EVALUATION OF THE OPTIMUM INCLUSION LEVELS OF MYCOFIX® AND BIOTRONIC® SE AS FEED ADDITIVES ON THE PERFOFRMANCE OF BROILER CHICKENS

BY

Wayebo Hannah KEHINDE
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A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,

FACULTY OF AGRICULTURE,

AHMADU BELLO UNIVERSITY,

ZARIA

December, 2017

DECLARATION

I declare that the work in this Thesis entitled "**Evaluation of the optimum inclusion levels of Mycofix® and Biotronic® SE as feed additives on the perfofrmance of broiler chickens"** was carried out by me in the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University Zaria under the supervision of Professor (Mrs.) A. A. Sekoni, Professor T. S. Olugbemi and Dr. P. A. Onimisi. The information derived from literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any institution.

Wayebo Hannah KEHINDE	________________	_____________
Name of Student	Signature	Date

CERTIFICATION

This thesis "Evaluation of the optimum inclusion levels of Mycofix® and Biotronic® SE as feed additives on the perfofrmance of broiler chickens" by Wayebo Hannah KEHINDE meets the regulation governing the award of Doctor of Philosophy (Animal Science) Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentations.

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DEDICATION

I dedicate this thesis to God Almighty for His ever present help to me during the period of the research.

I would like to also dedicate this work to my loving Husband Dr. E. A. Kehinde, who gave me all the necessary support to actualize this dream. Your sacrifices and that of our children, Erioluwa, Ewaoluwa, Eniara and my Ph.D. baby Ereoluwa, cannot be forgotten May the Lord reward you all.

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ABSTRACT

A preliminary study was carried out to determine the prevalence of Aflatoxin B1 (AfB1) contamination and common moulds growing in some selected poultry feed ingredients in Zaria town, Nigeria. Twenty-five (25) samples of five different feed ingredients, which included Maize (MZ), soybean cake (SBC), groundnut cake (GNC), brewers dried grain (BDG) and maize offal (M/O) were collected. Samples were collected in March from four commercial feed mills and the open market. The common moulds isolated from the samples were *Mucor spp., Aspergillus s pp., Fusarium spp., Penicillum spp., Curvularia spp.* and *Rhizopus spp*. Aflatoxin B1 contamination showed that maize and soya bean cake were less than the 20 parts per billion (ppb) permissible limits for AfB1 in poultry feed ingredients, while BDG, M/O and GNC were 40, 60 and 80 % respectively above 20 ppb permissible limits. Three feeding trials were conducted using broiler chickens. The first experiment was conducted to evaluate the effect of four levels of Mycofix® in diets of broiler chickens, experiment two evaluated the response of broiler chickens fed diets containing four levels of Biotronic ® SE and experiment three evaluated the response of broiler chickens fed diets containing single and combined levels of Mycofix® and Biotronic ® SE. In experiment one; 330 day old Ross broiler chicks were used for the feeding trial. The chicks were allotted in a completely randomized design (CRD) to five dietary treatments replicated thrice with 22 chicks per replicate. Treatment one was the control diet without Mycofix®, while treatments 2, 3, 4 and 5 had Mycofix® included at the rate of 100, 200, 300 and 400 g / 100kg diet respectively. In experiments two and three, a total of 396 day old Ross chicks each were used. In experiment 2, Treatment one was the positive control diet without Biotronic® SE and Oxytetracycline, treatments, 2, 3, 4, 5 had Biotronic® SE at the rate of 200, 300, 400, 500g Biotronic® SE / 100kg diet and Tretment 6 (negative control) had 100g Oxytetracycline/100kg feed. Experiment 3, Treatment one was the postive control diet without Mycofix®, Biotronic® SE and Oxytetracycline, treatments 2, 3, 4, 5 had 400g Mycofix®, 500g Biotronic® SE, 200gMycofix® + 250 Biotronic® SE, 400g Mycofix®+ 400g Biotronic® SE and treatment 6 (negative control) 100g Oxyteracycline/ 100kg of feed respectively. The diets were formulated to meet standard requirements for starter and finisher broiler chickens. Feed and water were given to the birds *ad libitum* for the experimentl periods. Data collected included the average of body weight gain, feed intake, feed conversion ratio, carcass percentage, nutrient digestibility; others were haematological, biochemical indices, kidney function test, microbial analysis of digesta, drug residue in meat samples and villi morphometerics. Data collected were subjected to Analysis of Variance (ANOVA) using General Linear Model procedure of SAS and significant differences among treatment means were compared using Dunnette test of significance. Experiment One, starter phase showed that dietary treatments had significant effect (P < 0.05) on final weight gain, feed conversion ratio, feed cost and feed cost per kilogram gain. Birds fed 400g/100kg diet Mycofix® had the best final body weight gain at both starter (839.67g) and finisher phases (2350.34g) respectively. The feed conversion ratio at the starter phase was significantly (P<0.05) lower in the experimental treatments and at the finisher phase, 400g Mycofix® treatment had a significantly (P<0.05) lower feed conversion ratio. There were no significant differences (P<0.05) across treatments for cut parts and organ weights of carcass. Haematological and biochemical indices were not significantly affected by dietary treatments as parameters were within the normal reference range for broiler chickens. Nutrient digestibility was significantly improved at 400g inclusion. Experiment Two, starter phase results showed similar (P>0.05) weight gain in birds on 500g Biotronic® SE and100g Oxytetracycline. The birds fed 500g Biotronic® SE had the best feed conversion ratio (1.7) and feed cost per kilogram gain (₦151.42) values. The finisher phase result showed no significant (P>0.05) differences in weight gain among Treatments 3, 4, 5 and 6. Birds on 400g Biotronic® SE had a lower (P > 0.05) FCR and feed cost per kilogram gain.The dressing percentage for the carcass was best in 500g Biotronic® SE and Oxytetracycline treatments. Prime cuts: breast, drumstick and wings were better in birds on 400g Biotronic® SE. Haematological parameters were not affected by dietary treatments. The values of alkaline phosphatase (ALP) were significantly (P>0.05) higher in the experimental treatments, alanine amino transferase (ALT) values was significantly (P>0.05) higher in the antibiotic treatment while aspartate aminotransferase (AST) values was significantly (P > 0.05) higher in birds on 400g Biotronic® SE and Oxytetracycline group. The crude protein values (86.54, 89.31 and 94.50 %) for nutrient digestibility was significantly (P<0.05) higher in 200, 300 and 400g Biotronic® SE groups respectively. The crude fibre (76.43 %) and ether extract (89.82 %) values were significantly (P<0.05) lower across treatments. The levels of Biotronic® SE was more effective in increasing aerobic plate count and coliform counts in both ileum and ceacum of broiler chicks. Birds fed the control diet showed no drug detected, while birds fed 200, 300, 400 and 500g Biotronic® SE were low and Oxytetracycline treatment showed high concentration of drug. In experiment three, results of the starter phase showed no significant (P>0.05) differences in most of the parameters measured across treatments. Birds on Oxytetracycline treatment had a significantly (P <0.05) higher feed intake (1920.44g) from the rest treatment groups and control. At the finisher phase, birds on 100g Oxytetracycline had a significantly (P < 0.05) higher weight gain, average daily weight gain and feed conversion ratio and least feed cost /kilogram gain Carcass result showed better breast weight for birds fed diets with Oxytetracycline and better drum stick for birds fed diets with 400g Biotronic® SE. No significant (P>0.05) differences for organ weights across treatments. No significant (P>0.05) differences were recorded across treatments for haematological profile, liver and Kidney function tests. Nutrient digestibility for birds fed 400g Biotronic® SE was better (P<0.05) in its percentage composition for dry matter, crude protein, crude fibre and ash content across treatments but similar to control. Results of villi morphometrics of sections of the jejunum showed that birds fed 400g® Mycofix and 400g Biotronic® SE had a significantly (P<0.05) higher villi crypt across treatment groups and control. Villi roundness for birds on control diet was significantly (P<0.05) higher other rest treatment groups. The study concludes the growth of fungi spp. in feed ingredients samples and presence of AfB1. The findings of the feeding trials concludes that the use of Mycofix® a toxin binder, improved performance significantly at both starter and finisher phases at the rate of 4kg/tone which was above the recommended level of 2-3kg /tone. The use of Biotronic® SE as a gut acidifier gave a better result for all the growth performance parameters above the control; at 500g/100kg for starter phase and 400g/kg at finisher phase. It had a positive effect on maintenance of normal microbial activity of ileum and ceacum and no residues in meat samples. The combined and single use of Mycofix® and Biotronic® SE did not significantly improve growth of broiler chickens, but performed comparable to the antibiotic treatment in all the parameters measured. The combinations had no adverse effect on measured performance parameters, haematological parameters, liver and kidney function tests and improved villi crypt an evidence of a positive synergy in their combination. It is recommended that Mycofix® can be singly used at 400g/100kg and 500g/100kg feed of Biotronic ® SE as a means of alleviating the incidences of

mycotoxins in feed and improving gut health.The combined inclusion recommended is at 200g Mycofix® and 200g Biotronic ® SE/100kg feed.

CHAPTER ONE

1.0 INTRODUCTION

Feed represents the greatest single expenditure associated with poultry production. Nutritional research in poultry has therefore centered on issues related to identifying barriers to effective digestion, utilization of nutrients, and on approaches for improving feed utilization (Ravindran, 2010). The quality of feed ingredients is very important as this will determine the quality of the feed and the end-products.Hence a more precise evaluation of the quality of dietary raw materials is needed (Kersten *et al.,* 2005). Feed materials may be contaminated at any time during growing, harvesting, processing, storage and distribution of the feed. Feeds may contain diverse microflora that are acquired from multiple environmental sources, including dust, soil, water, and insects (Maciorowski *et al*., 2006).

Mycotoxins are a historical problem in poultry, first recognised in the 1960s as the cause of 'turkey X disease' in England which resulted in the death of 100,000 turkey poults and many ducks, chickens and pheasants (Siska, 2013). Mycotoxins are toxins formed during fungi growth, myco means mould and toxin represents poison (Annongu, 2012). Mycotoxins are highly toxic secondary metabolic products of mould on almost all agricultural commodities worldwide. They occur under natural conditions in feed. Several studies proved that economic losses occur at all levels of food and production, including crop and animal production, processing and distribution (Robens and Cardwell, 2003; Wu, 2007; Bryden, 2012). According to the Food and Agriculture Organization (FAO), 25% of the world's crop harvests are contaminated with mycotoxins (FAO, 2012). There are currently more than 400 mycotoxins known. There are six major classes of mycotoxins that frequently occur namely, aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin and ergot alkaloids (CAST 2003). They are formed by different kinds of fungi and each fungi species can produce more than one type of mycotoxin. Surveys of mycotoxin levels in poultry feeds often reveal the presence of a number of different toxins; most samples in a recent survey contained at least 10 contaminants. Contamination of feeds with mycotoxins is a worldwide problem, in poultry particularly those produced by the genera, *Fusarium, Aspergillus and Penicillium* (Siska, 2013).

Mycotoxin binders or adsorbents are substances that bind to mycotoxins and prevent them from being absorbed through the gut and into the blood circulation (Jacela *et al.,* 2010). The addition of mycotoxin binders to poultry diets has been considered the most promising dietary approach to reduce the effects of mycotoxins (Galvano *et al.*, 2001). The theory is that the binder decontaminates mycotoxins in the feed by binding them strongly enough to prevent toxic interactions when the animal consumes the feed and to prevent mycotoxin absorption across the digestive tract.

Nutritional researchers have therefore implored the use of toxin binders as an approach to salvaging feed contamination with mycotoxins and protecting animals from disease problems and losses in performance. Another approach by researchers is the use of dietary supplementation with acidifiers, which are organic acids used to reduce bacterial growth. It helps to reduce colonization of pathogens on the intestinal wall, thus preventing damage to the epithelial cells. Acidifiers enhance increase in body weight and feed conversion ratio in broiler chicken (Skinner *et al.*, 1991).

The Mycofix® product line from BIOMIN is a range of specially developed feed additives that protect animal health by deactivating mycotoxins found in contaminated feed. Its modular system consists of three strategies: Adsorption – Elimination of toxins, Biotransformation – Elimination of toxicity and Bioprotection – Elimination of toxic effects. Mycofix is one of the new promising mycotoxins adsorbent that was successfully used to alleviate the negative effects of T-2 toxins in broilers (Aziz, 2005; Omar, 2010).

Biotronic® SE is a powerful combination of synergistically acting organic acid and their salts combined on a Sequential Release Medium (SRM). Besides decreasing pH, the selected acids penetrate the cell wall of gram – negative bacteria. The use of Biotronic® SE is indicated for the control of gram - negative bacteria including *E. coli* and *Salmonella i*n other to promote animal growth in pig and poultry. This highly effective synergism ensures its full economic benefit (Poultry Site, 2014).

1.1 Justification for the Study

One subject receiving much attention from researchers at present is that of mycotoxicity. It is an issue which has important implications for the global feed industry, bird performance and potential with negative consequences for the food chain (Stephen, 2008). Nigeria does not have standard regulationsand control on mycotoxins in poultry feed, consequently the risk of mycotoxins exists in the Nigerian poultry sector since the common feed ingredients, such as maize and groundnut cake, are known to contain high levels of mycotoxins (Kpodo and Bankole, 2008). Fungi are major spoilage agents of foods and feedstuffs. The proliferation of various fungi species in agricultural products leads to reduction in yield and quality with significant economic losses (Adejumo and Adejoro, 2014).

