levels of aflatoxin in diets show no clinical signs and abberations but are usually related with decreased in productivity such as weight gain, efficiency of the feed untilisation and suppressed immunity which eventually increased the risk of infection (Tedesco *et al.*, 2004; Shi *et al*., 2006; Bailey *et al*., 2006). Aflatoxicosis may be manifested in form of reduced biological activities such as feed intake, growth rates and feed efficiency and utilisation. Aside, toxicological effects on the animals, the carry-over through animal tissues, such as egg, meat and milk into the food chains are an important aspect of aflatoxicosis.

International standards had warranted attempt to reduce and remove aflatoxin in feed and feed sources. Attempted methods used are categorized as physical, chemical, phytochemical and biological. The use of herbs had been attempted for treatments of diseases with degrees of successes for bacterial and some fungi infections. Root from plants such as Garlic (*Alliumsativum*), Tumeric (*Curcumalonga*), and Ginger (*Zingiber officinale*) presents a potential alternative to attenuate Aflatoxin. Many plant roots have beneficial multifunctional aspects which are derived from their specific bio-active compounds. It has been used for decades to treat illness and improve health status and still accounts for over 75% of medical treatments of any kind in the developing countries (Fransworth *et al*., 1985). Herbs contain some complicated mixture of organic chemicals that may vary from species of plants to another, depending on many factors related to their establishment. There are documentations about their potentials as

antibiotics, antimicrobials, anti-inflammatory, antifungal and immunoreactive properties (Alade and Irobi, 1993; Brantner and Grein, 1994). The main objective of this study is to attempt some of the available herbs and atoxigenic *Aflatoxin flavus* to either dissociate, destroy or deactivate aflatoxicosis in aflatoxin induced broilers.

1.1 STATEMENT OF THE PROBLEM

According to the FAO (2004), about 30% of the world grains and cereals are poioned with different types of mycotoxins especially aflatoxin and as a result of dependence on grain cereals and legumes, poultry and pigs are the most susceptible livestock species to aflatoxin poisoning. There are dearth of literature on cheap and efffective methods of minimizing aflatoxin poisoning in poultry.These current studies were designed to explore the possibility of preventing and decontamination of Aflatoxin in poultry diet.

1.2 MAIN OBJECTIVE

The main objective of this study was to determine the interactions of temperature and moisture in the production of aflatoxins. Also this study sets out to investigate the effectiveness of different additives on aflatoxin contaminated diets on the overall productive performance of broiler chickens.

1.3 SPECIFIC OBJECTIVES

1) To determine the effects of moisture and temperature on the growth of toxigenic *Aspergillus prasiticus* and quantificationof Aflatoxins.

2) To determine the *in-vitro* binding ability of selected commercial enzyme and herbal products rich in antioxidants to attenuate the growth of toxigenic *Aspergillus prasiticus*.

3) To determine the effects of the additives screened in 2 above on performance, organs function, hematology and biochemical parameters of broilers fed Aflatoxin contaminated diet.

4) To determine the effects of non-toxigenic *Aspergillus flavus* on performance, organs function, haematology and biochemical parameters of broilers fed Aflatoxin contaminated diet.

5) To determine the effects of the additives screened in 2 above on performance, organs, serum haematology and biochemistry of broilers fed diets with High Moisture Maize (HMM), Medium Moisture Maize (MMM) and Low Moisture Maize (LMM).

CHAPTER TWO

LITERATURE REVIEW

2.1Discovery of Aflatoxin

Turkey "X" disease also called Moldy feed toxicosis was identified in 1960 to be caused by *Aspergillus spp of flavus and parasiticus* which was spread in the feed during the research.

Moldy feed toxicosis was identified in the 1950s as a livestock problem. Preceding the death of the organisms are early symptoms such as lethargy and muscular weakness. In 1962, the name Aflatoxin was given to bisfuranocoumarin metabolite group a toxic substance that was isolated from strains of the fungus *Aspergillus flavus* (Sargeant *et al.*, 1963).The spread and growth of toxigenic molds and the secondrary metabolites respectively occurs after harvest and storage at optimum moisture and temperature conditions. It is well-known that grains and cereals are susceptible to fungal colonization and hence, mycotoxin deposist especially afaltoxin production, although aflatoxin was earlier detected in the meal of peanut.

The cultivation region, moisture and temperature and the conditions under which crops are cultivated, harvested and stored are factors that influences the occurrence of Aflatoxin in

agricultural products. Aflatoxin is hepatoxic in man and animals could death. In the natural food poisoned by aflatoxin, B1 is most the most common, poisonous and most carcinogenic. Aflatoxins absorb ultra violet light and as a result fluoresce. That all Aflatoxins absorb ultra violet (UV) light in the range of 362nm to 363nm is a feature that is used as warm-up identification for aflatoxin. The main Aflatoxins members are B1, B2, G1 and G2. B1 and B2, Gl and G2 gives blue, green fluoresce respectively. Aflatoxin B1 and B2 in dairy cattle species are incompletely transformed to M1 and M2 in diary animal respectively which are the hydroxylated derivates. Aflatoxin P1 is a urinary metabolite of Bl in Monkeys.

2.1.2 Aflatoxin Presence in Feed and Food

Aflatoxin can be found in soil, animal's tissues, nuts, cereals and grains and edible oil (Fouzia Begum & Samajpati, 2000). Aflatoxins can occur in crops before harvest on the field or after harvest. Mold invasion of some stored crops also occurs if rodents or insect infests crops. Animal tissues are sometimes contaminated due to carryover from animal consumption of aflatoxin.

2.1.3 Kinds of Aflatoxins

World Health Organisation (WHO) in 1979 confirmed 17 isolated aflatoxins, with only 4 well studied and documented. Their intensely fluorescent in the UV light, aflatoxin are name and designated by letters B1, B2, G1 and G2 representing their blue and green fluorescence in UV light respectively. Two other similar aflatoxins are M1 and M2 which are found in milk. Toxigenic *Aspergillus flavus* isolates effectuate B1 and B2 while toxigenic *Aspergillus parasiticus* isolates effectuate B1, B2, G1 and G2 (Davis and Diener, 1983). Aflatoxicol, B2a,

aflatoxicol H1 and aflatoxins P1 and Q1 are other secondrary metabolites identified (FDA, 1979). Aflatoxins B1, B2, G1 and G2 are commonly together in the same food samples, where Aflatoxin B1 predominates. AFB2, AFG1 and AFG2 will usually not occur in the absence of AFB1 (Weidenborner, 2001).

2.1.4 The Conditions that enhance Biosynthesis of Aflatoxin Production.

Some factors have been found to influence the production of Aflatoxins. The factors can be physical, chemical or biological. Moisture and temperature are the physical factors. The other factors include air composition and substrate nature while the life factors are those factors that are company with the host (Hesseltine, 1983). Nutrients such as minerals e.g. zinc, fatty and amino acids, vitamins and energy source preferably starch are needed for aflatoxins synthesis (Wyatt, 1991). Aflatoxins concentrations are much in highly starched crops such as rice, maize and wheat and oil seeds such as soyabean and groundnut (Diener and Davis, 1968). Aflatoxins production by *Aspergillus parasiticus* were optimum at the temperature range of 25°C and 32°C and is limited at the temperature range of 12°C to 41°C (Lillehoj, 1983). Aflatoxins formation are increased in feeds at temperatures above 27°C and at least 62% humidity level, then moisture levels above 14% in the feeds and grains (Royes and Yanong, 2002).

2.1.5 Factors responsible for Aflatoxin Occurrence

Crops that are cultivated in sub-tropical and tropical countries are more tending to aflatoxin contamination due to moist and warm weather than those in temperate countries. Groundnuts and groundnut by-products have a very high risk of aflatoxin contamination. They are important substrates for the fungi to grow and aflatoxin contamination is the result of

synergies among the fungus, the host and the host's environment. The infestation and colonization of the substrate and the type and amount of aflatoxin that is produced is determined by the combination of these factors in a specific proportion. Mold infestation and toxin production can also be enhanced by water stress, high-temperature stress, and growth stage of specific crops, poor fertility, high crop densities, weed competition and damage of the host plant by insect.

Temperature and moisture are the most important of these factors. WHO 1979, established that moisture content of 18% and 9-10% for starchy cereals and oil-rich nuts respectively is optimum for maximum production of the toxin. It was also established that the minimum temperature for Aflatoxin production is 12°C - 27°C and maximum temperature is 40°C -42°C. Frequent contamination of crops with high levels of Aflatoxins has resulted into significant economic losses for farmers and has been detrimental to the health of humans and animals that consumes such contaminated crops all over the world.

2.1.6 Aflatoxin; Description of Structure

Aflatoxins B1, B2, G1 and G2 were chemically and physically described in 1963 by Asao *et al*; Van Dorp *et al* and Van der Zijden. Chemically, aflatoxins are difurocoumarolactones (difurocoumarin derivatives). The structural component is a bifuran ring attached to a coumarin nucleus with a pentenone ring. In the case of G Aflatoxins, there are a six membered lactone ring. It was reported by Devero (1999) that Aflatoxins B1, B2, G1 and G2 are separated by the color of their fluorescence under long wave ultraviolet light (B=blue, G= green). M1 and M2

Aflatoxins were extracted from urine and milk and discovered as mammalian metabolites of AFB1 and AFB2 (Patterson *et al*., 1978).

2.1.7 Aflatoxin; Physical and Chemical Properties

Aflatoxins B1, B2, G1 and G2 were originally isolated and extracted by groups of researchers in England. (Nesbitt *et al*., 1962, Sargeant *et al*., 1961). The molecular formula of aflatoxin B1 was established as $C_{17}H_{12}0_6$ and aflatoxin G1 as $C_{17}H_{12}0_7$; the Aflatoxins that are dihydro derivatives of the parent compounds were B2 and G2, $C_{17}H_{14}0_6$ and $C_{17}H_{14}0_7$ (Hartley *et al*., 1963). Some physical properties of the compounds are summarized in following Table 2.1

Table 2.1. Physical properties of Aflatoxin

Aflatoxin	Molecular Formula	Molecular Weight g	Melting Point ^{0}C	[α]D23
B1	$C_{16}H_{12}O_6$	312	268-269*	-559
B2	$C_{17}H_{14}O_6$	314	286-289*	-492
G1	$C_{17}H_{12}O_7$	328	244-246*	-533
G2	$C_{17}H_{14}O_7$	330	237-240*	-473

The chemical reactivity and behavior of the aflatoxins has received relatively little systematic study beyond work associated with structure elucidation. However, it has been shown that catalytic hydrogenation of aflatoxin B1 to completion results in the absorption of 3 molecules of hydrogen accompanied by the production of tetrahydrodeoxy derivative (Asao *et al*., 1963). If this procedure is interrupted after the absorption of 1 molecule of hydrogen, it results in the production of aflatoxin B2 in appreciable quantity. Under the influence of a strong acid as catalyst, Aflatoxin B1 will undergo addition reaction with hydroxyl group (Andrellos *et al*., 1964). Chromatographic property is altered when treated with formic acidthionyl chloride,

acetic acid thionyl chloride or trifluroacetic, while the fluorescence property is relatively unchanged. Levulinic, succinic, malonic and glutaric acids are Aflatoxin fragments gotten through the process of ozonolysis (Van Drop *et al*., 1963). Aflatoxin compound has been found to be unstable to alkaline hydrolysis due to the presence of lactone ring, incomplete recycling of the product of hydrolysis occurs after acidification. (De Iongh *et al*., 1962).

Based on the systematic studies on Aflatoxin stability, Aflatoxin seems to be a partially unstable compound, and this instability depends on some conditions. For example, Aflatoxin decomposes incompletely upon standing in solution of methanolic acid, and this is enhanced by the presence of light or increased temperature. Also, appreciable level of degradation of Aflatoxin occurs on chromatograms in the presence of air and ultraviolet (Wogan *et al*., 1966).

2.2 Aflatoxin; Biological effects

The discovery of Aflatoxins as feed contaminant is the motive behind the researches that has been carried out to know the biological effect of the contamination both on animals and humans health. The obvious signs of Aflatoxin toxicity are usually in different forms and depend on the test system, dosage and exposure period. In the case of instant application of large dosage of Aflatoxin, animal cells suffer damage and the whole animal is in turn affected, and when small dosages are gradually applied, it affects the histological make-up of the animals. Serious exposure for prolonged time causes tumor induction in many species of animal (Wogan *et al*., 1966).

2.2.1 Diseases in Animals induced by Aflatoxin

The disease caused by aflatoxin is known as Aflatoxicosis, it mainly has effect on animal liver which leads to injury of the liver. It also altered metabolism, decreased egg production and breaks down the immunity which makes the animal susceptible to infections and diseases. Also, prolonged ingestion of diet containing low fractions of aflatoxin can result in embryonic complications. Young animals are more sensitive to aflatoxin when compared to older animals. The clinical expressions of aflatoxin are digestive and metabolism disarray, reduced prolificacy and performance of reproductive organs, reduced feed efficiency that affects weight gain and anaemia. Aflatoxins not only cause reduction in milk production, but also the transformation of the milk containing aflatoxin M1 and M2.

2.2.2 Aflatoxin B1; Dynamics and Metabolism

The complete ingestion and absorbtion from the gastrointestinal tract is required since dose is needed in small concentration and to cause toxicity. The highest concentration of the aflatoxin is deposisted in the liver after ingestion and absorption (Mintzlaff *et al*., 1974). After AFB1 gets to the liver, it is metabolized and converted; the enzyme involved is microsomal and it produces different metabolites series of reaction such as hydration, hydroxylation, epoxidation and demethylation. Hydroxylation of AFB1 at C_{22} or C_2 effectuates AFQ1 and AFM1 respectively. Hydration of the $C_2 – C_3$ double leads to formation of AFB2 which is common in some species of poultry (Patterson and Roberts, 1970). Aflatoxicol is a metabolite of AFB1 formed by a soluble cytoplasmic reductase enzyme.

The impact of the toxicity of Aflatoxin B1 is felt on the liver being the target organ and this largely affect metabolism and digestion of lipids, fibers and proteins. In day-old chicks, AFB1 obstructs the activity of liver UDP glucose-glycogen transglucosylase bringing about depletion of hepatic glycogen stores (Shankaran *et al*., 1970).

Due to its toxicity on liver microsomal enzymes, the microsomal glucose-6-phosphatase is reduced by Aflatoxin B1 (Shankaran *et al*., 1970) while the activity of inducers to induce the activity of microsomal enzyme is unaffected by Aflatoxin B1 (Kato *et al*., 1970). Lipid also accumulates in chickens and ducklings liver that have been poisoned by aflatoxin (Carnaghan *et al*., 1966; Shank and Wogan, 1966). In rat *in vitro* assey, poisoning with the AFB1 often stimulates its own metabolism (Schabort and Steyn, 1969). Since aflatoxin inhibits protein synthesis, Aflatoxin increases the chance of poultry to infection with *Pasteurella multocida, Salmonella spp, Coccidia,* Marek disease virus and *Candida albicans* (Smith *et al*., 1969; Hamilton and Harris, 1971). Aflatoxin also causes anticoagulation of blood, this is probably because AFB1 obstructs synthesis of factors II and VII involved in prothrombin secretion and clotting mechanism (Bababunmi and Bassir, 1969).

2.2.3 Human Diseases induced by Aflatoxin

Aflatoxicosis in humans was mainly due to ingesting aflatoxin poisoned grains and cereals; the consumption of these grain was positively correlated to the incidence of cancer in the third world. Also, research done in Asian and African intitutions showed a straight line positive correlation between aflatoxin in feeds and liver cells cancer (L.C.C.). Prolonged consumption of aflatoxin poisoned diet even in low concentration was implicated in stomach, colon and liver

cancer and many other diseases. International Agency for Research on Cancer categorised AFB1 as a human carcinogen (IARC, 1988).

2.2.4 Aflatoxicosis in Animals

Aflatoxicosisis is caused by the ingestion of substances, feed or grains poisoned with aflatoxin. Aflatoxin can cause from acute, subacute and chronic toxicity, peracute signs (Smith, 2002). Animal are prone to aflatoxicosis, the effect is more mostly pronounced in pigs, poultry, cattle and sheep (Radostits *et al*., 2000). Cattle (beef and diary) are more proned to aflatoxicosis than sheep and horses. As a general rule, animals that are neonate, young and tender are more proned to aflatoxicosis than animals that are matured while pregnant (Cassel *et al*., 1988). Nursing animals are affected by exposure to aflatoxin metabolites secreted in the milk (Jones *et al*., 1994). There are differences in species with regards to how susceptible they are to aflatoxins, but in general most animals and humans are affected in the same manner.

2.2.5 Aflatoxin Toxicity

Animals which ingest sub-lethal doses of aflatoxin for a prolonged period a subacute toxicity syndrome (STS), it is usually results in moderately severe liver damage. There is also a reduction in growth rate and egg or milk production, suppression of the immune system low dosages of aflatoxins in the feed. The reactivity of aflatoxins with T-cells, decrease in Vitamin K activities and a decrease in phagocytic activity in macrophages causes Immunosuppression.

2.2.7Aflatoxins; Cellular Effects

Aflatoxins have a high affinity for nucleic acids and polynucleotides and therefore inhibit the synthesis of nucleic acid. They attach to guanine residues and form nucleic acid adducts. Aflatoxins also have been shown to decrease protein synthesis, lipid metabolism, and mitochondrial respiration. They also cause an accumulation of lipids in the liver, causing a fatty liver. This is as a result of impaired transport of lipids out of the liver after they are synthesized. This leads to high fecal fat content.

Carcinogenisis has been observed in rats, ducks, mice ,trout, and subhuman primates of which is rarely seen in poultry or ruminants. Trout are the most susceptible. In fact, 1ppb of aflatoxin B1 will cause liver cancer in trout. Carcinogenisis occurs due to the formation of 8, 9-epoxide, which binds to DNA and alters gene expression. There is a correlation with the presence of aflatoxins and increased liver cancer in individuals that are hepatitis B carriers. Animals of different species vary in their susceptibility to acute aflatoxin poisoning with LD50 values ranging from 0.3 to 17.9 mg/kg. In fact duckling liver metabolized Aflatoxin very rapidly in vitro (Patterson and Allcroft, 1970), although the species is sufficiently susceptible for day old birds to be used in a sensitive bioassay extensively (Patterson, 1973). It has been shown through studies that rabbit, duckling and guinea-pig make up a "fast metabolizing group" that is obviously capable of handling in less than 12 minutes an LD50 dosage. An intermediategroup consist of chick, mouse, pig and sheep, they carry out metabolism of an LD50 dosage in just few hours. The rat is the only example of a "slow metabolizing group" as at now, in which LD50 dose would likely disappear from the liver after days (Patterson, 1973).

Factors that affect the level of Aflatoxin toxicity residue in animals are; animals and poultry species,animals and poultry breeds, intensity and period of exposure, feeding and health of animals, age, sex and diseases, drugs and other mycotoxins. (FDA, 1979).

2.2.8 Aflatoxin; Toxicity in Ruminants

Aflatoxin level in the ration determines the effect of Aflatoxin on cattle. The duration of the feed ingestion and the age of animal are factors that also determine the effect of Aflatoxin on cattle (Jones *et al*., 1994). Once Aflatoxin is ingested in the feed by cattle, it is physically bound to ruminal contents and very little amount such as 2-5% reaches the intestine. AFB1 level which is in excess of about 100 μg/kg of feed is poisonous for cattle (Radostits *et al*., 2000).

2.2.9 Aflatoxin; Toxicity in Poultry

Aflatoxicosis has caused severe economic losses in the industry affecting broilers, layers, turkeys, ducklings, broilers and quails (CAST, 1989). The susceptibility of birds to aflatoxins varies among breeds, genetic lines and species. Comparative studies in poultry have shown that turkey and ducklings are the most sensitive to aflatoxin poisoning. Other poultry species are less sensitive when compared to turkey and ducklings while chickens are the most resistant (Leeson *et al*., 1995). The susceptibility ranges from ducklings, turkey poults, goslings, pheasant chicks, chickens (Muller *et al*., 1970). Ducklings are 5 to 15 times more sensitive to the effects of aflatoxins when compared to laying hens, while certain strain of hens may be 3 times more sensitive than other strainof hens (Jones *et al*., 1994; Calnek et al., 1997).

The direct and indirect impart of aflatoxicosis include high mortality index from heat stress, impaired and decreased performance in broilers, reduction in egg production in laying birds, anemia, hemorrhages and liver condemnations, paralysis and lameness, high mortality index in ducks, impaired ambulation and paralysis in quail, impaired immunization in turkeys and increased susceptibility to infectious diseases (Wilson *et al*., 1975; Lamont, 1979; Bryden *et al.*, 1980; Jones *et al.*, 1982; Dafalla *et al*., 1987a; Okoye *et al*., 1988;; Hegazy *et al*., 1991 and Calnek *et al.*, 1997)

2.2.9.1Aflatoxicosis in Ducks

Inappetence, reduced growth, abnormal vocalizations, feather picking, purple discolorations of legs and feet and lameness, ataxia, convulsion are forms of the lethal effect of Aflatoxicosis and ultimately death which is usually preceded by opisthotonus. Enlargement and paleness of liver and kidneys are symptoms osnecropsy. With chronicity, ascitis and hydropericardium developed accompanied by shrunken firm nodular liver, distention of the gall bladder and hemorrhages, bile duct hyperplasia and bile duct carcinoma in aflatoxicated Campbell ducks, distended abdomen due to liver tumors and secondary ascitis, microscopic lesions in the liver were fatty change in hepatocytes, proliferation of bile ductules and extensive fibrosis are accompanied by vascular and degenerative lesions in pancreas and kidney. (Asplin and Carnagham, 1961; Hetzel *et al*., 1984 and Calnek *et al*., 1997).

2.2.9.2 Aflatoxicosis in Chickens

Acute toxicity of aflatoxins in chickens may be characterized by hemorrhage in many tissues and liver necrosis with icterus. Although number of field cases of aflatoxicosis in

chickens has been diagnosed in various countries, the most severe spontaneous outbreak occurred in North Carolina, in which 50% of a flock of laying hens died within 48 hr of being fed highly toxic maize containing 100 ppm aflatoxin (Hamilton, 1971). The necropsy revealed that liver damage was the most important lesion showing paleness, occasional white pinhead-sized foci and petechial hemorrhages while gallbladder and bile ducts were distended. Levels of aflatoxin B1 in moldy feed normally vary from 0 to 10 ppm. At low levels of feed contamination, exposed chickens show, in general, weakness, failure to gain weight with concomitant decline in feed efficiency and egg production (Smith and Hamilton, 1970; Doerr *et al*.,1983). Hepatic damage is manifested by enlarged and putty-colored liver, petechial hemorrhages, marked vaccuolation of hepatic cells and bile duct proliferation. Feed levels of AFB1 as low as 250-500 ppb given to New Hampshire chickens have been reported to result in liver damage, decreased hemoglobin, and hypoproteinemia (Brown and Abrams, 1965).

Trials with naturally contaminated feed containing aflatoxin levels ranging from 1 to 1.5 g/tonne have caused growth retardation in chickens. Mortality was low but marked hepatic damage was manifested by enlarged and hemorrhagic liver, relatively, high dietary levels of aflatoxin B1 (0-10 g/tonne) given to Rock type broiler chickens have been reported to cause substantial decrease in weight gain, feed efficiency and hepatic microsomal drug metabolizing enzymes with concomitant increase in serum glutamic oxalacetic transaminase activity reflecting liver damage (Carnaghan *et al*., 1966; Dalvi and McGowan, 1984; Dalvi and Ademoyero, 1984).

Metabolic alterations caused by aflatoxins in chickens result in elevation of lipid levels, hepatic protein synthesis disruptions, different cases of blood coagulation, immunosuppression

and decrease in concentration of plasma amino acid ((Tung *et al*., 1972; Donaldson *et al*., 1972; Tung *et al*., 1975; Doerr *et al*., 1976; Voight *et al.*, 1980; Bababunmi and Bassir, 1982).

2.2.9.3 Aflatoxicosis in Turkeys

During the outbreak of Turkey“x" disease, the initial clinical signs wereanorexia, weight loss, depression, ataxia and recumbency in the order of precedence. Birds that have been affected eventually die within the period of one to two weeks. The manner of death is opisthotonos and is characterized by arched neck, head down back with their legs stretched backwards (Hamilton *et al*., 1972). Siller and Ostler, 1961; Wannop, 1961; Giambrone *et al*., (1985) ; Richard *et al*., (1987) also reported that the signs of Turkey “X” disease include decreased feed conversion, weight loss, reduced activities, unsteady gait, recumbency, anemia and consequently death. Though the body condition usually remains good, yet there was generalized congestion and edema at necropsy. Congestion occurs in the liver and kidney. The liver and kidney are also enlarged and rigid, the gall bladder was full, and the duodenum was swollen with catarrhal content (Siller and Ostler, 1961; Wannop, 1961; Calnek *et al*., 1997).

2.2.9.4 Aflatoxicosis in Broilers

Experimental trial and natural outbreak of aflatoxicosis in broilers, the following signs are frequently observed; decreased intake of feed and water, loss of weight, stunting, dullness, feathers becomes ruffled, unhealthy appearance and paleness, trembling, ataxia, lameness, legs and wings becomes paralyzed, gasping, prostration and death (Asuzu and Shetty, 1986; Okoye *et al*., 1988; Rao and Joshi, 1993; Leeson *et al*., 1995).

The liver shows distinguishing gross lesions that were expanded, pale yellow to grayish brown in colour and had a conspicuous reticular pattern. Some livers showpetecheal hemorrhages on the surface. Gall bladders became expanded and bile duct swollen, blood in the intestinal lumen. Enlargement of spleen, liver, and kidney, whereas the bursa of fabricius and thymus were decreased. Fading of colour occur in the liver turning dark red or yellow to congestion or fat accumulation respectively caused by lower dosesage of aflatoxin. In the chronic stage of Aflatoxicosis, livers shrink, firm and nodular and gall bladder was swollen. The kidney of the affected birds increases in size as it is congested. The spleen becomes enlarged and with spots in appearance. The liver histopathology showed congestion of hepatic sinusoids, fecal hemorrhages, centro lobular fatty cytoplasmic vacuolation and necrosis, biliary hyperplasia and nodular lymphoid infiltration. Kidney histopathology revealed that the epithelial cells of many tubules became vacuolated. ((Asplin and Carnaghan, 1961; Archibald *et al*., 1962; Smith and Hamilton, 1970; Tung *et al*., 1973; Tung *et al*., 1975a; Huff and Doerr, 1980; Slowik *et al*., 1985; Azuzu and Shetty, 1986; Dafalla *et al*., 1987b and Okoye *et al*., 1988)

2.2.9.5 Aflatoxicosis in Layinghens

The most obvious manifestations of experimental aflatoxicosis in layers are decreased in egg production and egg weight, enlarged liver and liver fat. High mortality and drastic reduction of egg production were reported to occur during a natural outbreak (Hamilton, 1971). Egg size, egg weight and yolk as percent of total egg size were decreased (Huff *et al*., 1975). In Japanese quail, reduction in feed efficiency, egg production, egg weight, hatchability and exterior and interior egg quality were detected (Sawhney *et al*., 1973a & b). Dhanasekaran *et al*., (2009) reported that histopathological analysis of aflatoxin ingested hens reveals that lesions were

observed in tissues of liver, kidney, intestine. (Nesheim and Lvy, 1971; Hamilton and Garlich, 1973; Leeson *et al.,* 1995)

Immunosuppression can be induced by Aflatoxin and it also makes toxicated birds to be moreprone to bacterial, viral and parasitic infections. AFB1 has been shown to cause immunosuppression in chickens, turkeys and laboratory animals (Sharma, 1993). The concentrations of immunoglobulins IgM, IgG and IgA in birds can also be reduced by Aflatoxin (Giambrone *et al.*, 1978a & b). When AFB1 is present in small quantity in animal feed, it reduces vaccinal immunity and as a result can lead to the occurrence of Turkey "X" disease even in well vaccinated flocks (Leeson *et al.,* 1995). For chickens that were fed with AFB1 and were vaccinated against Marek's disease, it was discovered that such chickens showed a relatively higher frequency of gross and microscopical lesions of Marek's disease than those fed Aflatoxin-free feed (Batra *et al*., 1991). Aflatoxicosis in chichens show decreased in antibody production after injection of sheep red blood cells (Thaxton *et al*., 1974).

It was observed that chickens poisoned with aflatoxin diets are more prone to Marek's disease, infectious bursal disease virus, congenitally acquired salmonellosis and duodenal and cecal coccidiosis than chickens receiving aflatoxin free feed (Edds *et al*., 1973; Wyatt and Hamilton, 1975; Giambrone *et al*., 1978a & b; Edds and Bortell, 1983). It is established that aflatoxin affects the normal functioning of B and T lymphocytes rather than causing destruction of these cells (Wyatt, 1991). The impairment of protein synthesis caused by dietary aflatoxin could account for the lack of humoral immunity without the necessity of B and T cell destruction (Wyatt, 1991). Regardless the atrophy of the bursa of fabricius and thymus gland, the apparent

alteration of splenic function is also of diagnostic significance and implies alteration in the immunocompetence of birds with aflatoxicosis (Richard *et al.*, 1975).

Aflatoxicosis also affects chicken external and common clotting pathways. It also causes changes in biochemical properties in thromboplastin clotting factors V, VII and X and causes reduction in plasma prothrombin and fibrinogen (Doerr *et al*., 1976). It as a result causes the whole blood to clot (Doerr *et al*., 1974). The elevated prothrombin time was considered to be the result of impaired hepatic synthesis of lotting factors caused by Aflatoxicosis on the liver cells (Huff et al., 1983). The activity of some digestive enzymes can be altered by Aflatoxin exposure. Aflatoxin exposure can also affect the absorption of carotenoid compounds from the gastrointestinal tract, and the metabolism of lipids (Leeson *et al*., 1995). Aflatoxin contaminated diets causes a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia and reduction in the concentrations of bile salts and pancreatic lipase, trypsin and amylase (Osbrone *et al*., 1982).Aflatoxin in some experiments, increased the activities of pancreatic chymotrypsin, amylase and lipase, but excludes trypsin (Richardson and Hamilton, 1987).

2.3. Aflatoxin; Biocontrol Strategy Development.

There were early studies that showed that aflatoxin production by *Aspergillus flavus* and *parasiticus* are reduced when cultivated with some particular fungi and bacteria. The fungi and bacteria affects the ability of the fungi to produce secondary metabolites by competitive exclusion (Ashworth *et al*., 1965; Weckbach and Marth, 1977; Wicklow *et al*., 1980, 1988; Horn and Wicklow, 1983; Ehrlich *et al*., 1985). In 1975, there was an outbreak of *Aspergillus flavus* in a maize field in Iowa, the outbreak led to reduction in the level of aflatoxin contamination

after harvest of the maize (Ehrlich *et al*., 1985). The synergy of *Aspergillus flavus* with *Aspergillus niger* caused a sharp decreased in the aflatoxin synthesis which was beyond a simple exclusion of one fungus by the other (Wicklow *et al*., 1980). The synergy of *Aspergillus flavus* with *Penicilin oxalicum* similar effect but, in this case, not only were aflatoxin amounts decreased to a level not by simple competitive exclusion, but the metabolite of *Penicilin oxalicum*, secalonic acid, were decreased (Ehrlich *et al*., 1985). It was speculated that the synergy of the two organisms caused a mopped up of the ATP needed for the oxidation steps in secondary metabolite production. Inoculation with the *Penicilin oxalicum* attenuated *Aspergillus flavus* synthesis of aflatoxins even when the growths were similar. It was also observed that *Aspergillus flavus* mutants which are being decreased in their abilities to produce aflatoxins also possessed similar competiting ability to that of *Penicilin oxalicum* (Ehrlich, 1987). Sweany *et al*., 2011 affirmed the concept that competition with *Aspergillus flavus* isolates incapable of aflatoxin production could attenuate aflatoxin contamination. This mode of biocontrol is currently the most widely used biocontrol method for decreasing aflatoxin contamination of grains (Wu and Khlangwiset, 2010a).