The poultry industry witnessed a tremendous growth with the best application of nutritional technologies. Today, animal feed production has been paid attention; however, feed safety is a concern for achieving productivity. Mycotoxin contamination of feed is a recurring problem, however the effective control is a challenge because the mycotoxins contamination occurs through various feed raw materials, which are used in poultry feed. Nevertheless the contamination of mycotoxins can come from the dust and leftover feed in the feeding channel; hence a rational approach has to be adopted for effective control of feed mycotoxins (Bhat, 2011).

On the other hand, gut health is currently gaining much more attention in literature especially in poultry and has been applied to coordinate the working efficiency of the gut (Laudadio *et al*., 2012). The gut is the most extensive exposed surface and is constantly exposed to a wide variety of potentially beneficial, non-infectious as well as harmful infectious pathogens (Lievin-Le Moal and Servin, 2006). This has necessitated the need to tackle the microbial population in the gut of the chicks as early as they are hatched in favour of the beneficial microorganism in the host. This is achievable by the use of acidifiers which plays the role of lowering the pH of the gut, thereby inhibiting the proliferation of pathogenic micro-organisms hence, reducing their adverse effect on the host animal.

The use of a toxin binder to mitigate the effect of mycotoxins present in feed and an acidifier to improve the gut health as a way of improving performance was considered in the study

1.2 Objectives of the Study

The objectives of the study were to;

1. determine fungi species and Aflatoxin B1 contamination in some common feed ingredients used in poultry finished feed.
2. know the optimum inclusion levels of Mycofix® and Biotronic® SE singly and in combination on growth performance, haematological parameters, serum biochemical indices and carcass characteristics, gut morphology and nutrient digestibility of broiler chickens.

3. evaluate the effects of various inclusion levels of Biotronic® SE on intestinal microbioata and drug residue in the meat of broiler chickens.

1.3 Research Hypotheses

Null Hypothesis (H_o): Some common feed ingredients used in poultry finished feed are not contaminated with fungi species and Aflatoxin B1.

Null Hypothesis (H_o): Inclusion of Mycofix® and Biotronic® SE as a toxin binder and organic acid respectively in the diets of broiler chickens do not enhance growth performance, apparaent nutrient digestibility, haematological profile, serum biochemical indices and carcass characteristics.

Null hypothesis (H_o): Inclusion of different levels of Biotronic® SE in the diets of broiler chickens does not enhance growth performance, apparent nutrient digestibility, haematological profile, biochemical indices, carcass ch aracteristics and growth of beneficial intestinal biodata.

Null hypotheses (H_o): Inclusion of single and different levels of Biotronic® SE, Mycofix® in the diets of broiler chickens does not enhance growth performance, apparent nutrient digestibility, haematological profile, liver function test, kidney function test, carcass characteristics and intestinal gut morphology of broiler chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxins

The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100,000 turkey poults died. When this mysterious turkey X disease was linked to a peanut (groundnut) meal contaminated with secondary metabolites from *Aspergillus flavus* (aflatoxins), it sensitized scientists to the possibility that other occult mold metabolites might be deadly (Bennett and Klich, 2003). While all mycotoxins were of fungal origin n, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolite are both important. Fungal products that are mainly toxic to bacteria (such as penicillin) are usually called antibiotics. Fungal products that are toxic to plants are called phytotoxins by plant pathologists. Mycotoxins are made by fungi and are toxic to vertebrates and other animal groups in low concentrations (Bennett, 1987).

The term "mycotoxin" was derived from "mykes" meaning fungi and "toxicon" meaning poison. Mycotoxins are secondary metabolites of low molecular weight produced by a wide range of fungi, principally molds. There are over 200 species of molds that produce mycotoxins. Aflatoxins (AF), zearalenone (ZEN**)**, ochratoxin A (OTA**)**, fumonisins (FUM), trichothecenes such as deoxynivalenol **(**DON**),** and T-2 toxin are some of the mycotoxins that can significantly impact the health and productivity of poultry species. Fungal growth and subsequent mycotoxin formation is dependent on a range of factors including seasons, location of grain cultivation, drought and time of harvest. Long term analysis of grain and feed samples worldwide has indicated that it is possible to have grains with extremely high concentrations of mycotoxins, although the overall mycotoxin contamination is low (Streit *et al*., 2013a). These data also revealed that mycotoxin contaminated grains typically contain more than just a single mycotoxin.

The mycotoxins are low weight metabolites which cause harm known as mycotoxicoses, in livestock, domestic animals and humans and therefore of public health significance (Ashiq, 2015). The production of mycotoxins is stimulated by certain environmental factors therefore the extent of contamination will differ with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods (Jonathan and Esho, 2010) Fungi that produce toxins in food are therefore classified into field fungi and storage fungi based on their ecological requirements for growth (Bayman and Baker, 2006). Mycotoxins are substances produced by fungi imperfecti which, in some cases, do protect plants or plant seeds against parasites. However, upon ingestion, inhalation or dermal contact, these mold-produced substances are poisonous to vertebrates. Even with more than 100,000 species of fungi known, only some of them are active mycotoxin producers. The fungi species which are known to produce the most hazardous mycotoxins in agriculture and in animal production are Fusarium, *Aspergillus* and *Penicillium* sp. (Schardl *et al*., 1996).

Three genera of fungi namely, *Aspergillus*, *Penicillium* and *Fusarium* have been found to produce most of the important mycotoxins. Other important producers of mycotoxins are the *Claviceps* and *Stachybotrys* (CAST, 2003). The formation of mycotoxins was a problem which occured in nature sporadically both seasonally and geographically under aerobic conditions in a wide variety of feedstuffs used in livestock feeds. The production of these mycotoxins usually took place under aerobic conditions (Shareef, 2010).

More than 300 mycotoxins have been identified and the major types, which are of most concern due to their toxicity and occurrence include; Aflatoxins, Vomitoxin, Fumonisins, Ochratoxin, T-2 Toxin and Zearalenone. They were known to have certain harmful effects on man and animals when ingested. These negative effects may be carcinogenic, mutagenic, immunosuppressive, nephrotoxic, neurotoxic and tetratogenic, hepatotoxic, hemorrhagic, depending on their precise nature (Akande *et al.*, 2006). When several mycotoxins occur together in food or feeds, the negative impact they exert on the health and productivity of livestock is greater than each toxin can exert individually. Also the effect of the loss caused by death due to mycotoxin poisoning is far less when compared with the economic impact resulting from reduced animal productivity, increased incidence of disease caused by immunosuppression, damage to vital organs and interference with the reproductive capacity of livestock (Smith and Seddon, 1998). Some important toxigenic species of filamentous fungi and related mycotoxins are presented in Table 2.1.

2.1.1 Impact of mycotoxins on feed

Mycotoxins are widely present in feed ingested by animals in different climate zones, which significantly contributes to the spread of mycotoxicoses. Exposure to moderate to high concentrations of mycotoxins produces various clinical symptoms. There is mounting evidence to indicate that exposure to no-observed-effect-level (NOEL) doses of mycotoxins, regarded as safe in modern feed production, may be far more detrimental to animal health and productivity than previously believed (Bryden, 2012). The commom mould species that may occur in feed materials and finished feed of broiler chickens are presented in table 2.1.

Table 2.1: Some important toxigenic species of filamentous fungi and related mycotoxins common in broiler chicken feed ingredients and finished feed

Fungal species	Toxins
Aspergillus flavus	Aflatoxins B1, B2, cyclopiazonic acid
A. parasiticus	Aflatoxins B1, B2, G1, G2
A. ochraceus	Ochratoxin A; Penicillic acid
A. versicolor	Sterigmatocystin, cyclopiazonic acid
Penicillium verrucosum	Ochratoxin A, citrinin
P. purpurogenum	Rubratoxins
P. expansum	Patulin, citrinin
Fusarium sporotrichiodes	T-2 toxin
F. verticilloides	Fumonisin B1
F. graminearum	Deoxynivalenol, nivalenol, zearalenone
Alternaria alternata	Tenuazonic acid
Stachybotrys atra	Satratoxins

Source: Wayne and Bryden (2007)

The moulds and mycotoxins that usually contaminate poultry feeds result from the contamination of the raw materials used in their production. During the pre- and post- harvest periods, the temperature and humidity, processing and handling of the raw materials and subsequently the animal feed play an important role in fungi contamination, growth and mycotoxin production (Adebajo *et al.*, 1994). The economic and commercial impact of mycotoxins on the productivity and nutritive value of infected cereal and forage is significant. Fungal contamination of feeds resulting in the production of mycotoxins affect feeds and entails a high risk of toxicosis to both man and animals. The mycotoxins produced are metabolized in the kidneys, liver and by microorganisms in the digestive tract and there is the possibility of the presence of the residues of these toxins in the edible animal products like eggs, milk and meat. However, these residues usually differ in associated toxicity and chemical structure from the parent molecule. Factors such as the number of occurring mycotoxins, ingested amount, animal sensitivity and duration of exposure to mycotoxin, determine the effects of the mycotoxin on the consumer (Ratcliff, 2002; Saleemi *et al*., 2010).

The result of a nationwide survey carried out in Nigeria by Nigerian Institute of Animal Science (NIAS) on feed ingredient and finished feed analysis for Aflatoxin is presented in Table 2.2.

2.1.2 Impact of mycotoxin on animals and humans

In farm animals, mycotoxins have negative effects on feed intake, animal performance, reproductive rate, growth efficiency, immunological defense as well as being carcinogenic, mutagenic, teratogenic, tremorgeni i.e cause tremor or damaged central nervous system, haemorrhagic, as well as causing damage to the liver and kidneys (Ratcliff, 2002).

Table 2.2: Feed ingredient and finished feed analysis for aflatoxin

Ingredients	Commercial millers	Toll millers	On farm millers	Toxin	Codex limit	Results
	Levels of Aflatoxin contamination in (ppb)					
GNC (Total of the three categories sampled)	511.559.7			Aflatoxin	20ppb	Very*** High
SBC	9.2	3.2	12.5	Aflatoxin		Low
Fish meal	21	26.4	14.3	Aflatoxin		Low
BDG	10.2	9.8	15.3	Aflatoxin		Low
BS	82	29.8	5.8	Aflatoxin		High
Grower mash	57.6	212.1	95.6	Aflatoxin		Very High
Layer Mash	142.4	100.2	139.4	Aflatoxin		Very High

Source: NIAS (2013), Nigerian Instute of Animal Science, GNC; Groundnut cake, SBC; Soyabean cake; BDG; Brewers dried grain, BS; Broiler starter ppb; parts per billion.

Overt mycotoxicoses in animals can occur as a result of the intake of very low levels of mycotoxins and this could lead to the impairment of immune and acquired resistance to infections which results in decreased productivity and economic losses (Dalcero *et al.,* 1998; Ratcliff, 2002; Paterson, 2006). Aflatoxin, ochratoxin, fumonisin, trichothecene, zearalenone and patulin are the most widespread mycotoxins in animal feed and human food (Huffman *et al*., 2010; Mazzoni *et al*., 2011).

The exposure of pregnant women, infants and children to high levels of mycotoxins is a major health concern. Aflatoxins are a major source of concern because they have been shown to be immunogenic, teratogenic, and also retard growth among experimental animals. Environmental conditions in developing countries favour their production and there are high exposures to aflatoxins throughout these regions. In West Africa, a study showed a significant correlation between aflatoxin exposure and stunted growth in children who were exposed to aflatoxin right from neonatal stages (Gong *et al.,* 2002). Aflatoxins also have the ability to cross the placental barrier and caused genetic defects at foetal stages (Maxwell *et al.,* 1998).

2.2. Some Mycotoxins Relevant to Poultry

2.2.1 Aflatoxins

Aflatoxins are a group of structurally related toxic compounds produced predominantly by certain strains of *Aspergillus flavus*, and *Aspergillus parasiticus* (Diaz *et al*., 2001). These molds are ubiquitous in nature and can grow and produce aflatoxins on any feed or grain used for poultry under favourable environmental conditions (Qazi and Fayyaz, 2006). United States Food and Drug Administration (USFDA) considered aflatoxins to be an unavoidable contaminant of foods and had set regulatory levels. The permissible level for poultry is 20 parts per billion (Aravind *et al*., 2003; Azab *et al*., 2005). Aflatoxins were the most prevalent and dangerous studied mycotoxins (Krnjaja *et al*., 2008). Ingestion of feed contaminated with aflatoxins caused high morbidity and mortality resulted in severe economic losses (Oguz *et al*., 2003; Diaz *et al*., 2008). Aflatoxins not only impaired weight gain, feed intake, feed conversion efficiency and egg production (Ortatatli *et al*., 2005), but also increased the susceptibility to environmental stress (Abdolamir *et al*., 2005) and severity of diseases like crop mycosis, salmonellosis, coccidiosis, aspergillosis, and Marek's disease (Ibrahim *et al*., 2000).