Cotty *et al.,* 1990s discovered that one atoxigenic *Aspergillus flavus* isolate Aflatoxin 36 (AF36) was isolated and purified from Arizona field of cottonseed, AF36 is a good atoxigenic agent which competes for reduction of cottonseed aflatoxin content (Cotty and Bhatnagar, 1994). Chang *et al*., (2012) also reported that this atoxigenic isolates were specialially good competitors against aflatoxin-producing isolates. It was later discovered that this isolate does not have the ability to produce aflatoxin due to a point mutation in the polyketide synthase gene that is necessary for the biosynthesis of aflatoxin (Ehrlich and Cotty, 2004).

2.3.1 *Aspergillus*; Diversity of Species and Lifecycle of *Aspergillus flavus*

The most common fungi species that is associated with aflatoxin poisoning of grains and cereals are *Aspergillus flavus* and *Aspergillus parasiticus* (Cotty *et al*., 1994; Cotty, 1997). *Aspergillus flavus* and *Aspergillus parasiticus* are distributed widely in temperate and tropical zones. *Aspergillus flavus* and *Aspergillus parasiticus* can be found in the soil and on plants especially, maize, cotton, and groundnuts (Samuel *et al.*, 2013) but it is rarely found in rice (Chen *et al.,* 2013). *Aspergillus flavus* and *Aspergillus parasiticus* populations are highly diverse. *Aspergillus flavus* is more invasive and it knocks out on competition *Aspergillus parasiticus* when both species are together in the soil. *Aspergillus nomius* is another Aflatoxin producing fungi that is rarely found in the soil, and it is also rarely associated with contamination of agricultural produce (Cotty *et al.,* 1994; Bhatnagar *et al*., 2001; Cardwell and Cotty, 2002).

Aspergillus flavus is a widely collection of strains and it include toxigenic and atoxigenic strains, sclerotial type variants, strains which varies in it response to light, strains that are dwelling in vegetative groups that possesses multiple compatibility (VCGs), and strains that are varied in their ability to colonize living tissues of plant. *Aspergillus flavus* lives in the soil as a saprophytic fungus but it readily colonizes most environments when there is opportunity of a rich source of carbon and nitrogen. *Aspergillus flavus* diversity is hence seen to be an evolutionary response to its distribution cosmopolitan.

Aspergillus flavus has a major mode of replication which is by asexual sporulation, this occurs only under certain conditions, *Aspergillus flavus* develops sclerotia, hardened masses of desiccated and melanized mycelia that can survive harsh environments. *Aspergillus flavus* in the

soil contains isolates from two morphologically different sclerotial variants, they are called the L-strain or *Aspergillus flavus* Group IB (Geiser *et al*., 2000), this is for isolates with average sclerotial size greater than 400 μm and the S-strain (Group IA) for isolates with sclerotial size less than 400 μm (Cotty, 1997). Both S-and L-strains of *Aspergillus flavus* can be found worldwide in regions where maize is grown. The sclerotial morphological difference between the S-and L-strain *Aspergillus flavus.* Soil populations in the soil are typically composed of isolates from numerous different VCGs. 30% of the *Aspergillus flavus* soil isolates in Arizona were identified on the average to belong to the S-strain (Cotty, 1997; Orum *et al*., 1997). S-strain isolates constantly produced more aflatoxin when compared to L-strain isolates yet the secretion of aflatoxin in this strain is not so much affected by nitrogen source.

2.4. Herbal Control

2.4.1 Turmeric

Medicinal plants are used as raw material for preparation of drugs used in combating diseases, from the start of civilization. It is shown in this study that *Curcuma longa* L. with the common name turmeric belonging to the Zingiberaceae family. Tumeric is regarded as a universal panacea in the herbal medicine with a diverse range of pharmacological activities. *Curcuma longa* is distributed in subtropical and tropical countries. *Curcuma longa* L is particularlly cultivated in China and India as well as other Asian countries. Turmeric can grow up to 1 m high with reduced stem. It is an important spice that has gained global acceptance especially among the people of the east (Ravindran *et al*., 2007). Turmeric and has been continuously used because of its flavoring, and medicinal properties (Govindarajan, 1980). Traditional medicine nowadays claims that it has power against diseases such as gastrointestinal

diseases, particularly biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza and cough (Ammon *et al*., 1992). Turmeric coloring principle is termed cucumin which gives turmeric it characteristic yellow color and is also the essential component of the plant (Ammon *et al*., 1992). Turmeric has been approved under different studies to be an anticancer, anti-diabetic, antioxidant, hypolipidemic, antiinflammatory, antimicrobial, anti-fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant, etc. The plant has also shown to possess anti HIV activity to fight against AIDS. Turmeric is hence considered as a spice as well as a medicinal plant with multiple functions.

2.4.1.1 Turmeric; plant components

The constituents of turmeric are 69.4% carbohydrates, 6.3% protein, 5.1% fat, 3.5% minerals, and 13.1%. moisture. The essential oil (5.8%) which is gotten by steam distillation has 53% Sesquiterpenes, 25% zingiberene, 1% *a*-phellandrene, 0.6% sabinene, 1% cineol, and 0.5% borneol. About 3-4% of Curcumin is found to be responsible for the yellow coloration, and consists of 94% curcumin I, 6% curcumin II and 0.3% curcumin III (Ammon and Wahl.,1991). Isolation of demethoxy and bisdemethoxy derivatives of curcumin have also been done from tumeric. The melting point of cucumin falls between 176–177 °C; at this point it forms a reddish- brown salt with alkali which is soluble in organic solvent such as acetic acid, ethanol, alkali, ketone and chloroform (Chattopadhyay *et al*., 2004). It has been reported that that the rhizome contains tumerone a, tumerone b, curzerenone, curdione, mono- and di-demethoxycurcumin. Gas Liquid Chromatography was used to analyze the essential oil present in the leaves of *C.longa* and was said to contain linalool, caryophyllene, geraniol, α -pinene, β-pinene, sabinene, myrcene, α-phellandrene, 1,8-cineole, *p*-cymene, C8-aldehyde, and methyl

heptanone (Bernard *et al*., 1982). A novel sesquiterpene, (6S)-2-methyl-6-(4- hydroxyphenyl-3-methyl)-2-hepten-4-one, two new bisabolane sesquiterpenes, (6S)-2-methyl-6- (4-hydroxyphenyl)-2-hepten-4-one, (6S)-2- methyl-6- (4-formylphenyl)-2-hepten- 4-one, and two calebin derivatives, 4''-(4'''-hydroxyphenyl-3'''-methoxy)- 2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate and 4''-(4'''-hydroxyphenyl)- 2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl- 3'-methoxy)-propenoate were isolated along with five known bisabolane sesquiterpenes from turmeric (Khajehdehi *et al*., 2012).

2.4.1.2 Turmeric; Antioxidant Property

It has been reported that curcumin is a strong remover of oxygen free radicals. Curcumin has antioxidant property which is comparable to that of vitamins C and E (Ammon *et al.*, 1992). It antioxidant property can also protect lipids or hemoglobin from oxidation and can hinder the generation of reactive oxygen species (ROS) such as H2O2, superoxide anions and nitrite radical by activated macrophages. Bisdemethoxycurcumin and demethoxycurcumin which are cucumin derivatives also possess antioxidant properties (Ammon *et al*., 1992). An *in vitro* study measuring the effect of curcumin on an inducible stress protein resulted in enhanced cellular resistance to oxidative damage (Bernard *et al*., 1982).

2.4.1.3 Turmeric; Effects on Hepatoprotective and Renoprotective

Turmeric possesses renoprotective and hepatoprotective properties that has been shown to be similar to that of silymarin. Animal studies have demonstrated renoprotective and hepatoprotective effects of turmeric from a variety of hepatotoxic insults. What is responsible for the hepatoprotective and renoprotective effects is the antioxidant property of turmeric, as well as

its ability to decrease the formation of pro-inflammatory cytokines (Govindarajan, 1980, Ammon *et al*., 1992, Ammon and Wahl.,1991). Curcumin in turmeric have also been found to reverse fatty changes, biliary hyperplasia and necrosis induced by aflatoxin production (Govindarajan, 1980). Sodium curcuminate, a salt of curcumin, also exerts choleretic effects by increasing biliary excretion of bile salts, cholesterol, and bilirubin, as well as increasing bile solubility, this can probably lead to preventing and treating cholelithiasis (Ammon *et al*., 1992).

2.4.1.4 Turmeric; Photo-Protector Activity

This activity is also dependent on the antioxidant property of tumeric. The larger part of skin surface lipids is unsaturated, this accounts for them being prone to attack by free radicals. The sun ultraviolet rays penetrate the skin and increases the hazard caused by these radicals. With exposure to ultraviolet radiations is being prolonged it may result in degradation of the lipids consequently causing deterioration in the skin texture. Laboratory studies showed that turmeric extract was effective in suppressing inflammation and protecting the cells of the epidermis from ultraviolet B radiation damage (Bernard *et al*., 1982). Small doses of turmeric in applying curcumin have been demonstrated to have a protective effect against chromosomal damage as a result of gamma radiation (Bernard *et al*., 1982).

2.4.2 Garlic

Garlic (*Allium sativum L*.) is one of the plants that have been investigated over the years in other to check for it ability to fight infectious diseases (Onyeagba *et al*., 2004). A very recent scheme of classification of garlic was class Liliopsida, subclass Liliidae, superorder Liliianae, order Amaryllidales, family Alliaceae, subfamily Allioideae, tribe Allieae and genus Allium

which was based on the sequences of nuclear ribosomal DNA (Friesen *et al*., 2006). Garlic was used by the early Egyptians to treat diarrhea (Bradley, 1992). It was also used by the Greek physicians Hippocrates and Galen for the treatment of intestinal diseases as well as other diseases. Also garlic was used by ancient Chinese and Japanese to treat headache, sorethroat, flu and fever. In Africa, Nigeria to be precise, it is used for the treatment of abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Jaber and AlMossawi, 2007). It was used in Europe and India, for the treatment of common colds, hay fever and asthma. The casual name given to garlic was Russian penicillin for its widespread use as a topical as well as systemic antimicrobial agent (Timbo *et al*., 2006).

2.4.2.1 Garlic; Active Chemical Constituents

About 33 Sulphur recognized compounds are contained in garlic including enzymes and minerals such as germanium, copper, iron calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, water and fiber. In garlic, 16 amino acids already been discovered, and they are lysine, glutamine, proline, glycine, alanine, cysteine, histidine, arginine, aspartic acid, threonine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005). Amongst other Allium species, garlic has a higher concentration of sulphur; this was found to be responsible for both pungent odor and medicinal effects of garlic. Allicin (diallyldisulphide) is one of the compounds in garlic that is most biologically active. In garlic, the most abundant sulphur compound is alliin (Sallylcysteine sulphoxide), and it is present at 10mg/g in fresh garlic and 30 mg/g in dry garlic. (Lawson, 1998). When garlic preparation such as mincing, chopping and crushing disturbs S-allyl cysteine sulphoxide is exposed to the allinase enzymes, it is converted to diallyl thiosulphinate, which gives a characteristic garlic aroma. The

allinase enzyme responsible for diallyl thiosulphanate conversion is inactivated pH level below 3.5 upon heating (Pedrazza-Chaverri *et al*., 2006). Allicin is considered the main antioxidant and scavenging compound and studies have revealed that other compounds can also play significant roles; such compounds include polar compounds of phenolic and steroidal origin, which offer various pharmacological properties without odor and are also heat stable (Lanzotti, 2006).

2.4.2.2 Garlic; Health Benefit

Garlic possesses the ability to both inhibit and destroy bacteria and fungi, it can lower blood pressure, blood cholesterol and blood sugar, it can also prevent the clotting of blood, and it contains anti-tumor properties. Garlic has the ability to enhance the immune system to fight off potential disease and maintain good state of health (Abdullah *et al*., 1988). It has the ability to induce the lymphatic system to action which is responsible for the removal of waste products from the body. The sulphur containing compounds that are present in garlic provides the body of the animal with protection by inducing the production of certain useful enzymes (Mansell and Reckless, 1991). The important role of garlic in coronary heart disease was experimented on rabbits and discovered that even atherosclerotic deposits and lesions that havepre-existing could actually be reversed if garlic was consistently taken (Bordia, 1981). In India, a study was carried out which revealed that 432 coronary artery patients were randomly grouped into two groups in which half of them consumed garlic juice in milk, while the other group patients did not consume garlic juice milk.

Within the three years of the trial, the observation was that many patients had died in the group not supplied with garlic juice (Yeh *et al*., 2006). Garlic is also reported to remove

oxidants, increase superoxide dismutase, catalase, glutathione peroxidase, glutathione levels, hinders lipid peroxidation and it reduces cholesterol synthesis by hindering 3-hydroxy-3-methylglutaryl-CoA. Garlic has been revealed to reduce platelet aggregation, arterial plaque formation, reduces homocysteine, reduce blood pressure, and increase microcirculation. Garlic can also help to prevent cognitive reduction by providing protection for the neurons from neurotoxicity and apoptosis, hence preventing ischaemia or fusion related neuronal death and by improving learning and memory (Borek, 2006). It reduces blood pressure when it is above normal. An *in vitro* study recently establish that the vasoactive potency of the Sulphur compounds found in garlic by which red blood cells converts organic polysulphides of garlic into hydrogen sulphide, a known cardio-protective vascular cell which acts as a signaling molecule and is endogenous (Benavides *et al*., 2007). Formation of fibrin may be reduced by constituents of garlic, these garlic constituents also help to reduce theexisting fibrin in the blood in a much better way than aspirin (Fukao *et al*., 2007).Adding garlic to the diet will help to enhance the breaking down of fibrin from 24 to 30% in humans (Ernst, 1994). Ajoene is a sulphur compound that can also be found in garlic, it is responsible for the anti-clotting effect of garlic. Ajoene is not viable at any temperature other than room temperature or above.

2.4.2.3 Garlic; Natural Immunity Booster

The introduction of deadly and infectious diseases that are viral like HIV/AIDS, such diseases which have no effective cure, hence strengthening the human immune system will be a more important and wise thing to do. Garlic is rich in sulphur which contains amino acids and other compounds that can improve the human immune system (Lau *et al*., 1991). Garlic makes macrophage cells more active hence initiates immune function (Salman *et al*., 1999). An

important immune system booster is Germanium and can be found in garlic. Other immune boosters found in garlic are thiamine, niacin, selenium and phosphorous (Morioka *et al*., 1993). Alliin containedin a standard preparation of garlic powder was used in a preliminary study in humans. This study has shown positive effects on human immune reactions and phagocytosis. Garlic is a potent treatment for herpes simplex virus and influenza B virus, this has been tested in the laboratory (Tsai *et al*., 1985). Recently, garlic has been shown to possess the ability offer protection from common cold virus (Josling, 2001).

2.4.2.4 Garlic; as Antibacterial

Gram positive and negative bacteria growth can be hindered by garlic, such bacteria are Staphylococcus, Streptococcus Klebsiella Escherichia, Salmonella, Proteus,, Lactobacillus, Pseudomonas, Shigella,Helicobacter pylori Micrococcus, and Enterobacter (Tsao and Yin, 2001). Allicin is responsible for the antimicrobial activity of garlic. Allinase carry out enzymatic activity on alliin to produce allicin. Allicin is found in garlic extracts, it is unstable, it breaks down within the period of 16hours at 23°C (Hahn, 1996). A way of stabilizing allicin is through the use of a water-based extract of allicin and this is due to the hydrogen bonding present in water to the reactive oxygen atom present in allicin (Lawson, 1996, Lawson and Wang, 1996).

2.4.2.5 Garlic; as Antifungal

Ajoene plays an important function as an antifungal agent (Ledezma and Apitz-Castro, 2006). Garlic has equal ability with the drug ketoconazole in inhibiting fungal growth, this was tested on the fungi Malassezia furfur, Candida albicans, Aspergillus and Cryptococcus (Shams-Ghahfarokhi *et al*., 2006). A report showed that garlic was used to treat a severe fungal brain

infection called Cryptococcus meningitis. It was further reported that, the researcher compared garlic with standard medical treatment (Amphotericin B) for effectiveness and it was shown intravenous garlic was more effective than the drug and it was not found to be toxic irrespective of its dosage (Lemar *et al*., 2007). Liquid garlic extract was used to treat mice that was infected by colonies of Candida, the colonies were greatly reduced. It has also been revealed thatphagocytic activities can be stimulated by garlic. Ringworm can be treated using oil extracted from garlic, the oil can also be used to treat skin parasites and warts (application will be external). Lesions rabbits and wild pigs caused by skin fungi were found to be treated by externally applying garlic extract and the lesions began to heal after seven days (Sabitha *et al*., 2005).

2.4.2.6 Garlic; as Antiparasitic

Garlic is globally recommended by traditional health workers as a treatment for parasites found in the intestines. Some cultural settings treat children that have been infested with helminthes using crushed garlic. The Chinese make use ofalcoholic extract of crushed garlic as medical treatments for intestinal diseases. Allicin in garlic shows activity that are antiparasitic against major human parasites that are found in the intestine such parasites are Entamoeba histolytica, Ascaris lumbricoides and Giardia lamblia (Kalyesa *et al*., 1975). Allicin dose of about 30 μg/ml is enough to inhibit Entamoeba histolytica in the heman intestine. Allicin also completely inhibit the growth of amoeba cultures (Mirelman *et al*., 1987). Furthermore, researchers have discovered that allicin even at lower concentrations of about 5 μg/ml inhibited 90% of trophozoites of E. histolytica virulence (Ankri *et al*., 1997).

2.4.3 Ginger

Ginger (Zingiber officinale, Rosc.) is another important crop, it is basically cultivated in Central Asia, China, India and Pakistan and it is globally distributed. Ginger is globally and traditionally used as a spice and treatment for some diseases (Larsen *et al*., 1999; Mohd-Yusof *et al*., 2002; Tapsell *et al*., 2006; Zhang *et al*., 2009; Manesh *et al*., 2012; Diarra *et al*., 2011). The root of ginger contains several compounds that possess biological activities, such activities are antioxidation, antimicrobial and pharmacological effects (Akoachere *et al*., 2002; Rabadah *et al*., 2004; Ali *et al*., 2008). Many active compounds are also contained in ginger, such include gingerol, shogaols, gingerdiol, and gingerdione (Kikuzaki and Nakatani, 1996; Zhang *et al*., 2009; Zhao *et al*., 2011).

2.4.3.1 Ginger; Effect on Feed Intake

The effect of ginger on feed intake is subjected to divided opinions. Using negative control broilers, 240 ppm of Z. officinale had a positive effect on feed intake(Tekeli *et al*. 2011). Nasiroleslami and Torki (2010) reported that when essential ginger oil was added, there was no effect observed on the feed intake level in laying hens. Zhang *et al*. (2009) also reported that there was no significant difference in feed intake in dailyon of ginger at the rate of 5 g/kg, though the feed intake was numerically more than it was in the control. Zhao *et al*. (2011) did not observe any difference in feed intake and feed conversion ratio (FCR) in his study between laying hens fed with ginger inclusion diet (5, 10, 15 and 20 g/kg), and Akbarian *et al*. (2011) reported that feeding laying hens with ginger diets at different levels (0.25, 0.5 and 0.75%) had no significant effect on feed intake and FCR. Incharoen and Yamauchi (2009) fed White

Leghorn laying hens with fermented ginger that has been dried (1 and 5%) and observed that feed intake and feed consumption rate tended to increase in the groups that were fed with ginger.

The use of 2% red ginger for broiler chickens has resulted in increased feed intakes and FCRs (Herawati, 2010). Onu (2010) showed that when ginger (0.25%) was added to the basal diet of broiler chicks, it resulted in increased food consumption rate, but the feed intake was not not affected. Ginger can also be administered in poultry through drinking water. Javed *et al*. (2009) showed that broiler chicks that were given aqueous extract dose of (15 ml/l of drinking) of a mixture of plants containing ginger enhanced feed intake and FCR. However, Kausar *et al*. (1999) reported that a carminative mixture having ginger at the dose rate of 2 and 4 ml/l of water improved FCR. Researchers have have tried to combined plant products and they included ginger. Moorthy *et al*. (2009) discovered that feed intake was not affected at the age of six weeks, but FCR was high in birds fed with a mixture of 0.2% ginger and 0.2% curry leaf powder. In this report the variation can be due to the ginger varieties used, their processing, dose and the periods of the experiments. Researchers assumed that what was responsible for the improved performance of the broiler birds fed with ginger was an enhanced palatability and the quick digestive effect of ginger. They further postulated that because of the quick digestive effect of ginger, the digestive tract would have been emptied earlier and feed consumption will have been enhanced. Ginger has been discovered to increase secretion of gastrointestinal enzymes including lipase, disaccharidase and maltase (Zhang *et al*., 2009). Herawati (2010) reported that the improved performance can be attributed to the two types of digestive enzymes that are present in ginger, they are protease and lipase and are present as part of the natural protective mechanisms of ginger plant (Zhang *et al*., 2009). Zhao *et al*. (2011) showed that ginger has the ability to

enhance the digestion, absorption and assimilation of animals' nutrient because it has positive effect on gastric secretion, enterokinesia and digestive enzyme activities.

2.4.3.2 Ginger; Effect on Poultry Production

According to AccoTekeli *et al*. (2011) the weight gain and growth performance was improved when *Z. officinale*was administered to broiler chickens at the rate of 120, 240 and 360 ppm, but Zhang *et al*. (2009) was unable to find any significant difference for daily weight gain by feeding broilers with ginger at the rate of 5 g/kg. According to Kausar *et al*. (1999) it was revealed that ginger contained in carminative mixture at the dose rate of 2 and 4 ml/l of drinking water resulted in body weight increase as at the week 5 of the experiment. There was improvement in body weight gain of broiler chicks that received dosage with aqueous extract of ginger contained in a plant mixture (Javed *et al*. 2009). El-Deek *et al*. (2002) detected that a ginger diet that contains 1 g/kg of ginger have no significant effect on growth performance, while Farinu *et al*. (2004) showed that supplementing diet with ginger at the levels of 5, 10, or 15 g/kg improved the growth a little in broilers. On the contrary Al-Homidan (2005) detected a reduction in the rate of growth starter broilers at age 1 to 4 week when ginger was used as a diet supplement at the rate of 60 g/kg body weight at the 6th week of age (Moorthy *et al*., 2009) this can be as a result of the toxicity (Zhang *et al*., 2009). The significant difference in the results on the growth performance of broilers can be because of the difference in the doses administered used in the experiments.

2.4.3.3 Ginger; Effect on Blood Biochemistry

Saeid *et al*. (2010) discovered that glucose in the serum, the total cholesterol, LDL-cholesterol and VLDL-cholesterol reduced greatly in broilers fed with 0.4 and 0.6% aqueous ginger extract but theconcentration HDL-cholesterol increased. Onu (2010) showed that ginger (0.25%) supplementation in the basal diet of broiler chicks did not cause any significant difference in total protein, albumin, globulin, urea and creatine. Rehman *et al*. (2011) recently show from study that the effect of placing broilers on dose (10 ml/l of the water they drink) adding aqueous extract of a mixture of three medicinal plants garlic, berberine and aloe vera, together with ginger, brought about a major reduction in serum glucose as well as the concentration of ALT (alanine aminotransferase), AST (aspartate aminotransferase) and ALP (alkaline phosphatase), but, the protein contained in serum experienced a major increase in the treated group. Same experiment, showed that the profile of the cholesterol including total cholesterol, triglyceride, LDL, VLDL showed major decrease in the treated group, while the concentration of HDL cholesterol improved. Zhang *et al*. (2009) foundin broilers treated with ginger powder, that totalconcentration of protein was increased at 21 days and 42 days of sampling but the concentration of cholesterol declined at these intervals. Al-Homidan (2005) discovered a decline in the total protein and globulin in the broiler chick plasma as a result of dietary supplementation of 60 g/kg which may be as a result of ginger toxicity. Farinu *et al*. (2004) showed that supplementation of ginger at rate range of 5, 10 and 15 g/kg showed no affect on the total protein and albumin in the serum of broiler chickens. The differences in these results may be as a result of doses difference and experimental conditions.

The particular pathway mechanisms by which alteration of blood metabolites occurred has not been discovered. A postulation was done that there was interfarance between (E)-8 beta, 17-epoxyllabed-12-ene-15, 16-dial, a compound isolated from ginger and cholesterol biosynthesis in liver homogenates of hypercholesterolaemic mice bringing about the reduction (Tanabe *et al*., 1993). It was revealed that feeding rats with ginger will greatly increase the activity of hepatic cholesterol 7-alpha-hydroxylase which usually limit the rate of enzyme activities in the biosynthesis of bile acids and initiates the conversion of cholesterol to bile acids resulting in excretion of cholesterol from the body (Srinivasan and Sambaiah, 1991).

2.4.3.4 Ginger; Toxicological Effects in Poultry Production

There are only few studies as regards the toxicological effects of ginger in poultry feed as supplement. But, Herawati (2010) showed that broilers fed diets that has 0.5, 1.0 and 1.5% red ginger showed oedema, necrosis and inflammation in muscles. Atsiri sescuiterpen oil is found in ginger and has the ability to toxic in animals. All phytobiotics have toxic features and how toxic it is can be determined by dose and period of feeding (Herawati, 2010). Consumption of such substances at higher quantity can cause symptoms of congestion, oedema, inflammation and necrosis (Ganiswarna, 1995).

CHAPTER THREE

EXPERIMENT ONE

THE EFFECT OF MOISTURE CONTENT AND TEMEPERATURE ON THE GROWTH RATE OF (*Aspergillus parasiticus)* AND AFLATOXIN PRODUCTION ON BROILERS FEED.

3.1 INTRODUCTION

The genus *Aspergilli* are widely distributed, available and vicious fungi in nature. These genera are implicated to spoil agricultural produce, especially animal feeds that are concoction of many grains and rich micro ingredients, producing toxic secondrary metabolites and causing aflatoxicosis (Klich 2007a). They produce aflatoxins, which are secondary metabolites, hepatotoxic, carcinogenic, immunosuppressive and antinutritional contaminants which reduce feed consumption and weight gain (Williams *et al*. 2004). These metabolites are known to be produced by several members of *Aspergillus* species (Klich 2007b). However, *Aspergillus flavus and Aspergillus parasiticus* are common and considered the major producer of aflatoxin being responsible for the contamination of several agricultural produce and livestock feeds (Klich 2007b).

Fungal growth on food commodities are influenced by synergy of extrinsic, intrinsic, implicit and processing factors (Sinha 1995). However, temperature, water activity and the gas composition are considered as the limiting factors for fungal growth and for mycotoxin production in the postharvest stage (Magan *et al*.2003).

Most livestock feeds can be contaminated by fungi during their preparation, especially during their storage because of varying moisture and storage temperature. *Aspergillus parasiticus* is of concern to the feed industry as potential spoilage organisms (Abarca *et al*, 2003; Al-Julaifi,

2003). Within this context, characterizing the optimal moisture levels and temperatures for fungal growth and mycotoxin production is prerequisite for any control strategy intended to deal with mycotoxigenic and spoilage fungi.

The aim of the present study was to determine the moisture and temperature level necessary for Aflatoxin production in poultry feed. Results from this trial will be used to predict and to prevent the germination and growth of *Aspergillus parasiticus* in formulated broilers diets.

3.2 MATERIALS AND METHODS

3.2.1 EXPERIMENTAL SITE

The research was carried out at University of Ilorin, Ilorin, Nigeria. It is situated in the Guinea Savannah belt. Location: "latitude 8° 20'N and longitude 4° 35'E and is at an approximate altitude of 306 m above sea level".

3.2.2 MICROORANISMS

The toxigenic strain of *Aspergillus parasiticus* was donated by International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The strain was tested for its capacity to produce Aflatoxin on Carrot-Potato Dextrose agar media (CPDAM).

3.2.3 BROILER FEED

Corn-Soybean broiler feed was formulated meeting requirement for broilers: 23% Crude Protein, 3200.18Kcal of energy. (NRC, 1994) (Table3.1).

Table 3.1: Feed composition and analyzed nutrients table.

Ingredients	**(%)**
Maize	50
Wheat Offal	13
Soya Bean Meal	20
Full Fat Soya Bean	10
Fish Meal	3
Oyster Shell	1
Bone Meal	2
Methionine	0.25
Lysine	0.25
Broilers Premix	0.25
Salt	0.25
Analyzed Nutrients	

Crude Protein	23.01
Crude Fiber	5.65
Ether Extract	6.23
Calcium	1.13
Phosphorus	0.89
Calculated Energy	3200.18Kcal

15kg of the broiler feed were sterilized at 121^0C for 15mins in an autoclave and the moisture contents was adjusted to 4, 8 12, 16 or 20% levels by adding the required amount of sterilized distilled water to the feed using Rapid Tester (Harvest Hand Moisture tester, Dickey-John) . The moisturized feed were then stored anaerobically at 15,20,25,30, 35 or 40^0C. There were 30 treatments made up of 5x6 factorial combinations of moisture and temperature. All treatments were set up in triplicates.

Table 3.2: Experimental layout.

Moisture (%)	Temperature (ºC)
4	15
	20
	25
	30
	35
	40
8	15
	20
	25
	30
	35
	40
12	15
	20

	25
	30
	35
	40
16	15
	20
	25
	30
	35
	40
20	15
	20
	25
	30
	35
	40

3.2.4 INOCULATION

The toxigenic *Aspergillus parasiticus* were cultured on Carrot Potato Dextrose agar for 72 h at 28ºC. An heamatocytometer was used in counting the conidial/ml, *Aspergillus parasiticus* spore suspension was adjusted until 1 mL of each produced 1.022×10^7 conidial. The animal feed adjusted to 4, 8, 12, 16 and 20% moisture (90g) were aseptically inoculated with 5 mL (5×10^7) of the spore suspension from the two microorganisms, thoroughly shaken to evenly distribute spores, and incubated for 14 days anaerobically at 15, 20, 25, 30, 35 and 40^0C temperature in different incubators. The development of the *Aspergillus parasiticus* strain was stopped by sterilizing the cultured feeds for 15 min at 121ºC after the 14days of incubation.

3.2.5 AFLATOXIN EXTRACTION AND DETERMINATION

"Broiler feed (50 g) were wet-milled with 70% methanol (1:5 v/w) using an electric blender (Waring Commercial, Model-HGBTWTG4, Torrington, Connecticut) for 3 min at high speed. The blended feed was filtered using Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK). To each volume of extract were added 100 mL of distilled water and 25 mL of dichloromethane in a separating funnel. The mixture was shaken vigorously for about 2 min in a fume chamber. The lower layer of the dichloromethane was withdrawn and allowed to pass through a funnel with a bed of anhydrous sodium sulphate. Again 100 mL of distilled water and 25 mL of dichloromethane were added and the procedure repeated. The extract was allowed to evaporate to dryness in the fume hood. The filtrate was diluted as appropriate, spotted alongside standards of aflatoxin B1, B2, G1, and G2 (Supelco, Bellefonte, Pennsylvania), and separated on thin layer chromatography plates (silica gel 60, 20 mm) with the development solvent diethyl ether– methanol–water (96:3:1)" Garber and Cotty, 1997. Aflatoxins were quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with winCATS 1.4.2 software).

3.2.6 PROXIMATE ANALYSIS:

Crude protein (N x 6.25) was determined by the Kjeldahl method after acid digestion. Ether extract was determined by the petroleum ether- extraction method using a Soxhlet apparatus. Crude fibre was determined from the organic residue left after sequential extraction of feed with petroleum ether. Dry Matter was determined by oven drying at 70°C for 24h and drying was continued until a constant weight was achieved. (AOAC, 2002)

3.2.7 STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance using the model for factorial design (Steel and Torrie, 1980) and using General Linear Model procedure SAS Institute (2002). Significant differences between treatments means were separated using the Duncan multiple range test (Duncan, 1955).

3.3 RESULTS

Table 3.3 shows the effects of moisture and temperature on the weight of broiler feed inoculated with *Aspergillus parasiticus* placed in the oven for 14days. Broiler feeds with moisture levels 4 and 8% were comparable and significantly higher in final weight than those of 12, 16 and 20% ($p<0.05$). Moisture level 16 and 20% were not significantly different in final weight. Broiler feeds with moisture level 4, 8 and 12% were comparable and significantly higher in weight gain than 16 and 20% which were also comparable ($p<0.05$). Feeds with moisture level 4, 8 and 12% had comparable weight gain and significantly higher in weight gain (%) than 16 and 20% that were also comparable ($p<0.05$). There were significant increase in weight gain with increase in temperature ($p<0.05$).

The highest weight gain was recorded in feed incubated at 25^0C while the least was recorded at feed incubated at 40^0C ($p<0.05$). Weight gain of feed incubated at 4, 12, 30 and 35^0C were comparable. Also, increase in temperature caused increased in percentage ($p<0.05$) with

the highest at 25^0C and lowest at 40^0C ($p<0.05$), 15^0C, 20^0C, 30^0C, and 35^0C are statistically comparable for percentage weight gain ($p<0.05$). There were no significant interactions between temperature and moisture for all the parameters measured ($p>0.05$).

Table 3.3: The effects of moisture and temperature on weight gain by *Aspergillus parasiticus* broiler feed placed in the oven for 14days.

Source of Variation	Initial (g)	Final (g)	Weight Gain (g)	Change in Weight gain (%)
Moisture (%)				
4	90.00	95.00^{a}	5.00^{a}	5.56^{a}
8	90.00	94.99^{ab}	4.99^{a}	5.54^{a}
12	90.00	94.16^{b}	4.16^{b}	5.46^{a}
16	90.00	94.30^{c}	4.30^{b}	4.78^{b}
20	90.00	94.31^{c}	4.31^{b}	4.78^{b}
Temperature (^{0}C)				
15	90.00	94.68^{ab}	4.68^{bc}	5.19^{bc}
20	90.00	94.76^{ab}	4.76^{ab}	5.29^{ab}
25	90.00	94.80^{a}	4.80^{a}	5.33^{a}
30	90.00	94.76^{ab}	4.76^{ab}	5.29^{ab}
35	90.00	94.65^{b}	4.65^{bc}	5.17^{bc}
40	90.00	94.60^{b}	4.60^{c}	5.11^{c}
Moisture*Temperature	NS	NS	NS	NS
SEM±	0.00	1.318	1.413	1.960

^{abcde}: means in the same column with different superscripts are significantly different ($P<0.005$)

Table 3.4 shows the effects of moisture and temperature on proximate composition of broiler feed inoculated with *Aspergillus parasiticus* placed in the oven for 14days. Increase in moisture content of the feeds resulted in significant decrease of the dry matter content ($p< 0.05$), 4% moisture had the highest value while 20% had lower value. Compared to the initial moisture in the diets, there were significant decrease with 4%, 8% and 12% comparable and higher than those of 16% and 20% that were also statistically the same but lower ($p< 0.05$). Protein content of feeds with 4%, 8% or 12% water were comparable ($p>0.05$) and significantly lower than feeds with 16% or 20% water ($p>0.05$).

Protein content of 16% or 20% were also comparable ($p>0.05$). An increase were observed in the values compared to initial crude protein placed in the oven, the increase were also across board (11.86% -13.35%), the values were significantly comparable and lower for levels 4%, 8% and 12% against 16% and 20% that were higher but were statistically the same ($p< 0.05$). Values of crude fiber followed the same trend for crude protein ($p< 0.05$), but when adjusted with initial values of the crude fiber placed in the oven, there were decrease across board (-25.30 to – 30.13%) with 4% 8% and 12% were comparable and higher than 16% and 20% that are also statistically comparable ($p< 0.05$). Broiler feeds with moisture level 4% 8% and 12% were comparable and significantly lower in ether extract than those of 16% and 20% ($p< 0.05$). But increase in Ether Extract was observed when compared to initial values placed in

the oven (16.80% - 18.77%), 16% and 20% were comparable and higher to those of 4%, 8% and 12% that were comparable.

Increase in temperature (10^0C to 40^0C) had no significant effect on the feed. Thus, there were no significant effects of storage temperature of feed inoculated with *Aspergillus flavus and parasiticus* on dry matter, crude protein, crude fiber and ether extract ($p>0.05$). There were no significant interactions between storage temperature and moisture content of feed inoculated with *Aspergillus parasiticus* on the proximate composition of broiler feed ($p>0.05$).

Table 3.4: The effects of moisture and temperature on proximate composition of broiler feed inoculated with *Aspergillus parasiticus* placed in the oven for 14days.

Source of Variation	Dry Matter (%)	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)	Change to Initial (DM)[a] (%)	Change to Initial (CP)[b] (%)	Change to Initial (CF)[c] (%)	Change to Initial (EE)[d] (%)
Moisture (%)								
4	93.29[a]	24.60[b]	4.51[a]	7.49[b]	-2.91[a]	11.86[b]	-25.30[a]	16.80[b]
8	89.42[b]	24.58[b]	4.52[a]	7.48[b]	-2.88[a]	11.78[b]	-25.04[a]	16.68[b]
12	85.47[c]	24.64[b]	4.49[a]	7.51[b]	-2.95[a]	12.01[b]	-25.75[a]	16.99[b]
16	81.28[d]	25.02[a]	4.34[b]	7.67[a]	-3.35[b]	13.35[a]	-30.13[b]	18.77[a]
20	77.42[e]	25.01[a]	4.35[b]	7.67[a]	-3.34[b]	13.33[a]	-30.02[b]	18.74[a]
Temperature (^{0}C)								
15	85.35	24.80	4.43	7.57	-3.11	12.57	-27.58[b]	17.74
20	85.42	24.72	4.46	7.54	-3.03	12.29	-26.66	17.36
25	85.43	24.70	4.47	7.53	-3.02	12.23	-26.48	17.28
30	85.40	24.74	4.46	7.55	-3.05	12.35	-26.88	17.45
35	85.34	24.81	4.42	7.58	-3.13	12.61	-27.76	17.79
40	85.31	24.85	4.41	7.60	-3.17	12.74	-28.12	17.96
Moisture*Temperature	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.109	0.052	0.479	0.605	0.133	0.058	1.561	0.138

Intial Values: Dry Matter 4^0C=96%, 8^0C=92%, 12^0C=88%, 16^0C=84%, 20^0C=80%. Crude Protein: 21.68%, Crude Fiber: 5.65%, Ether Extract: 6.23
a: Dry Matter, b: Crude Protein, c: Crude Fiber, d: Ether Extract [abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table3.5 shows the effects of moisture and temperature on *Aspergillus* count and Aflatoxin quantification of broiler feed inoculated with *Aspergillus parasiticus* placed in the oven for 14days. There were significant interactions between temperature and moisture on all the parameters measured ($p<0.05$), the details of the interactions is represented in tables 3.5a, 3.5b, 3.5c, 3.5d and 3.5e.

Table 3.5: The effects of moisture and temperature on *Aspergillus* count and Aflatoxin quantification of broiler feed inoculated with *Aspergillus parasiticus* placed in the oven for 14days.

Source of Variation	Aspergillus Count (10^6) CFU	*AFB1 (ng)	*AFB2 (ng)	*AFG1 (ng)	*AFG2 (ng)

Moisture (%)					
4	3.66^{a}	35.42^{c}	19.16^{a}	15.32^{a}	3.14^{a}
8	2.80^{b}	23.54^{d}	8.88^{b}	9.38^{b}	2.12^{a}
12	1.36^{d}	27.27^{d}	8.02^{b}	6.24^{c}	2.12^{b}
16	1.77^{c}	73.12^{a}	7.70^{b}	6.83^{c}	3.37^{a}
20	1.18^{d}	55.91^{b}	5.71^{c}	3.20^{d}	0.96^{c}
Temperature (^{0}C)					
15	2.86^{a}	57.24^{a}	5.62^{d}	6.27^{b}	1.33^{b}
20	2.29^{b}	45.79^{b}	4.50^{d}	5.01^{bc}	1.06^{b}
25	1.97^{c}	39.31^{c}	7.86^{c}	2.95^{cd}	0.88^{b}
30	2.00^{c}	40.07^{c}	3.93^{d}	2.65^{d}	0.80^{b}
35	1.96^{c}	39.14^{bc}	11.74^{b}	5.29^{b}	1.59^{b}
40	1.84^{c}	36.74^{c}	25.72^{a}	27.01^{a}	8.10^{a}
Moisture*Temperature	S	S	S	S	S
SEM±	0.313	6.253	2.698	3.077	1.171

*AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2
^{abcde}: means in the same column with different superscripts are significantly different (P<0.005)

Table 3.5a shows the interaction between moisture and temperature on *Aspergillus* count by *Aspergillus parasiticus* inoculated broiler feed placed in the oven for 14days. Broiler feed inoculated at 4% moisture and 15^{0}C temperature level had the highest concentration CFU for *Aspergillus* while the lowest was at 20% moisture and 40^{0}C temperature level ($p<0.05$). At 4% moisture a decrease in *Aspergillus* count with an increase in temperature was observed up to 25^{0}C, while an increase was observed at 30^{0}C but statistically lower than 15^{0}C that had the

highest values in the group, a dip of value was also observed at 35^0C then an increase lower to peak values in the group ($p<0.05$). The same trend was observed for 8% moisture. At 12% moisture a decrease in the CFU of *Aspergillus* with an increase in temperature up to 30^0C, the decrease was followed by an increase in *Aspergillus* count at 35 and 40^0C of temperature levels ($p<0.05$). The values observed were higher than 15^0C. 16% and 20% of moisture followed a different pattern, every increase is accompanied by lower values as the temperature levels increased down the group ($p<0.05$). As the moisture levels increased, there were decrease in the *Aspergillus* count up to 12% moisture level then an increase at 18% and a decrease at 20% ($p<0.005$). These trends were observed for temperature levels 15, 20, 30 and 35^0C. At 25^0C of temperature an increase was followed by a decrease as the moisture level increases. As the moisture levels increase there was reduction in the values of *Aspergillus* count for 40^0C.

Table 3.5a: Details of interaction between moisture and temperature on *Aspergillus* count by *Aspergillus parasiticus* inoculated broiler feed

	(CFU)		Moisture (%)		
Temperature(^{0}C)	4	8	12	16	20
15	1.15^{j}	2.13^{f}	1.35^{i}	4.15^{b}	5.52^{a}
20	0.92^{k}	1.71^{h}	1.08^{jk}	3.32^{d}	4.42^{b}
25	2.22^{f}	2.21^{f}	1.00^{k}	2.56^{f}	1.84^{h}
30	0.81^{k}	1.49^{i}	0.95^{k}	2.90^{e}	3.87^{c}
35	1.33^{i}	2.00^{h}	1.76^{h}	1.78^{h}	2.90^{e}

40	0.63[l]	1.07[jk]	2.03[gh]	2.07[g]	3.38[d]

[abcde]: means in the same column and rows with different superscripts are significantly different (P<0.005)

Table 3.5b shows the interaction between moisture and temperature on AFB1 in *Aspergillus parasiticus* inoculated broiler feed placed in the oven for 14days. Broiler feed inoculated at 20% moisture and 15^0C temperature level had the highest level of AFB1while the lowest was at 4% moisture and 40^0C temperature level ($p<0.05$). 4% and 8% of moisture showed the same trend, every increase in value of AFB1 was followed by a decrease in value of AFB1 as the temperature levels increased down the group ($p<0.05$). At 12% moisture a decrease in the AFB1with an increase in temperature up to 30^0C, the decrease was followed by an increase in AFB1 at 35 and 40^0C of temperature levels ($p<0.05$). The values observed were higher than 15^0C. At 16% moisture a decrease in AFB1 with an increase in temperature was observed up to 25^0C, while an increase was observed at 30^0C but statistically lower to 15^0C that had the highest values in the group, a dip value was also observed at 35^0C then an increase lower to peak values in the group ($p<0.05$). The same trend was observed for 20% moisture.As the moisture levels increase, there were decrease in the AFB1 up to 12% moisture level then an increase at 18% followed by a decrease at 20% ($p<0.005$). These trends were observed for temperature levels 15, 20, 30 and 35^0C. At 25^0C of temperature an increase was followed by a decrease as the moisture levels increase. As the moisture levels increase there was decrease in the values of AFB1 for 40^0C.

Table 3.5b: Details of interaction between moisture and temperature on AFLATOXIN B1 (AFB1) in *Aspergillus parasiticus* inoculated broiler feed.

	AFB1		Moisture (%)		
Temperature(^{0}C)	4	8	12	16	20
15	23.07^{g}	42.68^{e}	27.08^{g}	82.93^{bc}	110.45^{a}
20	18.45^{h}	34.15^{f}	21.67^{g}	66.35^{d}	88.36^{b}
25	44.32^{e}	44.32^{e}	20.05^{g}	51.13^{d}	36.73^{f}
30	16.15^{h}	29.88^{g}	18.96^{h}	58.05^{d}	77.32^{c}
35	26.63^{g}	40.00^{e}	35.22^{f}	35.72^{f}	58.15^{cd}
40	12.60^{h}	21.52^{g}	40.61^{e}	41.29^{e}	67.68^{d}

^{abcde}: means in the same column and rows with different superscripts are significantly different (P<0.005)

Table 3.5c shows interaction between of moisture and temperature interactions on Aflatoxin B2 (AFB2) in *Aspergillusflavus* and *parasiticus* inoculated broiler feed placed in the oven for 14days. Broiler feed inoculated at 4% moisture and 40^{0}C temperature level had the highest level of AFB2 (47.38ng) while the lowest (0.96ng) was at 8% moisture and 30^{0}C temperature level ($p<0.05$). At 4% moisture a decrease in AFB2 with an increase in temperature was observed up to 25^{0}C, while an increase was observed at 30^{0}C but statistically lower to 15^{0}C. An increase in AFB2 was observed from 30^{0}C to 40^{0}C. Every increase in AFB2 for 8% was accompanied by a decrease in value as the temperature increases down the group. The observations at 12, 16 and 20% were similar as the temperature levels increased down the group. An increase was followed by a decrease up to 30^{0}C, the reverse of it was observed for 35^{0}C and 40^{0}C.As the moisture level rises, there were decrease in the AFB2 up to 8% moisture level then an increase at 12-16% followed by a decrease at 20% ($p<0.005$). These trends were observed for temperature levels 15, 20, 30 and 35^{0}C. At 25^{0}C of temperature decrease in value was followed

by an increase as the moisture level rises. As the moisture level increased there were decrease in the values of AFB2 for 40^0C.

Table 3.5c: Details of interaction between moisture and temperature on AFLATOXIN B2 (AFB2) in *Aspergillus parasiticus* inoculated broiler feed.

Temperature(^{0}C)	Moisture (%)				
	4	8	12	16	20
15	17.13^{c}	1.37^{j}	2.06^{h}	4.10^{g}	3.43^{h}
20	13.71^{cd}	1.10^{l}	1.64^{i}	3.29^{gh}	2.74^{h}
25	7.340^{f}	10.23^{de}	4.01^{g}	8.86^{e}	8.86^{e}
30	11.99^{d}	0.96^{l}	1.44^{j}	2.88^{h}	2.40^{h}
35	17.45^{c}	10.72^{d}	10.57^{de}	12.00^{d}	7.99^{e}
40	47.38^{a}	28.91^{b}	28.43^{b}	15.06^{cd}	8.82^{e}

^{abcde}: means in the same column and rows with different superscripts are significantly different ($P<0.005$)

Table 3.5d shows the details of interaction between moisture and temperature on Aflatoxin G1 (AFG1) in *Aspergillus parasiticus* inoculated broiler feed placed in the oven for 14days. Broiler feed inoculated at 4% moisture and 40^0C temperature level had the highest level

of AFG1 (49.75ng)while the lowest (0.00ng) were at 12% moisture-15^{0}C temperature, 12% moisture-20^{0}C temperature, 20% moisture-15^{0}C temperature and 20% moisture-20^{0}C temperature levels ($p<0.05$). At 4%, 8% and 16% moisture all increase were followed by a decrease with an increase in temperature up to 30^{0}C, a reversed of the trend was observed between 35 and 40^{0}C temperature levels ($p<0.05$). The observations at 12 and 20% were similar, no AFG1 was detected in 15 and 20^{0}C ($p>0.05$), an increase followed by a decrease in AFG1 were observed as temperature levels increased down the group.

With increase in moisture level, there were decrease in the AFG1 up to 12% moisture level then an increase at 16% followed by a decrease at 20% ($p<0.005$). These trends were observed for temperature levels 15, 20 and 35^{0}C. At 25 and 30^{0}C increase in values were observed followed by decrease as the moisture level increased. The values were constant at 16 and 20% moisture ($p<0.05$). As the moisture level increased there were reduction in the values of AFG1 for 40^{0}C ($p<0.05$).

Table 3.5d: Details of interaction between moisture and temperature on AFLATOXIN G1 (AFG1) in *Aspergillus parasiticus* inoculated broiler feed.

Temperature(^{0}C)	AFG1(ng)		Moisture (%)		
	4	8	12	16	20
15	16.17^{c}	7.69^{de}	0.00^{f}	7.50^{de}	0.00^{f}
20	12.93^{de}	6.15^{e}	0.00^{f}	6.00^{e}	0.00^{f}
25	2.76^{f}	3.83^{ef}	1.50^{f}	3.30^{ef}	3.32^{ef}
30	2.48^{f}	3.40^{ef}	1.35^{f}	2.99^{f}	2.99^{f}
35	7.85^{de}	4.82^{ef}	4.75^{ef}	5.40^{ef}	3.60^{ef}
40	49.75^{a}	30.35^{b}	29.85^{b}	15.81^{dc}	9.26^{e}

[abcde]: means in the same column and rows with different superscripts are significantly different (P<0.005)

Table 3.5e shows the interaction between moisture and temperature on Aflatoxin G2 (AFG2) in *Aspergillus parasiticus* inoculated broiler feed placed in the oven for 14days. Broiler feed inoculated at 4% moisture and 40^0C temperature level had the highest level of AFG2 (14.92ng)while the lowest (0.00ng) were at 4% moisture-15^0C temperature, 4% moisture-20^0C temperature, 8% moisture-15^0C temperature, 8% moisture-20^0C temperature levels, 12% moisture-15^0C temperature, 12% moisture-20^0C temperature, 20% moisture-15^0C temperature, 20% moisture -20^0C temperature ($p<0.05$). At 4%, 8%, 12% and 20% moisture, no AFG2 was detected at temperatures 15 and 20^0C. An increase was observed at 25^0C, followed by decrease at 30^0C, the reversed trends were observed for temperatures 35 and 40^0C. The observation at 16% was that of an increase in temperature that led to a decrease in AFG2 up to 30^0C, at 35^0C an increase was observed up to 40^0C.

There were no detection of AFG2 for moisture levels 4, 8, 12 and 20% ($p>0.05$), AFG2 was detected at 16% ($p<0.005$). These trends were observed at 15^0C and 20^0C. For every decrease in AFG2 across the moisture level, it was followed by an increase of AFG2 for temperatures 25, 30 and 35^0C. The values were constant at 16 and 20% moisture ($p<0.05$). As the moisture level rises there was reduction in the values of AFG2 for 40^0C.

Table 3.5e: Details of interaction between moisture and temperature on AFLATOXIN G2 (AFG2) in *Aspergillus parasiticus* inoculated broiler feed.

Temperature(^{0}C)	Moisture (%)				
	4	8	12	16	20
15	0.00^{g}	0.00^{g}	0.00^{g}	6.65^{bc}	0.00^{g}
20	0.00^{g}	0.00^{g}	0.00^{g}	5.32^{bc}	0.00^{g}
25	0.83^{fg}	1.15^{fg}	0.45^{g}	0.99^{f}	0.99^{f}
30	0.74^{fg}	1.04^{f}	0.41^{g}	0.90^{fg}	0.90^{fg}
35	2.36^{e}	1.45^{ef}	1.43^{ef}	1.62^{e}	1.08^{fg}
40	14.92^{a}	9.11^{b}	8.96^{b}	4.75^{c}	2.78^{d}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 3.6 shows the correlation between proximate composition, Aflatoxin quantification, weight gain and *Aspergillus* Count. The correlation was significant and positive between Aspergillus and dry matter, crude fiber, ether extract, biomass, AFB1 and AFB2 (r=0.34-0.99), while a negative correlation but significant relationship exist between Dry Matter and Crude Protein.

Table 3.6: Correlation between Proximate Parameters, Aflatoxin Quantification, Biomass and *Aspergillus* Count.

	Aspergillus Count	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Weight gain	AFB1	AFB2	AFG1	AFG2
Aspergillus Count	1.000*	0.697*	-0.415*	0.420*	0.419*	0.459*	0.989*	0.340*	0.337	0.049
Dry Matter		1.0000*	-0.762*	0.763*	0.761*	0.831*	0.698*	0.379*	0.337	0.116
Crude Protein			1.000*	-0.999*	0.999*	-0.979*	-0.416*	-0.088	-0.071	0.097
Crude Fiber				1.000*	-0.999	0.978*	0.420*	0.082	0.067	-0.105
Ether Extract					1.000*	-0.979*	-0.419*	-0.087	-0.067	0.101
Weight Gain						1.000*	0.460*	0.125	0.098	-0.099
AFB1							1.000*	0.340*	0.337*	0.049
AFB2								1.000*	0.921*	0.796*
AFG1									1.000*	0.867*
AFG2										1.000*

3.4 DISCUSSION

Results from this study showed that moisture, temperature and *Aspergillus parasiticus* increased the weight gain of feed stored anaerobically in the oven for 14days. As the moisture and temperature levels increased the weight gain decreased. Moisture content at is one of the key factors at whatever inclusion affect the metabolite production in Solid State Fermentation (Archana and Satyanarayana, 1997). Moisture affects the physical properties of any solid substrate by causing swelling of the substrate. (How and Ibrahim, 2004).Fermentation is also implicated; moisture, temperature and storage time and good factors necessary for fermentation of grains and *Aspergillus* biodegradation.

The increase in the weight gain also leads to changes in the nutritional content of the feed. These could be attributed to the ability of *Aspergillus parasiticus* to break down the crude fiber to build up their biomass resulting in increased crude protein, ether extract and reduction in the crude fiber (Duarte *et al*. 2008). The results of proximate composition and analyzed fiber components in all treatment showed an increase in the protein contents, this was partly due to the ability of the *Aspergillus parasiticus* to liberate protein hitherto encapsulated in the cell. Decrease in fiber may be due to the ability of *Aspergillus parasiticus* to break polysaccharides into single sugars which are easily available for utilization by the organisms. (Iyayi and Aderolu., 2004). The results from this research confirm that fermentation improves the nutritional value of substrates (Carvalheiro *et al*., 2007).

Higher moisture contents from this trial resulted in reduction in weight gain, this may be due to oxygen limitation within the substrates which consequently affected the oxygen transfer within the substrate, therefore resulting in poor growth (Ramesh and Lonsane, 1990; Sandhya *et*

al., 2005). Temperature has a significant effect in the germination of spores which could determine the success of the Solid State Fermentation system. Therefore, temperature has become one of key parameters in SSF system (How and Ibrahim, 2004; Pang and Ibrahim, 2005). Lower temperature can prevent the increasing of excessive temperature due to the gradient temperature which generating during fermentation (Syarifah *et al*. 2011). The results from this trial showed the feed stored at higher temperature has reduced weight gain when compared to feed stored at lower temperature and this might likely be linked to aerobic mature of molds (Syarifah *et al*. 2011). Decline in spores at higher temperature also can be caused by the denaturation of the organisms or its inactivation at higher temperatures, high-moisture concentrations that exclude adequate oxygen can prevent mold growth. However, in practical situations, molds will grow in wet feeds such as silage or wet byproducts, when oxygen is available.

The entire modified atmosphere tested allowed the production of AFB1, AFB2 and AFG1. High moisture favors the synthesis of AFB1, AFB2 and AFG1 in this trial. Temperature and moisture are the physical factors necessary for Aflatoxin synthesis (Hesseltine, 1983). Specific nutrients which are available in the broiler feed such as amino acids, vitamins, fatty acids, minerals (especially zinc) and energy source preferably starch and soil nutrients such as zinc are required for aflatoxins formation are also present in the broiler feed (Wyatt, 1991). Aflatoxins yield are much in highly starched ingredients such as animal feed, the diet composition is maize soybeans diets (Diener and Davis, 1968). Aflatoxins production by *Aspergillus parasiticus* is optimum at the temperature range of 15°C and 20°C and 20% moisture in this trial and is limited at the temperature 40°C and moisture of 4%, the limit of aflatoxin

production is inline with the report of Lillehoj, 1983 while the optimum synthesis of aflatoxin did not support the work done by (Royes and Yanong, 2002).

3.5 CONCLUSION

It can be concluded from this trial that Aspergillus count is directly proportional to Aflatoxin B1 synthesis. Low temperature (15^0C) and high Moisture (20%) increased the synthesis of AFB1. High Temperature and Low Moisture increased the Synthesis of AFB2, AFG1 and AFG2. From this trial, *Aspergillus* prone feed could be stored at temperature of 40^0C and moisture level of 4%.

CHAPTER FOUR

EXPERIMENT 2

IN-VITRO EFFECTS OF FEED ADDITIVES ON GROWTH OF FUNGI (*Aspergillus spp*).

4.1 INTRODUCTION

Poultry feeds by virtue of their high nutrients generously support the growth of molds and fungi. These molds and fungi produce secondrary toxic metabolites which are known as mycotoxins. OchratoxinA (OA), Aflatoxin (AF), and T-2 toxin (T2) are few examples of

secondary metabolites of *Aspergillus spp* which are often found in poultry feeds and feedstuffs. Ingestion of these mycotoxins may show greater negative effects on the wellness and performance of birds than when consumed alone (Raju and Deve- gowda, 2002; Denli and Okan, 2006; Yegani *et al*., 2006). Several approaches have been used to neutralize Aflatoxicosis in poultry, using single or synergy of physical, chemical, nutritional and biological methods. Herbal components like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and green algae (*Spirulina plantesis*) and other mineral materials have shown a good prospect to neutralise mycotoxin and they also act as good antioxidants and binder. Though some of them have proved effective on some mycotoxins, the search is still on for a natural, cost effective and available solution to this problem in poultry.

There is hence the need to ascertain the effectiveness of selected commercial enzyme and herbal products and other products that are rich in antioxidants to inhibit *Aspergillus parasiticus*.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL SITE

The research was carried out at University of Ilorin, Ilorin, Nigeria. It is situated in the Guinea Savannah belt. Location: “latitude 8° 20’N and longitude 4° 35’E and is at an approximate altitude of 306 m above sea level”.

4.2.2 MICROORGANISMS

The toxigenic strain of *Aspergillus parasiticus* was donated by International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The strain was tested for its capacity to produce Aflatoxin on Carrot-Potato Dextrose agar media (CPDAM)

4.2.3 PREPARATION OF HERB EXTRACTS

"Herb materials of three plant species belonging to three botanical families (Table 4.1) included in this study were collected, washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite and dried in shade. The shade-dried material of each plant species was grounded into a powdered material using a blender to pass 100 mm sieve and the mince was sealed in polyethylene bags until extraction. For preparation of aqueous extracts, 50 g of dry powder plant material from each plant species was soaked in sterilized water (10 ml of water/g of plant material) with stirring for 48 h then filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to remove debris and obtain a clear filtrate. The filtrates were evaporated and dried under reduced pressure and temperature below 40°C. Each of the plants extracted were diluted to meet up the concentration needed to inhibit *Aspergillus* growth".

Table 4.1: Plants identification

English Name	Botanical Name	Family
Ginger	*Zingiber officinale*	Zingiberaceae
Tumeric	*Curcuma longa*	Zingiberaceae
Garlic	*Alliumsativum*	Amaryllidaceae

4.2.4 ANTIFUNGAL SCREENING TEST OF PLANT EXTRACTS AND XYLANASE

"Antifungal activity was evaluated on the toxigenic *A. flavus and Parasiticus* strain using Czapek dox broth medium (sucrose, 30g; sodium nitrate, 3g; dipotassium phosphate, 0.5g; magnesium sulfate, 0.5g; potassium chloride, 0.5g; ferrous sulfate, 0.01g; distilled water, 1000ml; pH, 6.5). The plant extract residues and nutrase xylanase were re-dissolved in 5 ml of

Czapek broth, sterilized in disposable Millipore filter (0.22 µm pores) and mixed with 45ml, 40ml, 35ml, 30ml and 25ml of sterile Czapek broth in 150 ml Erlenmyer flasks to obtain final concentration of 10mg/ml**,** 20mg/ml**,** 30/mg ml**,** 40mg/ml and 50mg/ml respectivelyof each plant extract and xylanase. The control (positive and negative) set were kept parallel to the treatment sets without plant extracts. The flasks were inoculated with 1.02 x 10^7 conidial/ml with the toxigenic *Aspergillus parasiticus* isolate and incubated at 25 ± 2°C for 7 days. After incubation, content of each flask was filtered (Whatman No.1) and Mycellia weight of the filtered mycelium was determined after drying at 70°C for 4 days till their weights remains constant". The percentage of mycelial inhibition was calculated using the following formula:

Percentage of Mycelial inhibition = [C – T / C] x 100.

Where, C and T are the mycelial dry weight (mg) in control and treatment respectively.

Table 4.2: Experimental Layout:

	10mg/ml	20mg/ml	30mg/ml	40mg/ml	50mg/ml
Control	-	-	-	-	-
Nutrase Xylanase®	+	+	+	+	+
Zingiber officinale	+	+	+	+	+
Curcuma longa	+	+	+	+	+
Alliumsativum	+	+	+	+	+

4.2.5 STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance using the factorial design (Steel and Torrie, 1980) and General Linear Model procedure SAS Institute (2000). Significant differences between treatments means were separated using the Duncan multiple range test (Duncan, 1955).

4.3 RESULTS

Table 4.3 shows the effects of plant extracts and nutrase xylanase on the mycellia weight and inhibition of *Aspergillusflavus* and *parasiticus*. The different plants extracts (*Zingiberofficinale, Curcuma longa and Allium Sativum*) reduces the Mycellia weight of the mycelia of the tested organisms, the effects of the three of them were statistically comparable ($p< 0.05$) and statistically lower to the control and nutrase xylanase ($p< 0.05$). The same trend was noticed for the inhibition rate of this plant extracts as compared to the control and Nutrase Xylanase ($p< 0.05$). The concentration levels (10, 20, 30, 40 and 50 mg/ml) had similar effects on the mycellia weight of the mycelia but statistically lower to the control (0mg/ml) ($p< 0.05$). 30, 40, 50mg/ml were comparable but lower compared to 10 and 20mg/ml for the inhibition rate ($p< 0.05$).