Aflatoxin contamination of food and feeds is serious all over the world. Shundo *et al*. (2009), Soubra *et al*. (2009); Bankole *et al*. (2010), reported aflatoxin contamination of foodstuffs in many countries especially in Asia and Africa. Aflatoxin was more often found in corn, peanuts, and cottonseed grown in warm and humid climates (Russell *et al*., 1991). Aflatoxin contamination had been found to develop both in the pre- and post-harvest periods. The highest levels are often associated with post-harvest spoilage of food commodities which were stored under inappropriate high moisture and temperature conditions which facilitated the rapid growth of moulds. The level of contamination of the produce depended on the plant stress, temperature, water activity, culture and storage conditions (Moss, 2002). Quite a large number of foods had showed susceptibility to aflatoxin contamination, some of the most commonly affected include, peanuts, maize, dried fruit, nuts, spices, figs, vegetable oils, cocoa beans, corn, rice and cotton seeds as documented in the Reports on Carcinogens (ROC, 2003). Among these agricultural commodities usually infected by aflatoxigenic fungi some were food sources while others were animal feeds. Aflatoxin affects the health of the humans and livestock that consume these commodities and the related products (Moss, 2002)**.**

2.2.2 Ochratoxin A

Ochratoxin A (OTA) was discovered in 1965 in South Africa (Van der Merwe *et al*., 1965). At that time it was isolated as a toxic metabolite of *Aspergillus ochraceus* from corn meal artificially inoculated with the fungus. In 1969, naturally occurring OTA was isolated from a commercial corn sample in the United States (Shotwell *et al*., 1969). Later, it was recognized as a secondary metabolite of several *Aspergillus* and *Penicillium* species that were widespread (Duarte *et al.,* 2010). OTA is one of the most important mycotoxins, with great public health concerns because, it had been confirmed to be nephrotoxic, genotoxic, neurotoxic, immunotoxic, embriotoxic and teratogenic and it was suspected of being carcinogenic (JECFA, 2008). OTA mainly contaminated cereals and their products such as foods for human consumption and by products for animal feeds. Ochratoxin contamination occured under a variety of environmental conditions in both temperate and tropical climates thus was found in many countries (Battaccone *et al.,* 2010; Cabanes *et al.,* 2010). According to Vega *et al.* (2009), 50% of man's daily intake of OTA came from the consumption of cereals. *Aspergillus* and *Penicillium* species are two storage fungi mainly responsible for the post-harvest production of OTA in cereals (Magan and Aldred, 2005). OTA could have several effects, such as nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic effects on several species of animals, and could cause kidney and liver tumours in mice and rats (El Khoury and Atoui, 2010).

2.2.3 Trichothecenes

Trichothecenes was first isolated from *Trichothecium roseum* by Freeman and Morrison (1949). This was followed by the isolation and description of other trichothecenes (TCTs), which included diacetoxyscirpenol (DAS), T-2 toxin (T-2), nivalenol (NIV) and deoxynivalenol (DON). Deoxynivalenol (DON) is the most prevalent and the most studied mycotoxin produced by *Fusarium*. DON, also known as vomitoxin showed great stability during storage/milling and in the processing and cooking of food (Sobrova *et al.,* 2010). *F. graminearum* and *F. culmorum* were the predominant toxigenic *Fusarium* species found in infected grains are the most important producers of Deoxynivalenol (DON). Cereal grains and associated by-products constitute important sources of energy for poultry. There is increasing evidence that global supplies of cereal grains for animal feedstuffs were commonly contaminated with *Fusarium* mycotoxins (Placinta *et al*., 1999; Binder *et al*., 2007; Griessler *et al*., 2010). *Fusarium* mycotoxins were likely the most economically significant grain mycotoxins globally (Placinta *et al.*, 1999), and annual economic losses in animal production industries have been estimated to be as much as several hundred million dollars (Hussein and Brasel, 2001).

Fusarium species are pathogens found on cereal crops and they produce mycotoxins before, or immediately after, harvesting grains and cereals (Yazar and Omurtag, 2008). Many of the trichothecenes**,** fumonisine and zearalenone which were mainly found in cereal grains were produced by the *Fusarium* genus, and this genus also includes a number of important plant pathogens.

Apart from cereals and grains, trichothecenes (TCTs) have been found in soybeans, potatoes, sunflower seeds, peanuts and bananas and also in processed foods, especially those produced from cereals such as; bread, breakfast cereals, noodles, and beer. DON and nivalenol are the most dominant TCTs in grains (Foroud and Eudes, 2009; Karlovsky, 2011). Acute exposure of animals to DON resulted in decreased feed consumption and vomiting, while exposure for longer periods causes reduced growth, and adverse effects on the thymus, spleen, heart, and liver (Sobrova *et al.,* 2010). Prolonged daily exposure, which led to chronic toxicity, was the real concern as it had been observed that DON deregulated the immune response (Merhej *et al.,* 2011). In animals the ingestion of DON with contaminated feeds and food led to growth retardation, and reproductive disorders (Pestka, 2010; Sobrova *et al.,* 2010).

2.2.4 Fumonisins

Bezuidenhout *et al.* (1988) discovered and characterized Fumonisins in 1988 and to date twenty- eight fumonisins have been isolated and they can be divided into four groups (A, B, C and P). The B groups is further divide into FB1, FB2 and FB3 and these three are the principal fumonisins analyzed as natural contaminants of cereals (CAST, 2003; Yazar and Omurtag, 2008).

Fumonisin B1 is the most abundant member of this mycotoxin family; it comprises about 70 per cent of the total fumonisin content of *Fusarium* cultures (Reddy *et al.,* 2010). Fumonisins found in food are produced mainly in the field; insect damage of corn ears and kernels, temperature and moisture conditions is important factors that affect *Fusarium* infection and toxin synthesis (Richard, 2007; Yazar and Omurtag, 2008). Fumonisins have been found to be a very common contaminant of corn-based food and feeds in several parts of the world including Africa and the USA (Kumar *et al.*, 2008). The occurrence of fumonisins have also been reported in products such as rice and sorghum (CAST, 2003), wheat noodles, curry, beer and corn-based brewing adjuncts (Yazar and Omurtag, 2008).

2.3 Mycotoxin binders

Mycotoxin binders or adsorbents are substances that bind to mycotoxins and prevent them from being absorbed through the gut and into the blood circulation (Jacela *et al*., 2010). Depending on their mode of action, these feed additives may act by reducing the bioavailability of the mycotoxins or by degrading them or transforming them into less toxic metabolites known as toxin binders (Abrunhosa *et al*., 2009).

The contamination of feed with mycotoxins is a continuing feed safety issue, leading to economic losses in animal production (Wu, 2007). Consequently, a variety of methods for the decontamination of feed has been developed, but the addition of mycotoxin detoxifiers to the feed is the most commonly-used method (Jard *et al.,* 2011; Kolosova and Stroka, 2011). Sequel to this, the Commission Regulation of the European communities in 2009, in collaboration with European parliament established a new functional group of feed additives for use in animal nutrition. This new feed additives (mycotoxin binder) have been developed which suppressed or reduced the absorption, promote the excretion of mycotoxins or modify their mode of action and thereby mitigate possible adverse effects of mycotoxins on animal health (European Commission, 2009).

Low levels of mycotoxins may exist in poultry feed even with excellent management. Several mycotoxin binders have been developed that prevent the toxic effects of mycotoxins on animals consuming contaminated feed. These materials bind with the mycotoxin(s) and prevent the negative effects on the animals consuming them. Potential mycotoxin binders include activated carbon aluminosilicates (e.g., clay, bentonite, montmorillonite, zeolite, phyllosilicates) and complex indigestible carbohydrates (e.g., cellulose, polysaccharides in the cell walls of yeast and bacteria) as well as some synthetic polymers. The diversity in chemistry of mycotoxins influenced the effectiveness of mycotoxin binders. Mycotoxin control measures may require multiple approaches to solve the problems associated with mycotoxin consumption. More recent approaches include the use of a combination of binders, microbial enzymes, yeast cell walls, and natural antioxidant (Jacquie, 2015).

2.4 Types of mycotoxin adsorbent / binder

Depending on their mode of action, mycotoxin binders / adsorbents act in diverse ways as documented by Abrunhosa *et al* (2009). It is commonly known that mycotoxins vary in their chemical structures, which results in vast differences regarding their chemical, physical, and biochemical properties. While the biochemical properties define the toxicity of mycotoxins, chemical and physical properties determine the methods that can be used to detoxify them. Considering the great variety of mycotoxin structures it is very obvious that there is no single method, which can be used to deactivate mycotoxins in feed. Therefore, different strategies have to be combined in order to specifically target individual mycotoxins without reducing/affecting the quality of feed. The best known method for mycotoxin deactivation is "binding" with the use of binding agents, which are referred to as mycotoxin binders, adsorbents, or enterosorbents (Murugesan *et al.,* 2015)**.**

An effective binder or sequestering agent is one that prevents or limits mycotoxin absorption from the gastro-intestinal tract of the animal. In addition, they should be free from impurities and odours. Many can impair nutrient utilisation and are mainly marketed, based on *in-vitro* data only (www.KnowMycotoxin.com*,* 2008). They can be of organic (microbial) or inorganic (mainly clay minerals) in nature. Another method is "bio-protection," which uses different substances such as algae and plant ingredients that protect vulnerable organs such as the liver and strengthen the immune system of animals. Enzymatic or microbial detoxification sometimes referred to as "biotransformation" or "biodetoxification" utilizes microorganisms or purified enzymes thereof to catabolize the entire mycotoxin or transform or cleave it to less or non-toxic compounds (Murugesan *et al.,* 2015)**.**

2.4.1 Inorganic binders (clay group)

Clays are natural adsorbents chemically made of silicates or aluminosilicates. They include a large range of products such as hydrated sodium calcium aluminosilicates (HSCAS), phyllosilicates (of which montmorillonite or magnesium hydrated HSCAS is one of the major compounds in this group), bentonite and zeolite (the latter two are clays of volcanic origin).

Much of the pioneering work with mycotoxin binders was done with silicates and specifically with the HSCAS material studied at Texas A and M University by the Phillips' research group. These binders have the property of adsorbing organic substances either on their external surfaces or within their inter-laminar spaces, by the interaction with/or substitution of the exchanged cations within these spaces. Therefore, mycotoxins can be adsorbed into this porous structure and be trapped by elementary, electric charges (Grenier and Applegate, 2013).

2.4.2 Organic binders

Substances investigated as potential organic mycotoxin-binding agents include activated charcoal, synthetic polymers, yeast cell walls and components thereof, and bacterial cells (Grenier and Applegate, 2013). Organic mycotoxin adsorbents are carbon based polymers. Examples would include: fibrous plant sources such as: oat hulls, wheat bran, alfalfa fibre, extracts of yeast cell wall, cellulose, hemicelluloses and pectin. Such materials are biodegradable but can, in some cases be vectors of mycotoxin contamination. Benefits of yeast cell wall are low inclusion, high surface area and certainly no toxic contaminants.

The efficacy of glucomannan-containing yeast products as mycotoxin adsorbents in feeds had been investigated globally with several studies with all animals. Research conducted in France at the National Institute for Agricultural Research (NIRA) identified four *Saccharomyces cerevisiae* yeast strains that differed greatly in their glucan/mannan ratio. It was found that large differences existed in adsorptive capacity between the yeast strains with the amount of mycotoxin adsorbed strongly related to the beta-D-glucan content. Advanced molecular techniques were used to elucidate the spatial conformation and molecular sites of interaction between zearalenone and glucomannan-containing yeast product (www.KnowMycotoxin.com 2008).

2.4.3 Charcoal or activated carbon

Activated carbon is a general adsorptive material with a large surface area and excellent adsorptive capacity. It has been recommended as a general toxin adsorbing agent and was routinely recommended for various digestive toxicities (Merck Manual, 2005). Activated carbon is a general adsorptive material with a high surface to mass ratio (500-3500 m^2/g) (Whitlow, 2006). In one of the first studies to test the concept of mycotoxin binding, activated charcoal at a high dosage was shown to reduce aflatoxicosis in goats (Hatch *et al.*, 1982). In subsequent studies, the effects of activated charcoal have been variable. Galvano *et al.* (1996) showed reduced aflatoxin residues in milk of cows consuming different sources of charcoal, but responses to charcoal did not exceed that seen with a clay based binder a hydrated sodium calcium aluminosilicate (HSCAS).

2.4.4 Silicate binder

Silicates are divided into subclasses, by their structures (Bingham *et al.*, 2003). Minerals in different subclasses may have similar chemistry. The silicate subclasses included neosilicates (single tetrahedrons), sorosilicates (double tetrahedrons), inosilicates (single and double chains), cyclosilicates (rings), phyllosilicates (sheets), and tectocilicates (frameworks). Silicates investigated as adsorbent materials were classified primarily as phyllosilicates and tectosilicates. The most extensively studied of these materials was HSCAS and several reviews were available (Ramos and Hernandez, 1997; Phillips, 1999; Bingham *et al.*, 2003).

Other silicates that have been studied include bentonites, zeolites, clinoptilolites and various others that were often not completely characterized. The clay group is a sub-category of the phyllosilicates. Bentonite is a general clay material originating from volcanic ash and containing primarily montmorillonite as the main constituent. The pioneering work with mycotoxin binders was done with silicates and specifically with the HSCAS material studied at Texas A and M University. Phillips *et al.* (1988) screened a large number of silicates and selected one of the best materials for further study. That specific HSCAS included at 0.5 to 2.0% of the diet was well documented to adsorb aflatoxin and to prevent aflatoxicosis across species, including chickens and swine (Colvin *et al.*, 1989; Kubena *et al.*, 1991), lambs (Harvey *et al.*, 1991a), dairy cows (Harvey *et al.*, 1991b), dairy mink (Bonna *et al.*, 1991) goats (Smith *et al.*, 1994) and turkeys (Ledoux *et al.*, 1999). Responses to HSCAS appeared to be dose dependent (Smith *et al.*, 1994).

This HSCAS is characterized as an "aflatoxin-selective clay," was not a good adsorbent of other mycotoxins (Phillips, 1999), and therefore, was not expected to be protective against feeds containing multiple mycotoxins (Dwyer *et al.*, 1997). Watts *et al.* (2003) showed that 1% HSCAS was not protective to chicks and poults receiving diets containing 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B1, 100 μg aflatoxin B11 mg zearalenone and 0.5 mg ochratoxin per kg of diet.

2.4.5 Organic polymers as binders

Some complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans and others), and synthetic polymers such as cholestryamine and polyvinylpyrrolidone can also adsorb mycotoxins. Indigestible dietary fiber has adsorbance potential for mycotoxins. Alfalfa fiber had reduced the effects of zearalenone (Stangroom and Smith, 1984) in rats and swine and T-2 toxin in rats (Carson and Smith, 1983). *Saccharomyces cerevisiae* live yeast was shown to reduce the detrimental effects of aflatoxin in broiler diets (Stanley *et al.*, 1993). The aflatoxin protective effect of live yeast was confirmed in rats, but thermolysed yeast was shown ineffective (Babtista *et al.*, 2002). Fibrous material from the yeast cell wall was shown to have a potential to bind several mycotoxins (Devegowda *et al.*, 1998). Yeast cell walls, particularly the cell wall of Saccharomyces cerevisiae, were an environmentally friendly alternative to inorganic adsorbents, which were not extensively biodegradable and were associated with the risk of contaminants. In addition to the beneficial effects observed in counteracting aflatoxin these organic binders would be efficient against a large range of mycotoxins, which make them more adapted to the most frequent cases of multi-contaminated feed (Jans *et al*., 2012).

Esterified glucomannan polymer extracted from the yeast cell wall was shown to bind with aflatoxin, ochratoxin, and T-2 toxin, individually and combined (Raju and Devegowda, 2000). Additions of esterified glucomannan at 0.5 or 1.0 g/kg to diets supplying 2 mg of total aflatoxin resulted in dose-dependent responses in broiler chicks (Basmacioglu *et al*., 2005). The esterified glucan polymer may have the capability to bind several mycotoxins. Yiannikouris *et al*. (2004) demonstrated the mechanism of binding with zearalenone. A glucan polymer bound both T-2 toxin and zearalenone *in vitro* (Freimund *et al.,* 2003). The glucan polymer product was protective against depression in antioxidant activities resulting from T-2 toxin consumed by growing quail (Dvorska and Surai, 2001). A glucan polymer product has protected swine (Swamy *et al.,* 2002), broilers (Swamy *et al.,* 2004), and hens (Chowdhury and Smith, 2004) against some of the detrimental effects of multiple mycotoxins but without restoring growth rate. Aravind *et al*. (2003), using dietary additions of 0.5% esterified glucomannan, alleviated growth depression in broilers associated with naturally contaminated diets containing aflatoxin, ochratoxin, zearalenone, and T-2 toxin.

Certain bacteria, particularly strains of lactic acid bacteria, propionibacteria, and bifidobacteria, appear to have the capacity to bind mycotoxins, including aflatoxin and some *Fusarium*-produced mycotoxins (El-Nezami *et al*., 2000a). The binding appears to be physical with deoxynivalenol, diacetoxyscerpenol, nivalenol, and other mycotoxins associated with hydrophobic pockets on the bacterial surface.

2.5 Impact of Mycotoxins on Animal Nutrition and Health

Mycotoxins, which can be present in animal feed, can have serious negative effects on animal health. Mycotoxins are produced by moulds, which are filamentous fungi that frequently contaminate grains and improperly stored feeds. Mold growth typically is associated with extremes in weather conditions, and their spores are found almost everywhere, including soil and plant debris. Crops can be contaminated with mold in the field, at harvest, or during storage, processing, or feeding. The effects of mycotoxins in poultry feed depended on the specific mycotoxin or mycotoxins present, the level of contamination, the length of time the animal has been consuming the mycotoxin(s), the animal's age, sex, and level of stress (Jacquie, 2015).

Mycotoxins have significant economic and commercial impact, both productivity and nutritive value of the infected cereal and forage were affected (Ratcliff, 2002). The effect of mould contamination on nutritional value of stored maize is presented in Table 2.3. The nutritive value drops after contamination by mould. Contamination by moulds affected both the alimentary value and organoleptic characteristic of feed with a risk of toxicosis. The biological effects of mycotoxins depended on ingested amounts, number of occurring toxins, duration of exposure to mycotoxins and animal sensitivity. Also mycotoxins can induce health problems that are specific to each toxin or affect the immune status of animals, favouring infections.

Table 2.3: Effect of mould contamination on the nutritional value of stored maize

	ME (Kcal/kg)	CP (%)	Fat (%)
*Good corn	3,410	8.9	4.0
Mouldy corn	3,252	8.3	1.5
Loss of nutrient	158	0.6	2.5
% Loss in nutrient	4.6	6.7	62.5

Source: O'Keeffe (2003). ME = Metabolisable energy. CP = Crude protein
*Good corn; Non-contaminated corn

This was the major reason for the difficulty of diagnosing mycotoxicoses (Yiannikouris and Jouany, 2002) because mycotoxins produce a wide range of harmful effects in animals.

The economic impact of reduced animal productivity, increased incidence of disease due to immunosuppression, damage to vital organs and interference with reproductive capacity were many times greater than the impact caused by death due to mycotoxin poisoning. Mycotoxins in combination appear to exert greater negative impact on the health and productivity of livestock in comparison to their individual effects (Smith and Seddon, 1998).

2.6 Effect of Toxin Binder on Broiler Chickens

2.6.1 Effect of toxin binders on growth performance

Study conducted by Jelena *et al.* (2015) to determine the efficacy of three different adsorbents namely inorganic (modified zeolite), organic (esterified glucomannans) and mixed (inorganic and organic components, with the addition of enzymes), in protecting broiler from toxic effects of ochratoxin A in their feed. Broilers were fed diets containing 2 mg/kg of ochratoxin A (OTA) and supplemented with adsorbents at the recommended concentration of 2 g/kg for 21 days. The presence of OTA led to a notable reduction in body weight, lower weight gain, increased feed conversion. The presence of inorganic, organic and mixed adsorbents in contaminated feed only partially reduced the negative effects of OTA on the broiler performances. The broilers that were fed with adsorbent-supplemented feed reached higher body weights (17.9, 19.09 and 13.59 %) compared to the group that received only OTA. Ani *et al.* (2015) reported better weight gains when birds fed 5 % dietary clay used as toxin binders compared to those on other diets and the similarity between birds in the control and those of other treatments in these traits suggested that inclusion of clay in the diest improved the performance of the birds. Hedayati *et al.* (2014) reported that when a toxin binder was varied in diets of broiler it gave the highest body weight which was significantly better than the control group. Agboola *et al*. (2015) reported in their findings that supplementation with Mycotoxin binder and probiotics resulted in improved body weight gain over the control diet which is an indication of the positive effect of mycotoxin binder on broiler performance through the control of the gut microbiota.

2.6.2 Effect of toxin binders on feed conversion efficiency

Feed conversion ratio (FCR) was documented not to be significantly influenced by clay supplementation (Damiri *et al*., 2010; Eser *et al*.; 2012). However, Katouli *et al*. (2010) reported significant effect of bentonite, kaolin and zeolite on the FCR of broiler chicks. They reported that FCR of birds fed 3 % Kaolin in their 1st and 2nd weeks of life and 3 % zeolite in their 1st week significantly (P <0.05) reduced compared to the control. Girish and Devegowda (2004) did not detect any differences in body weight and feed conversion ratio between the broilers fed non-contaminated feeds containing toxin binders and without them.

2.6.3 Efeect of toxin binders on feed intake

Acosta *et al*. (2005) reported that the addition of 1% zeolite in diet of broilers decreased feed intake while Eser *et al*. (2012) found no apparent relationship between doses of Sepiolite and feed intake in broilers. Talat *et al*. (2009) reported no significant difference in feed intake and feed conversion ratio in both the control diet and other treatments with the toxin binders sodium bentonite and HSCAS. Similarly, Ologhobo *et al.* (2015) reported no significant difference in feed intake and feed conversion raito in both the control and montmorillonite group.

2.6.4 Effect of toxin binders on carcass characteristics

Ologhobo *et al.* (2015) reported a decrease in dressing percentage when dietary aflatoxin contamination with binders was used in different broiler diets. These results were in line with findings of Awan (1997) who reported a decrease in dressing percentage due to the Aflatoxin (AF) in the diet. However, these result did not agree with the report of Ibrahim *et al.* (1998) who observed consistency in growth rate of chicks fed 2·5mg AF kg–1 feed with chicks receiving similar levels of dietary AF.

2.6.5 Effect of toxin binders on nutrient digestibility

Dibner and Buttin (2002) suggested that organic acids improved protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of sub-clinical infections and secretion of immune mediators, through reduced production of ammonia and other growth depressing microbial metabolites.

2.7 Haematology

Haematology refers to the study of the numbers and morphology of the cellular elements of blood. These include red cells (erythrocytes), white cells (leucocytes), the platelets (thrombocytes) and the use of their results in the diagnosis and monitoring of disease (Merck Manual, 2012).

Haematology studies were useful in the diagnosis of many diseases and the extent of the damage to blood in animal and human (Ovuru and Ekweozor, 2004). Haematological parameters were good imdicators of the physical status of exposed animals (Khan and Zafar, 2005). Blood acts as a pathological reflector of the status of exposed animals to disease and other conditions (Olafedehan *et al.,* 2010). Isaac *et al.* (2013) reported that animals with good blood composition were likely to show good performance. Test of blood in the laboratory could help to detect any deviation from normal in farm animals and humans (Ogunbanjo *et al*., 2009). The examination of blood gave the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it played a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004; Doyle, 2006). The blood transports or conveys nutrient from one part of the body to another therefore, whatever affects the blood will adversely or moderately affects the animal in terms of health, growth, maintenance and reproduction (NseAbasi *et al*., 2014). Blood analysis is a fast means of assessing clinical and nutritional health status of animal in feeding trials (Olabanji *et al*., 2007). Haematological parameters of animals were significantly influenced by reproductive cycle, sex, age and season (Yakub *et al*., 2013). The range of values for haematological parameter of chicken is shown in Table 2.4.

2.7.1 Significance of haematological parameters

Haematological parameters were parameters related to the blood and blood-forming organs (Stenesh, 1975). Haematological parameters affect both the health and nutritional state of an animal. The nutritional value of a feed stuff could therefore be reflected through parameters such as: white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), lymphocytes and neutrophils. The full blood count (FBC), also referred to as a full blood examination or complete blood count, is one of the most commonly performed blood tests because it could tell the animals state of health. It is important for diagnosing conditions in which the number of blood cells was abnormally high or abnormally low, or the cells themselves were abnormal. A full blood count measured the status of a number of different features of the blood, including the amount of haemoglobin in the blood the number of red blood cells the

Table 2.4 Haematological parameters of chickens

Haematological Types	Units – International Standard (SI)	Normal Range
RBC	$x10^{12}/L$	2.5 – 3.5
Hb	g/dl	7.0 – 13.0
PCV	%	22 – 35.0
MCV	Fl	90.0 –140.0
MCH	Pg	33.0 – 47.0
MCHC	g/l	26.0 – 35.0
WBC	$x10^{9}/L$	1.2 – 3.0
Eosinophils	$x10^{9}/L$	1.5 – 6.0
Basophils	$x10^{9}/L$	Rare
Lymphocytes	$x10^{9}/L$	45.0 – 70.0
Monocytes	$x10^{9}/L$	5.0 – 6.0

Source: Reference values of Jain (1993)

percentage of blood cells as a proportion of the blood volume (haematocrit or packed cell volume). The volume of red blood cells (mean cell volume); the average amount of haemoglobin in the red blood cells number (known as mean cell haemoglobin), the number of white blood cells (white cell count), the percentages of the different types of white blood cells (leucocyte differential count) and the number of platelets.

2.7.2 Haematological components and their functions

2.7.2.1 Red blood cells

The red blood cells also known as erythrocytes, are the most common types of blood cell in animals and is the principal means of transporting oxygen to the body tissues through the circulatory systems (Chineke *et al.*, 2006). Isaac *et al*. (2013) reported that the red blood cell is involved in the transport of oxygen and carbon dioxide in the body. NseAbasi *et al*. (2014) reported that a decrease in the red blood cell count indicated a reduction in the level of oxygen that would be carried to the body tissues. Red blood test could help diagnose anaemia and other conditions affecting red blood cells (Bunn, 2011). The red blood cell test provided information about the haemoglobin content and size of red blood cells (Gernsten, 2009). Abnormal values of the red blood cells indicate the presence of anaemia (Gernsten, 2009). Lower than normal red blood cells may be due to anaemia, bone marrow failure, haemolysis (red blood cells destruction due to blood vessel injury or other causes) haemorrhage and malnutrition (Bunn, 2011).

2.7.2.2 White blood cells

White blood cell also known as leukocytes and its differentials functions to defend the body against infection and disease by destroying infectious agents (Isaac *et al*., 2013). Animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies and have high counts degree of resistance to diseases (Soetan *et al*., 2013).

Neutrophil counts generally increase in acute infectious and non-infectious inflammations, during intoxication and in certain metabolic diseases. An increase in the counts of acidophilic granulocytes was observed in parasitic diseases, allergies and mycotoxicoses. Eosinophils can provoke an inflammatory response to environmental allergens, such as fungi, by identifying toxic metabolites (Matsuwaki *et al*., 2011).