There were significant interactions between concentrations and types of extract for mycellia weight and percentage inhibition ($p<0.05$). The detailed interactions is shown in Tables 4.1a and 4.1b

Table 4.3: The effects of plant extracts and their concentrations on mycellia weight of *Aspergillusflavus* and *parasiticus.*

	Mycellia Weight (g)	Inhibition (%)
Extracts		
Control	0.120[a]	0.00[d]
Nutrase Xylanase®	0.104[a]	13.48[c]
Zingiber officinale	0.040[b]	67.42[a]
Curcuma longa	0.036[b]	70.21[a]
Alliumsativum	0.044[b]	63.21[b]
Concentration (mg/ml)		
0	0.120[a]	0.00[c]
10	0.045[b]	62.67[b]
20	0.046[b]	61.74[b]
30	0.045[b]	63.55[ab]
40	0.041[b]	66.63[a]
50	0.040[b]	66.91[a]
Extracts*Concentration	S	S
SEM±	0.008	5.158

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 4.3a shows the interactions of plant extracts and their concentrations to reduce mycellia weight of *Aspergillusflavus* and *parasiticus* in a liquid broth for 7days. At 0 mg/l concentrations, the Mycellia weight were comparable for all the plant extracts ($p>0.05$). *Zingiber officinale* showed to be more effective at 10mg/l and lower compared to *Curcuma longa, Alliumsativum* that are statistically comparable ($p<0.05$). *Zingiber officinale* and *Alliumsativum* were comparable but less effective to*Curcuma longa* at 20, 30 and 40mg/l ($p<0.05$). The three plants extracts were comparable at 50mg/l. Nutrase Xylanase® was all time higher at whatever concentrations used ($p<0.05$).

Table 4.3a: Details of interaction of plant extracts and their concentrations on mycellia weight of *Aspergillusflavus* and *parasiticus.*

Plant Extracts	Concentration (mg/l)					
	0	10	20	30	40	50
Nutrase Xylanase®	0.120^{a}	0.09^{b}	0.10^{ab}	0.11^{ab}	0.09^{b}	0.10^{ab}
Zingiber officinale	0.120^{a}	0.02^{d}	0.03^{c}	0.03^{c}	0.03^{c}	0.02^{d}
Curcuma longa	0.120^{a}	0.03^{c}	0.02^{d}	0.02^{d}	0.01^{e}	0.02^{d}
Alliumsativum	0.120^{a}	0.03^{c}	0.03^{c}	0.03^{c}	0.03^{c}	0.02^{d}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 4.3b shows the interactions of plant extracts and their concentrations on growth of *Aspergillusflavus* and *parasiticus* in a liquid broth for 7days. At 0 mg/l concentrations, the inhibition was statistically the same for all the plant extracts. *Zingiber officinale* showed to be more effective at 10mg/l followed *Curcuma longa* then *Alliumsativum* ($p<0.05$). At 20mg/l and 40mg/l *Curcuma longa* showed to be more effective compared to *Zingiber officinale* that is statistically more effective than *Alliumsativum* ($p<0.05$). 30mg/l and 50mg/l showed the same trend. The inhibition percentage was higher in *Curcuma longa* while *Zingiber officinale* and *Alliumsativum* were statistically comparable ($p<0.05$).Nutrase Xylanase® was all time lower at all the concentrations tested ($p<0.05$).

Table 4.3b: The Details of interaction between plant extracts and their concentrations on growth of *Aspergillusflavus* and *parasiticus*.

Plant Extracts	Concentration (mg/l)					
	0	10	20	30	40	50
Nutrase Xylanase®	0.00^{g}	22.09^{f}	13.71^{f}	8.37^{f}	22.81^{f}	13.93^{f}
Zingiber officinale	0.00^{g}	83.26^{bc}	78.12^{c}	79.30^{d}	80.59^{c}	83.26^{bc}
Curcuma longa	0.00^{g}	74.88^{d}	80.23^{c}	87.21^{c}	91.75^{a}	87.21^{b}
Alliumsativum	0.00^{g}	70.46^{e}	74.88^{d}	79.30^{d}	71.39^{de}	83.26^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

4.4 DISCUSSION

The results obtained from the present investigation revealed that the highest antifungal activities were exhibited by the plant extracts (*Zingiberofficinale, Curcuma longa* and *Allium Sativum*) than the commercial enzyme. The basis of the reaction might be due to the nature and combinations of phytocompounds presents in the crude extracts. At different concetrations of extracts that were added to the fungal cultures in liquid media, a remarkable reduction in mycelia was noticed and which in turn will reduce the aflatoxin concentration (Razzaghi-abyaneh *et al*., 2008; Sindhu *et al*. 2011; Komala *et al*.,2012)

Turmeric, ginger and garlic in this study inhibited mycelia growth and spore formation, there is a positive correlation between spore count and aflatoxin production from the previous study. It has been suggested that the regulation of aflatoxin synthesis and conidiogenesis may be interlinked, since the loss of aflatoxigenic capabilities in the nona-flatoxigenic variant strains of *Aspergillus parasiticus* was correlated with alterations in the conidial morphology (Kale *et al*.,1996). Curcumin is an activie ingredient in turmeric which has been implicated to inhibit mycelia growth and spore formation (Soni *et al*.,1992 and Gowda *et al.*, 2004). The inhibition observed by treatment with Ginger extracts might be linked to volatile essential oils which include zingeberene, curcumene and farnesene and nonvolatile bioactive polyphenol compounds (gingerol, shogaol and their derivatives), which have a high antioxidant activity. This was attributed to the presence of α, β- unsaturated ketones moieties of (6)–shogaol, and the presence of short carbon chains of (6)–gingerol and (6)–shogaol, which made their antioxidants more potent than the other four long carbon chain compounds (El-Ghovab *et al*; 2010., Mesomo *et al*., 2012, Oboh *et al*., 2012; Guo *et al*., 2014; Offei-Oknye *et al*., 2015). Garlic (*Allium sativum*) has inhibitory activity on the growth of fungi *invitro*. The antimicrobial activity of garlic is believed

to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase and ajoene (Yoshida, 1987 and Ankri *et al.,* 1999). Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi.

The possible mechanism of action of extracts components on the growth of fungi was reported in several studies. It is generally accepted that the extracts components act on the functionality and the structure of the cell membrane. Low concentrations result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane, whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death (Canson *et al*; 2002 and Viuda-Marthos *et al.* 2008). Conner and Beuchat, (1984) suggested that the antifungal activity is the product of extracts components' interaction with enzymes responsible for energy production and the synthesis of structural compounds of the cell. On the other hand, Omidbeygi *et al*; (2007) suggested that the extracts components pass through the cell membrane, integrating with enzymes and proteins of membranes, causing loss of macromolecules from the interior of the cell, leading to changes in the cell and ultimately to its death. Cristani *et al*; 2007 reported that the antifungal activity of terpene relates to their ability to act not only on the permeability, but also on other functions of cell membranes. These components can pass through the cell membrane and interact with intracellular structures. Daferera *et al*; (2000) reported that fungitoxic effect of extracts could results to hydrogen bonds formation between hydroxyl groups of phenolic compounds and active sites of cellular enzymes.

4.5 CONCLUSION

Based on the results of this study it can be concluded that the three extracts at 30mg/l (Tumeric *Curcuma longa*, Ginger *Zingiber officinale* and Garlic *Allium sativum*) in the order

listed were effective in controlling the growth of *Aspergillus parasiticus* The plants extracts had a reducing effect on mycelial dry weight, indicating whatever the concentration of the plant extracts increases not only the mycelia growth is prevented but also the ability of aflatoxin production. These extracts must be subjected to further study to characterize the active compound define toxicity and evaluate economic feasibility.

CHAPTER FIVE

EXPERIMENT 3

EFFICACY OF FEED ADDITIVES TO CONTROL THE TOXIC EFFECTS OF AFLATOXICOSIS IN BROILER CHICKENS

5.1 INTRODUCTION

Poultry industry has gained much attention as one of the important economic activity across the world. Many activities and researches are going to ensure the optimization of profit but despite all of these activities there is a major challenge of contaminants that reduces the

profit margin, decreases the efficiency of feed to gain ratio. The wellnesses of the birds are also hampered by these contaminants which sometimes are retained in vital organs and tissues of the animals. Aflatoxins toxin are produced by *Aspergillus flavus and Aspergillus parasiticus* on foods and feeds. The disease which aflatoxin causes is called aflatoxicosis. The factors influencing occurrence of aflatoxins are certain environmental conditions such as moisture level and temperature among others (Wan *et al*., 2013; Manafi and Khosravinia, 2013).

Aflatoxins are known to have strong hepatotoxic and carcinogenic effects. There are reports on effects of aflatoxins on performance of birds, nutrient retention, full blood count and serum chemistry have been previously reported (Raju and Devegowda 2002; Dersjant-Li *et al*., 2003; Tedesco *et al*., 2004). From previous trail, it was clear that both the level and length of AFB1 exposure affect weight gain of broilers. Next to weight gain, effects on liver and vital organs. Recent data indicates high correlation between outbreaks of Newcastle disease (ND) and aflatoxin contamination in broilers (Yunus *et al*., 2008).

Some feed additives from plant sources have been tested to be antifungal and are also known to be effective in reducing the toxic effects of Aflatoxicosis. The active ingredients found in turmeric are curcumin, demethoxycurcumin, bisdemethoxycurcumin (Wuthi-Udomler *et al*., 2000), and tetrahydrocurcuminoids (Osawa *et al*., 1995). The extracts from turmeric are found to have antifungal (Wuthi-Udomler *et al*., 2000) and antioxidant value (Osawa *et al*., 1995). Some pharmacological activities of turmeric also include nematocidal (Kinchi *et al*., 1993) and anti-inflammatory (Ammon & Wahl, 1991) has been demonstrated. Moreover, Soni *et al*. (1997) proved the protective effects of turmeric as feedd additives on aflatoxin induced mutagenicity and hepatocarcinogenicity. The use of *Zingiber officinale* rhizome has gained attention among

modern health practitioners in recent years (Abo-Ghanema *et al*.,2012). *Z.officinale* has been used as feed additive to improve the health state, performance and productivity of many farm animals (El makki *et al*., 2013).

Aqueous extract of *Zingiber officinale* selectively ameliorated the deviation induced in both kidney and heart of animals administrated AFB1 (Azza and Nadia, 2009). Najafi and Taherpour (2014) reported that using 0.8% ginger could be suggested as effective replacement for virginimycin with respect to feed efficiency and health parameters, adding of ginger rhizome powder at 3% concentration to diet significantly decreased total cholesterol in the serum, Aspartate amino transferase (AST) and Alanin amino transferase (ALT) (Zhang *et al*.2009). Garlic has shown to possess important antioxidant properties through its ability to scavenge free radicals (Borek, 2001), inhibit lipid peroxidation, mutations and oxidative stress (Shaarawy *et al*., 2009). Garlic powder has considerable effects on the production performance of broiler chickens (Pourali *et al*.,2010). The use of garlic as a feed additive in broiler diets has been shown to improve feed conversion ratios and to reduce mortality (Tollba and Hassan,2003) in contrast to other studies showing that garlic paste had no effect on feed intake, body weight gain, or feed efficiency in broiler chickens (Horton *et al*.,1991; Choi *et al*.,2010).

However, the effectiveness of each of the additives is still a subject of research in the situation of Aflatoxicosis in broilers.

The aims of this study were to:

- To determine the activity of feed additives from experiment 2 to evaluate the protective effects of the on performance of broilers fed with infected diets.

- To determine the activity of the feed additives from experiment 2 to evaluate the protective effects of the on broilers organs weight of broilers fed with infected diets.

- To determine the activity of feed additives from experiment 2 to evaluate the protective effects on the hematology and serum biochemistry of broilers fed with infected diets.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL SITE

The research was carried out at University of Ilorin, Ilorin, Nigeria. It is situated in the Guinea Savannah belt. Location: "latitude 8° 20'N and longitude 4° 35'E and is at an approximate altitude of 306 m above sea level".

5.2.2 MICROORGANISMS

The toxigenic strain of *Aspergillus parasiticus* was donated by International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The strain was tested for its capacity to produce Aflatoxin on Carrot-Potato Dextrose agar media (CPDAM).

5.2.3 PREPARATION OF PLANT EXTRACTS

"Plant materials of three plant species belonging to three botanical families (Table 1) included in this study were collected, washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite and dried in shade. The shade-dried material of each plant species was grounded into a powdered material using a blender to pass 100 mm sieve and the mince was sealed in polyethylene bags until extraction. For preparation of aqueous extracts, 50 g of dry powder plant material from each plant species was soaked in sterilized water (10 ml of water/g of plant material) with stirring for 48 h then filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to remove debris and obtain a clear filtrate. This procedure was repeated until the quantity needed was gotten. The filtrates were evaporated and dried under reduced pressure and temperature below 40°C".

Table 5.1: Plants identification

English Name	Botanical Name	Family
Ginger	*Zingiber officinale*	Zingiberaceae
Tumeric	*Curcuma longa*	Zingiberaceae
Garlic	*Alliumsativum*	Amaryllidaceae

5.2.4 EXPERIMENTAL ANIMALS AND DESIGN

One hundred and ninety two, unsexed one-day old commercial broiler chicks was weighed and assigned to an arrangement of two factors of Aflatoxin AF (0 and 500ppb) treated

with best level of screened plant extracts from experiment 2 in a Factorial Design manner, forming a total of 8 dietary treatments each with 3 replicates and 8 birds per replicate.

Table 5.2: Outline of Experimental Diets.

Aflatoxin	Control	Garlic	Ginger	Tumeric
0	+	+	+	+
500ppb	+	+	+	+

5.2.5 AFLATOXIN EXTRACTION AND DETERMINATION

"Rice bran (50 g) were wet-milled with 70% methanol (1:5 v/w) using an electric blender (Waring Commercial, Model-HGBTWTG4, Torrington, Connecticut) for 3 min at high speed. The blended feed was filtered using Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK). To each volume of extract were added 100 mL of distilled water and 25 mL of dichloromethane in a separating funnel. The mixture was mixed and shaken vigorously for about 2 min in the fume chamber. The lower layer of the dichloromethane was withdrawn and allowed to pass through a funnel with a bed of anhydrous sodium sulphate. Again 100 mL of distilled water and 25 mL of dichloromethane were added and the procedure repeated. The extract was allowed to evaporate to dryness in the fume hood. The filtrate was diluted as appropriate, spotted alongside standards of aflatoxin B1, B2, G1, and G2 (Supelco, Bellefonte, Pennsylvania), and

separated on thin layer chromatography plates (silica gel 60, 20 mm) with the development solvent diethyl ether– methanol–water (96:3:1)" Garber and Cotty, 1997. Aflatoxin was quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with winCATS 1.4.2 software).

5.2.6 EXPERIMENTAL DIETS

The experimental diets were prepared by the addition of required quantities of rice bran containing aflatoxin to arrive at the levels of 0 and 500ppb of aflatoxin B1. Basal diet was formulated and compounded to meet the NRC (1995) nutrient requirements of commercial broilers during the trial. Chicks were provided *ad libitum* supply of feed and water throughout the trial. Feeding of test diets commenced at day old of age and continued until the termination of the experiment at five weeks of age.

Table 5.3: Diets Composition Table

	Basal and Plant Extracts				Aflatoxin and Plant Extracts			
Ingredients	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
Basal	100	100	100	100	100	100	100	100
Aflatoxin(500ppb)	-	-	-	-	+	+	+	+
Garlic (3mg/g)	-	+	-	-	-	+	-	-
Ginger (3mg/g)	-	-	+	-	-	-	+	-
Tumeric (3mg/g)	-	-	-	+	-	-	-	+
AnalyzedNutrients								
Crude Protein (%)	23.01							
Crude Fiber (%)	5.65							
Ether Extract (%)	6.23							
Calcium (%)	1.13							
Phosphorus (%)	0.89							
Calculated Energy Kcal/kg	3200.18Kcal							

Basal (%): Maize 50, Wheat Offal 13, Soyabean Meal 20, Full Fat Soya Bean 10, Fish Meal 3, Oyster Shell 1, Bone Meal 2, Methionine 0.25, Lysine 0.25, Salt 0.25, #Broilers Premix 0.25. # (*Vitamin/ mineral premix contained the following: (Univit. 15 Roche) 1500 I.U, Vit A, 1500 I.U, Vit D, 3000 I.U, Vit E, 3.0g, Vit K, Vit. B_2 0.3g, Vit.B_6, 8.0mg, Vit.B_{12}, 8.0g, Nicotinic Acid, 3.0g, Ca-Pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO_2, 0.01g, Se.

5.2.7. EVALUATION PARAMETERS

5.2.7.1. Zoo technical performance:

Birds in each group were weighed on a weekly basis and feed consumption were recorded. Feed Conversion Rate (FCR) and cost benefit analysis.

5.2.7.2. Nutrient Retention:

At the end of third week, nutrient retention was carried out using the total collection method. Weighed qualities of feed were supplied and excreta samples collected over 72hrs period. Excreta samples were oven dried and ground prior to proximate analysis.

5.2.7.3. Proximate Analysis:

The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2000). Samples were oven dried at 70^{o}c, weighed and ground prior to analysis. Crude protein (N*6.25) was determined by the Kjeldahl method after acid digestion. Ether extract was determined by the petroleum ether- extraction method using a Soxhlet apparatus. Crude fibre was determined from the organic residue left after sequential extraction of feed with petroleum ether. Dry Matter was determined by oven drying at 70°C for 24h and drying was continued until a constant weight was achieved

5.2.7.4. Absolute and relative organ weight:

At 5 weeks of age, two birds from each replicate were selected randomly, weighed, slaughtered and the liver, kidney, gizzard, proventriculus and bursa of Fabricus were excised and weighed for determination of relative organ weight expressed on a relative to body weight basis (organ to body weight index) as described by Montgomery *et al*. (1986).

5.2.7.5. Blood Collection:

Two birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial. Blood samples were collected in heparinized (complete blood count) and non-heparinized tubes from six birds in each treatment by puncturing the brachial vein during the 5th week of age. Serum samples were separated after 8 to 10 hours as per the standard procedures and will be stored at –20 ºC for subsequent analysis. The full blood counts were analyzed using the auto Hematology analyzer at the Faculty of Veterinary Medicine, University of Ilorin, Ilorin. The mean corpuscular values– Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) – were calculated using the standard formular (Coles 1986).

5.2.7.6 Serum bio-chemical variables:

At the end of the study (5 weeks of age), serum samples were collected for detection of some bio-chemical parameters. Serum creatinine was determined by the modified Jaffe method (Blass *et al*.1974), Serum Urea Nitrogen was determined by the method of (Chawla, 1999). SerumALP activity was determined by the phenolphthalein monophosphate method (Babson *et al*., 1966). Liver enzymatic activities including Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities by Reitman and Frankle (1957) were measured colorimetrically.

5.2.8 STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance using the factorial design (Steel and Torrie, 1980), using the General Linear Model procedure of the Statistical Analysis System (SAS ®) software (2002). The Duncan multiple range tests were used to compare means (Duncan, 1955).

5.3 RESULTS

Table 5.4 shows effects of dietary inclusion of plant extracts with or without Aflatoxin on performance of broilers (0-5wks). There were significant interactions between dietary inclusion of Aflatoxin with or without Plant extracts additives on performance of broilers ($p<0.05$). The details of the interactions is shown in tables' 5.4a- 5.4c.

Table 5.4: Effects of dietary inclusion of plant extracts with or without Aflatoxin on performance of broilers (0-5wks).

Source of Variation	**Feed Intake (g/bird/day)**	**Weight Gain (g/bird/day)**	**Feed Conversion Ratio**
Aflatoxin (ppb)			
0	39.53	21.66	1.83
500	38.54	21.44	1.80
Types of Additives (3mg/g)			
Control	38.22[b]	18.96[c]	2.02[a]
Garlic	45.67[a]	25.07[a]	1.82[b]

Ginger	39.00[b]	21.40[b]	1.83[b]
Tumeric	33.26[c]	20.75[b]	1.60[c]
Aflatoxin*Types of Additives	S	S	S
SEM±	1.825	1.105	0.074

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 5.4a shows the details of interaction between diet with or without aflatoxin and the three types of plant additives on feed intake. Aflatoxin supplementation reduced the feed intake when compared with the control ($p<0.05$). Additive effect of garlic reduced feed intake for birds on diets with no Aflatoxin supplementation, although an increase for birds with aflatoxin supplementation, the value was statistically comparable for birds on aflatoxin supplementation. Additive effect of Ginger also reduced feed consumption for birds with no aflatoxin supplementation, additive effect of ginger improved the feed consumption for birds on diets supplemented Aflatoxin which was comparable with control diet without aflatoxin supplementation ($p>0.05$). Additive effect of tumeric improved the feed intake for the diets with

or without Aflatoxin supplementation and the value were comparable with the control without aflatoxin supplementation (p>0.05)

Table 5.4a: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on feed intake.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	46.16[a]	33.61[b]	33.67[b]	46.70[a]
500	30.29[b]	32.61[b]	46.34[a]	44.64[a]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005

Table 5.4b shows the details of interaction between diet with or without Aflatoxin and the three types of plant additives on weight gain. Aflatoxin supplementation reduced the weight gain of birds when compared with to birds on diet without aflatoxin supplementation (p<0.05). Additive effect of garlic reduced weight gain for birds on diet without aflatoxin supplementation, although it increased the weight gain of birds on aflatoxin supplementation when compared to birds on control with aflatoxin supplementation (p<0.05). The same trend was observed for diets with ginger with or without Aflatoxin supplementation (p<0.05). Additive effect of tumeric improved the weight gain of birds with or without Aflatoxin supplementation and the values were comparable with the control without Aflatoxin supplementation (p>0.05). Additives effect

of the plant extracts improved the weight gain of birds on diet with Aflatoxin supplementation while it reduced the weight gain of diets without Aflatoxin supplementation ($p>0.05$).

Table 5.4b: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on weight gain.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	24.82^{a}	20.71^{b}	16.63^{c}	24.47^{a}
500	13.10^{d}	20.79^{b}	25.68^{a}	26.17^{a}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.4c shows the details of the interaction between diet with or without Aflatoxin and the three types of plant additives on feed conversion ratio. Aflatoxin supplementation increased the feed conversion ratio for birds when compared with the control to birds that fed on the diet without Aflatoxin ($p<0.05$). Additive effect of garlic increased the feed conversion ratio for birds on diet without Aflatoxin, it was a decrease for birds on garlic and Aflatoxin diet, the value was comparable with control without aflatoxin supplementation ($p<0.05$). The same trend was observed for birds on ginger with or without aflatoxin diet ($p<0.05$). Additive effect of tumeric reduced the feed conversion ratio for the birds that were fed with or without Aflatoxin diets and the values were comparable with the control without aflatoxin supplementation ($p>0.05$). Additives effect of the plant extracts reduced the feed conversion ratio of diets with aflatoxin

supplementation while it increased the feed conversion ratio of diets without aflatoxin supplementation with the exception of Tumeric ($p>0.05$).

Table 5.4c: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on feed conversion ratio

Aflatoxin (ppb)	Types of Additives **(3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	1.87^{b}	1.91^{b}	1.91^{b}	1.62^{c}
500	2.31^{a}	1.74^{cb}	1.77^{cb}	1.58^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different ($p<0.005$)

Table 5.5 shows cost benefit analysis for diet with or without aflatoxin and the three types of plant extracts supplementation. Aflatoxin supplementation increased the cost of raising 1kg of broilers which in return decreased the percentage saving to raise 1kg per broilers ($p<0.05$). Additives effects of plant extracts except tumeric without aflatoxin supplementation increased the cost of raising 1kg of broilers which also decreased the percentage savings for 1kg of broilers ($p<0.05$). Additive effect of turmeric decreased and increased the cost of raising 1kg of broilers and percentage savings respectively ($p<0.05$). Additives effects of plant extracts to diets with aflatoxin supplementation reduced the cost of producing 1kg of broilers and it increased the percentage savings when compared with diets with aflatoxin supplementation ($p<0.05$). Tumeric as an additives gave a comparable higher percentage savings than other plant extracts ($p<0.05$) to raise 1kg of broilers.

Table 5.5: Cost Benefit Analysis of dietary inclusion of plant extracts with or without Aflatoxin.

Source of Variation	**Cost of Feed/kg (N)**	**Cost of Raising 1kg of Broiler (N)**	**Percentage Savings (%)**
0 (ppb)			
Control	179.8^{c}	336.23^{c}	-
Garlic	194.8^{a}	372.07^{b}	-10.65^{c}
Ginger	189.8^{b}	362.52^{b}	-7.81^{c}
Tumeric	189.8^{b}	307.48^{d}	8.55^{a}
500 (ppb)			
Control	179.8^{c}	415.34^{a}	-23.53^{d}
Garlic	194.8^{a}	338.95^{c}	-0.81^{b}
Ginger	189.8^{b}	335.95^{c}	0.10^{b}
Tumeric	189.8^{b}	296.09^{e}	11.94^{a}
SEM±	0.00	14.025	3.801

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

The effects of dietary inclusion of plant extracts with or without Aflatoxin on nutrient retention of Broilers (21-24days) as shown intable 5.4. Ether extract retention were comparable for birds fed diet with or without Aflatoxin supplementation, additive effects of Tumeric extracts improved the ether extract retention ($p<0.05$), additive effects of garlic and ginger were statistically comparable with control ($p>0.05$). There were significant interactions between diets with or without Aflatoxin and dietary additives of plant extracts on dry matter, moisture content, crude protein and crude fiber retentions ($p>0.05$). The details of the interactions is shown in tables 5.4a to 5.4c.

Table 5.6: Effects of dietary inclusion of plant extracts with or without Aflatoxin on nutrient retention of Broilers (21-24days)

Source of Variation	**Dry Matter (%)**	**Crude Protein (%)**	**Crude Fiber (%)**	**Ether Extract (%)**
Aflatoxin (ppb)				
0	62.39[a]	87.24[a]	65.99[a]	53.05
500	60.73[b]	77.78[b]	61.55[b]	52.79
Types of Additives (3mg/g)				
Control	60.74[b]	70.71[c]	49.80[d]	52.68[b]
Garlic	61.34[ab]	84.07[b]	61.12[c]	51.32[b]
Ginger	60.70[b]	88.24[a]	74.10[a]	50.88[b]
Tumeric	63.45[a]	87.02[a]	70.09[b]	56.79[a]
Inclusion*Types of Additives	S	S	S	NS
SEM±	1.897	1.879	2.260	2.187

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 5.6a shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant extracts additives on dry matter retention. Birds on Aflatoxin based diet has a reduced intake of dry matter of when compared with diet without Aflatoxin ($p<0.05$). Additive effects of garlic and ginger deceased the dry matter of the intake of birds when compared with the control without aflatoxin, additive effect of tumeric increased the dry matter intake and had the highest value for dry matter intake for birds. The reverse trend were observed with the additive effect of plant extracts to diet with aflatoxin supplementation ($p<0.05$). Additive effect of garlic exert an increase effect for birds on diet with Aflatoxin supplementation than diet without aflatoxin supplementation ($p<0.05$), the same trend was observed for Ginger, the reverse of ginger and garlic was observed for turmeric ($p<0.05$).

Table 5.6a: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on dry matter of intake.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	63.07^{ab}	57.07^{c}	57.64^{c}	65.14^{a}
500	58.40^{bc}	65.60^{a}	63.77^{a}	61.77^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.6b shows the summary of the interaction of diet with or without Aflatoxin diets and the three types of plant extracts additives on crude protein retention. In the absence of

aflatoxin supplementation, there was an increase in crude protein retention by the birds on control and plant extracts diets ($p<0.05$). Aflatoxin supplementation decreased crude protein retention ($p<0.05$), additives inclusion of the plant extracts increased the crude protein retention, although their values were statistically comparable ($p<0.05$)

Table 5.6b: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on crude protein retention.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	85.44^{ab}	85.58^{ab}	89.55^{a}	88.39^{a}
500	55.97^{c}	82.57^{b}	86.94^{ab}	85.65^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.6c shows the summary of the interaction between Basal and Aflatoxin diets and the three types of plant extracts additives on crude fiber retention. Diet without aflatoxin supplementation had an improved crude fiber retention compared with diet with aflatoxin supplementation ($p<0.005$). Additive effect of plant extracts to diet with Aflatoxin supplementation improved the crude fiber retention, ginger and turmeric exerted a comparable advantage than garlic ($p<0.05$). The same trend was observed for birds on ginger and turmeric diet without Aflatoxin ($p<0.05$), garlic exerted a lower influence, lower to other plant additives and the control ($p<0.05$).

Table 5.6c: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on crude fiber retention.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Tumeric
0	64.94^{cd}	59.87^{d}	72.22^{ab}	66.97^{bc}
500	34.65^{e}	62.37^{cd}	75.97^{a}	73.21^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.7 shows effects of dietary inclusion of plant extracts with or without Aflatoxin supplementation on organs of Broilers expressed as the percentage live body weight (5wks). Aflatoxin supplementation increased the size of gizzard of birds. The addition of plants extracts reduced the organs except for ginger that has a higher value compared to the control ($p<0.05$). There were significant interactions between dietary inclusion of Aflatoxin with or without Plant extracts Supplementation for all the other organs weighed ($p<0.05$). The detail of the interactions is shown in Tables 5.7a - 5.7f.

Table 5.7: Effects of dietary inclusion of plant extracts as additives with or without Aflatoxin on organs of Broilers expressed as percentage body weight (5wks).

	Liver (%)	Kidney (%)	Proventriculus (%)	Gizzard (%)	Lung (%)	Bursa of Fabricius (%)	Intestine (%)
Aflatoxin (ppb)							
0	1.88^{b}	0.61^{b}	0.54^{b}	2.63^{a}	0.46^{a}	0.05^{b}	4.03^{b}
500	2.67^{a}	0.73^{a}	0.70^{a}	2.55^{a}	0.46^{a}	0.10^{a}	5.55^{a}
Types of Additives (3mg/g)							
Control	3.29^{a}	0.69^{a}	0.86^{a}	2.62^{ab}	0.41^{b}	0.09^{a}	6.12^{a}
Garlic	2.06^{b}	0.68^{a}	0.60^{b}	2.64^{ab}	0.46^{ab}	0.09^{a}	4.74^{b}
Ginger	2.01^{b}	0.73^{a}	0.50^{b}	2.70^{a}	0.49^{a}	0.05^{b}	4.45^{b}
Tumeric	1.74^{b}	0.59^{b}	0.52^{b}	2.41^{b}	0.47^{ab}	0.06^{b}	3.86^{c}
Inclusion*Types of Additives	S	S	S	NS	S	S	S
SEM±	0.263	0.060	0.105	0.217	0.051	0.017	0.314

^{abcde}: means in the same column and row with different superscripts are significantly different ($p<0.005$)

Table 5.7a shows the summary of the interaction between diets with or without Afaltoxin and the three types of plant extracts additives on percentage liver weight. Aflatoxin

supplementation increased the percentage weight of liver when compared with the diet with no aflatoxin ($p<0.05$), plant additives reduced the absolute weight of liver ($p<0.05$), their values were comparable. The reverse trend was observed when plant extracts were added as additive to diet without Aflatoxin ($p<0.05$). Tumeric supplementation had the least value, followed by ginger and garlic, the values of the additives were comparable ($p>0.05$).

Table 5.7a: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on liver weight expressed as percentage body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	1.49^{b}	2.20^{b}	2.05^{b}	1.78^{b}
500	5.08^{a}	1.97^{b}	1.97^{b}	1.70^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.7b shows the summary of the interaction between Basal and Aflatoxin diets and the three types of plant extracts additives on kidney weight expressed as percentage body weight. Aflatoxin supplementation increased the percentage of the kidney when compared with the diet with no aflatoxin ($p<0.05$). The plant additives supplementation reduced the percentage weight of kidney in the order of turmeric, garlic and ginger ($p<0.05$). The reversed result was observed when the diets with no afaltoxin were supplemented with three types of plant additives.

Table 5.7b: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on kidney weight expressed as percentage body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**

	Control	Garlic	Ginger	Turmeric
0	0.45^{d}	0.65^{bc}	0.69^{bc}	0.65^{bc}
500	0.92^{a}	0.70^{bc}	0.76^{ab}	0.54^{cd}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005

Table 5.7c shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on proventriculus expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of proventriculus when compared with the diet without aflatoxin ($p<0.05$). The supplementation of plant additives in the diet reduced the absolute weight of proventriculus ($p<0.05$). The same trend was observed for diet with no aflatoxin supplemented with plant additives ($p<0.05$). The result for plant extracts additives with or without aflatoxin were comparable ($p>0.05$).