2.7.2.3 Packed cell volume

Packed cell volume also called haematocrit is the percentage of red blood cells in blood (Purves *et al*., 2004) or the proportion of blood volume that is occupied by red blood cells (Wikihow, 2013). Packed cell volume is involved in the transportation of oxygen and absorbed nutrients (Isaac *et al*., 2013). Increased packed cell volume indicated a better transportation of oxygen and absorbed nutrients (NseAbasi, 2014). Peters *et al.* (2011) stated that packed cell volume was significant in the diagnosis of anaemia and also serve as a useful index of the bone marrow capacity to produce red blood cells (Awodi *et al*., 2005; Chineke *et al*., 2006). Chineke *et al*. (2006) stated that high packed cell volume reading indicated either an increase in numbers of red blood cells or reduction in circulating plasma volume. A low level of packed cell volume was an indication of anaemia (Aster, 2004).

2.7.2.4 Haemoglobin

Haemoglobin is the protein molecule in the red blood cells that carries oxygen from the lungs to the body’s tissues and returns carbon dioxide from the tissues back to the lungs (Ugwuene, 2011; Soetan *et al*., 2013; Isaac *et al*., 2013). Peters *et al.* (2011) stated that haemoglobin and mean corpuscular haemoglobin were major indices for evaluating circulating erythrocytes and were significant in the diagnosis of anaemia. Haemoglobin deficiency decreased blood oxygen carrying capacity (NseAbasi, 2013). Haemoglobin concentrations are also indicative of adaptation of adverse environmental conditions (Kumar and Pachaura, 2000). According to Minka and Ayo (2007) haematological values were used to assess stress and welfare in animals.

2.7.2.5 Mean corpuscular volume

Mean cell volume is the expression of the average volume of individual erythrocytes (red blood cells) calculated with the following formula:

MCV (femtoliters (fl)) = (PCV × 10) ÷ RBC

It is useful for determining the type of anaemia an animal might have. A low MCV may indicate iron deficiency, chronic disease, pregnancy, a haemoglobin disorder such as thalassaemia, anaemia due to blood cell destruction or bone marrow disorders. A high MCV may indicate anaemia due to nutritional deficiencies, bone marrow abnormalities, liver disease, alcoholism, chronic lung disease or therapy with certain medications (Jaime and Howlett, 2008).

2.7.2.6 Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration

The mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are further guides to investigate anaemia. The MCH is the haemoglobin content of the average red cell. The MCHC is the average haemoglobin concentration in a given volume of packed red cells. The MCH may be low in types of anaemia where the red blood cells are abnormally small or high in other types of anaemia where the red blood cells are enlarged for example, as a result of folic acid or vitamin B12 deficiencies. The MCHC is low in iron deficiency, blood loss, pregnancy and anaemia caused by chronic disease. MCH is calculated with the following formula:

MCH (pictograms (pg)) = (Hb × 10) + RBC

MCHC is calculated with the following formula:

MCHC (g/l) = (Hb × 100) ÷ PCV (Jaime and Howlett, 2008).

2.7.2.7 Blood platelets

Platelets are the smallest of the blood cells. Blood platelets are responsible in blood clotting. Low platelet concentration is an indication that the process of blood clotting will be prolonged resulting in excessive loss of blood in the case of injury (NseAbasi, 2013).

2.7.2.8 Leucocytes (White Cell) differential count

Leucocytes differential count provides an estimate of the numbers of the 5 main types of white blood cells. These are neutrophils, monocytes, lymphocytes, eosinophils and basophils. Each of the 5 types has a specific role in the body (Mitruka and Rawnsley, 1977). Neutrophils and monocytes protect the body against bacteria and eat up small particles of foreign matter. Lymphocytes are of large and small morphology, which are two types; the B and T-forms. The B is derived from the bone marrow and T from the thymus. The B form produces antibodies which combine with foreign materials or antigens, while the T is responsible for the regulation of the antigen and the cell mediated response of the animal. Aggregates of lymphocytes are found within the bone marrow of the birds, although major sites of lymphopoiesis in adult birds are located in the spleen, liver, intestines and caecal tonsils. An increase in the cell count of lymphocytes is an indication of viral infection (Dieterian-Lievre, 1988). Lymphocytes are involved in the immune process, producing antibodies against foreign organisms, protecting against viruses and fighting cancer. Eosinophils kill parasites and are involved in allergic responses. High numbers of eosinophils may be associated with worm infections or exposure to substances that cause allergic reactions. Basophils also take part in allergic responses and increased basophil production may be associated with bone marrow disorders or viral infection (Ritz *et al*., 2005).

2.8 Effect of Toxin Binder on Blood Constituents

Haematological parameters of animal are determined as an index of their health status. In a study conducted by Che *et al*. (2011) the addition of three mycotoxin adsorbents (EGM, HSCAS and CMA) alleviated the alteration of RBC, WBC and Hb caused by mould contaminated diet. Furthermore, supplementation of 0.05% HSCAS or 0.1% CMA to the diet containing mycotoxins significantly decreased Haematocrit ($P<0.05$). The mold contaminated diet increased the levels of white blood cells (WBC), haemglobin (Hb) and packed cell volume (PCV), and decreased red blood cell (RBC) level. Similarly, Abbès *et al.* (2006) reported that mice treated with 500 mg/kg ZEN caused a significant increase in WBC, PCV, Hb, Mean Corpscular Volume (MCV), Mean Corpscular Haemoglobin (MCH), Mean Corpsular Haemoglobin Concentartion (MCHC) and Mean Platelets Volume (MPV) levels, and decrease in RBC level. Their results indicated that haemostasis blood system damage was induced by mycotoxins (Abbès *et al.*, 2006).

2.8.1 Effect of toxin binders on serum biochemistry

Serum biochemical parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities provide a sensitive and specific measure of hepatic function or injury (Abbès *et al*., 2006). Serum alkaline phoshatase (ALP) and γ-glutamyl transferase (GGT) activities (Kubena *et al.*, 1997), and total protein (TP), albumin (ALB), globulin (GLB) and glucose (GLU) concentrations (Mathur *et al*., 2001) have also been described as valuable parameters of hepatic injury and function. In a study conducted, mould contaminated feed resulted in an increase in GGT and AST activities, and a decrease in the concentration of blood urea nitrogen (BUN) and GLB as compared to the control diet. Aravind *et al.* (2003) reported broilers fed a naturally contaminated diet were associated with significant decrease in BUN concentration and increase in GGT mactivity along with unaltered total blood concentration at 21d of age. Sharma *et al.* (2008) reported serum AST activity was increased in chicks fed with fumonisin B1 or moniliformin. Similar results were observed by other studies (Gowda *et al*., 2008), which suggested that mycotoxins exert a direct toxic effect on animal liver. Increased activity of liver enzymes such as ALT, AST, AP, gamma GT and LDH was used for evaluation of aflatoxicoses in chickens, duckling and turkey poults (Yildirim *et al.,* 2011).

Eraslan *et al.* (2006) reported a moderate increase in the albumin: globulin ratio of broilers by addition of 0.3 per cent hydrated sodium bentonite in aflatoxin mixed feed of broilers. They also reported that histo-pathological finding in liver sections of broiler fed aflatoxin plus hydrated sodium bentonite indicated a non-protective effect of this adsorbent. Due to their montmorillonite content, bentonites swell and form thixotropic gels, as result of their ion exchange capabilities, they are widely used as mycotoxin sequestering agent (Duarte and Smith, 2005). Eraslan *et al.* (2006) reported the effectiveness of sodium bentonite in reliving the damages due to the presence of aflatoxins (1ppm) in 45 day old broiler chickens.

2.9 Acidifiers

Antibiotics have been used worldwide in poultry industry in the past 60 years for preventing diseases and improvement of growth performance. The continuous and misuse of antibiotics in livestock production and specially poultry industry has resulted in concerns about development of drug-resistant bacteria, drug residues in the body of the birds, and imbalance of normal microflora. As a result, the expanded use of antibiotics, in particular for growth promotion, had led to a total ban of antibiotic application to feed in a number of European countries (Choct, 2001). Therefore, animal researchers and animal food producers were looking for suitable feed additives to improve poultry natural immune system and hereby increase poultry performance. Several scientific reports demonstrated that acidifiers and organic acids could stimulate the natural immune response of poultry, reduce the activity of pathogenic bacteria and balance bacteria population in poultry (Cross, 2002; Dalloul *et al.*, 2003). Acidifiers constitute an important component of modern feeding strategies without antibiotics. Acidifiers are used in three ways in a poultry operation. Firstly they were added to the poultry feed in a solid form. This fights mould development in the feed and reduced the pH in the birds' crops. Secondly, when sprayed into the poultry litter and this attacked the bacteria that facilitate the breakdown of uric acid, limiting the amount of ammonia released. Lastly by injection into the water to kill bacteria, facilitate chlorine in killing bacteria and lowering the pH in the birds' gut (Technical Team, 2014).

The term acidification included the reduction of pH in feeds and in the animal digestive tract, mainly in the stomach (Peris and Calafat, 2001). Acidifiers can be in organic or inorganic acids or associated salts. As a group of chemicals, organic acids are considered to be any organic carboxylic acid of the general structure R- COOH including fatty acids and amino acids (Partanen and Mroz, 1999). Organic acids are widely distributed in plants and animals. They are also produced by microbial fermentation of carbohydrates and other fermentable material, predominantly in the large intestine of pigs (Partanen and Mroz, 1999). Acidifiers are acids included in feeds in order to lower the pH of the feed, gut, and microbial cytoplasm thereby inhibiting the growth of pathogenic intestinal microflora. This inhibition reduces the microflora competing for the host nutrients and results in better growth and performance of the chicken. They also act as mould inhibitors. They are added up to 0.25% of the diet. Most acids are efficacious and their effect remained as long as the acid is not volatilized. Organic acids have been used extensively for more than 25 years in swine production and more recently in poultry (Avitech, 2004).

2.10 Organic Acids

The term ‘organic acids’ refers to all those acids built on a carbon skeleton, known as carboxylic acids, which can alter the physiology of bacteria, caused metabolic disorders that prevent proliferation and cause death (Theobald, 2012).

The European Union allowed the use of organic acids and their salts in poultry production because these were generally considered safe (Adil *et al*., 2010a). Organic acids have been used for decades in commercial compounded feeds, mostly for feed preservation, for which formic and propionic acids were particularly effective (Lückstädt, 2014). In the European Union, these two organic acids and several such as (lactic, citric, fumaric and sorbic acids and their salts e.g. calcium formate, calcium propionate were used under the classification ‘feed preservative’ (Lückstädt and Mellor, 2011). The short-chain acids (C_1–C_7) were associated with anti-microbial activity. They were either simple mono-carboxylic acids such as formic, acetic, propionic and butyric acids or carboxylic acids with the hydroxyl group such as lactic, malic, tartaric and citric acids or short-chain carboxylic acids containing double bonds like fumaric and sorbic acids (Shahidi *et al.,* 2014).

Organic acids are weak acids and are only partly dissociated. Most organic acids with anti-microbial activity have a pKa (the pH at which the acid is half dissociated) between 3 and 5. A wide range of organic acids with variable physical and chemical properties exists, of which many are used as drinking water supplements or as feed additives (acidifiers). The use of organic acids have been reported to protect the young chicks by competitive exclusion (Mansoub *et al*., 2011), enhancement of nutrient utilization, growth and feed conversion efficiency (Lückstädt and Mellor, 2011).The supplementation of organic acids at the right high doses in animal feed can increase the bod yweight, improves feed conversion ratio and reduce colonization of pathogens in the intestine.

Specifically, Kirchgessner and Roth (1988) differentiated the various actions of organic acids which include, decrease of pH value and buffering capacity. Antibacterial and antifungal effects in the feed are achieved by reduction of pH value and the release of hydrogen ions in the stomach. This action activated pepsinogen to form pepsin to improve protein digestibility, inhibits gram-negative indigenous microflora in the gastro-intestinal tract and improved energy utilization in the intermediate metabolism.

The efficiency of an organic acid to inhibit the growth of a microorganism depended on its pKa value. In its un-dissociated form, organic acids have anti-microbial power as they can pass through the walls of bacteria and fungi and alter their metabolism. Therefore, the lower the pKa of the organic acids, which implies higher proportion of its dissociated forms, the greater the reduction of stomach pH and the lower its antimicrobial effect in the more distal portions during its transit through the digestive tract. A strong acid (with low pKa) will acidify the feed and the stomach, but would not have strong direct effects on the microflora in the intestine. They were also formed through microbial fermentation by organic acids produced by microbial fermentation constitute a major part of the host animals' energy supply especially in ruminant. In monogastric animals organic acids were important source of energy for cells of the intestinal wall (Hink and Patrick, 2003).

2.10.1. Formic acid

Formic acid (HCO_2H**),** also called methanoic acid, is the simplest of the carboxylic acids, used in processing textiles and leather. Formic acid was first isolated from certain ants and was named after the Latin word *formica*, meaning "ant." It was made by the action of sulphuric acid on sodium formate, which was produced from carbon monoxide and sodium hydroxide (Brown, 2009).

Formic acid is a colourless, transparent liquid with a pungent smell. It is commonly used as a preservative in ensiling forage and various by-products which contain less substrate for the desirable production of lactic acid by *Lactobacilli*. Formic acid is an effective acidulant, but it could also inhibit microbial decarboxylase and enzymes such as catalase (Partanen and Mroz, 1999). The antibacterial activity of formic acid was primarily against yeasts and some bacteria, while lactic acid bacteria and moulds were resistant to its effect (Lueck, 1980).

2.10.2 Propionic acid

Propionic acid is a colourless, oily liquid with a pungent, rancid smell. It occurs naturally in dairy products and is a by-product of human metabolism (RED, 1991). Propionic acid is manufactured primarily for use as a preservative and anti-mould agent in animal feeds and grains. It is also used as a preservative and flavouring agent in packaged foods such as baked goods and cheese. As a food additive, propionic acid is listed as “Generally Recognized as Safe” (GRAS) by the U.S. Food and Drug Administration (FDA) as an anti-microbial agent and flavouring agent. It is technically a fungicide (moulds) and bactericide. Over 75% of propionic acid used for this purpose in form of the salt called ammonium propionate, because it is less corrosive to farm equipment. Specific agricultural applications were high when used for grains with high moisture content in indoor storage of hay, oats, corn, barley, wheat, and sorghum as antibacterial additive in livestock and poultry drinking water. It is also used as surface sanitizer for storage areas for silage and grain and as poultry-litter additive (RED, 1991).

2.10.3 Acetic acid

Acetic acid (CH_3COOH), also called ethanoic acid, is the most important of the carboxylic acids. A dilute (approximately 5 percent by volume) solution of acetic acid produced by fermentation and oxidation of natural carbohydrates is called vinegar a salt, ester, or acylal of acetic acid is called acetate. Biologically, acetic acid is an important metabolic intermediate, and it occurs naturally in body fluids and in plant juices (Brown, 2014).

2.10.4 Citric acid

Citric acid ($C_6H_80_7$) is a weak organic acid and has been used as a natural preservative. Citric acid has been widely used as an organic acid supplement for pigs and chickens (Kim *et al*., 2015). Citric acid (anhydrous and monohydrate) is approved as a food additive for use as a preservative (*quantum satis*) in a wide range of commonly consumed foods and is authorised as a preservative in feed for all animal species without restrictions. The use of citric acid in animal nutrition is safe for the consumer (EFSA, 2015). The addition of citric acid reduces the pH of aqueous suspensions of compound feedingstuffs and feed materials. Citric acid might have the potential to act as an acidity regulator in feedingstuffs (EFSA, 2015). Citric acid occurs in all living organisms as an intermediate in the tricarboxylic acid or Krebs cycle. It is a natural component of fruit, vegetables and plants (roots and leaves). The acid is found in significant quantities in lemon and lime juices (41 and 39 mg/kg for ready-to-consume juice and 31 and 30 mg/kg for concentrates, respectively). Citric acid is currently listed in the European Union Register of Feed Additives as a technological additive (functional group: preservative) for use in feed for all animal species.

2.10.5 Lactic acid

Lactic acid is produced by many bacterial species, primarily those of genera *Lactobacillus, Bifidobacterium, Streptococcus, Pediococcus and Leuconostoc*. It is a natural constituent of some feedstuffs and among the oldest of the preservatives of food. The anti-microbial action of lactic acid is directed primarily against bacteria, whereas many moulds and yeasts can metabolize it (Kim *et al*., 2005)

2.10.6 Butyric acid

In the past decade, butyric acid ($C_4H_8O_2$) has been the most intensively studied by many poultry researchers. Butyric acid is considered important for the normal development of epithelial cells because it can be used as a direct energy source by epithelial cells and has bactericidal activity in the GIT (Pryde *et al.*, 2002).

2.11 Acidifiers in Animal Nutrition

Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry. The EU has banned all antibiotic growth promoters (AGP) from livestock production with effect from January 2006. Alternatives to AGP were sought in a variety of forms. The ban on AGPs created significant opportunity for feed acidification (Frost and Sullivan, 2004).

Young animals have limited ability to produce hydrochloric acid in the stomach. At birth, hydrochloric acid production is negligible, but it increases with advancement in age. The greater the production of acid in the stomach, the lower the gastric pH (Peris and Calafat, 2001). In the stomach, pH can regulate the movement of viable bacteria and molds from the animal's environment to the small intestine and is involved in the activation of pepsin, a proteolytic enzyme. Pepsin is secreted as an inactive zymogen, pepsinogen, and its conversion to the active pepsin is catalyzed by the action of acid on any existing pepsin in the stomach. Additionally, the optimal pH for pepsin activity is 2.0. At higher pH, activity is severely reduced. The initial proteolytic activity carried out by pepsin is necessary for the subsequent activity of trypsin in the small intestine. An additional important role for stomach acid is the protection of the lower part of the digestive tract from bacterial invasion (Easter, 1988). The low pH (2.0 or less) commonly found in normal growing and adult animals have been shown to have a pronounced bacteriostatic effect. Thus, viable microorganisms entering the digestive system via the mouth are unable to pass through the stomach and successfully colonize in the small intestine.

Organic acids and salts have a long-history in the food and the feed industries, which commonly use them as preservatives. Some authors such as Hebeler *et al.,* (2000); Overland *et al.*, (2000); Walsh *et al*., (2003); Hellweg *et al*., (2006) also considered them to be a viable alternative to antibiotics in pig feeds where they already have had considerable success. According to Partanen and Mroz (1999), formic, acetic, propionic, butyric, lactic, sorbic, fumaric, tartaric and citric were the most promising of organic acids in this regard. Organic acids are weak acids that are commonly found in fruit juices and fermented foods that are added to foods as preservative agents (Luck and Jager, 1997). Organic acids such as propionic acid have been used for more than 30 years to reduce bacteria and mould growth in foodstuffs and thus preserve hygienic quality. In feed legislation they were registered as preservatives, but their positive effects on animal health and performance, if they were added to feed in sufficient amounts, are well documented. Acids used as feed additives were predominantly compounds that naturally occur in cell metabolism, thus they are natural products with low toxicity (Kirchgessner and Roth, 1988).

Acidification of diets with weak organic acids have been reported to decrease colonization of pathogen, production of toxic metabolites, improved digestibility of protein, calcium, phosphorous, magnesium, zinc and served as substrate in the intermediary metabolism (Isabel and Santos, 2009). Acidification of feed or water consumption by animals, using organic acids, represent a frequent practice to control digestive micro-flora, improve feed utilization, production performance and maintaining the health state of animals (Dhama *et al*., 2014). Acidifiers, particularly, the short chain fatty acid salts like acetate, propionate and butyrate have contributed greatly to the profitability in poultry business that provides people with nutritious products (Patterson and Waldroup, 1998). Moreover, acidifiers improved growth performance through establishment of low GIT pH condition by supporting endogenous digestive enzymes and reducing undesired gut micro organisms (Richards *et al*., 2005). Acidifiers constitute an important component of modern feeding strategies without antibiotics. Organic acids are known to have strong antibacterial effects and they have been used in the protection of feed from microbial and fungal destruction (Hedayati *et al.,* 2013).

2.12 Impact of acidifiers on digestive system of chickens

Organic acids are beneficial in practical studies. The efficacy of poultry digestion depends on micro-organisms, which live naturally in the digestive tracts. Inclusion of formic and propionic acids reduced pH in crop and gizzard alone but not in the entire intestinal tract. Organic acids in crop reduced *Salmonella* populations. Organic acids reduced production of toxic components by bacteria and a change in the morphology of the intestinal wall and reduce colonization of pathogens on the intestinal wall, thus preventing damage to the epithelial cells (Avitech, 2004).

Various studies revealed that body weight gain, feed intake, feed conversion ratio, carcass weight, abdominal fat weight, abdominal fat percentage and intestinal weight were affected significantly by giving organic acid mixtures. Organic acids enhanced growth performance and carcass quality of broiler chickens. One of the first reports of improved broiler performance when diets were supplemented with single acids was by formic acid (Vogt *et al.,* 1981). Subsequently, Izat *et al.* (1990a) reported reduced levels of *Salmonella* spp. in carcass and caecal samples after including calcium formate in broiler diets. Izat *et al.* (1990b) showed that buffered propionic acid could be used to counteract pathogenic microflora in the intestine of broiler chickens, which resulted in significant reduction in carcass contamination with *Escherichia coli* and *Salmonella* spp. Improvements in broiler performance and hygiene in response to organic acids were often reported. However, an important limitation is that organic acids are rapidly metabolised in the foregut (from crop to gizzard), which reduced their impact on growth performance. Double salts of organic acids, such as potassium diformate and sodium diformate, which reach the small intestine, demonstrated the effects of potassium on nutrient utilization (Selle *et al.,* 2004).

There were a body of literature for poultry, on use of organic acid however much smaller than in pig production. Several studies have been done using fumaric acid, an early study by Vogt and Matthes (1981) reported on the effect of fumaric acid in broilers and laying hens. Fumaric acid improved feed efficiency by 3.5 to 4% in broilers. Layers feed efficiency was also improved but rate of lay was not affected. Patten and Waldroup (1988) reported a significant improvement in weight gain of broilers using 0.5 and 1.0% fumaric acid, but there was no effect on feed intake. Higher acid concentrations were associated with reductions in feed intake and body weights. Skinner *et al*. (1991) reported a significant improvement of 49-d body weight and feed utilization in male broilers fed 0, 0.125, 0.25, or 0.5% fumaric acid. Mortality rates, abdominal fat percentages, and dressing percentages were not affected. A similar study was reported by Runho *et al.* (1997) in which 0.25 to 1.0% fumaric acid was compared to an antibiotic growth promoter (Nitrovin) fed to Hubbard broilers. Growth was not affected, but feed consumption was reduced, resulting in a significant improvement in feed to gain ratio. Feed efficiencies for 0.5, 0.75, and 1% fumaric acid were comparable to the antibiotic control Runho *et al.* (1997). Associated with this performance improvement was a significant improvement in apparent metabolizable energy.

In the study of (Vogt and Matthes, 1981), when 2% citric acid was added to feed, coliform bacteria increased in the small intestine. The use of citric acid at dietary concentrations of up to 1% in relation to the caecal colonisation with *Salmonella typhimurium* and carcass contamination following oral challenge. The number of birds colonised with *Salmonella typhimurium* increased following supplementation with citric acid as compared to control, which indicated that citric acid may not be reliable with respect to the prevention of Salmonella colonisation of the caeca (Waldroup *et al*., 1995).

2.13 Broiler Gut Microbiota

The gut is a pivotal organ system which mediates nutrient uptake and use by the animals. The gut is also a major site of potential exposure to environmental pathogens (Yegani and Korver, 2008). Hence, a well-functioning and healthy gut is the cornerstone of the optimum performances of the birds. When the gut function and health were impaired, digestion and absorption of nutrients were affected thus the health and performance of birds would be compromised (Sugiharto, 2014). Besides being responsible for the absorption of nutrients from the lumen, intestinal mucosa of broiler chicken plays an important role in providing an effective barrier between the hostile luminal content and the host internal tissues. In this notion, intestinal mucosa was an important determinant of gut health and performance of chicken (Rinttila¨ and Apajalahti, 2013).

The gut microbiota aid in the digestion of feed and feed components like essential amino acids and vitamins which otherwise may remain unavailable to the host to provide essential amino acids and vitamins. Furthermore, by-product of bacterial metabolism (short chain fatty acids), fuel epithelial cells and suppress expression of pathogen virulence factors. To support the intestinal mucosal barrier functions, the dynamic balance between the mucus layer, epithelial cells, microbiota and immune cells in the intestine are of importance (Schenk and Mueller, 2008). A number of factors associated with diet and infectious diseases agents have been reported to affected this dynamic balance, and subsequently affect the health status and production performance of the chicken (Yegani and Korver, 2008).

A subtherapeutic use of antibiotics had been widely practiced in poultry industry for decades to maintain the balance of ecosystem in the gut as well as to improve the growth performance of chicken (Huyghebaert *et al.*, 2011). However, this practice had been questioned, given the increasing prevalence of resistance to antibiotics in chicken (Kabir, 2009). Hence, alternatives to antibiotics were needed in poultry industry to maintain the gut health and promote the performance of birds. Of the factors that may be responsible for the gut health and performance of chicken, commensal micro-organism in the gut seemed to have pivotal roles as they may help to direct the development of gut structure and morphology, modulate the immune responses, offer protection from luminal pathogens as well as aid digestion and utilization of the nutrients (Rinttila¨ and Apajalahti, 2013).

Similar to mammals, the immune system of birds is complex and composed of several cells and soluble factors that work together to produce a protective immune response (Yegani and Korver, 2008). It has been known that commensal gut microbiota was important inducers for the development and maturation of both innate defence mechanisms and adaptive immune responses of chicken (Brisbin *et al.*, 2008). Based on the studies in mammals, specific commensal bacterial species may also have a vital role in inducing the accumulation of certain immune cell populations in the intestine (Kogut, 2013). Lactobacilli are a group of commensal bacteria that have long been known for their ability to activate the intestinal immune system and increase the resistance to diseases, in part through the release of low-molecular weight peptides which induce immune activation (Muir *et al*., 2000). These bacteria have also been reported to produce a wide variety of short chain fatty acids (SCFAs), which are bacteriostatic for a subset of bacterial species either directly or by reducing pH of the intestinal environment, produce bacteriocins with microbicidal or microbiostatic properties and contribute to the colonization resistance against pathogenic microbes by modifying the receptors used by the pathogenic bacteria (Rinttila¨ and Apajalahti, 2013). Moreover, SCFAs produced by lactic acid bacteria (LAB) favour the renewal and barrier function of the gastro-intestinal epithelium (Kogut, 2013).