Table 5.7c: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on proventriculus weight expressed as percentage live body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	0.61^{b}	0.54^{b}	0.51^{b}	0.49^{b}
500	1.12^{a}	0.67^{b}	0.49^{b}	0.54^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($p<0.005$)

Table 5.7d shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant extracts as additives on percentage of lung expressed as percentage

of live body weight. Aflatoxin supplementation increased the percentage of the lung when compared with the diet with no aflatoxin ($p<0.05$). The plant additives supplementation to the diet with aflatoxin had a comparable value statistically ($p>0.05$). Plant additives to the diet with no aflatoxin increased value of the lung, although the three types of plant additives were comparable to each other ($p>0.05$).

Table 5.7d: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on lung weight expressed as percentage live body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	0.35^{b}	0.45^{ab}	0.58^{a}	0.47^{ab}
500	0.47^{ab}	0.47^{ab}	0.42^{ab}	0.47^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.7e shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on Bursa of fabricius weight expressed as percentage body weight. Aflatoxin supplementation increased the percentage of bursa of fabricius when compared with the diet with no aflatoxin ($p<0.05$). The plant additives supplementation reduced the

percentage weight of bursa of fabricius ($p<0.05$). The reverse was observed when the diet with no Afaltoxin were supplemented with three types of plant additives ($p<0.05$).

Table 5.7e Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on absolute Bursa of Fabricius weight expressed as percentage live body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	0.03^{b}	0.04^{b}	0.05^{b}	0.07^{b}
500	0.15^{a}	0.14^{ab}	0.05^{b}	0.05^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.7f shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on percentage intestine weight expressed as live body weight. Aflatoxin supplementation increased the percentage of intestine when compared with the diet with no aflatoxin ($p<0.05$). The plant additives supplementation reduced the percentage of intestine ($p<0.05$) for diets with Aflatoxin. The reverse was observed when the diet with no Afaltoxin was supplemented with three types of plant additives ($p<0.05$). The addition of plant

extracts as additives increased the percentage weight of intestine in this order turmeric, ginger and garlic, thou, the three were statistically comparable (P>0.05).

Table 5.7f: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on percentage intestine weight expressed as live body weight.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	3.67^{c}	4.31^{bc}	4.31^{bc}	3.73^{c}
500	8.57^{a}	5.16^{b}	4.49^{bc}	3.98^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

The effects of dietary inclusion of plant additives with or withoutAflatoxin on complete blood count of Broilers (5 weeks) is shown inTable 5.8. Aflatoxin inclusion had significant decreased effects on RBC, MCV and MCHC (p<0.05). Dietary plant additives Garlic, Ginger and Tumeric increased the volume of RBC and MCHC (p<0.05).The volume per liter for RBC was highest for Tumeric. The values of the plant additives for MCHC were higher when compared with control (p<0.05). There were significant interactions between Aflatoxin inclusion and dietary additives of plant extracts on WBC, Heterophils, Lymphocytes, Monocytes, Hgb, PCV, MCH and Platelets (P>0.05). The details of the interactions is shown in tables 5.8a to 5.8h.

Table 5.8: Effects of dietary inclusion of Aflatoxin with or withoutplant extracts as additives on complete blood count of Broilers (5wks).

	#WBC $*10^9$/L	Heterophils %	Lymphocyte %	Monocyte %	#RBC $*10^{12}$/L	#HGB (g/dl)	#PCV %	#MCV (fl)	#MCH (pg)	#MCHC (g/dl)	Platelet $*10^{12}$/L
Aflatoxin (ppb)											
0	6.49^{b}	29.25^{a}	68.63^{a}	2.13^{b}	8.81^{a}	13.15^{a}	40.00^{a}	47.82^{a}	14.90^{a}	32.67^{a}	178.25^{b}
500	10.69^{a}	22.25^{b}	63.75^{b}	14.00^{a}	6.30^{b}	7.95^{b}	29.88^{b}	45.82^{b}	12.89^{b}	27.03^{b}	266.67^{a}
Types of Additives (3mg/g)											
Control	11.88^{a}	23.25^{c}	60.75^{d}	16.00^{a}	6.09^{d}	8.35^{b}	29.00^{c}	47.90^{ab}	13.84^{ab}	28.98^{b}	258.67^{a}
Garlic	7.85^{b}	28.00^{a}	64.75^{c}	7.25^{b}	7.13^{c}	11.08^{b}	36.50^{ab}	51.66^{a}	15.39^{a}	30.07^{ab}	238.50^{b}
Ginger	7.55^{b}	26.75^{b}	67.75^{b}	5.50^{c}	7.85^{b}	10.33^{b}	35.00^{b}	44.50^{b}	13.05^{b}	29.21^{ab}	208.58^{c}
Turmeric	6.93^{c}	25.00^{bc}	71.50^{a}	3.50^{d}	9.15^{a}	12.45^{a}	39.25^{a}	43.24^{b}	13.31^{b}	31.14^{a}	184.08^{d}
Aflatoxin*Types of Additives	S	S	S	S	NS	S	S	NS	S	NS	S
SEM±	0.386	1.581	1.649	0.984	0.378	0.867	2.404	4.431	1.391	1.633	5.879

#WBC: White Blood Cell, RBC: Red Blood Cell, HGB: Hemoglobin, PCV: Packed Cell Volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

^{abcde}: means in the same column with different superscripts are significantly different ($P<0.005$)

Table 5.8a shows the summary of the interaction between diets with or without and the three types of plant additives on WBC volume. Aflatoxin supplementation increased the volume of WBC when compared with the diet with no aflatoxin ($p<0.05$). The three plant additives reduced the volume of WBC either with or without Aflatoxin ($p<0.05$). The dietary additives of plant extracts on diet with no Aflatoxin were statistically comparable. Diets with Aflatoxin ginger and garlic were statistically comparable while turmeric was lowered when compared to the others. The values of the diets with Aflatoxin for plant extracts as additives were comparable the same ($p>0.05$).

Table 5.8a: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on WBC.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	7.60^{c}	6.35^{d}	6.50^{d}	5.50^{d}
500	16.15^{a}	9.35^{b}	8.60^{bc}	8.35^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.8b shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on heterophils volume. Aflatoxin supplementation reduced the volume of heterophils when compared with the diet with no aflatoxin ($p<0.05$). The three plant additives increased the heterophils volume ($p<0.05$) for Aflatoxin supplemented diets. The reversed trend was observed except for Garlic that is higher when compared to the control for diet with no Aflatoxin ($p<0.05$).

Table 5.8b: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Heterophils.

Aflatoxin (ppb)	Types of Additives (3mg/g)			
	Control	Garlic	Ginger	Turmeric
0	29.50^{a}	31.00^{a}	29.00^{ab}	27.50^{c}
500	17.00^{e}	25.00^{cd}	24.50^{cd}	22.50^{d}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.8c shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on volume of lymphocyte. Aflatoxin supplementation reduced the volume of lymphocyte when compared with the diet with no aflatoxin ($p<0.05$). The plant additives increased the volume of lymphocyte without aflatoxin, turmeric exerted much more effect than ginger and garlic ($p<0.05$). The trend observed in diet without aflatoxin was closely similar to that of diet with Aflatoxin except for garlic additive that was numerically lower than that of other additives and the control; all the values were statistically similar.

Table 5.8c: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Lymphocyte

Aflatoxin (ppb)	Types of Additives (3mg/g)			
	Control	Garlic	Ginger	Turmeric
0	68.00^{b}	67.00^{bc}	69.50^{ab}	70.00^{ab}
500	53.50^{d}	62.50^{c}	66.00^{bc}	73.00^{a}

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.8d shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on volume of monocyte. Aflatoxin supplementation increased the volume of Monocyte when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the volume of monocyte for the diet with Aflatoxin ($p<0.05$). The plant additives combined with diet without aflatoxin were statistically similar to each other ($p>0.05$).

Table 5.8d: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Monocyte.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	2.50^{de}	2.00^{de}	1.50^{e}	2.50^{ed}
500	29.50^{a}	12.50^{b}	9.50^{c}	4.50^{d}

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.8e shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on volume of HGB. Aflatoxin supplementation reduced the volume per liter of HGB when compared with the diet with no aflatoxin ($p<0.05$). The plant additives with the diets increased the volume of HGB either with or without Aflatoxin ($p<0.05$). The values of HGB for plant additives were higher across board for diet without aflatoxin while it lower across board for diet with aflatoxin, although all the values were statistically comparable ($p>0.05$).

Table 5.8e: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Hemoglobin

Aflatoxin (ppb)	Types of Additives (3mg/g)			
	Control	Garlic	Ginger	Turmeric
0	10.35^{cd}	13.35^{b}	12.50^{bc}	16.40^{a}
500	6.35^{e}	$8.80d^{e}$	$8.15d^{e}$	$8.50d^{e}$

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.8f shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on volume of PCV. Aflatoxin supplementation reduced the volume of PCV when compared with the diet with no aflatoxin ($p<0.05$). The plant additives increased the volume of PCV either with or without Aflatoxin ($p<0.05$). PCV values were higher for diets without Aflatoxin than for diet with Aflatoxin ($p<0.05$).

Table 5.8f: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on on PCV

Aflatoxin (ppb)	Types of Additives (3mg/g)			
	Control	Garlic	Ginger	Turmeric
0	36.50^{abc}	39.50^{abc}	41.00^{ab}	43.00^{a}
500	21.50^{e}	33.50^{cd}	29.00^{d}	35.50^{bcd}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.8g shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on the volume of MCH. Aflatoxin supplementation increased the volume of MCH when compared with the diet with no aflatoxin ($p<0.05$). The plant additives increased the volume of MCH for diet with no Aflatoxin ($p<0.05$). The reversed trend was observed except for garlic that is higher when compared to the control for diet with Aflatoxin. ($p<0.05$).

Table 5.8g: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on MCH

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	13.61^{bc}	16.43^{a}	13.88^{bc}	15.70^{ab}
500	14.06^{b}	14.36^{b}	12.20^{bc}	10.92^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.8h shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on Platelet volume. Aflatoxin supplementation increased the volume of Platelet when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the Platelet volume for diet with aflatoxin ($p<0.05$). The same trend was observed except for Garlic that is higher when compared to the control for diet with no aflatoxin ($p<0.05$).

Table 5.8h: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Platelet.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric

0	206.00[d]	210.00[cd]	153.50[e]	143.50[e]
500	311.33[a]	267.00[b]	263.67[b]	224.67[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

The effects of dietary inclusion plant additives with or without Aflatoxin on some Serum Biochemical Indices (5 weeks) as shown intable 5.9. There were significant interactions between Aflatoxin inclusion and dietary additives of plant extracts on urea, creatinine, ALT, AST, ALP (p>0.05). The details of the interactions are shown in tables 5.9a to 5.9e.

Table 5.9: Effects of dietary inclusion of plant extracts with or without Afaltoxin on some Serum Biochemical Indices of Broilers (5wks).

	Urea mM/L	Creatinine mg/ml	ALT U/l	AST U/l	ALP U/l
Aflatoxin (ppb)					
0	68.90[b]	0.91[b]	26.78[a]	62.63[b]	114.40[b]
500	88.94[a]	1.38[a]	73.39[a]	91.94[a]	171.50[a]
Types of Additives (3mg/g)					
Control	101.00[a]	2.07[a]	73.55[a]	104.63[a]	230.22[a]
Garlic	78.62[b]	1.02[b]	44.25[b]	73.63[b]	129.53[b]
Ginger	73.26[c]	0.76[b]	39.45[b]	67.96[bc]	109.43[b]
Turmeric	62.80[d]	0.73[b]	43.83[bc]	62.92[c]	102.63[c]

Aflatoxin* Types of Additives	S	S	S	S	S
SEM±	4.060	0.278	3.296	5.650	7.373

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 5.9a shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on urea. Aflatoxin supplementation increased the concentration of Urea when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the Urea concentration for diet with Aflatoxin ($p<0.05$). Tumeric had the lowest value followed by ginger and then garlic ($p<0.05$). The same trend were observed for diet with no Aflatoxin ($p<0.05$). Tumeric reduced the concentration of urea more than the other two plant extracts additives for the two diets ($p<0.05$).

Table 5.9a: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Urea.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric

0	85.26^{bc}	60.33^{e}	70.06^{d}	59.48^{e}
500	116.74^{a}	96.43^{b}	76.47^{cd}	66.12^{ed}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.9b shows the summary of the interaction between diets with or without and the three types of plant additives on creatinine. Aflatoxin supplementation increased the concentration of creatinine when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the creatinine concentration for diet with Aflatoxin ($p<0.05$). Garlic had the lowest value, thou, ginger and tumeric had comparable statistically value with garlic ($p<0.05$). The same trend were observed for diet with no Aflatoxin ($p<0.05$). Tumeric reduced the concentration of creatinine more than the other two plant extracts additives for diet with Aflatoxin, thou, it was significant comparable with ginger ($p<0.05$). For diets with aflatoxin, all the plant additives extract had values that were statistically comparable ($p>0.05$).

Table 5.9b: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on Creatinine.

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	1.45^{b}	0.60^{c}	0.68^{bc}	0.90^{c}
500	2.69^{a}	1.44^{b}	0.77^{bc}	0.63^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 5.9c shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on ALT. Aflatoxin supplementation increased the concentration of ALT when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the ALT concentration for diet with Aflatoxin ($p<0.05$). Ginger had the lowest value, thou, garlic and tumeric had comparable statistically value with ginger ($p<0.05$). The same trend was observed for diet with no Aflatoxin ($p<0.05$). Tumeric reduced the concentration of ALT more than the other two plant extracts additives and the values were statistically comparable for diet with Aflatoxin ($p<0.05$).

Table 5.9c: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on ALT

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	81.00^{d}	64.25^{cd}	52.25^{d}	53.00^{d}
500	128.25^{a}	83.00^{b}	83.67^{b}	72.83^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.9d shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on AST. Aflatoxin supplementation increased the concentration of AST when compared with the diet with no aflatoxin ($p<0.05$). The plant additives reduced the AST concentration for diet with Aflatoxin ($p<0.05$). Tumeric had the lowest value, thou, garlic and ginger had comparable statistically value with tumeric ($p>0.05$).

The same trend was observed for diet with no Aflatoxin ($p<0.05$). Tumeric reduced the concentration of urea, although statistically comparable with ginger and garlic ($p>0.05$).

Table 5.9d: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on AST

Aflatoxin (ppb)	**Types of Additives (3mg/g)**			
	Control	Garlic	Ginger	Turmeric
0	166.61^{b}	106.95^{cd}	95.03^{d}	89.01^{d}
500	293.83^{a}	152.11^{b}	123.83^{c}	116.24^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 5.9e shows the summary of the interaction between diets with or without Aflatoxin and the three types of plant additives on ALT. Aflatoxin supplementation increased the concentration of ALT when compared with the diet with no Aflatoxin ($p<0.05$). The plant additives reduced the ALT concentration for diet with Aflatoxin ($p<0.05$). Garlic had the lowest value, the values of ginger and turmeric were higher thou, statistically comparable ($p>0.05$). The same trend was observed for diet with no Aflatoxin ($p<0.05$). The values of the plant additives were statistically comparable ($p>0.05$).

Table 5.9e: Details of interaction between diet with or without Aflatoxin and the three types of plant extracts additives on ALT

Aflatoxin (ppb)	Types of Additives (3mg/g)			
	Control	Garlic	Ginger	Turmeric
0	48.60^{d}	18.00^{c}	20.00^{e}	20.50^{e}
500	98.50^{a}	70.50^{b}	58.90^{c}	66.67^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

5.4 DISSCUSSION

The depression in growth upon feeding aflatoxin could be attributed to reduce protein retention, impaired nutrient absorption and reduced appetite. The results from this trial are in line with earlier reports on the performance depressing effects of Aflatoxin B1 (Sharlin *et al*; 1980; Santurio., 1999; Tedesco *et al*., 2004; Shi *et al*., 2006; Denli *et al*., 2009). Feed consumption was reduced and this is suggestive of reduced appetite during aflatoxicosis, this might be a protective mechanisms adopted by the birds to ingest less aflatoxin or on the other hand it might be due to impaired liver metabolism caused by liver damage as indicated by liver enzymes in this trial (Johri and Majundar, 1990).

The growth promoting effect exerted by turmeric in the diets with and without aflatoxin could be linked to curcumin, the major antioxidant ingredient of turmeric which is known to inhibit the biotransformation of aflatoxin B1 to Aflatoxicol in liver and is also responsible for its antimutagenic and anticarcinogenic action (Chun *et al*., 1999; Rangsaz and Ahangaran, 2001). Garlic exerted both positive and negative effect on diets with and without aflatoxin respectively. The reduction in the feed intake which eventually leads to reduction in weight gain might be attributed to higher doses which might lead to repulsive odour and taste of garlic (Pourali *et al*., 2010). Otunola *et al*. (2010) stated that garlic contained anti nutrient factors (ANFs) up to 4.6% of saponin and 1.2% of flavonoid in dry matter, which is one of the factors causing the depressive effect on production performance of broilers. ANFs such as saponin and falvonoids have therapeutic advantage in a diseased situation and such as be used as pharmacologically active bio-ingredients (Schopke and Hiller, 1990). This might explain the reason why it exerted a positive influence on Aflatoxin supplemented diet. Also, improvement in weight gain might be due the fact that allicin (an antibiotic substance found in garlic), inhibits growth of intestinal

bacteria and inhibit aflatoxins producing fungi resulting in lowering the load of these organisms n the intestine and birds may absorb more nutrients leading to the improvement in weight gain of the birds using rations supplemented with garlic. These findings were in agreement with the research work of (Pourali *et al*., 2010; Fayed *et al*., 2011). Growth performance was not significantly affected by ginger for diet without Aflatoxin supplementation. These results suggest that growth performance of broilers may respond to ginger supplementation in a dose-dependent manner. For the diet with Aflatoxin diet, supplementation with ginger led to increase in body weight due to increase in feed intake, this might be possible because ginger contains volatile oils as zingibaine, zingiberol, gingrol and resin which speed digestion and enhance protein digesting enzymes, these results agree with that obtained by (Demir *et al*., 2003 and Iqbal *et al*., 2011). Apart from ginger exerting its influence as doses dependent, it might be possible the activeness might be disease dependent as explained for garlic.

Aflatoxin supplementation in diet in this current trial increased the moisture content in the feaces. Gary and Richard, (1995) reported that broilers that were provided moldy feed ingredients, consumption of mycotoxins may cause the droppings to be excessively wet. Mycotoxins are known to irritate the digestive tract and to cause marked pathological changes in the kidneys. Ochratoxin, Aflatoxin, Oosporin and Citrinin are mycotoxins known to cause these changes. Such changes can lead to increased water consumption and wet droppings. Plant extracts intervention reduce the moisture content, this might further corroborate that bioactive ingredients in this plants extracts are advantageous to the digestive tract and keeping the integrity of the Kidney. Although, the plant extracts had a negative effect on birds without aflatoxin, overdose of the plant extracts that has been corroborated by previous workers might be reason for the negative feedback instead of the positive impart in the disease condition. The plant

extracts improved the nutrient retention for crude protein and crude fiber, many reasons has been proposed, Lee and coworkers in 2003 and Jang *et al*. (2007) observed that plant extracts such as turmeric, garlic and ginger enhance the activity of digestive enzymes such as trypsin and amylase and also bile salts. Furthermore, it has been reported that plant extracts improve liver and metabolism functions and increase the concentration of the pancreatic digestive enzymes (Al-Kassie, 2009).

Aflatoxin exerted potentiated depressing effect on bursa, liver, kidney, gizzard, proventriculus and intestine weight when fed. Although, Huff and Doerr (1981) did not report any influence of the combined feeding of AF and OA on bursa weight in broilers. The increase weight of bursa of Fabricius in the AF group during five weeks trial might be due to the mild oedema and on prolonged exposure the bursal atrophy. The toxicosis leading to liver enlargement and the hypertrophy of liver to enhance its detoxification role, were attributed for the increase in the liver weight due to Aflatoxin (Swaminathan, 2002). Aflatoxin caused comparatively more hepatic damage than other mycotoxin group which concurred with the findings of Kumar *et al*. (2005). In Aflatoxin fed birds, the change in weight observed in the kidneys, liver, proventriculus, gizzard and intestine concurred with the observations of Kumar and Balachandran (1998). The inflation in the intestine might have resulted in venous congestion in the systemic circulation leading to anoxia and poor supply of nutrients to the vital organs like liver and kidneys, which would have further reduced their function and caused degeneration of their cells.

Feeding the plant extracts in combination with the Aflatoxin was suggestive of beneficial additive effects. Turmeric, garlic and ginger have been confirmed to have anti-inflammatory (Hara-Kudo *et al*., 2004) and many of its therapeutic effects have been confirmed by modern

scientific research. Such effects include antioxidant (Moreira *et al*., 2005), antiinflammatory (Glass and Johnson, 2004, Gutierrez *et al*., 2008, Gutierrez *et al*., 2009), anticarcinogenic and antimicrobial (Juven *et al*., 1994), hepatoprotective (Lopez *et al*., 2005), thrombosuppressive (Škrinjar *et al*., 2000), cardiovascular (i.e., as protection against myocardial infarction) (Škrinjar *et al*., 2000), hypoglycemic (Škrinjar, 2005) and antiarthritic (i.e., as protection against rheumatoid arthritis) (Šarić and Škrinjar, 2008).

Results of hematology revealed that hemoglobin content, red blood cell, and packed cell volume were reduced. This reduction is linked to reduced feed intake, especially reduced protein intake, malnutrition and disturbances in protein metabolism. Aflatoxins inhibit DNA- dependent RNA polymerase and causes impairment of nuclear DNA template function, resulting in general inhibition of protein synthesis (Gelboin *et al*.1966; Yu, 1977). The reduction in the mean values of hematology tissue and the increased value of WBC and increase of its component in the present study indicate the deleterious effects of aflatoxin and the result is in accordance with the studies of Cravens *et al* .2015. The ability of the plant extracts to improve the feed intake and digestibility and synthesis of protein improved the complete blood count when supplemented. The toxicity of aflatoxin was expressed as significant alterations in serum indices. The reason of elevation in hepatic enzyme activities (ALT and AST) in chickens receiving aflatoxin could be the hepatic damage and leakage of enzymes in the bloodstream (Quezada *et al*., 2002; Tedesco *et al*., 2004; Fatemi *et al*., 2006; Hashem & Mohamed, 2009). Although in the present study, the diet with aflatoxin significantly increased serum AST and ALT but this finding is in contrast to the finding of Huff *et al*. (1992) that has reported aflatoxin caused a decrease in the activity of AST. Several previous findings are in accordance with the results obtained in the present study that showed that aflatoxin treatment significantly increased ALT and AST enzyme activities

(Jindal *et al*., 1994; Amer *et al*., 1998; Kubena *et al*., 1998; Allameh *et al*., 2005). In this study, a significant increase in the serum level of uric acid and creatinine was observed in chickens fed diet containing aflatoxin. This finding indicates that aflatoxin can affect renal tissue. This finding and previous finding about the effect of aflatoxin on liver enzyme activity emphasize previous report in the literature in which liver and kidney are the primary target organs in the metabolism of aflatoxin. The plant extracts exerted the protective attributes especially that of their antioxidant properties to mopped up some of the leakages in the serum.

5.5 CONCLUSION

In conclusion, the improvement of some performance indices, digestibility, visceral organs, complete blood count, and some serum biochemical indices showed the ability of these plant extracts in the order (Tumeric, Ginger and Garlic) to diminish the inhibitory effect of aflatoxin in broilers up to 5weeks of age. Findings from this trial showed that only Tumeric exerted a beneficial influence to a diet without Aflatoxin.

CHAPTER SIX

EXPERIMENT 4

EFFICACY OF ATOXIGENIC FUNGI (*Aspergillus spp*) TO CONTROL THE TOXIC EFFECTS OF AFLATOXICOSIS IN BROILER CHICKENS

6.1 INTRODUCTION

Atoxigenic *A. flavus* has been researched to be a potential bio-control agent for management of aflatoxin contamination in grains especially maize. AspergiIlus species occur in nature in varied communities consisting of varied genetic make ups called vegetative compatibility groups. Each differs in their capacity to produce aflatoxins in that, some produce variable amounts of toxins and they are called toxigenic strains whereas others produce no toxins hence atoxigenic or nontoxigenic strains. Tampering with the structures of fungal communities to favor the growth of atoxigenic strains is implicated to result in drastic reduction of aflatoxins because the chief causal agent of contamination has been reduced (Mehl *et al*., 2012).

Aspergillus species as effective bio-control agents operate under the following principles: When application of selected atoxigenic strains at suitable stages in crop development (just before local Aspergillus populations begin to increase), then the community configuration within the production area will shift from aflatoxin producers dominated area to one in which beneficial atoxigenic strains dictate. This leads to reduced crop aflatoxin contamination. This has been termed as shift of strain profile from toxigenic to atoxigenic. Also, atoxigenic strains competitively exclude the toxigenic strains in the field, which gives a carryover advantage in storage. Now that there are fewer toxigenic strains during storage and also the atoxigenic strains dwell in the crop and continue to offer protection until they are used, the atoxigenic strains are selected from the environment through a powerful process like; microbiological procedures,

DNA, and field-based methodologies to ensure that they are environmentally friendly and improved to provide effective, long-lasting, and area-wide reductions in aflatoxins (Mehl *et al*., 2012).

While Atoxigenic *A. flavus* has been developed, there is little or no information on the effectiveness of such Atoxigenic *A. flavus* to reduce Aflatoxicosis in broilers. This experiment focused on the use of Atoxigenic *A. Flavus* in the control of growth of fungi in animal diet and subsequent effect on performance, serum biochemistry and hematology on broiler fed contaminated diet.

6.2. MATERIALS AND METHODS

6.2.1 EXPERIMENTAL SITE

The research was carried out at University of Ilorin, Ilorin, Nigeria. It is situated in the Guinea Savannah belt. Location: “latitude 8° 20’N and longitude 4° 35’E and is at an approximate altitude of 306 m above sea level”.

6.2.2 MICROORGANISMS

The strain of *Aspergillusflavus* 32G-28 and *A. parasiticus* and atoxigenic Aspergillus species were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The strains were tested for its capacity to produce Aflatoxin on Carrot-Potato Dextrose agar media (CPDAM).

6.2.3 INNOCULATION

The toxigenic *Aspergillusflavus*32G-28 and *A. parasiticus*and atoxigenic Aspergillus specieswere cultured on Carrot Potato Dextrose agar for 72 h at 28ºC. A heamatocytometer was used in counting the conidial/ml *A. flavus* and *A. parasiticus*spore suspension made with 1 mL producing 1.022 x 10^7 conidial and 1ml of Aspergillus speciesproducing 2.23 x 10^8 conidial. Rice husk adjusted 12% moisture (100 g) were aseptically inoculated with 5 mL (5 x 10^7) of the spore suspension. The diet with Atoxigenic diet was also inoculated with 5ml (1.12 x 10^9). Diet that has a combination of Atoxigenic and toxigenic had 5ml each aseptically inoculated. The mixture was thoroughly shaken to evenly distribute spores, and incubated for 14 days 20^0C temperature in different ovens. The development of the *A. flavus, A. parasiticus* and Aspergillus specie strains was stopped by sterilizing the cultured rice rusk for 15 min at 121ºC.

6.2.4 AFLATOXIN EXTRACTION AND DETERMINATION

Rice rusk (50 g) were wet-milled with 70% methanol (1:5 v/w) using an electric blender (Waring Commercial, Model-HGBTWTG4, Torrington, Connecticut) for 3 min at high speed. The blended feed was filtered using Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK). To each volume of extract were added 100 mL of distilled water and 25 mL of dichloromethane in a separating funnel. The mixture was mixed and shaken vigorously for about

2 min in the fume chamber. The lower layer of the dichloromethane was withdrawn and allowed to pass through a funnel with a bed of anhydrous sodium sulphate. Again 100 mL of distilled water and 25 mL of dichloromethane were added and the procedure repeated. The extract was allowed to evaporate to dryness in the fume hood. The filtrate was diluted as appropriate, spotted alongside standards of aflatoxin B1, B2, G1, and G2 (Supelco, Bellefonte, Pennsylvania), and separated on thin layer chromatography plates (silica gel 60, 20 mm) with the development solvent diethyl ether– methanol–water (96:3:1) (Garber and Cotty, 1997). Aflatoxin was quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with winCATS 1.4.2 software).

6.2.5 EXPERIMENTAL ANIMALS AND DESIGN

Ninety six (96), unsexed one-day old broiler chicks of commercial strains were housed in electrically heated battery brooder and fed with the experimental diet that met the nutrient requirement for broilers as recommended by NRC, 1994. The diets consisted of a 2 x 2 factorial combination (0 and 500ppb). There were four treatments in all with three replicates and eight chicks per replicates. Water and feed were supplied ad libitum during the trial that lasted for 5 weeks.

Table 6.1: The experimental layout

Treatments	Control	Test diet
0ppm	Basal diet	Basal diet + Atogenic Strain
500ppb	Basal diet	Basal diet + Atogenic Strain

6.2.6 EXPERIMENTAL DIETS

The experimental diets were prepared by the addition of required quantities of rice bran containing aflatoxin to arrive at the levels of 0 and 500ppb of aflatoxin B1. Basal diet was formulated and compounded to meet the NRC (1995) nutrient requirements of commercial broilers during the trial. Chicks were provided *ad libitum* supply of feed and water throughout the trial. Feeding of test diets commenced at day old and continued until the termination of the experiment at five weeks old.

Table 6.1b: Diets composition table

Diets	**(%)**	**(%)**	**(%)**	**(%)**
Basal	100	100	100	100
Toxigenic	-	-	+	+
Atoxigenic	-	+	-	+
AnalyzedNutrients				
Crude Protein (%)			23.01	
Crude Fiber			5.65	
Ether Extract (%)			6.23	
Calcium (%)			1.13	
Phosphorus (%)			0.89	
Energy Kcal			3200.18Kcal	

Basal (%): Maize 50, Wheat Offal 13, Soyabean Meal 20, Full Fat Soya Bean 10, Fish Meal 3, Oyster Shell 1, Bone Meal 2, Methionine 0.25, Lysine 0.25, Salt 0.25, #Broilers Premix 0.25. # (*Vitamin/ mineral premix contained the following: (Univit. 15 Roche) 1500 I.U, Vit A, 1500 I.U, Vit D, 3000 I.U, Vit E, 3.0g, Vit K, Vit. B_2 0.3g, Vit.B_6, 8.0mg, Vit.B_{12}, 8.0g, Nicotinic Acid, 3.0g, Ca-Pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO_2, 0.01g, Se.

6.2.7 EVALUATION PARAMETERS

6.2.7.1 Zoo technical performance:

Birds in each group were weighed on a weekly basis and feed consumption were recorded. Feed Conversion Rate (FCR) and cost benefit analysis were also determined.

6.2.7.2 Nutrient Retention:

At the end of third week, nutrient retention was carried out using the total collection method. Weighed qualities of feed were supplied and excreta samples collected over 72hrs period. Excreta samples were oven dried and ground prior to proximate analysis.

6.2.7.3 Proximate Analysis:

The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2000). Samples were oven dried at 70^{o}c, weighed and ground prior to analysis. Crude protein (N*6.25) was determined by the Kjeldahl method after acid digestion. Ether extract was determined by the petroleum ether- extraction method using a Soxhlet apparatus. Crude fibre was determined from the organic residue left after sequential extraction of feed with petroleum ether. Dry Matter was determined by oven drying at 70°C for 24h and drying was continued until a constant weight was achieved.