The intestine contains both bacteria that are beneficial for the health, such as gram-positive lactobacilli and bifidobacteria and potential pathogenic bacteria, such as Clostridium spp., Salmonella and Escherichia coli. It is generally accepted that a proper bacterial balance between the number of beneficial bacteria and pathogenic bacteria in the intestine (at least 85% of total bacteria should be good bacteria) was vital for the host, and the impact on gut health often came from microbial imbalance in the gut of chicken (Choct, 2001).

It was suggested that commensal intestinal bacteria were important in digestion and synthesis of dietary compounds and involved in the development of gastrointestinal tract. These bacteria also play important roles in the regulation of intestinal epithelial proliferation, host energy metabolism and vitamin synthesis. Based on the study in mammals, commensal bacteria contribute to the regulation of digestion by mediating the bile acid synthesis, lipid absorption, amino acid metabolism, vitamin synthesis and SCFA production (Brestoff and

Artis, 2013). Moreover, commensal bacteria influenced the activity of digestive enzymes and gut mucosal morphology of the chicken (Lan *et al.*, 2005).

In turn, the gut microbiota were affected by the flow of nutrients from the diets, host derived substrates, immunological responses of the host and the gut anatomy (Rehman *et al*., 2007). Any disturbance in the gut microbial balance brought about by changes in diets composition, host immunity or gut physiology could lead to dybiosis and / or enteritis. Dybiosis may impact host metabolism by either altering the ratio of beneficial and detrimental species, hence affecting the host's availability to nutrients (Kogut, 2013).

Therefore, a favourable gut microbiota was important for the optimal growth and performance of chickens, while an unfavourable microbiota may promote enteric infection, leading to decreased growth rates and increased mortality. However, gut bacteria need not be pathogenic to impact negatively on bird's performance and production. A normal poultry gut microbiota was described as being dominated by *Firmicutes* and *Bacteriodetes*, while intestinal dybiosis was associated with elevated *Enterobacteriaceae* (Kogut, 2013)

2.14 Effect of organic acid on the gastro-intestinal tract

Good intestinal health in the poultry industry is of great importance to achieve target growth rates and feed efficiency. Organic acid (1.0% sorbic acid and 0.2% citric acid) supplementation significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks at 14 days of age (Rodríguez-Lecompte *et al*., 2012). Garcıá *et al.* (2007) reported that broilers fed diets containing formic acid had the longest villi (1273 and 1250 µm for 0.5 and 1.0% formic acid, respectively) compared with control (1088 µm). Similarly, crypts of jejunum were significantly deeper in birds fed the formic acid diet (1.0%) than birds fed the antibiotic diets (266 vs. 186 µm, respectively; $P < .05$) in the same experiment. Thus, formic acid supplementation increased both the villus height and crypt depth. Short-chain fatty acids have been demonstrated to stimulate the proliferation of normal crypt cells, enhancing healthy tissue turnover and maintenance. This reduction in the muscularis thickness was helpful in improving the digestion and absorption of nutrients as reported by Teirlynck *et al.* (2009) that the thickening of mucous layer on the intestinal mucosa contributed to the reduced digestive efficiency and nutrient absorption.

Paul *et al*. (2007) found that the histology of intestinal parts revealed that organic acid salt (ammonium formate and calcium propionate) supplementation increased the villus height of different segments of the small intestine than the control group possibly by reducing intestinal colonization of pathogenic and non-pathogenic bacteria. The increase of villus height of different segments of the small intestine may be attributed to the role of the intestinal epithelium as a natural barrier against pathogenic bacteria and toxic substances that were present in the intestinal lumen.

The addition of organic acids to broiler diets resulted in the pH reduction of digesta in various parts of the GIT. In general, the degree of pH reduction was usually greater in the upper part of GIT (crop, proventriculus and gizzard) as compared to the lower part of the GIT (duodenum, jejunum, ileum and caecum). In seven experiments conducted, 11 of 13 organic acid-supplemented groups showed decreased crop pH compared with the control groups, with 7 of 11 observations being significant. Three experiments reported that the pH reductions in the crop were dose dependent and it is likely that of all locations in the GIT the crop showed the greatest pH reduction. This observation may be related to the short transit of the acids to the crop in addition to the less acidic conditions of the crop (Thompson and Hinton, 1997).

2.15 Effect of Organic Acid on Broiler Chickens Performance

In poultry production, organic acids have not gained as much attention as in pig production (Langhout, 2000). High levels of production and efficient feed conversion were the need of the modern broiler industry which to a certain extent could be achieved by the use of specific feed additives. Organic acids have growth-promoting properties and can be used as alternatives to antibiotics (Fascina *et al.*, 2012). Dietary supplementation of organic acids increased the body weight and feed conversion ratio (FCR) in broiler chicken. Panda *et al.* (2009) reported that 0.4% butyrate in the broiler diet (646g) was similar to antibiotics (642g) in maintaining body weight gain but superior for FCR. No added advantage on these parameters was obtained by increasing the concentration of butyrate from 0.4% to 0.6% in the diet. Brzóska *et al.* (2013) reported that organic acid (0.3–0.9%) had a growth-enhancing and mortality-reducing effect in broiler chickens, with no significant influence on carcass yield or proportion of individual carcass parts.

Improvements in broiler performance in response to organic acids were often reported. However, an important limitation was that organic acids were rapidly metabolized in the foregut (the crop to the gizzard), which reduced their impact on growth performance (Lückstädt and Mellor, 2011). Double salts of organic acids, such as potassium diformate and sodium diformate, which reach the small intestine, have been shown to have a significant impact on nutrient utilization (Lückstädt and Mellor, 2011).

Dibner and Buttin (2002) suggested that organic acids and their salts improved protein and energy digestibility. This was achieved by reducing microbial competition with the host for nutrients, endogenous nitrogen losses, lowering the incidence of sub-clinical infections, secretion of immune mediators, by reducing the production of ammonia and other growth suppressing microbial metabolites. Probably these could be the reasons that organic acids or their salts improved feed utilization leading to better performance in broilers.

2.16 Anti Microbial activity of Organic Acids

An increased population of pathogenic bacteria in the gastro intestinal tract (GIT) often resulted in reduced growth performance of broiler chickens. Therefore, the prevention of pathogenic bacterial over-growth in the GIT may be one of the most important strategies for enhancing growth performance when supplemental Antibiotic growth promoters (AGPs) were not used in animal diets. Organic acids can easily penetrate the bacteria cell wall and disrupt normal cellular functions, including replication and protein synthesis of bacteria (Denyer and Stewart, 1998; Davidson, 2001). The sequential mechanisms of bactericidal action were followed as proposed by Mani-Lopez *et al.,* (2012), proposed the following sequential mechanism of bactericidal action. (1) Acid form of organic acids (protonated form) can penetrate across the bacteria cell wall, (2) penetrated organic acids within bacterial cells dissociate into the conjugated base form (non-protonated form) with a concomitant reduction in cellular pH, and (3) decreased pH created a stressful environment leading to cellular dysfunctions, and thus prevented bacterial growth. Such reactions were likely to occur mainly with pH-sensitive bacteria species, which included the wide range of pathogenic bacteria. Akyurek *et al.* (2011) reported that broiler chickens fed diets containing organic acid blends had less pathogenic bacterial loads such as coliforms and clostridia but greater beneficial bacteria such as Lactobacilli in the ileum compared with those fed diets containing AGPs. It was also likely that the decreased pH in the GIT induced by dietary organic acids may play a role in preventing bacterial transfer from the diet or environment.

The addition of organic acids in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria. Most common bacteria that affect the intestinal health of poultry are *Salmonella, Campylobacter* and *Escherichia coli* which could be controlled by supplementation of an organic acid in diet (Van Immerseel *et al.,* 2006; Naseri *et al.,* 2012). *Salmonella* is a human pathogen that is commonly found in poultry products. From a public health point of view, it is necessary to control this biological hazard. It is possible to decrease chicken carcass and egg contaminations by adding organic acids to the feed or drinking water at appropriate times, which could hinder its multiplication (Russell *et al*., 1998). *Salmonella* infection in poultry was mainly spread by contaminated feed (Koyuncu *et al*., 2013). The presence of *Salmonella* in poultry feed as well as feed ingredients such as grains, oilseed meals, feathers, fishmeal, blood meal, meat by-products and broiler feed has been documented (Petkar *et al*., 2011; Hald *et al*., 2012; Andino, 2014). *Salmonella* can multiply in the GIT of birds and potentially be excreted in the faeces during growing phase (Kušar *et al.,* 2010).

2.17 Effect of Organic acid on Nutrient Utilisation

Reduced pH in the upper part of the GIT may increase nutrient digestibility, and therefore, nutrient utilization in diets. In the stomach, a reduction in gastric pH activated pepsinogen and other zymogens by adjusting gastric acidity closer to that required for optimal activity (Jongbloed *et al.,* 2000). This increased enzyme activity can improve the digestion of proteins and possibly other nutrients. Furthermore, acidic digesta may decrease gastric emptying, and therefore provide more time for nutrient digestion in the GIT (Mayer, 1994). Several researchers have demonstrated that dietary supplementation of organic acids could improve the retention of protein and other nutrients.

Organic acids used as an acidifier in poultry feeds have been considered to be attractive alternatives for improving nutrient digestibility. Ghazala *et al*. (2011) reported that dietary 0.5% of either fumaric or formic acid and 0.75% of acetic or 2% citric acid improved both

ME and nutrient digestibility, of, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) of broiler diets. Moreover, Hernández *et al.* (2006) and Garciá *et al.* (2007) reported that supplementation of formic acid (0.5 or 1.0%) in broiler finisher diet was found to improve apparent ileal digestibility (AID) of dry matter (DM) by 67.8 or 68.8%, respectively and CP by 72.5 or 73.5%, respectively as compared with control by 56.4% DM and 60.7% CP. Similarly, 2% citric acid in the broiler diet also increased the retention of DM, CP and neutral detergent fibre (Ao *et al.*, 2009).

2.18 Effect of Acidifier on Digestion

The influence of organic acids in the digestive tract can be split into two parts, the acidification and the action of the anion of the organic acids. The addition of organic acids induced a more rapid reduction of the pH value in the stomach, which resulted in a shorter time to reach pH of 3. This range of pH was needed for an optimal activation of pepsinogen and pepsin. The response led to an improvement of the digestibility of protein, which had been proven several times (Schöner, 2001).

Acidification with various weak organic acids to diet such as formic – lactic, fumaric-propionic and ascorbic acids have been reported to improve digestibility of protein, while Ca, P, Mg and Zn serve as substrate in the intermediary metabolism (Kirchgessener and Roth, 1988) Partanen and Morz (1999) indicated that acidifier stimulated the endogenous enzymes in digestion. The efficiency of poultry digestion depended on the micro organisms which were naturally in its digestive tract. Certain feed additives products which were incorporated into animal feed created favourable conditions in the animal's intestine for the digestion of the feed (Denli *et al.*, 2003).

Acidification of the gut is by stimulated enzyme activity and optimized digestion and the absorption of nutrients and minerals. Un-dissociated forms of organic acids penetrated the lipid membrane of bacterial cells and dissociate into anions and cations. After entering the neutral pH of the cell's cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine triphosphate in organic phosphate interactions. Improved broiler performance by supplementation with single acids was noticed for formic acid (Vogt and Matthes, 1981) and fumaric acid (Kirchgessner, 1991). Izat *et al*., (1999) found significantly reduced levels of Salmonella spp. in carcass and caecal samples after including calcium formate to broiler diets. In another trial by Izat *et al*., (1990a) buffered propionic acid was used to counteract pathogenic microflora in the intestine and carcass of broiler chickens, that resulted in a significant reduction in *E. coli* and *Salmonella spp*.

Acidification with various organic acids had been reported to reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells (Langhout, 2000; Green and Sainsbury, 2001; Denli *et al*., 2003) also improved the digestibility of proteins, calcium, phosphorus, magnesium, and zinc, and also served as substrate in the intermediary metabolism (Kirchgessner and Roth, 1988).

2.19 Effect of Organic Acid on Feed

Feed could also be infected with fungi, bacteria or yeast in a hygienic environment. Under favourable conditions, microbes could multiply rapidly during storage especially at higher moisture level and in a warm environment. Conserving agents reduced microbial growth and depending on the agent, microbial numbers, could lower the uptake of pathogenic organisms by the animal. For conserving purposes, acid concentration was in general lower than for performance promotion. However, for each acid, its specific inhibiting effect on bacteria, yeast or mould had to be considered when recommendations for feed supplementation were made (Strauss and Hayler, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey of Feedstuff for Aflatoxin

3.1.1 Sample collection

This was done using purposive sampling technique, the feed mills that samples were collected from were selected by choice. Samples of Maize (MZ), soybean cake (SBC), groundnut cake (GNC), brewers dried grain (BDG) and maize offal (M/O) were collected from four major feed mills in Zaria metropolis and a batch from Giwa grain market for evaluation of Aflatoxin B1 contamination and mycobiota analysis. A total 500 grams of each ingredient were collected from the designated feed millers and put into medium size transparent polythene bags and labelled. Thereafter, samples were taken to the Mycotoxin laboratory of the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria for evaluation of Aflatoxin B1 detection and fungi isolation and identification.