6.2.7.4 Absolute and relative organ weight:

At 5 weeks of age, two birds from each replicate were selected randomly, weighed, slaughtered and the liver, kidney, gizzard, proventriculus and bursa of Fabricus were excised and weighed for determination of relative organ weight expressed on a relative to body weight basis (organ to body weight index) as described by Montgomery *et al*. (1986).

6.2.7.5 Blood Collection:

Two birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial. Blood samples were collected in heparinized (complete blood count) and non-heparinized tubes from six birds in each treatment by puncturing the brachial vein during the 5th week of age. Serum samples were separated after 8 to 10 hours as per the standard procedures and were stored at –20 ºC for subsequent analysis. The full blood counts were analyzed using the auto Hematology analyzer at the Faculty of Veterinary Medicine, University of Ilorin, Ilorin. The mean corpuscular values– mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Coles 1986).

6.2.7.6 Serum bio-chemical variables:

At the end of the study (5 weeks of age), serum samples were collected for detection of some bio-chemical parameters. Serum creatinine was determined by the modified Jaffe method (Blass *et al*.1974), Serum Urea Nitrogen was determined by the method of (Chawla, 1999). SerumALP activity was determined by the phenolphthalein monophosphate method (Babson *et al*., 1966). Liver enzymatic activities including Aspartate aminotransferase (AST) and Alanine

aminotransferase (ALT) activities by Reitman and Frankle (1957) were measured colorimetrically.

6.2.8 STATISTICAL ANALYSIS:

Data collected were subjected to analysis of variance using the factorial design (Steel and Torrie, 1980), using the General Linear Model procedure of the Statistical Analysis System (SAS ®) software (2002). The Duncan multiple range tests were used to compare means (Duncan, 1955).

6.3 RESULTS

Table 4.2 shows effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on Performance of Broilers (0-5wks). There were significant interactions between dietary inclusion of Aflatoxin with or without Atoxigenic organisms on Performance of Broilers ($p<0.05$). The details of the interactions are shown in Tables 6.2a- 6.2c.

Table 6.2: The effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on Performance of Broilers (0-5wks)

	Feed Intake (g/bird/day)	Weight Gain (g/bird/day)	FCR
Toxigenic (ppb)			
0	48.70[a]	26.66[a]	2.09[a]
500	39.40[b]	17.73[b]	2.22[b]
Atoxigenic			
Control	38.06[b]	18.80[b]	2.11[a]
Atoxigenic	50.05[a]	27.60[a]	1.81[b]
Toxigenic*Atoxigenic	S	S	S
SEM±	1.562	1.275	0.870

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 6.2a shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for feed intake. In the absence of aflatoxin supplementation, there was an increase in feed intake by the birds in control diets ($p<0.05$). The reverse was the case for the diet with Aflatoxin ($p<0.05$). Supplementation of the diets with Atoxigenic organisms improved the feed intake of the birds however, it was higher for diet without aflatoxin ($p>0.05$).

Table 6.2a: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on feed intake.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	46.16^{b}	51.25^{a}
500	29.95^{c}	48.84^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($p<0.005$)

Table 6.2b shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for weight gain. In the absence of aflatoxin supplementation,

there was an increase in weight gain by the birds in control diets ($p<0.05$). The reverse was the case for the diet with Aflatoxin ($p<0.05$). Supplementation of the diets with Atoxigenic organisms improved the weight gain of the birds however, it was higher for diet without aflatoxin ($p>0.05$).

Table 6.2b: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight gain.

	Atoxigenic	
Toxigenic (ppb)	Control	Atoxigenic
0	24.82^{b}	28.50^{a}
500	12.77^{c}	26.70^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.2c shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for feed conversion ratio. In the absence of aflatoxin supplementation, there was decrease in feed conversion ratio by the birds in control diets ($p<0.05$). The reverse was the case for the diet with Aflatoxin ($p<0.05$). Supplementation of the diets with Atoxigenic organisms improved the feed conversion ratio of the birds and it was lower for diet without aflatoxin ($p>0.05$).

Table 6.2c: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on feed conversion ratio.

	Atoxigenic	
Toxigenic (ppb)	Control	Atoxigenic
0	1.87^{b}	1.80^{c}
500	2.35^{a}	1.83^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.3 shows cost benefit analysis for basal and aflatoxin diets with or without Atoxigenic organisms. Aflatoxin increased the percentage cost price of raising 1kg per broilers numerically and it was statistically significant. Including the Atoxigenic organisms reduced the percentage reduction in raising 1kg of broilers. Atoxigenic organisms inclusion gave a comparable higher percentage savings with or without Aflatoxin ($P<0.05$) to raise 1kg of broilers.

Table 6.3: Cost Benefit Analysis for Basal and Aflatoxin diets with or without Atoxigenic organisms.

	Cost of Feed/kg (N)	Cost of Raising 1kg of Broiler (N)	Percentage (%)
Basal	178.50^{c}	333.80^{b}	-
Basal + Atoxigenic	180.50^{b}	324.90^{c}	2.67
Aflatoxin	180.50^{b}	424.18^{a}	-27.08
Aflatoxin + Atoxigenic	181.00^{a}	331.23^{b}	0.79
SEM±	0.00	12.123	

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

The effects of dietary inclusion of Aflatoxin with or withoutAtoxigenic organisms on nutrient retention of Broilers (21-24days) as shown inTable 6.4. Aflatoxin inclusion had significant increased effects on moisture content while a significant decreased effect on dry matter, crude protein, ether extract and crude fiber ($p<0.05$). Dietary additives of atoxigenic organisms increased the dry mater and decreased the moisture content ($p<0.05$). There were significant interactions between dietary inclusion of Aflatoxin with or without Atoxigenic organisms on nutrient retention of Broilers for Crude Protein, Ether Extract and Crude Fiber ($P>0.05$).The details of the interactions is shown in tables 6.4a to 6.4c.

Table 6.4: The effects of dietary inclusion of Aflatoxin with or withoutAtoxigenic organisms on nutrient retention of Broilers (21-24days).

	Dry Matter	Moisture Content	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)
Toxigenic (ppb)					
0	62.46[a]	37.54[b]	77.67[a]	68.02[a]	63.62[a]
500	58.59[b]	41.41[a]	59.72[b]	47.11[b]	47.01[b]
Atoxigenic					
Control	60.74[b]	39.37[b]	60.04[b]	49.80[b]	57.94[a]
Atoxigenic	63.44[a]	36.56[a]	77.35[a]	65.34[a]	52.68[b]
Interaction	NS	NS	S	S	S
SEM±	2.345	1.435	2.593	2.666	2.173

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.4a shows the summary of the interaction between basal and aflatoxin diets with or without Atoxigenic organisms on crude protein retention. In the absence of aflatoxin

supplementation, there was an increase in crude protein retention, thou, additives effect of Atoxigenic organisms reduced the crude protein retention ($p<0.05$). Aflatoxin supplementation decreased crude protein retention ($p<0.05$), additives effect of atoxigenic organisms improved crude protein retention, although their values were statistically comparable ($p<0.05$).

Table 6.4a: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on crude protein retention.

	Atoxigenic	
Toxigenic (ppb)	Control	Atoxigenic
0	74.11^{b}	61.25^{a}
500	45.97^{c}	58.84^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.4b shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on crude fiber retention. In the absence of aflatoxin supplementation, there was an increase in crude fiber retention with the additives effect of Atoxigenic organisms ($P<0.05$). Aflatoxin supplementation decreased crude fiber retention ($P<0.05$), additives effect of atoxigenic organisms improved crude fiber retention ($P<0.05$).

Table 6.4b: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on crude fiber retention.

	Atoxigenic	
Toxigenic (ppb)	Control	Atoxigenic
0	64.94^{ab}	71.10^{a}

500	34.65[c]	59.58[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.4c shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on ether extract retention. In the absence of aflatoxin supplementation, there was an increase in ether extract retention with the additives effect of Atoxigenic organisms ($p<0.05$). Aflatoxin supplementation decreased ether extract retention ($p<0.05$), additives effect of atoxigenic organisms improved ether extract retention ($p<0.05$).

Table 6.4c: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on ether extract retention.

	Atoxigenic	
Toxigenic (ppb)	Control	Atoxigenic
0	64.13[a]	63.11[a]
500	41.23[c]	52.78[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5 effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on absolute organs of Broilers (5wks). There were significant interactions between dietary inclusion of Aflatoxin with or without Plant extracts Supplementation for all the organs weighed ($p<0.05$). The detail of the interactions is shown in Tables 4.5a - 4.5g.

Table 6.5: The effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on some weight of organs of Broilers expressed as percentage live body weight (5 weeks).

	Liver (%)	Kidney (%)	Proventriculus (%)	Gizzard (%)	Lung (%)	Bursa of Fabricius (%)	Intestine (%)
Toxigenic (ppb)							
0	1.70[b]	1.18[b]	0.59[b]	2.49[b]	0.38[b]	0.10[a]	4.01[b]
500	3.39[a]	1.38[a]	0.79[a]	2.71[a]	0.57[a]	0.05[b]	5.50[a]

Atoxigenic							
Control	3.29[a]	1.69[a]	0.86[a]	2.77[a]	0.53[a]	0.09[a]	6.12[a]
Atoxigenic	1.81[b]	0.88[b]	0.51[b]	2.42[b]	0.43[b]	0.06[a]	3.39[b]
Toxigenic*Atoxigenic	S	S	S	S	S	S	S
SEM±	0.363	0.084	0.130	0.103	0.044	0.023	0.831

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5a shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on liver weight expressed as percentage live body weight. Aflatoxin supplementation increased percentage of liver when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage liver ($p<0.05$) for diet supplemented with Aflatoxin. The reversed was observed when atoxigenic organisms was supplemented with diet with no Aflatoxin ($p<0.05$).

Table 6.5a: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of liver expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	1.49^{b}	1.92^{b}
500	5.08^{a}	1.71^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.5b shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of kidney expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of kidney when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage of kidney ($p<0.05$) for the two diets.

Table 6.5b: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of kidney expressed as percentage live body weight

	Atoxigenic

Toxigenic (ppb)	Control	Atoxigenic
0	1.45[b]	0.92[c]
500	1.92[a]	0.84[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5c shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of proventriculus expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of proventriculus when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage of proventriculus ($p<0.05$) for the two diets though, statistically comparable.

Table 6.5c: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of proventriculus expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	0.61[b]	0.56[b]
500	1.12[a]	0.47[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5d shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of gizzard expressed as percentage live body weight.

Aflatoxin supplementation increased the percentage of gizzard when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage of gizzard ($p<0.05$) for the two diets though, statistically comparable.

Table 6.5d: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of gizzard expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	2.57^{b}	2.41^{b}
500	2.97^{a}	2.44^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.5e shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of lung expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of lung when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage of lung ($p<0.05$) for the diet with Aflatoxin and increased for diet with Aflatoxin ($p<0.05$), although their values were statistically comparable ($p>0.05$).

Table 6.5e: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of lung expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	0.35^{b}	0.42^{b}
500	0.70^{a}	0.44^{b}

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5f shows the summary of the interaction between basal and aflatoxin diets with or without Atoxigenic organisms on weight of Bursa of fabricius expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of Bursa of fabricius when compared with the diet with no aflatoxin ($p<0.05$). Additive effect of Atoxigenic organisms reduced the percentage of Bursa of fabricius ($p<0.05$) for the diet with Aflatoxin and increased for diet with Aflatoxin ($p<0.05$), although their values were statistically comparable ($p>0.05$).

Table 6.5f: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of bursa of fabricius expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	0.03^{b}	0.07^{b}
500	0.15^{a}	0.05^{b}

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.5g shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of intestine expressed as percentage live body weight. Aflatoxin supplementation increased the percentage of intestine when compared with the diet with no aflatoxin ($p<0.05$). Additive effect of Atoxigenic organisms reduced the percentage of intestine ($p<0.05$) for the diet with Aflatoxin and increased for diet with Aflatoxin ($p<0.05$), although their values were statistically comparable ($p>0.05$).

Table 6.5g: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on weight of intestine expressed as percentage live body weight.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	3.67^{b}	4.35^{b}
500	8.57^{a}	2.43^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

The effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on complete blood count of Broilers (5wks)is shown inTable 6.6. Aflatoxin inclusion had significant decreased effect on RBC and an increased effect on MCV ($p<0.05$). Dietary atoxigenic organisms increased the volume of RBC and MCV ($p<0.05$). There were significant interactions between Aflatoxin inclusion with or without Atoxigenic organisms on WBC, Heterophils, Lymphocytes, Monocytes, Hgb, PCV, MCH, MCHC and Platelets ($P>0.05$). The detail of the interactions is shown in tables 6.6a to 6.6h.

Table 6.6: Effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on complete blood count of Broilers (5wks)

	WBC $*10^9$/L	Heterophils %	Lymphocyte %	Monocyte %	RBC $*10^{12}$/L	HGB (g/dl)	PVC %	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet $*10^{12}$/L
Toxigenic (ppb)											
0	6.70^{b}	26.67^{a}	69.33^{a}	4.33^{b}	7.11^{a}	11.48^{a}	33.59^{a}	47.28^{a}	10.04^{b}	34.02^{a}	194.84^{b}
500	11.13^{a}	22.83^{b}	58.67^{b}	18.50^{a}	4.83^{b}	6.61^{b}	23.14^{b}	50.35^{a}	14.39^{a}	28.75^{b}	257.67^{a}
Atoxigenic											
Control	10.51^{a}	24.00^{a}	62.50^{b}	13.67^{a}	$5,42^{b}$	7.43^{b}	25.81^{b}	47.90^{a}	13.84^{b}	28.98^{b}	241.34^{a}
Atoxigenic	7.46^{b}	25.50^{a}	65.50^{a}	9.17^{b}	6.32^{a}	10.66^{a}	30.93^{a}	49.74^{a}	16.60^{a}	$33'79^{a}$	211.17^{b}
Toxigenic*Atoxigenic	S	S	S	S	NS	S	S	NS	S	S	S
SEM±	0.276	1.979	2.363	0.866	0.443	0.369	1.766	6.329	1.481	1.041	5.459

^{abcde}: means in the same column with different superscripts are significantly different (P<0.005)

Table 6.6a shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on White Blood Cell (WBC). Aflatoxin supplementation elevated the volume of WBC per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the volume of WBC ($p<0.05$) for diet supplemented with Aflatoxin. The value of WBC were statistically comparable ($p>0.005$) for diet with or without atoxigenic organisms for diets with no Aflatoxin ($p<0.05$).

Table 6.6a: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on White Blood Cell.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	6.76^{c}	7.03^{c}
500	14.37^{a}	7.88^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.6b shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for Heterophils. Aflatoxin supplementation reduced the percentage of Heterophils in WBC per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms increased the percentage of Heterophils in WBC ($p<0.05$) for diet supplemented with Aflatoxin although, the values were statistically comparable for diet with or without atoxigenic organisms supplemented no Aflatoxin ($p>0.05$).

Table 6.6b: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Heterophils.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	27.33[a]	26.00[a]
500	20.67[b]	25.00[ab]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.6c shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for Lymphocytes. Aflatoxin supplementation reduced the percentage of Lymphocytes in WBC per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms increased the percentage of Lymphocytes in WBC ($p<0.05$) for diet supplemented with Aflatoxin although, the values were statistically comparable for diet with or without atoxigenic organisms supplemented with Aflatoxin ($p>0.05$).

Table 6.6c: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Lymphocytes

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	70.33[a]	68.33[ab]
500	54.67[c]	62.67[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.6d shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Monocyte. Aflatoxin supplementation elevated the percentage of monocyte in the volume of WBC per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms reduced the percentage of monocyte ($p<0.05$) for diet supplemented with Aflatoxin. The value Monocyte was higher for diet with Atoxigenic organisms in the control with no Aflatoxin ($p<0.05$).

Table 6.6d: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Monocyte.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	2.67^{d}	6.00^{c}
500	24.67^{a}	12.33^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.6e shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for Heamoglobin. Aflatoxin supplementation reduced the volume of Heamoglobin per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms increased the volume of heamoglobin ($p<0.05$) for diet supplemented with Aflatoxin. The value heamoglobin was higher for diet with Atoxigenic organisms compared to the control with no Aflatoxin ($p<0.05$).

Table 6.6e: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Heamoglobin.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**

0	9.21[b]	13.75[a]
500	5.65[d]	7.57[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.6f shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for PCV. Aflatoxin supplementation reduced the percentage of PCV per liter of blood when compared with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic organisms increased the percentage of PCV ($p<0.05$) for diet supplemented with Aflatoxin. The value PCV was higher for diet with Atoxigenic organisms although, statistically comparable to the control with no Aflatoxin ($p<0.05$).

Table 6.6f: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Packed Cell Volume (PCV)

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	32.49[a]	34.71[a]

500	19.14[c]	27.14[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.6g shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for MCH. Aflatoxin supplementation increased the percentage of MCH per liter of blood thou, statistically comparable with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic was also comparable with the percentage of MCH ($p<0.05$). The value MCH was higher for diet with Atoxigenic organisms and it was statistically comparable to the control with no Aflatoxin ($p<0.05$).

Table 6.6g: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on MCH

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	13.61[b]	18.48[a]
500	14.06[b]	14.72[ab]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.6h shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for Platelet. Aflatoxin supplementation increased the percentage of Platelet per liter of blood thou, statistically comparable with the diet with no aflatoxin ($p<0.05$), additive effect of Atoxigenic was also comparable with the percentage of platelet ($p<0.05$). The value platelet was higher for diet with Atoxigenic organisms and it was statistically comparable to the control with no Aflatoxin ($p<0.05$).

Table 6.6h: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Platelet

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	183.34[c]	206.33[b]
500	299.33[a]	216.00[b]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

The effect of dietary inclusion of Aflatoxin with or without Atoxigenic organisms in some serum biochemical indices (5 weeks) is shown in Table 6.7. There were significant interactions between Aflatoxin inclusion with or without atoxigenic organisms on Urea, Creatinine, ALT, AST, ALP (P>0.05). The details of the interactions is shown in tables 6.7a to 6.7e.

Table 6.7: Effects of dietary inclusion of Aflatoxin with or without Atoxigenic organisms on some serum biochemical indices of Broilers (5wks)

	Urea mM/L	Creatinine mg/ml	ALT U/l	AST U/l	ALP U/l
Toxigenic (ppb)					
0	69.57[b]	1.01[b]	102.90[b]	159.90[b]	215.25[b]
500	156.86[a]	2.47[a]	198.42[a]	252.71[a]	303.47[a]
Atoxigenic					
Control	128.89[a]	2.15[a]	211.65[a]	276.16[a]	312.61[a]
Atoxigenic	97.54[b]	1.33[b]	89.67[b]	136.45[b]	206.09[b]

Toxigenic*Atoxigenic	S	S	S	S	S
SEM±	2.234	0.348	9.259	17.111	24.287

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 6.7a shows the summary of the interaction between Basal and Aftoxin diets with or without Atoxigenic organisms for Urea. Aflatoxin supplementation increased the concentration of Urea when compared with the diet with no aflatoxin ($p<0.05$). Atoxigenic organisms reduced the Urea concentration for diet with Aflatoxin ($p<0.05$). The same trend were observed for diet with no Aflatoxin ($p<0.05$).

Table 6.7a: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Urea.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	76.30^{c}	62.84^{d}
500	181.47^{a}	132.24^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.7b shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for Creatinine. Aflatoxin supplementation increased the concentration of Creatinine when compared with the diet with no aflatoxin ($p<0.05$). Atoxigenic organisms reduced the Creatinine concentration for diet with Aflatoxin ($p<0.05$). The same trend were observed for diet with no Aflatoxin, although they were statistically comparable ($p<0.05$).

Table 6.7b: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on Creatinine.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	1.17^{bc}	0.84^{c}
500	3.12^{a}	1.83^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.7c shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for ALT. Aflatoxin supplementation increased the concentration of ALT when compared with the diet with no aflatoxin ($p<0.05$). Atoxigenic organisms reduced the ALT concentration for diet with Aflatoxin ($p<0.05$). The same trend were observed for diet with no Aflatoxin. ($p<0.05$).

Table 6.7c: Detail of interactions between Basal and Aflatoxin diets with or withoutAtoxigenic organisms on ALT.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	145.80^{b}	60.00^{d}
500	277.50^{a}	119.33^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 6.7d shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for AST. Aflatoxin supplementation increased the concentration of AST when compared with the diet with no aflatoxin ($p<0.05$). Atoxigenic organisms reduced the AST concentration for diet with Aflatoxin ($p<0.05$). The same trend was observed for diet with no Aflatoxin. ($p<0.05$).

Table 6.7d: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on AST.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	194.40^{b}	180.56^{c}

500	357.92[a]	147.50[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 6.7e shows the summary of the interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms for ALP. Aflatoxin supplementation increased the concentration of ALP when compared with the diet with no aflatoxin ($p<0.05$). Atoxigenic organisms reduced the ALP concentration for diet with Aflatoxin ($p<0.05$). The same trend was observed for diet with no Aflatoxin. ($p<0.05$). Atoxigenic organisms increased the concentration of ALP with toxigenic organisms ($p<0.05$).

Table 6.7e: Details of interaction between Basal and Aflatoxin diets with or without Atoxigenic organisms on ALP.

	Atoxigenic	
Toxigenic (ppb)	**Control**	**Atoxigenic**
0	249.89[b]	180.56[c]
500	375.32[a]	231.63[bc]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

5.4 DISCUSSION

The depression in growth upon feeding aflatoxin could be attributed to reduce protein retention, impaired nutrient absorption and reduced appetite. The results from this trial are in line with earlier reports on the performance depressing effects of Aflatoxin B1 (Sharlin *et al*; 1980; Santurio., 1999; Tedesco *et al*., 2004; Shi *et al*., 2006; Denli *et al*., 2009). Feed consumption was reduced and this is suggestive of reduced appetite during aflatoxicosis, this might be a protective mechanisms adopted by the birds to ingest less aflatoxin or on the other hand it might be due to impaired liver metabolism caused by liver damage as indicated by liver enzymes in this trial (Johri and Majundar, 1990).

Aflatoxin exerted potentiated depressing effect on bursa, liver, kidney, gizzard, proventriculus and intestine weight when fed. Although, Huff and Doerr (1981) did not report any influence of the combined feeding of AF and OA on bursa weight in broilers. The increase weight of bursa of Fabricius in the AF group during five weeks trial might be due to the mild oedema and on prolonged exposure the bursal atrophy. The toxicosis leading to liver

enlargement and the hypertrophy of liver to enhance its detoxification role, were attributed for the increase in the liver weight due to Aflatoxin (Swaminathan, 2002). Aflatoxin caused comparatively more hepatic damage than other mycotoxin group which concurred with the findings of Kumar *et al*. (2005). In Aflatoxin fed birds, the change in weight observed in the kidneys, liver, proventriculus, gizzard and intestine concurred with the observations of Kumar and Balachandran (1998). The inflation in the intestine might have resulted in venous congestion in the systemic circulation leading to anoxia and poor supply of nutrients to the vital organs like liver and kidneys, which would have further reduced their function and caused degeneration of their cells.

Results of hematology revealed that hemoglobin content, red blood cell, and packed cell volume were reduced. This reduction is linked to reduced feed intake, especially reduced protein intake, malnutrition and disturbances in protein metabolism. Aflatoxins inhibit DNA- dependent RNA polymerase and causes impairment of nuclear DNA template function, resulting in general inhibition of protein synthesis (Gelboin *et al*.1966; Yu, 1977). The reduction in the mean values of hematology tissue and the increased value of WBC and increase of its component in the present study indicate the deleterious effects of aflatoxin and the result is in accordance with the studies of Cravens *et al* .2015.

The toxicity of aflatoxin was expressed as significant alterations in serum indices. The reason of elevation in hepatic enzyme activities (ALT and AST) in chickens receiving aflatoxin could be the hepatic damage and leakage of enzymes in the bloodstream (Quezada *et al*., 2002; Tedesco *et al*., 2004; Fatemi *et al*., 2006; Hashem & Mohamed, 2009). Although in the present study, the diet with aflatoxin significantly increased serum AST and ALT but this finding is in

contrast to the finding of Huff *et al*. (1992) that has reported aflatoxin caused a decrease in the activity of AST. Several previous findings are in accordance with the results obtained in the present study that showed that aflatoxin treatment significantly increased ALT and AST enzyme activities (Jindal *et al*., 1994; Amer *et al*., 1998; Kubena *et al*., 1998; Allameh *et al*., 2005). In this study, a significant increase in the serum level of uric acid and creatinine was observed in chickens fed diet containing aflatoxin. This finding indicates that aflatoxin can affect renal tissue.

Atoxigenic organisms were used for bio-control and they exerted a positive influence on the performance, cost benefit, digestibility, reducing toxicity in some organs, hematology and serum biochemistry indices this trial. This present trial was not detailed on the molecular study of the atoxigenic *Aspergillus flavus* used as an additive. The species used for this trial has been detailed by Bandyopadhyay *et al*., (2005) that reported the benefit of a single use of atoxigenic strains that benefitted the treated crop contaminated with Aflatoxin, the implication is that it can competes with toxigenic strains in the ecosystem. Also, the biology of the production of Atoxigenic organisms that reduced Aflatoxin production has been reported by many researchers (Bayman and Cotty, 1991; Leslie, 1993; Grubisha and Cotty, 2010, 2015). The use of atoxigenic isolates of *Aspergillus flavus* as biocontrol agents in the broiler diet is to displace aflatoxigenic fungi (Cotty, 2006; Dorner, 2004, 2009). This biocontrol applications in the diet increase the frequencies of atoxigenic vegetative compatibility groups (VCGs) in the treated diet and in so doing less aflatoxins accumulate in treated feed (Cotty, 2006; Cotty and Jaime-Garcia, 2007; Dorner, 2004; 2009). The bio-control additives provides atoxigenic VCGs with both reproductive and dispersal advantages over resident aflatoxin-producers. After applications in the feed, the atoxigenic VCGs contained in the bio-control formulation produce conidia on the feed

and disperse to other nutrient sources including the target nutrient to create their own founder populations and shift the community composition within the treated area and to some extent in neighbouring area from one dominated by aflatoxin producers to one in which atoxigenic VCGs dominate (Cotty and Mellon, 2006). The overall amount of *Aspergillus flavus* in the environment nor the frequency of feed infection increased as a result of applications of atoxigenic VCGs in biocontrol formulations (Atehnkeng *et al*., 2014; Bock *et al*., 2004; Cotty, 2006; Dorner, 2004, 2009). Attenuating the effect of toxigenic organisms was implicated for the positive and promising obtained from this trial.

6.5 CONCLUSION

Evidences from the performance, heamatology, serum biochemical indices show that Bio-control use of atoxigenic organisms is effective as a decontaminant for aflatoxin and also profitable for diet without Aflatoxin.

CHAPTER SEVEN

EXPERIMENT 5

EFFICACY OF FEED ADDITVES ON BROILERS FED HIGH, MEDIUM AND LOW MOISTURE MAIZE.

7.1 INTRODUCTION

Energy is an expensive component of feedstuffs for poultry, 50-55% of maize are used for the formulation of feeds in Nigeria and some Nations of the world. Australia dispenses about 10 million tonnes of cereal grains yearly for feeding livestocks, 19% of which are used in the poultry meat industry. Energy consumption and utilisation is very paramount in poultry and it is estimated by the amount of energy from the feed metabolised in the animal (Apparent Metabolisable Energy; AME). The AME values of maize, however varies, especially at the time of harvest. “The use of newly harvested maize as energy source in poultry diets is more problematic compared to stored grains, often depressing bird performance and causing wet droppings”. (Meyers Strategy Group Report 1995)

High moisture grains can be an economical feed source due to high grain yields and elimination of the cost for drying grain. Rather than simply a processing system, high moisture grain requires alteration in harvest and storage of the grain but by processing the grain into storage, it can eliminate processing of grain at the time of feeding. Biological and biochemical activities occur only when moisture is present. Hence, for safe storage of grain, both the moisture content of the grain and that of the surrounding air should be reduced and monitored (Jayas and White, 2003). Maize grains, like other stored products, are hygroscopic materials. They consist of a constant amount of dry matter but water content will vary (Devereau *et al*., 2002). Moisture

content plays a significant role in the storage of grain; when grain has more moisture, it heats up and can have mold spoilage (Brewbaker, 2003). As a general expression, the higher the moisture content, the more susceptible the maize grain is to mold and insect deterioration.

Molds growing on maize grains present a great threat, especially through production of secondary metabolites (mycotoxins) (Weinberg *et al*., 2008). Mycotoxins are a chronic problem for maize grown in warm, humid, tropical, and sub-tropical regions (Kaaya and Kyamuhangire, 2006). Molds and fungal infections can result in mycotoxin contamination in all stage from growing, harvesting, storage to processing (Chulze, 2010). The most important mycotoxins that frequently occur in cereal grains are aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone (Pitt, 2000). The two most common and toxic mycotoxin compounds encountered on maize in tropical and subtropical regions are aflatoxins and fumonisins (Krska, 2008).

Nigeria as a country has two types of maize, the early maize dried by sun and the late maize dried by the combinations of sun and dried. The late maize is always dry before harvest. This experiment is to find out if wet maize is safe in Nigeria and if not safe will the intervention that has shown potential in the previous trials be able to make this maize available for use.

The aims of this study are:

- To determine the activity of the mycotoxin binder and absorbents from experiment 2 on High moisture maize (HMM), Medium Moisture Maize (MMM) and Low moisture maize (LMM) used as energy source to evaluate on performance of broilers.

- To determine the activity of the mycotoxin binder and absorbents from experiment 2 on High moisture maize (HMM), Medium Moisture Maize (MMM) and Low moisture maize (LMM) used as energy source to evaluate on organ weights of broilers

- To determine the activity of the mycotoxin binder and absorbents from experiment 2 on High moisture maize (HMM), Medium Moisture Maize (MMM) and Low moisture maize (LMM) used as energy source to evaluate on hematology and serum biochemistry of broilers fed with Aflatoxin.

7.2 MATERIALS AND METHODS

7.2.1 EXPERIMENTAL SITE

The research was carried out at University of Ilorin, Ilorin, Nigeria. It is situated in the Guinea Savannah belt. Location: "latitude 8° 20'N and longitude 4° 35'E and is at an approximate altitude of 306 m above sea level".

7.2.2 SOURCE OF MAIZE

The maize for this trial was sourced from an Agronomy farm in Ilorin. The maize was harvested when the moisture of the maize was 20%. After which it was dried until the moisture was reduced to 15 and 10%.

7.2.3 EXPERIMENTAL ANIMALS AND DESIGN

Two hundred and sixteen, unsexed one-day old commercial broiler chicks were weighed and assigned in an arrangement of 3 x 3 factorial combination of type of maize and treatments. Birds were fed either of High Moisture Maize (HMM), Medium Moisture Maize (MMM) or Low Moisture Maize (LMM), each of this was tested with a control, atoxigenic fungus and Tumeric. Thus, there were nine treatments, each with three replicates cages of eight chicks. Experimental diets and water were supplied ad libitum during the trial period that lasted for five weeks.

Table 7.1: Outlay of Experiment

Moisture level	T1 (Control)	T2 (Atoxigenic fungi)	T3 (Tumeric)
HMM (20%)	+	+	+
MMM (15%)	+	+	+

LMM (10%)	+	+	+

7.2.4 EXPERIMENTAL DIETS

The experimental diets were prepared by the addition of required quantities of Maize. Basal diet was formulated and compounded to meet the NRC (1995) nutrient requirements of commercial broilers during the trial. Feed and water were supplied *ad libitum* throughout the trial. Feeding of test diets commenced at day old of age and continued until the termination of the experiment at five weeks of age.

Table 7.1b: Diets composition table

	DIETS								
Diets	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
Basal	50	50	50	50	50	50	50	50	50
LMM	50	50	50	-	-	-	-	-	-
MMM	-	-	-	50	50	50	-	-	-
HMM	-	-	-	-	-	-	50	50	50
Control	+	-	-	+	-	-	+	-	-
Atoxigenic	-	+	-	-	+	-	-	+	-

Tumeric (3mg/g))	-	-	+	-	-	+	-	-	+
Total	100	100	100	100	100	100	100	100	100
AnalyzedNutrients									
Crude Protein (%)	22.68			21.97			20.10		
Calculated Energy Kcal	2967.09			2967.09			2967.09		
Crude Fiber (%)	5.15			5.04			4.95		
Ether Extract (%)	6.98			6.29			6.13		
Calcium (%)	1.19			1.14			1.12		
Phosphorus (%)	0.90			0.88			0.79		

Basal (%): Wheat Offal 13, Soyabean Meal 20, Full Fat Soya Bean 10, Fish Meal 3, Oyster Shell 1, Bone Meal 2, Methionine 0.25, Lysine 0.25, Salt 0.25, #Broilers Premix 0.25. # (*Vitamin/ mineral premix contained the following: (Univit. 15 Roche) 1500 I.U, Vit A, 1500 I.U, Vit D, 3000 I.U, Vit E, 3.0g, Vit K, Vit. B_2 0.3g, Vit.B_6, 8.0mg, Vit.B_{12}, 8.0g, Nicotinic Acid, 3.0g, Ca-Pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO_2, 0.01g, Se.

7.2.5.EVALUATION PARAMETERS

7.2.5.1. Zoo Technical Performance

Birds in each group were weighed on a weekly basis and feed consumption were recorded. Feed Conversion Rate (FCR) and cost benefit analysis were also determined.

7.2.5.2. Nutrient Retention

At the end of third week, nutrient retention was carried out using the total collection method. Weighed qualities of feed were supplied and excreta samples collected over 72hrs period. Excreta samples were oven dried and ground prior to proximate analysis.

7.2.5.3. Proximate Analysis

The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2000). Samples were oven dried at 70^{o}c, weighed and ground prior to analysis. Crude protein (N x 6.25) was determined by the Kjeldahl method after acid digestion. Ether extract was determined by the petroleum ether- extraction method using a Soxhlet apparatus. Crude fibre was determined from the organic residue left after sequential extraction of feed with petroleum ether. Dry Matter was determined by oven drying at 70°C for 24h and drying was continued until a constant weight was achieved.

7.2.5.4. Absolute and relative organ weight

At 5 weeks of age, two birds from each replicate were selected randomly, weighed, slaughtered and the liver, kidney, gizzard, proventriculus and bursa of Fabricus were excised and weighed for determination of relative organ weight expressed on a relative to body weight basis (organ to body weight index) as described by Montgomery *et al*. (1986).

7.2.5.5. Blood Collection

Two birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial. Blood samples were collected in heparinized (complete blood count) and non-heparinized tubes from six birds in each treatment by puncturing the brachial vein during the 5th week of age. Serum samples were separated after 8 to 10 hours as per the standard procedures and were stored at –20 ºC for subsequent analysis. The full blood counts were analyzed using the auto Hematology analyzer at the Faculty of Veterinary Medicine, University of Ilorin, Ilorin. The mean corpuscular values– mean corpuscular volume (MCV), mean corpuscular haemoglobin

(MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Coles 1986).

7.2.5.6 Serum bio-chemical variables

At the end of the study (5 weeks of age), serum samples were collected for detection of some bio-chemical parameters. Serum creatinine was determined by the modified Jaffe method (Blass *et al*.1974), Serum Urea Nitrogen was determined by the method of (Chawla, 1999). SerumALP activity was determined by the phenolphthalein monophosphate method (Babson *et al*., 1966).Liver enzymatic activities including Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities by Reitman and Frankle (1957) were measured colorimetrically.

7.2.6 STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance using the factorial design (Steel and Torrie, 1980) and the General Linear Model procedure of the Statistical Analysis System (SAS®) software (2002). The Duncan multiple range tests were used to compare means (Duncan, 1955).

7.3 RESULTS

Table 7.1b shows effects of types of maize treated with Atoxigenic organisms and turmeric on mold formation in broiler diet. There was an increase in the formation of mold as the moisture in the maize increased and also with the inclusion of atoxigenic organims. There was reduction in the formation of mold with the inclusion of turmeric in the diets with different moisture level.

Table 7.1b: The effects of types of maize treated with Atoxigenic organisms and Tumeric on mold formation in broiler diet.

	Mold
Low Moisture (LMM)	
Control	--
Atoxigenic	+
Tumeric	--
Medium Moisture (MMM)	
Control	+
Atoxigenic	++
Tumeric	-
High Moisture (HMM)	

Control	+++
Atoxigenic	+++
Tumeric	+

+: presence of mold, -: absence of mold

Table 7.2 shows effects of types of maize treated with Atoxigenic organisms and Tumeric on performance of broilers (0-5wks). There was a significant difference in the types of maize used, the values of LMM and HMM were statistically comparable, thou, lower to MMM ($p<0.05$) for the feed conversion ratio (FCR). Addition of Atoxigenic organisms and Tumeric reduced the feed conversion ratio ($p<0.05$) compared to the control and atoxigenic organisms had lower feed to gain ratio compared to tumeric, the two intervention were better than the control ($p<0.05$). There were significant interactions between types of maize and the treatments on feed intake and weight gain ($p<0.05$). The details of the interactions is shown in tables 7.2a - 7.2b.

Table 7.2: The effects of types of maize treated with Atoxigenic organisms and Tumeric on Performance of Broilers (0-5wks)

	Feed Intake (g/bird/day)	Weight Gain (g/bird/day)	FCR
Types of Maize			
Low Moisture (LMM)	57.54[b]	29.66[b]	1.94[a]
Medium Moisture (MMM)	57.25[b]	30.03[b]	1.91[b]
High Moisture (HMM)	63.23[a]	32.18[a]	1.94[a]
Treatments			
Control	62.69[a]	29.47[b]	2.11[a]
Atoxigenic	59.52[b]	33.12[a]	1.80[c]
Tumeric	55.80[c]	29.27[b]	1.90[b]
Types of Maize*Treatments	S	S	NS
SEM±	1.244	1.275	0.870

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 7.2a shows the summary of the interactions between types of maize treated with atoxigenic organisms and tumeric for feed intake. Feed intake was high for HMM, low for MMM for birds fed the control diet ($p<0.05$), the same trend was observed for diets treated with Tumeric. Birds on atoxigenic treated types of maize had MMM and HMM with increased feed intake, although they are statistically comparable. The value of LMM was statistically lower to MMM and HMM for birds on atoxigenic organisms. There was a decrease in feed intake for LMM with the intervention of feed additives, atoxigenic organisms was lower compared to Tumeric ($p<0.05$). There was an increase in feed intake by birds fed MMM atoxigenic treated compared to control, the birds fed on Tumeric was the lowest compared to the control ($p<0.05$). The reverse of MMM was observed for HMM ($p<0.05$).

Table 7.2a: Details of the interaction between types of maize treated with Atoxigenic organisms and Tumeric on feed intake.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low Moisture	62.73^{b}	51.25^{a}	53.89^{ef}
Medium Moisture	58.72^{cd}	61.53^{cb}	51.48^{f}
High Moisture	66.61^{a}	61.05^{cb}	62.04^{b}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.2b shows the summary of the interactions between types of maize and the treatments on for weight gain. The birds fed the control for the types of maize had the highest feed intake for LMM, followed by HMM although statistically comparable with LMM, MMM

was lowest but statistically comparable with MMM. Treatment with Atoxigenic and Tumeric improved the weight gain as the moisture content increased. There was increase in weight gain for birds fed LMM supplemented with intervention of feed additives, atoxigenic organisms although, the values obtained were lower compared to Tumeric ($p<0.05$). The same trend were observed for MMM and HMM ($p<0.05$).

Table 7.2b: Details of the interaction between types of maize treated with Atoxigenic organisms and Tumeric on weight gain.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low Moisture	30.46^{dc}	31.29^{bc}	27.22^{f}
Medium Moisture	27.87^{ef}	33.92^{a}	28.31^{ef}
High Moisture	29.48^{de}	34.16^{a}	32.89^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.3 shows cost benefit analysis to raise 1kg of broilers for types of maize treated with atoxigenic organisms and tumeric. Increasing the moisture of the maize reduce the cost of producing 1kg of broilers ($p<0.05$). Additive effects of atoxigenic organisms and tumeric reduced the percentage for diets with LMM, although atoxigenic organism with LMM was better than turmeric. The same trend was observed for MMM and HMM.

Table 7.3: Cost Benefit Analysis to raise 1kg of broilers for types of maize treated with Atoxigenic organisms and Tumeric.

Types of Maize	**Treatments**	**Cost of Feed/kg (N)**	**Cost of Raising 1kg of Broiler (N)**	**Percentage (%)**
LMM	Control	178.50^{b}	367.71^{a}	-

LMM	Atoxigenic	180.50[a]	323.10[c]	12.13[cd]
LMM	Tumeric	180.50[a]	357.39[b]	2.81[f]
MMM	Control	171.00[c]	360.81[b]	1.91[f]
MMM	Atoxigenic	173.50[c]	314.04[d]	14.60[c]
MMM	Tumeric	174.50[c]	317.59[d]	13.63[c]
HMM	Control	160.00[d]	344.00[b]	6.89[e]
HMM	Atoxigenic	162.50[d]	290.88[e]	20.89[a]
HMM	Tumeric	163.50[d]	309.02[f]	15.96[b]
SEM±		3.432	4.980	1.675

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 7.4 shows effects of types of maize treated with atoxigenic organisms and tumeric on nutrient retention of broilers (21-24days). Protein retention by birds fed the LMM diet and those fed MMM were comparable ($p<0.05$) and significantly higher than those of birds fed HMM ($p<0.05$). There was significant variation of prptein with the use of additives, the order being atoxigenic turmeric and control ($p<0.05$). There were significant interactions between

types of maize and the treatments on for dry matter, crude fiber and ether extract ($p<0.05$). The detail of the interactions is shown in tables' 7.2a- 7.2c.

Table 7.4: Effects of types of maize treated with atoxigenic organisms and tumeric on nutrient retention of Broilers (21-24 days)

	Dry Matter (%)	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)
Types of Maize				
Low Moisture	71.13[a]	71.12[a]	76.01[a]	88.31[a]
Medium Moisture	67.98[b]	72.00[a]	71.79[b]	75.80[c]

High Moisture	66.47[c]	61.47[b]	69.85[c]	88.31[c]
Treatments				
Control	69.12[a]	62.89[c]	73.57[a]	80.68[b]
Atoxigenic	68.29[b]	72.55[a]	75.03[a]	89.07[a]
Tumeric	68.17[b]	69.14[b]	69.06[b]	79.49[b]
Types of Maize*Treatments	S	NS	S	S
SEM±	0.791	2.580	1.920	2.184

[abcde]: means in the same column with different superscripts are significantly different (P<0.005)

Table 7.4a shows the summary of the interactions between types of maize treated with atoxigenic organisms and tumeric for dry matter in feaces. Dry matter was high for LMM, low for HMM for birds on control, the value of MMM was in between ($p<0.05$), Treatment with atoxigenic organisms increased the dry matter for LMM and MMM but statistically comparable, while that of HMM was lower. Treatment with turmeric on the other hand was statistically comparable for LMM, HMM but lower for MMM ($p>0.05$). Additive effect of Atoxigenic and turmeric reduced the dry matter of feaces for LMM, although the values were statistically comparable ($p<0.05$). Additive effect of atoxigenic organisms increased the dry matter of feaces.

Turmeric additive was statistically comparable with the control of MMM. Tumeric treatment for HMM increased the dry matter of feaces, while Atoxigenic and control for HMM were statistically comparable ($p<0.05$).

Table 7.4a: Details of interactions between types of maize treated with atoxigenic organisms and tumeric for dry matter in feaces.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low Moisture	74.66^{a}	69.96^{b}	68.78^{bc}
Medium Moisture	67.77^{cd}	69.01^{bc}	67.16^{ed}
High Moisture	64.92^{e}	65.88^{ed}	68.56^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.4b shows the summary of the interactions between types of maize treated with atoxigenic organisms and tumeric for crude fiber retention. For the control diet, crude fiber retention were comparable for LMM and MMM ($p<0.05$) and siginificantly higher than HMM ($p<0.05$). Treatment with atoxigenic organisms increased the crude fiber retention for LMM ($p<0.05$) while that of MMM and HMM were statistically comparable ($p>0.05$). For birds fed with turmeric for crude fiber retention on the other hand was statistically comparable for LMM, HMM and MMM ($p<0.05$). Additive effect of Atoxigenic increased the crude fiber retention for LMM ($p<0.05$), while treatment with turmeric reduced the crude fiber retention when compared to control for LMM ($p<0.05$). Treatment of MMM with atoxigenic organisms and turmeric

reduced crude fiber retention for MMM ($p<0.05$) compared to other treatment. There was no significant effect of the additive on crude fiber retention for birds fed HMM ($p<0.05$).

Table 7.4b: Details of interactions between types of maize treated with atoxigenic organisms and tumeric for crude fiber retention.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low Moisture	77.31^{ab}	79.28^{a}	71.45^{de}
Medium Moisture	75.72^{bc}	72.75^{cd}	66.90^{e}
High Moisture	67.67^{de}	73.04^{cd}	68.84^{de}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.4c shows the summary of the interactions between types of maize treated with atoxigenic organisms and tumeric ether extract retention. Ether extract retention were statistically comparable for LMM and HMM, while it was lower for MMM for birds fed the diet control ($p<0.05$). Treatment with atoxigenic organisms increased the ether extract retention for LMM and MMM resulted in comparable ether extract retention ($p>0.05$) which were siginificantly higher than those of birds fed HMM ($p<0.05$). Treatment with turmeric for ether extract retention on the other hand was statistically comparable for LMM and HMM ($p>0.05$), while it was lower for MMM ($p<0.05$). Additive effect of Atoxigenic organisms and tumeric increased the ether extract retention for LMM when compared to the control ($p<0.05$). Additive effect of atoxigenic organisms increased the ether extract retention for MMM ($p<0.05$), turmeric additive reduced the ether extract retention for MMM when compared to the control ($p<0.05$). Tumeric treatment for

HMM increased the ether extract retention, while atoxigenic organisms and control were statistically comparable ($p>0.05$).

Table 7.4c: Details of interactions between types of maize treated with atoxigenic organisms and tumeric for ether extract retention.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low Moisture	87.10^{bc}	91.98^{a}	85.85^{bc}
Medium Moisture	72.34^{d}	91.53^{a}	63.49^{e}
High Moisture	82.55^{c}	83.73^{bc}	89.13^{ab}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.5 shows effects of types of maize treated with Atoxigenic organisms and Tumeric on some absolute organs of Broilers (5weeks). There was no significant effects of types of maize on the bursa of fabricius of broilers ($p>0.05$). There was also no significant effects of the additives on the bursa of fabricius ($p>0.05$). There was no significant interaction between the type of maize and additives on the bursa of fabricius. There were significant interactions between types of maize and the treatments on for liver, kidney, proventriculus, gizzard, lung and intestine ($p<0.05$). The details of the interactions is shown in tables' 7.5a- 7.5f.

Table 7.5: The effects of types of maize treated with Atoxigenic organisms and Tumeric on some organs of Broilers.

	Liver (%)	Kidney (%)	Proventriculus (%)	Gizzard (%)	Lung (%)	Bursa of Fabricius (%)	Intestine (%)
Types of Maize							
Low Moisture (LMM)	2.52^{b}	0.09^{b}	0.57^{b}	3.52^{c}	0.44^{c}	0.05	6.67^{c}
Medium Moisture (MMM)	3.10^{a}	0.11^{b}	0.70^{a}	3.76^{b}	0.53^{b}	0.06	8.52^{b}
High Moisture (HMM)	3.19^{a}	0.20^{a}	0.69^{a}	4.77^{a}	0.75^{a}	0.07	9.10^{a}
Treatments							
Control	2.80^{b}	0.12^{b}	0.65^{b}	4.15^{a}	0.57^{b}	0.08	8.60^{a}
Atoxigenic	3.02^{a}	0.12^{b}	0.60^{c}	3.75^{b}	0.54^{b}	0.06	7.47^{b}
Tumeric	2.98^{a}	0.16^{a}	0.71^{a}	4.15^{a}	0.62^{a}	0.06	8.22^{a}
Types of Maize*Treatments	S	S	S	S	S	NS	S
SEM±	1.105	0.023	0.030	0.217	0.036	0.012	0.398

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.5a shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on liver. The percentage value of liver were statistically for types of maize for birds on control ($p<0.05$). Treatment with atoxigenic organisms increased the absolute weight of liver MMM and HMM, although the values of LMM and MMM were statistically comparable ($p>0.05$) for birds on atoxigenic organisms treatments ($p>0.05$). Treatment with turmeric on liver reduced the value for MMM and HMM ($p<0.05$), while the value for LMM was higher compared with MMM and HMM ($p>0.05$). Additive effect of Atoxigenic organisms reduced the value on liver, while that of turmeric increased the percentage compared to control for birds on LMM ($p<0.05$). The same trend was observed for MMM ($p<0.05$). Additive effect of atoxigenic organisms and turmeric reduced the percentage value of liver weight compared to birds on control for HMM ($p<0.05$).

Table 7.5a: Details interactions between types of maize treated with atoxigenic organisms and turmeric on liver.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	3.16^{b}	2.28^{d}	3.61^{a}
Medium moisture	3.33^{ab}	2.41^{d}	3.21^{b}
High moisture	3.06^{bc}	2.86^{c}	2.49^{d}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.5b shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on Kidney. The percentage value of kidney were statistically

comparable for LMM and MMM, also statistically comparable between MMM and HMM ($p<0.05$). Treatment with atoxigenic organisms increased the percentage of the kidney MMM and HMM when compared with LMM although the values were statistically comparable ($p>0.05$) for birds on atoxigenic organisms treatments ($p>0.05$). Treatment with turmeric for kidney increased the value for MMM, while LMM and HMM were statistically comparable ($p>0.05$). Additive effect of Atoxigenic organisms reduced the percentage of kidney, while that of turmeric increased the percentage compared to control for birds on LMM ($p<0.05$). The same trend was observed for MMM ($p<0.05$). Additive effect of atoxigenic organisms and turmeric reduced the percentage of liver weight compared to birds on control for HMM ($p<0.05$).

Table 7.5b Details interactions between types of maize treated with atoxigenic organisms and turmeric on Kidney.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	0.24^{a}	0.05^{e}	0.07^{e}
Medium moisture	0.21^{ab}	0.10^{cde}	0.17^{bc}
High moisture	0.15^{bc}	0.12^{c}	0.08^{de}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.5c shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on proventriculus. The percentage of proventriculus were statistically comparable for LMM, MMM and HMM for birds on control, also statistically comparable between MMM and HMM ($p<0.05$). Treatment with atoxigenic organisms increased

the percentage value of proventriculus of birds on MMM and HMM when compared with LMM although the values were statistically comparable ($p>0.05$). Treatment with turmeric on proventriculus increased the value for MMM, while LMM and HMM were statistically comparable ($p>0.05$).

Additive effect of Atoxigenic organisms reduced the percentage value of proventriculus, while that of turmeric was comparable to the control for birds on LMM ($p<0.05$). Additive effect of atoxigenic organisms reduced the percentage value of proventriculus while the additive effect of turmeric caused an increase when compared to the control for birds on MMM ($p<0.05$). Additive effect of atoxigenic organisms was statistically comparable to the control for birds on HMM ($p>0.05$), while additive effect of turmeric was statistically lower compared to the control and atoxigenic organisms addition ($p<0.05$).

Table 7.5c: Details interactions between types of maize treated with atoxigenic organisms and turmeric on proventriculus.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	0.67^{bc}	0.49^{e}	0.63^{bc}
Medium moisture	0.72^{b}	0.53^{de}	0.89^{a}
High moisture	0.68^{b}	0.68^{b}	0.59^{cd}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.5d shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on gizzard. The percentage value of gizzard were statistically comparable for LMM, MMM and HMM for birds on control, also statistically comparable between MMM and HMM ($p<0.05$). Treatment with atoxigenic organisms increased the percentage value of gizzard of birds on HMM while that of MMM and LMM were statistically comparable but lower to HMM ($p>0.05$). Treatment with turmeric on gizzard increased the value for MMM, although statistically comparable with birds on LMM, the value of HMM was lower ($p<0.05$).

Additive effect of Atoxigenic organisms and tumeric reduced the perecntage value of gizzard when compared to the control for birds on LMM ($p<0.05$). Additive effect of atoxigenic organisms reduced the percentage value of gizzard while the additive effect of turmeric also reduced the percentage value gizzard, although higher when compared to atoxigenic organisms but lower to the control for birds on MMM ($p<0.05$). Additive effect of atoxigenic organisms was statistically comparable to the control for birds on HMM ($p>0.05$), while additive effect of turmeric was statistically lower compare to the control and atoxigenic organisms addition ($p<0.05$).

Table 7.5d: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Gizzard.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	4.59^{ab}	2.96^{e}	3.71^{cd}

Medium moisture	5.06[a]	3.25[de]	4.13[bc]
High moisture	4.66[ab]	4.35[b]	3.44[de]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.5e shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on lung. The percentage value of lung were statistically comparable for LMM and MMM, while HMM was statistically higher for birds on control ($p<0.05$). Treatment with atoxigenic organisms were statistically comparable for LMM and MMM, while HMM was statistically lower when compared to LMM and MMM ($p<0.05$). Treatment with turmeric on lung increased the value for MMM, followed by HMM while LMM was lower for turmeric treatment ($p<0.05$). Additive effect of Atoxigenic organisms and tumeric reduced the value and were statistically comparable on lung when compared to the control for birds on LMM ($p<0.05$). Additive effect of atoxigenic organisms reduced the percentage value of lung while the additive effect of turmeric also increased the percentage value of lung but lower to the control for birds on MMM ($p<0.05$). Additive effect of atoxigenic organisms and tumeric was statistically lower although atoxigenic organisms addition was lower when compared with turmeric addition, the two were lower when compared to the control for birds on HMM ($p<0.05$).

Table 7.5e Details interactions between types of maize treated with atoxigenic organisms and turmeric on Lung.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	0.71[b]	0.50[cd]	0.41[de]

Medium moisture	0.71^{b}	0.49^{cd}	0.67^{b}
High moisture	0.83^{a}	0.35^{e}	0.52^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.5f shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on intestine. The percentage value of intestine were statistically comparable for MMM and HMM, while HMM was statistically lower for birds on control ($p<0.05$). Treatment with atoxigenic organisms were statistically comparable for LMM and MMM, while HMM was statistically higher when compared to LMM and MMM ($p<0.05$).

Treatment with turmeric on intestine increased the value for LMM and MMM that were statistically comparable, while HMM was lower for turmeric treatment ($p<0.05$). Additive effect of Atoxigenic organisms reduced the percentage value of intestine, turmeric intervention increased the percentage value of the intestine when compared with the control for birds on LMM ($p<0.05$). Additive effect of atoxigenic organisms and tumeric reduced and were comparable for intestine than the control for birds fed with MMM ($p<0.05$). Additive effect of atoxigenic organisms and tumeric were statistically lower and comparable when compared to the contol for birds on HMM ($p<0.05$).

Table 7.5f Details interactions between types of maize treated with atoxigenic organisms and turmeric on absolute weight Intestine.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric

Low moisture	7.93[bc]	5.51[d]	8.97[ab]
Medium moisture	9.48[a]	6.30[d]	8.67[ab]
High moisture	9.88[a]	8.19[bc]	7.72[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.6 shows effects of types of maize treated with Atoxigenic organisms and Tumeric on complete blood count of broilers (5weeks). There were significant difference in the types of maize for Red blood cell (RBC) as the moisture level increased the RBC increased also ($p<0.005$). Addition of additive turmeric increases the RBC while additive inclusion of atoxigenic organisms reduced the RBC when compared to the control ($p<0.005$). MCV was statistically comparable for LMM and MMM, while MMM and HMM were also statistically comparable ($p>0.05$), additive inclusion of atoxigenic organisms and turmeric increased the volume of MCV when compared to the control ($p>0.05$). There were significant interactions between types of maize and the treatments on WBC, Heterophils, Lymphocyte, Monocyte, HGB, PCV, MCH, MCHC and platelets ($p<0.05$). The details of the interactions is shown in tables 7.6a- 7.6i.

Table 7.6: Effects of types of maize treated with Atoxigenic organisms and Tumeric on complete blood count of Broilres

	WBC $*10^9$/L	Heterophils %	Lymphocyte %	Monocyte %	RBC $*10^{12}$/L	HGB (g/dl)	PCV %	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet $* 10^{12}$/L
Types of Maize											
Low Moisture (LMM)	14.34^{a}	28.00^{a}	62.89^{b}	9.00^{a}	7.94^{b}	11.05^{c}	39.65^{b}	50.15^{a}	14.01^{ab}	28.08^{b}	308.38^{a}
Medium Moisture (MMM)	10.19^{b}	29.33^{a}	67.67^{a}	3.00^{b}	8.37^{b}	12.77^{b}	39.37^{b}	47.66^{ab}	15.14^{a}	31.89^{a}	241.86^{b}
High Moisture (HMM)	8.82^{c}	28.44^{a}	68.78^{a}	2.89^{b}	11.84^{a}	16.21^{a}	51.78^{a}	43.99^{b}	13.50^{b}	30.87^{a}	218.49^{c}
Treatments											
Control	8.72^{c}	28.00^{a}	69.22^{a}	2.78^{c}	11.08^{a}	17.55^{a}	49.49^{a}	45.24^{a}	15.93^{a}	35.39^{a}	230.11^{c}
Atoxigenic	10.60^{b}	29.33^{a}	66.00^{a}	4.56^{b}	7.62^{c}	12.78^{b}	44.85^{b}	47.89^{a}	13.49^{b}	28.23^{b}	251.58^{b}
Tumeric	14.03^{a}	28.44^{a}	64.11^{b}	7.56^{a}	9.46^{b}	9.71^{c}	36.46^{c}	48.67^{a}	13.23^{b}	27.21^{b}	287.05^{a}
Types of Maize*Treatments	S	S	S	S	NS	S	S	NS	S	S	S
SEM±	0.505	2.419	2.090	1.171	0.554	1.077	3.152	4.893	1.452	1.409	16.758

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.6a shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on WBC. The volume of the WBC was increasing as the moisture level increased ($p<0.05$). Additive effect of Atoxigenic organisms reduced the volume of WBC, while turmeric reduced the volume compared to atoxigenic organisms, the two were lower compared to the control for birds on LMM diets ($p<0.05$). The same trend was observed for birds on MMM ($p<0.05$). Additive effect of atoxigenic organisms and tumeric was statistically lower and comparable when compared to the contol for birds on HMM ($p<0.05$).

Table 7.6a: Details interactions between types of maize treated with atoxigenic organisms and turmeric on WBC.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	9.88^{cde}	9.14^{de}	7.15^{f}
Medium moisture	12.16^{b}	11.18^{bc}	8.45^{ef}
High moisture	21.00^{a}	10.24^{cd}	10.86^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.6b shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on Heterophils. The volume of the Heterophils were statistically comparable as the moisture level increased ($p>0.05$). Additive effect of Atoxigenic organisms and turmeric were statistically comparable with the control for birds on LMM diets ($p>0.05$). The same trend was observed for birds on MMM ($p<0.05$). Additive effect of atoxigenic organisms and tumeric was statistically higher and comparable while the control and Atoxigenic were also comparable for birds on HMM ($p<0.05$).

Table 7.6b: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Heterophils.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	28.67^{ab}	29.67^{ab}	25.67^{ab}
Medium moisture	30.67^{ab}	29.67^{ab}	27.67^{ab}
High moisture	24.67^{b}	28.67^{ab}	32.00^{a}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.6c shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on lymphocyte. The volume of the lymphocyte were statistically comparable for MMM and HMM, the volume of lymphocyte for LMM was higer when compared with MMM and HMM. Additive effect of Atoxigenic organisms and turmeric increased the volume and were statistically comparable with the control for birds on LMM diets (p>0.05). The same trend was observed for birds on MMM (p<0.05). Additive effect of atoxigenic organisms and tumeric was statistically higher and comparable while the control and Atoxigenic were also comparable for birds on HMM (p<0.05).

Table 7.6c: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Lymphocyte.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	67.67^{ab}	69.00^{ab}	71.00^{a}
Medium moisture	60.67^{c}	67.00^{ab}	70.33^{ab}

High moisture	60.33[c]	67.00[ab]	65.00[bc]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.6d shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on monocyte. The volume of the monocyte were statistically increasing as the moisture level is increasing ($p<0.05$). Additive effect of Atoxigenic organisms and turmeric were statistically comparable with the control for birds on LMM diets ($p>0.05$). Additive effect of Atoxigenic organisms and turmeric reduced the volume of monocyte, although they were statistically comparable ($p<0.05$) for birds on MMM diets. The same trend was observed for birds on HMM ($p<0.05$).

Table 7.6d: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Monocyte.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	3.33[c]	1.67[c]	3.33[c]
Medium moisture	8.33[b]	3.33[c]	2.00[c]
High moisture	15.33[a]	4.00[c]	3.33[c]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.6e shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on HGB. The volume of the HGB was statistically decreasing as the moisture level is increasing ($p<0.05$). Additive effect of Atoxigenic organisms increased the volume while that of turmeric increase but more than previous for the volume of HGB when compared with control for birds on LMM diets ($p>0.05$). Additive effect of Atoxigenic organisms reduced the volume of HGB while that of turmeric increased the volume of HGB when compared to the control ($p<0.05$) for birds on MMM diets. The volume of HGB were statistically comparable for birds on HMM ($p<0.05$).

Table 7.6e: Details interactions between types of maize treated with atoxigenic organisms and turmeric on HGB.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	13.46^{cd}	17.88^{b}	21.32^{a}
Medium moisture	11.44^{de}	10.60^{def}	16.25^{bc}
High moisture	8.26^{f}	9.83^{ef}	11.05^{def}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.6f shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on PCV. The volume of the PCV was statistically decreasing as the moisture level is increasing ($p<0.05$). Additive effect of Atoxigenic organisms decreased the volume while that of turmeric increase the volume of PCV when compared with control for birds on LMM diets ($p>0.05$). Additive effect of Atoxigenic organisms reduced the volume of PCV while that of turmeric increased the volume of PCV when compared to the control ($p<0.05$) for birds on MMM diets. Both additive effect of atoxigenic organisms and turmeric increased the volume and was higher compared to the control for birds on HMM ($p<0.05$).

Table 7.6f: Details interactions between types of maize treated with atoxigenic organisms and turmeric on PCV

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	47.45^{abc}	45.12^{bcd}	55.90^{a}
Medium moisture	43.53^{de}	37.70^{de}	53.30^{ab}
High moisture	27.95^{f}	35.29^{ef}	46.15^{bcd}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.6g shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on MCH. The volume of the MCH were statistically

comparable for the types of maize ($p>0.05$). Additive effect of Atoxigenic organisms increased the MCH, although statistically comparable with turmeric when compared with control for birds on LMM diets ($p>0.05$). Additive effect of Atoxigenic organisms and tumeric were statistically comparable reduced the volume of MCH when compared to the control ($p<0.05$) for birds on MMM diets. Additive inclusion of atoxigenic organisms was statistically comparable and higher to turmeric inclusion when compared to control for birds on HMM ($p<0.05$).

Table 7.6g: Details interactions between types of maize treated with atoxigenic organisms and turmeric on MCH.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	13.61^{bc}	18.48^{a}	15.70^{ab}
Medium moisture	14.36^{abc}	12.22^{bc}	13.88^{bc}
High moisture	14.06^{b}	14.72^{ab}	10.92^{c}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.6h shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on MCHC. The volume of the MCHC were statistically comparable for the types of maize ($p>0.05$). Additive effect of Atoxigenic organisms increased the MCHC, although statistically comparable with turmeric when compared with control for birds on LMM diets ($p>0.05$). Additive effect of Atoxigenic organisms increased MCHC, although comparable to control, turmeric inclusion was statistically higher than the two ($p<0.05$)

for birds on MMM diets. Additive inclusion of atoxigenic organisms and turmeric were statistically comparable and lower to the control for birds on HMM ($p<0.05$).

Table 7.6h: Details interactions between types of maize treated with atoxigenic organisms and turmeric on MCHC

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	28.36^{bc}	38.14^{a}	38.14^{a}
Medium moisture	26.29^{cd}	28.10^{cd}	30.33^{b}
High moisture	29.60^{b}	27.90^{cd}	24.14^{d}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.6i shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on platelet. The volume of the platelet were statistically comparable for LMM and HMM while it was highest for HMM ($p<0.05$). Additive effect of Atoxigenic organisms decreased the platelet, although statistically comparable with control, turmeric was the least when compared with control for birds on LMM diets ($p<0.05$). The same trend was observed ($p<0.05$) for birds on MMM diets. Additive inclusion of atoxigenic organisms and turmeric were statistically comparable and lower to the control for birds on HMM ($p<0.05$).

Table 7.6i: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Platelet

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	267.80^{bc}	235.97^{cd}	186.55^{e}
Medium moisture	292.91^{b}	255.71^{bc}	206.11^{de}
High moisture	364.43^{a}	233.91^{cde}	262.82^{bc}

^{abcde}: means in the same column and row with different superscripts are significantly different (P<0.005)

Table 7.7 shows effects of types of maize treated with Atoxigenic organisms and Tumeric on some biochemical indices of Broilers(5weeks). There were significant difference in the types of maize for AST. LMM and HMM were statistically comparable and lower to MMM ($p<0.005$). Additive addition of turmeric and atoxigenic organisms reduced the concentration AST in the serum ($p<0.005$). There were significant interactions between types of maize and the treatments on for Urea, Creatinine, ALT and ALP ($p<0.05$). The detail of the interactions are shown in tables 7.7a- 7.7d.

Table 7.7: Effects of types of maize treated with Atoxigenic organisms and Tumeric on some serum biochemical indices of Broilers. (5wks).

	Urea mM/L	Creatinine mg/ml	ALT U/l	AST U/l	ALP U/l
Types of Maize					
Low Moisture (LMM)	71.60^{b}	0.84^{b}	29.70^{a}	62.08^{b}	116.88^{a}
Medium Moisture (MMM)	92.74^{a}	1.33^{a}	18.97^{b}	75.25^{a}	117.46^{a}
High Moisture (HMM)	69.11^{c}	0.17^{c}	18.80^{b}	58.25^{b}	111.30^{a}
Treatments					
Control	90.11^{a}	0.80^{b}	28.50^{a}	85.92^{a}	184.58^{a}
Atoxigenic	77.54^{b}	0.45^{c}	19.83^{b}	54.92^{b}	71.13^{c}
Tumeric	65.81^{c}	1.09^{a}	19.13^{b}	54.75^{b}	89.93^{b}
Types of Maize*Treatments	S	S	S	NS	S
SEM±	2.416	0.136	1.085	4.051	7.127

^{abcde}: means in the same column with different superscripts are significantly different (P<0.005)

Table 7.7a shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on urea. The concentration of urea in the serum was high for MMM and HMM which were statistically comparable, although higher to LMM ($p<0.05$). Additive effect of atoxigenic organisms decreased the concentration of urea, although statistically higher to additive intervention with turmeric. The two were lower compared to control for birds on LMM diets ($p<0.05$). The same trend was observed ($p<0.05$) for birds on HMM diets. Birds on MMM diets had an elevated value of urea for atoxigenic organisms, although statistically comparable with the control, turmeric addition was the lowest but also comparable with the control ($p>0.05$).

Table 7.7a: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Urea.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	85.26^{c}	70.06^{d}	59.48^{e}
Medium moisture	94.31^{ab}	96.43^{a}	87.49^{bc}
High moisture	90.74^{abc}	66.12^{de}	50.47^{f}

^{abcde}: means in the same column and row with different superscripts are significantly different ($P<0.005$)

Table 7.7b shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on creatinine. The concentration of creatinine in the serum was high for MMM and HMM which were statistically comparable, although higher to LMM ($p<0.05$). Additive effect of atoxigenic organisms decreased the concentration of urea, although

statistically comparable to additive intervention with turmeric. The two were lower compared to control for birds on LMM diets ($p<0.05$). The same trend was observed ($p<0.05$) for birds on MMM and HMM diets.

Table 7.7b: Details interactions between types of maize treated with atoxigenic organisms and turmeric on Creatinine.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	0.95^{b}	0.68^{d}	0.90^{c}
Medium moisture	1.28^{a}	0.44^{de}	0.46^{e}
High moisture	1.29^{a}	0.23^{e}	0.11^{e}

^{abcde}: means in the same column with different superscripts are significantly different ($P<0.005$)

Table 7.7c shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on ALT. The concentration of ALT in the serum decreased the moisture level increased, although statistically comparable for MMM and HMM ($p<0.05$). Additive effect of atoxigenic organisms and turmeric decreased the concentration of AST, although statistically comparable for birds on LMM diets ($p<0.05$). The values were statistically comparable for birds on MMM and HMM diets ($p>0.05$).

Table 7.7c: Details interactions between types of maize treated with atoxigenic organisms and turmeric on ALT.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric

Low moisture	48.60[a]	20.00[b]	20.50[b]
Medium moisture	18.40[b]	20.50[b]	18.00[b]
High moisture	18.50[b]	19.00[b]	18.90[b]

[abcde]: means in the same column and rows with different superscripts are significantly different (P<0.005)

Table 7.7d shows the summary of the interactions between types of maize treated with atoxigenic organisms and turmeric on ALP. The concentration of ALP in the serum was high for MMM and HMM which were statistically comparable and they were higher when compared to LMM ($p<0.05$). Atoxigenic organisms and turmeric intervention reduced the concentration of ALP and were also statistically comparable for birds on LMM diets ($p>0.05$). Additive effect of atoxigenic organisms and turmeric decreased the concentration of ALP, although statistically atoxigenic organisms were lower compared to additive intervention with turmeric. The two were lower compared to control for birds on MMM diets ($p<0.05$). The same trend was observed ($p<0.05$) for birds HMM diets.

Table 7.7d: Details interactions between types of maize treated with atoxigenic organisms and turmeric on ALP.

	Treatments		
Types of Maize	Control	Atoxigenic	Tumeric
Low moisture	166.61[b]	95.03[c]	89.01[cd]
Medium moisture	193.31[a]	52.11[f]	106.95[c]
High moisture	193.83[a]	66.24[ef]	73.83[de]

[abcde]: means in the same column and row with different superscripts are significantly different (P<0.005)

7.4 DISCUSSION

Results from this trial, there was no indication of reduction in feed intake using high moisture feed in the diet. Birds on HMM had an increased feed intake and this might be suggestive of the moisture diluting the energy content of the maize and birds will always eat to satisfy their energy requirement (Hott *et al*., 2008). Also, the variation in the feed intake as the moisture level increases was suggestive to an increased mold growth. Molds need moisture, time and room temperature to grow (Diekman and Green, 1992). The presence of might be suggestive of mycotoxins especially Aflatoxin which might still be at the tolerance level. When conditions

are optimal for molds to colonize grains in the field or in storage, their first effect is utilization of nutrients for their metabolism and propagation and this results in decreased nutritional value of energy, crude protein, and crude fat (Tindall, 1983).

The use of atoxigenic *Aspergillus flavus* improved the weight gain and the feed to gain ratio, this might be suggestive of the probiotic potential of this organisms (Saleh *et al*, 2011, 2012). Alternatively, it may be related to prebiotic potential of the organism to bring a more balanced microbial population in the gastrointestinal tract of the broilers resulting from making available food for the beneficial organisms such as lactose for lactosbacillus which has an important role in health and performance. Birds do not produce endogenous enzymes such as xylanase and cellulase, which are required for the digestion of soluble non-starch polysaccharides. These enzymes can be produced by spp of *Aspergillus* and yeast and thus digestibility might have been improved (Thongsong *et al*., 2008). The use of atoxigenic isolates of *Aspergillus flavus* as biocontrol agents in the diet was to displace aflatoxigenic fungi. These biocontrol applications in the diet increase the frequencies of atoxigenic VCGs in the treated diet to lessen aflatoxins in treated feed (Cotty, 2006; Cotty and Jaime-Garcia, 2007; Dorner, 2004; 2009). Beneficial effects of turmeric in this study was exerted via the moisture level of the diet, birds fed diet with high moisture had the highest feed intake and weight gain. The observation agreed with previous assertion that turmeric stimulate appetite and increase feed intake, activation of immune responses and antibacterial, antiviral and antioxidant actions (Toghyani *et al*., 2010 and 2011). Additionally, antimycotic function of turmeric was suggestive to the reason while it served as mold inhibitor in the trial (Polasa *et al*., 1991 and Anwarul *et al*., 2006).

Wet litter as observed from this trial poses is a threat to chickens and one of the many key contributing factors is increased moisture of the diet (Dunlop *et al*., 2016). It is suggestive that

mycotoxins like Aflatoxin have neprotoxic potential (Hoer, 2008 and Collett, 2012). In this study, contamination of feed by molds is suggestive and where there are molds co-occurrence of several mycotoxins is common as most fungi have the ability to produce several mycotoxins and as commodities may be infected by several fungi (Streit *et al*.2012). This could potentially lead to synergistic or additive effects (Streit *et al*. 2012). Effect of turmeric was able to reduce the moisture in the feaces as suggestive by the dry matter because of its function as mold inhibitor (Polasa *et al*.1991 and Anwarul *et al*.,2006).

The additive inclusion of atoxigenic organisms and turmeric in this trial were suggestive of the protective potential of the duo on the vital organs. Mold will always impair metabolisms by enlarging the liver, proventriculus, intestine and kidney (Kumar and Balachandran, 1998; Swaminathan, 2002).

Results of hematology revealed that hemoglobin content, red blood cell, and packed cell volume were reduced for dietary increase in moisture of maize. This reduction is linked to partial reduced feed intake, especially reduced protein intake and disturbances in protein metabolism. The reduction in the mean values of hematology tissue and the increased value of WBC and increase of its component in this study indicate the deleterious effects of increased moisture of the maize which aided mold growth and the result is in accordance with the studies of Cravens *et al* .2015.

The toxicity of increased moisture of diet was expressed as significant alterations in serum indices. The reason of elevation in hepatic enzyme activities (ALT and AST) in chickens receiving high moisture diets could be the hepatic damage and leakage of enzymes in the bloodstream. Similar data have been reported previously (Fatemi *et al*., 2006; Hashem &

Mohamed, 2009; Quezada *et al*., 2002). These results also agree with the reports of earlier studies (Amer *et al*., 1998; Jindal *et al*., 1994) which used moldy dietary aflatoxin in broiler. It seems that hepatobiliary hyperplasia results in a significant increase of ALT (Zaky *et al*., 1998). Although in previous studies Allameh *et al*., (2005) and Kubena *et al*., (1998) expressed ALT and AST activities were considered as sensitive indicators of liver lesions, it seems that the level of these enzymes in serum mostly varied in aflatoxin-treated chickens and maybe found reduction or elevation ALT or AST in experimental aflatoxicosis. In this study, a significant increase in the serum level of uric acid and creatinine were observed in chickens fed diet containing high moisture. This finding indicates that high moisture diet can affect renal tissue. Tumeric exerted its protective attributes especially that of its antioxidant properties to mop up some of the leakages in the serum.

7.5 CONCLUSION

High moisture maize reduced the cost of raising 1kg of broilers.Results from Hematology and Serum biochemical indices were suggestive to feeding high moisture maize might be detrimental to the wellness of broilers.

Additive effect of atoxigenic organisms and turmeric improved the wellness of the birds, performance and also reduce the cost of raising 1kg of broilers.

CHAPTER EIGHT

8.1 GENERAL DISCUSSION

Aflatoxicosis is mostly caused by species of *Aspergillus*. Aflatoxins are field, processing and storage mycotoxins. *Aspergillus spp* thrive favorably under humid conditions and ambient temperature. It is for this reason that aflatoxins are limited to tropical climates and are mostly found in grain, legumes and cereals (Hell *et al*., 2000). *Aspergillus* may invade plant species such as maize during growth and may remain on cereal grains even after the maize is harvested. Such infected maize when used to formulate poultry diet contaminate the whole diet, although some ingredients in the diets are also susceptible to Aflatoxin contamination from the source. (Hell *et al*., 2000; Hawkins *et al*., 2005; Turner and Sylla, 2005).

Results from experiment 1 showed that moisture, temperature and *Aspergillus parasiticus* increased the weight gain of feed stored anaerobically in the oven for 14days. As the moisture and temperature levels increased the weight gain decreased. Moisture content at is one of the key factors at whatever inclusion affect the metabolite production in Solid State Fermentation (Archana and Satyanarayana, 1997). Moisture affects the physical properties of any solid substrate by causing swelling of the substrate. (How and Ibrahim, 2004).Fermentation is also implicated; moisture, temperature and storage time and good factors necessary for fermentation of grains and *Aspergillus* biodegradation.

The increase in the weight gain also leads to changes in the nutritional content of the feed. These could be attributed to the ability of *Aspergillus parasiticus* to break down the crude fiber to build up their biomass resulting in increased crude protein, ether extract and reduction in the crude fiber (Duarte *et al*. 2008). The results of proximate composition and analyzed fiber

components in all treatment showed an increase in the protein contents, this was partly due to the ability of the *Aspergillus parasiticus* to liberate protein encapsulated in the cell. Decrease in fiber may be due to the ability of *Aspergillus parasiticus* to break polysaccharides into single sugars which are easily available for utilization by the organisms. (Iyayi and Aderolu., 2004). The results from this research confirm that fermentation improves the nutritional value of substrates (Carvalheiro *et al*., 2007).

Higher moisture contents from this trial resulted in reduction in weight gain, this may be due to oxygen limitation within the substrates which consequently affected the oxygen transfer within the substrate, therefore resulting in poor growth (Ramesh and Lonsane, 1990; Sandhya *et al*., 2005). Temperature has a significant effect in the germination of spores which could determine the success of the Solid State Fermentation system. Therefore, temperature has become one of key parameters in SSF system (How and Ibrahim, 2004; Pang and Ibrahim, 2005). Lower temperature can prevent the increasing of excessive temperature due to the gradient temperature which generating during fermentation (Syarifah *et al*. 2011). The results from this trial showed the feed stored at higher temperature has reduced weight gain when compared to feed stored at lower temperature and this might likely be linked to aerobic mature of molds (Syarifah *et al*. 2011). Decline in spores at higher temperature also can be caused by the denaturation of the organisms or its inactivation at higher temperatures, high-moisture concentrations that exclude adequate oxygen can prevent mold growth. However, in practical situations, molds will grow in wet feeds such as silage or wet byproducts, when oxygen is available.

The entire modified atmosphere tested allowed the production of AFB1, AFB2 and AFG1. High moisture favors the synthesis of AFB1, AFB2 and AFG1 in this trial. Temperature and

moisture are the physical factors necessary for Aflatoxin synthesis (Hesseltine, 1983). Specific nutrients which are available in the broiler feed such as amino acids, vitamins, fatty acids, minerals (especially zinc) and energy source preferably starch and soil nutrients such as zinc are required for aflatoxins formation are also present in the broiler feed (Wyatt, 1991). Aflatoxins yield are much in highly starched ingredients such as animal feed, the diet composition is maize soybeans diets (Diener and Davis, 1968). Aflatoxins production by *Aspergillus parasiticus* is optimum at the temperature range of 15°C and 20°C and 20% moisture in this trial and is limited at the temperature 40°C and moisture of 4%, the limit of aflatoxin production is inline with the report of Lillehoj, 1983 while the optimum synthesis of aflatoxin did not support the work done by (Royes and Yanong, 2002).

Results from experiment two revealed that the highest antifungal activities were exhibited by the plant extracts (*Zingiberofficinale*, *Curcuma longa* and *Allium Sativum*) than the commercial enzyme. The basis of the reaction might be due to the nature and combinations of phytocompounds presents in the crude extracts. At different concetrations of extracts that were added to the fungal cultures in liquid media, a remarkable reduction in mycelia was noticed and which in turn will reduce the aflatoxin concentration (Razzaghi-abyaneh *et al*., 2008; Sindhu *et al*. 2011; Komala *et al*.,2012)

Turmeric, ginger and garlic in this study inhibited mycelia growth and spore formation, there is a positive correlation between spore count and aflatoxin production from the previous study. It has been suggested that the regulation of aflatoxin synthesis and conidiogenesis may be interlinked, since the loss of aflatoxigenic capabilities in the nona-flatoxigenic variant strains of *Aspergillus parasiticus* was correlated with alterations in the conidial morphology (Kale *et al*., 1996). Curcumin is an activie ingredient in turmeric which has been implicated to inhibit

mycelia growth and spore formation (Soni *et al*., 1992 and Gowda *et al*., 2004). The inhibition observed by treatment with Ginger extracts might be linked to volatile essential oils which include zingeberene, curcumene and farnesene and nonvolatile bioactive polyphenol compounds (gingerol, shogaol and their derivatives), which have a high antioxidant activity. This was attributed to the presence of α, β- unsaturated ketones moieties of (6)–shogaol, and the presence of short carbon chains of (6)–gingerol and (6)–shogaol, which made their antioxidants more potent than the other four long carbon chain compounds (El-Ghovab *et al*; 2010., Mesomo *et al*., 2012, Oboh *et al*., 2012; Guo *et al*., 2014; Offei-Oknye *et al*., 2015). Garlic (*Allium sativum*) has inhibitory activity on the growth of fungi *invitro*. The antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase and ajoene (Yoshida, 1987 and Ankri *et al.,* 1999). Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi.

The possible mechanism of action of extracts components on the growth of fungi was reported in several studies. It is generally accepted that the extracts components act on the functionality and the structure of the cell membrane. Low concentrations result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane, whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death (Canson *et al*; 2002 and Viuda-Marthos *et al*. 2008). Conner and Beuchat, (1984) suggested that the antifungal activity is the product of extracts components' interaction with enzymes responsible for energy production and the synthesis of structural compounds of the cell. On the other hand, Omidbeygi *et al*; (2007) suggested that the extracts components pass through the cell membrane, integrating with enzymes and proteins of membranes, causing loss of

macromolecules from the interior of the cell, leading to changes in the cell and ultimately to its death. Cristani *et al*; 2007 reported that the antifungal activity of terpene relates to their ability to act not only on the permeability, but also on other functions of cell membranes. These components can pass through the cell membrane and interact with intracellular structures. Daferera *et al*; (2000) reported that fungitoxic effect of extracts could results to hydrogen bonds formation between hydroxyl groups of phenolic compounds and active sites of cellular enzymes. The decrease in the mycelia weight will reduce aflatoxin production. From experiment one there was a strong positive correlation between the counts of *Aspergillus* and aflatoxin production.

The depression in growth upon feeding aflatoxin in experiment 3 and 4 could be attributed to reduce protein retention, impaired nutrient absorption and reduced appetite. The results from this trial are in line with earlier reports on the performance depressing effects of Aflatoxin B1 (Sharlin *et al*; 1980; Santurio., 1999; Tedesco *et al*., 2004; Shi *et al*., 2006; Denli *et al*., 2009). Feed consumption was reduced and this is suggestive of reduced appetite during aflatoxicosis, this might be a protective mechanisms adopted by the birds to ingest less aflatoxin or on the other hand it might be due to impaired liver metabolism caused by liver damage as indicated by liver enzymes in this trial (Johri and Majundar, 1990). Aflatoxin supplementation in diet in this current trial increased the moisture content in the faeces. Mycotoxins are known to irritate the digestive tract and to cause marked pathological changes in the kidneys and liver. Such changes can lead to increased water consumption and wet droppings (Gary and Richard, 1995).

Aflatoxin exerted depressing effect on bursa, liver, kidney, gizzard, proventriculus and intestine weights when fed (Kumar and Balachandran (1998). Although, Huff and Doerr (1981)

did not report any influence of the combined feeding of aflatoxin and ocratoxin on bursa weight in broilers. The increase weight of bursa of fabricius in the aflatoxin group during five weeks trial might be due to the mild oedema. The toxicosis leading to liver enlargement and the hypertrophy of liver to enhance its detoxification role, were attributed for the increase in the liver weight due to Aflatoxin (Swaminathan, 2002).

Results of haematology revealed that haemoglobin content, red blood cell, and packed cell volume were reduced. This reduction is linked to reduced feed intake, especially reduced protein intake, malnutrition and disturbances in protein metabolism. The reduction in the mean values of hematology tissue and the increased value of WBC and increase of its component in the present study indicate the deleterious effects of aflatoxin (Cravens *et al* .2015).

The toxicity of aflatoxin was expressed as significant alterations in serum indices. The reason of elevation in hepatic enzyme activities (ALT and AST) in chickens receiving aflatoxin could be the hepatic damage and leakage of enzymes in the bloodstream. Similar data have been reported previously (Fatemi *et al*., 2006; Hashem & Mohamed, 2009; Quezada *et al*., 2002). Although in the present study, the diet with aflatoxin significantly increased serum AST and ALT which is in contrast to the finding of Huff *et al*. (1992) that has reported aflatoxin caused a decrease in the activity of AST. Also, Tedesco *et al*. (2004) reported that the serum activity of ALT significantly was lower in aflatoxin treated birds. The finding in this study support the suggestion about the high level of the enzymes after treatment. In this study, a significant increase in the serum level of uric acid and creatinine was observed in chickens fed diet containing aflatoxin. This finding indicates that aflatoxin was nephrotoxic and could affect the effciency of kidney function in the bird.

The growth promoting effect exerted by turmeric in the diets with and without aflatoxin could be linked to curcumin, the major antioxidant ingredient of turmeric which is known to inhibit the biotransformation of Aflatoxin B1 to Aflatoxicol in liver (Chun *et al*., 1999). Garlic exerted both positive and negative effect on diets with and without aflatoxin respectively. The reduction in the feed intake which eventually leads to reduction in weight gain might be attributed to higher doses which might lead to repulsive odour and taste of garlic (Pourali *et al*., 2010; Choi*et al*., 2010 and Stanaćev *et al*., 2011)

Otunola *et al*. (2010) stated that garlic contained anti nutrient factors (ANFs) such as saponin and flavonoid are one of the factors causing the depressive effect on production performance of broilers. ANFs such as saponin and falvonoids have therapeutic advantage in a diseased situation and such as be used as pharmacologically active bio-ingredients (Schopke and Hiller, 1990). This might explain the reason why it exerted a positive influence on Aflatoxin supplemented diet. Also, improvement in weight gain might be due the fact that allicin (an antibiotic substance found in garlic), inhibits growth of intestinal bacteria and inhibit aflatoxins producing fungi resulting in lowering the load of these organisms n the intestine and birds may absorb more nutrients leading to the improvement in weight gain of the birds using rations supplemented with garlic. These findings were in agreement with previous researches of (Pourali *et al*., 2010; Fayed *et al*., 2011).

Growth performance was not significantly affected by ginger for diet without Aflatoxin supplementation. These results suggest that growth performance of broilers may respond to ginger supplementation in a dose-dependent manner. For the diet with Aflatoxin diet, supplementation with ginger led to increase in body weight due to increase in feed intake, this might be possible because ginger contains volatile oils as zingibaine, zingiberol, gingrol and

resin which speed digestion and enhance protein digesting enzymes, these results agree with that obtained by (Demir *et al*., 2003 and Iqbal *et al*., 2011). Apart from ginger exerting its influence as doses dependent, it might be possible the activeness might be disease dependent as explained for garlic.

Plant extracts intervention reduce the moisture content, this might further corroborate that bioactive ingredients in this plants extracts are advantageous to the digestive tract and keeping the integrity of the Kidney. Although, the plant extracts had a negative effect on birds without aflatoxin, overdose of the plant extracts that has been corroborated by previous workers might be reason for the negative feedback instead of the positive impact in the disease condition. The plant extracts improved the nutrient retention for crude protein and crude fiber. Plant extracts such as turmeric, garlic and ginger enhance the activity of digestive enzymes such as trypsin and amylase and also bile salts. Furthermore, it has been reported that plant extracts improve liver and metabolism functions (Lee *et al*., 2003 and Jang *et al*., 2007).

The ability of the plant extracts to improve the feed intake and digestibility and synthesis of protein also will lead to improve the complete blood count when supplemented. This finding and previous finding about the effect of aflatoxin on liver enzyme activity emphasize previous report in the literature in which liver and kidney are the primary target organs in the metabolism of aflatoxin. The plant extracts exerted the protective attributes especially that of their antioxidant properties to mopped up some of the leakages in the serum..

Atoxigenic organisms was used for bio-control and it has exerted a positive influence on the performance, cost benefit, digestibility, reducing toxicity in some organs, hematology and serum

biochemistry indices this trial. Bandyopadhyay *et al*., (2005) reported the benefit of a single use of atoxigenic strains that benefitted the treated crop contaminated with Aflatoxin, the implication is that it can competes with toxigenic strains in the ecosystem. (Bayman and Cotty, 1991; Leslie, 1993; Grubisha and Cotty, 2010, 2015). This bio-control applications in the diet increase the frequencies of atoxigenic VCGs in the treated diet and in so doing less aflatoxins accumulate in treated feed (Cotty, 2006; Cotty and Jaime-Garcia, 2007; Dorner, 2004; 2009). Atoxigenic VCGs are dispersed with a carrier (i.e. wheat, sorghum or barley grain) that also serves as a nutrient source (Cotty and Mellon, 2006). The bio-control additives provides atoxigenic VCGs with both reproductive and dispersal advantages over resident aflatoxin-producers. After applications in the feed, the atoxigenic VCGs contained in the bio-control formulation produce conidia on the feed and disperse to other nutrient sources including the target nutrient. Attenuating the effect of toxigenic organisms was implicated for the positive and promising obtained from this trial.

Result from experiment 5 showed no indication of reduction in feed intake using high moisture maize in the diet. Birds on HMM had an increased feed intake and this might be suggestive of the moisture diluting the energy content of the maize and birds will always eat to satisfy their energy requirement (Hott *et al*., 2008). Also, the variation in the feed intake as the moisture level increases was suggestive to an increased mold growth. Molds need moisture, time and room temperature to grow (Diekman and Green, 1992). The presence of might be suggestive of mycotoxins especially Aflatoxin which might still be at the tolerance level. When conditions are optimal for molds to colonize grains in the field or in storage, their first effect is utilization of nutrients for their metabolism and propagation and this results in decreased nutritional value of energy, crude protein, and crude fat (Tindall, 1983).

The use of atoxigenic *Aspergillus flavus* improved the weight gain and the feed to gain ratio, this might be suggestive of the probiotic potential of this organisms (Saleh *et al*, 2011, 2012). Alternatively, it may be related to prebiotic potential of the organism to bring a more balanced microbial population in the gastrointestinal tract of the broilers resulting from making available food for the beneficial organisms such as lactose for lactosbacillus which has an important role in health and performance. Birds do not produce endogenous enzymes such as xylanase and cellulase, which are required for the digestion of soluble non-starch polysaccharides. These enzymes can be produced by spp of *Aspergillus* and yeast and thus digestibility might have been improved (Thongsong *et al*., 2008). The use of atoxigenic isolates of *Aspergillus flavus* as biocontrol agents in the diet was to displace aflatoxigenic fungi. These biocontrol applications in the diet increase the frequencies of atoxigenic VCGs in the treated diet to lessen aflatoxins in treated feed (Cotty, 2006; Cotty and Jaime-Garcia, 2007; Dorner, 2004; 2009). Beneficial effects of turmeric in this study was exerted via the moisture level of the diet, birds fed diet with high moisture had the highest feed intake and weight gain. The observation agreed with previous assertion that turmeric stimulate appetite and increase feed intake, activation of immune responses and antibacterial, antiviral and antioxidant actions (Toghyani *et al*., 2010 and 2011). Additionally, antimycotic function of turmeric was suggestive to the reason while it served as mold inhibitor in the trial (Polasa *et al*., 1991 and Anwarul *et al*., 2006).

Wet litter as observed from this trial poses is a threat to chickens and one of the many key contributing factors is increased moisture of the diet (Dunlop *et al*., 2016). It is suggestive that mycotoxins like Aflatoxin have neprotoxic potential (Hoer, 2008 and Collett, 2012). In this study, contamination of feed by molds is suggestive and where there are molds co-occurrence of several mycotoxins is common as most fungi have the ability to produce several mycotoxins and

as commodities may be infected by several fungi (Streit *et al*.2012). This could potentially lead to synergistic or additive effects (Streit *et al.* 2012). Effect of turmeric was able to reduce the moisture in the feaces as suggestive by the dry matter because of its function as mold inhibitor (Polasa *et al*.1991 and Anwarul *et al*.,2006).

The additive inclusion of atoxigenic organisms and turmeric in this trial were suggestive of the protective potential of the duo on the vital organs. Mold will always impair metabolisms by enlarging the liver, proventriculus, intestine and kidney (Swaminathan, 2002, Kumar and Balachandran, 1998). The ability of the turmeric and atoxigenic organisms to improve the feed intake and digestibility retention of protein also will lead to improve the complete blood count when supplemented This finding indicates that high moisture diet can damage liver and renal tissue as suggestive by the values of the the serum indices. Tumeric exerted its protective attributes especially that of its antioxidant properties to mop up some of the leakages in the serum.

8.2 CONCLUSION

It can be concluded from this trial that Aspergillus count is directly proportional to Aflatoxin B1 synthesis. Low temperature (15^0C) and high Moisture (20%) increased the synthesis of AFB1. High Temperature and Low Moisture increased the Synthesis of AFB2, AFG1 and AFG2. From this trial, *Aspergillus* prone feed could be stored at temperature of 40^0C and moisture level of 4%.

Based on the results from experiment two it can be concluded that the three extracts at 30mg/l (Tumeric *Curcuma longa*, Ginger *Zingiber officinale* and Garlic *Allium sativum*) in the order listed were effective in controlling the growth of *Aspergillus parasiticus* The plants

extracts had a reducing effect on mycelial dry weight, indicating whatever the concentration of the plant extracts increases not only the mycelia growth is prevented but also the ability of aflatoxin production.

The improvement of some performance indices, digestibility, visceral organs, complete blood count, and some serum biochemical indices in experiment three showed the ability of these plant extracts in the order (Tumeric, Ginger and Garlic) to diminish the inhibitory effect of aflatoxin in broilers up to 5weeks of age. Findings from this trial showed that only Tumeric exerted a beneficial influence to a diet without Aflatoxin.

Evidence from the performance, heamatology, serum biochemical indices in experiment four showed that Bio-control use of atoxigenic organisms is effective as a decontaminant for aflatoxin and also profitable for diet without Aflatoxin.

Results from experiment five showed that high moisture maize reduced the cost of raising 1kg of broilers. Results from Hematology and Serum biochemical indices were suggestive to feeding high moisture maize might be detrimental to the wellness of broilers. Additive effect of atoxigenic organisms and turmeric improved the wellness of the birds, performance and also reduce the cost of raising 1kg of broilers.

8.3 RECOMMENDATION

It was concluded from this study that poultry feed are best stored at 4% moisture and 40^{o}C to control aflatoxin and in case of aflatoxicosis outbreak Tumeric, Garlic and Ginger can be used as treatment while addition of atoxigenic *Aspergiluus spp* can be used as biological control.

When a farmer has to use HMM addition of Tumeric and atoxigenic *Aspergiluus spp* is recommended.

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