3.1.2 Aflatoxin determination

Aflatoxin B1 (AfB1) was analysed using the Enzyme Linked Immune Sorbent Assay (ELISA) method. An indirect competitive ELISA protocol was used for the quantitative analysis of aflatoxin. Aflatoxin extraction and determination were performed according to the manufacturer’s procedure following the AOAC (1995) laboratory procedures for aflatoxin determination. Twenty grammes of each sample were blended in a blender to obtain a uniform mixture. A total of 100ml of 70% Methanol and 30 ml distilled water was added to 0.5 % of Potassium Chloride (KCl) which was then added to the sample and shaken thoroughly.The 100 mls of 70 % Methanol was added to the ground sample in a conical flask and shaken. The mixture was shaken in an orbital shaker for 30 minutes at 150 rpm and the mixture was filtered using filter paper, after which an ELISA reader was used to take the readings. All reagents and standards were from Trilogy analytical laboratory Washington. An ELISA READER; model; Bio – rad (404-750nm) was used to take the readings and samples were incubated in Techmel and Techmel USA, model; TT – 9082 incubator.

Collected samples were categorized into low (<20ppb), medium (20-50ppb) and high (>50ppb) levels of contamination of AfB1 following the general guidelines of FDA (Hanif *et al*., 2006).

3.1.3 Fungal cultivation and isolation

The growth media used was potato dextrose agar (PDA) amended by streptomycin. The media were prepared and thereafter sterilized by autoclaving at a temperature of 121 °C for 15 minutes at 6.5kg per square inch. They were then allowed to cool to 45 °C on the workbench before plating out into petri dishes. The dishes were inoculated with feed ingredient samples and incubated at room temperature for 5 days at the end of which they were examined for fungal growth. Fungi were isolated and cultured according to the method described by Pitt and Hocking (2009). Growths were further sub-cultured onto fresh media for another 5 days to obtain pure cultures. After isolation, identification of the pure isolates shown on the plates was confirmed using macroscopic and microscopic morphology (light microscope) observation and the interpretative key of some common genera of moulds (Samson *et al*, 2004; Pitt and Hocking, 2009).

3.2 Experimental Site and Location

The experiment was carried out at the poultry unit of the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Ahmadu Bello University, Samaru, Zaria. The site is located in the guinea savannah zone of Nigeria at Latitude 11^{o} 9’ 46’’ N, Longitude 7^{o}37’45’’E and at an altitude of 610m above sea level. The temperature ranges between 26-40^{o}C depending on the season while the relative humidity during the dry and wet seasons are 21 and 72% respectively. The wet period in Zaria is between May and October with annual rainfall of about 1500mm (Institute for Agricultural Research Meteorological Unit, 2016).

3.2.1 Source of experimental birds

Ross breed of broiler chicks were purchased from a reputable hatchery located in Ibadan, South Western Nigeria.

3.2.2 Source of toxin binder, organic acid and oxytetracycline

Mycofix®; a toxin binder along with Biotronic® SE, an organic acid and Oxytretracycline were purchased from a commercial dealer in poultry products in Kaduna metropolis.

3.3 Laboratory Studies

Chemical analysis: Analysis of the proximate chemical composition of the experimental diets were done according to the method described by AOAC (1995). Nitrogen Free Extract (NFE) was determined by subtracting % CP+ %CF + ASH + EE from 100 and Metabolizable Energy (ME) was calculated according to the procedure of Pauzenga (1985) as: ME (kcal kg^{-1}) = 37 x Protein (%) + 81.8 x Fat (%) + 35.5 x NFE (%). The analyses were carried out at the Biochemistry Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria.

3.4 Experiment 1: Effect of Varying Levels of Mycofix® on Feed Utilization byBroiler Chicken

3.4.1 Experimental diets

Five isonitrogenous and isocaloric diets were formulated to meet the nutrient requirements recommended by NRC (1994) for broiler starter and finisher diets. Experimental diets in each phase had the same ingredients and nutrient composition.

3.4.2 Starter phase (0 – 4 weeks)

The experimental starter diets (Table 3.1) consisted of 23.19% crude protein and 2902kcal/ kg diet of metabolisable energy (ME). There were 5 treatments, designated as treatment 1 (control) contain no Mycofix®,while treatments 2, 3, 4, and 5 had varying inclusion levels of Mycofix® at 100, 200, 300 and 400g respectively per 100kg feed.The manufacturers' recommendations were 2 – 3kg/Tonne (200-300g/100kg feed) for both broiler starter and finisher feeds.

3.4.3 Finisher phase (5 – 8 weeks)

The experimental finisher diets (Table 3.2) had 20.54% crude protein and 2929kcal/kgME. There were 5 treatments, treatment 1 (control) had no Mycofix®,while treatments 2, 3, 4, and 5 had Mycofix® included at 100, 200, 300, and 400g respectively per 100kg feed.

Table 3.1: Ingredient composition of experimental broiler starter diets supplemented with varing inclusion levels of Mycofix® (0 – 4 weeks)

	Mycofix® Levels of Inclusion g/100kg				
Ingredients	0	100	200	300	400
Maize	56.00	56.00	56.00	56.00	56.00
Soya bean cake	29.70	29.70	29.70	29.70	29.70
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME Kcal/kg	2902	2902	2902	2902	2902
Crude protein (%)	23.19	23.19	23.19	23.19	23.19
Crude fibre (%)	3.59	3.59	3.59	3.59	3.59
Ether extract (%)	3.16	3.16	3.16	3.16	3.16
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Avail Phosphorous (%)	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.46	1.46	1.46	1.46	1.46
Methionine (%)	0.56	0.56	0.56	0.56	0.56
AfB1(ppb)	72.24	72.24	72.24	72.24	72.24
Cost/kg diet (₦)	83.82	83.88	83.94	84.00	84.06

[A]Biomix Broiler starter premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 30mg. AfB1= Aflatoxin B1, ppb = parts per billion. ME = Metabolisable energy

Table 3.2: **Ingredient composition of experimental broiler finisher diets supplemented with varing inclusion levels of Mycofix® (5 – 8 weeks)**

	Levels of Mycofix® g/100kg				
Ingredients	0	100	200	300	400
Maize	58.00	58.00	58.00	58.00	58.00
Soya bean cake	20.00	20.00	20.00	20.00	20.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00
Maize offal	4.70	4.70	4.70	4.70	4.70
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME Kcal/kg	2929	2929	2929	2929	2929
Crude protein (%)	20.54	20.54	20.54	20.54	20.54
Crude fibre (%)	4.11	4.11	4.11	4.11	4.11
Ether extract (%)	3.35	3.35	3.35	3.35	3.35
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.50	0.50	0.50	0.50	0.50
AfB1(ppb)	99.03	99.03	99.03	99.03	99.03
Cost/ kg diet(₦)	79.44	79.50	79.56	79.62	79.68

[A]Biomix Broiler Finisher premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 50mg. AfB1 = Aflatoxin B1, ppb = parts per billion.

3.5 Experimental Design and Management of Birds

A total of 330 unsexed day-old Ross breed broiler chicks were used for the study. The chicks were brooded together for three days and fed the control diet. On the third day, the chicks were weighed and randomly allocated to 5 dietary treatments with three replicates consisting of 22 birds per replicate. The design of the experiment was a completely randomized design. The chicks were raised in a deep litter poultry house with feed and water provided *ad libitum*. Routine vaccines against Newcastle and gumboro diseases were given according to the vaccination schedule of Veternarinay Teaching Hospital of Ahmadu Bello University, Zaria.

3.6 Growth Study

Feed intake was calculated as the difference between supplied feed and feed leftover in each pen. Weight gain was determined as difference between the weight of birds at the week under consideration and the previous one.

Feed conversion ratio was calculated as the ratio of feed intake per unit weight gain each week. Mortality was recorded and mortality percentages calculated by dividing the number of birds that died within a period by the initial number of birds placed in a pen and multiplying by 100. Feed and water were given *ad libitum* throughout the study period. At the end of the 4^{th} week, the birds were randomized within treatments using the average weight of the birds within treatments to achieve uniform weights thereafter, the finisher diets were fed to birds for another four weeks.

3.7 Haematological Analysis

At the 8^{th} week of the feeding trial in the finisher phase, 2 ml of blood each was taken at slaughter from six birds per treatment into sterile sample bottles containing ethylene diamine tetra acetic acid (EDTA). Samples were taken within an hour after collection to the clinical pathology laboratory of the Veterinary Teaching Hospital of Ahmadu Bello University, Samaru, Zaria for haematological analysis**.** The haematologic profile of the whole blood samples were estimated using the Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL), which was standardized for chicken blood (Balog *et a*l., 2003). The samples were analysed for packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (WBC) and haemoglobin concentration (Hbc), the percentage of heterophils (HET**)**, lymphocytes (LYM), following standard procedures by Davies *et al*, (1984). While mean cell volume (MCV), mean corpuscular hemoglobin (MCH**),** mean corpuscular haemoglobin concentration (MCHC) were calculated as derivatives.

3.8 Evaluation of Blood Serum Constituents

At the 8th week of the feeding trial, 2ml of blood samples were taken from 2 birds per replicate into heparinized tubes and taken to the clinical pathology laboratory of the Ahmadu Bello University Teaching Hospital, Zaria to determine parameters related to liver function. Plasma was separated by centrifugation at 3500 rpm for 15 minutes and frozen at - 20^0C. Biuret method of total serum protein determination was employed for this assay as described by Reinhold (1995). Glucose was calorimetrically determined using Randox Kit. Liver enzymes such as Serum transaminases that included aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase were assayed using Randox Colometric kit United Kingdom as described by Bush (1991). The kidney function parameters such as urea, creatinine, sodium ion, chloride ion and hydrogen carbonate were determined using Agape colometric kit, made in Switzerland following the procedure of Bush (1991).

3.9 Carcass Evaluation

At the end of the 8th week, two birds from each pen having representative weights for the group (6 birds per treatment) were selected. The liveweight of the selected birds were recorded, birds were starved of feed overnight but water was provided. The birds were sacrified by severing the jugular vein and hung upside down for proper bleeding. Each of the carcasses was scalded, de-feathered and eviscerated. The prime cuts such as thigh, drum sticks, wings and breast were separated, weighed individually and expressed as percentage of carcass weight. The internal organs such as heart, liver, gizzard, spleen and pancreas were expressed as percentages of the live weight while the viscera: abdominal fat, intestinal weight and length were expressed as percentages of their live weights.

3.10 Digestibility Study

A digestibility trial was carried out on the 8^{th} week of the experiment to determine nutrient digestibility. Six (6) birds per treatment having representative average weights for the group were used. The birds were kept individually in metabolic cages for three days to allow them acclimatize to the cage. Trays were placed under each cage for faecal collection.The birds were given one kilogram of feed each for the five day collection period, water was also given *ad libitum* throughout the period. Total feacal samples collected for five days were weighed and oven-dried at 65^{o} C for 24 hours. The dried faecal samples were then analysed for nutrient contents using methods described by AOAC (1995) at the Biochemistry Laboratory of the Department of Animal Science, Ahmadu Bello University Zaria. Nutrient digestibility was determined for dry matter, crude protein, ether extract, crude fibre, ash retention and nitrogen free extract. The percentage digestibility was calculated using the equation below:

$$\text{Digestibility} = \frac{\text{Nutrient intake - Nutrient output}}{\text{Nutrient Intake}} \times 100$$

Where: Nutrient intake (g) = Feed intake x Nutrient in diet; Nutrient output (g) = Faecal output x Nutrient in faeces.

3.11 Data Analysis

All the data collected from the experiment were subjected to analysis of variance (ANOVA) using general linear model of Statistiscal Analysis System (SAS, 2003) Software package. Significant differences between means were separated using Dunnetts Test.

3.12 Experimental Model

The linear Model used for the experiment was:

$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{t}_i + \mathbf{e}_{ij}$

Where:

$\mathbf{Y}_{ij}$= Dependent variable

$\boldsymbol{\mu}$= Overall mean

$\mathbf{t}_i$= i^{th} Effect of treatment

$\mathbf{e}_{ij}$ = Random error

3.13 Experiment 2: Effect of four inclusion Levels of Biotronic® SE on Feed Utilization by Broiler Chickens

3.14 Experimental treatments

A total of 396 day-old Ross broiler chicks of mixed sexes were used for this study. The chicks were weighed on arrival and randomly alloted to 6 dietary treatments with three replicates consisting of 22 birds per replicates in a completely randomized design. They were housed in deep litter pens. The experimental starter diets (Table 3.3) had Treatment one without Biotronic® SE, that represented the positive control, Treatments 2, 3, 4 and 5 had 200, 300, 400 and 500g Biotronic® SE inclusion while, Treatment 6 had 100g Oxytetracycline this represented the negative control. At the end of the 4th week the feed was changed to the finisher diet (Table 3.4), for another four weeks.The control diet was without Biotronic® SE and Oxytetracycline,

Table 3.3: Ingredient composition of experimental broiler starter diets supplemented with varing inclusion levels of Biotronic ® SE (0 – 4weeks)

	Levels of Inclusion (g/100kg) Biotronic® SE					
Ingredients	0	200	300	400	500	100g Oxytr
Maize	56.00	56.00	56.00	56.00	56.00	56.00
Soya bean cake	29.70	29.70	29.70	29.70	29.70	29.70
Groundnut cake	10.00	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME Kcal/kg	2902	2902	2902	2902	2902	2902
Crude protein (%)	23.19	23.19	23.19	23.19	23.19	23.19
Crude fibre (%)	3.59	3.59	3.59	3.59	3.59	3.59
Ether extract (%)	3.16	3.16	3.16	3.16	3.16	3.16
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.87	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.46	1.46	1.46	1.46	1.46	1.46
Methionine (%)	0.56	0.56	0.56	0.56	0.56	0.56
Cost/ kg diet(₦)	83.82	83.88	84.00	84.06	84.12	91.32

[A]Biomix Broiler starter premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium, Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 30mg, Bio: Biotronic, Oxyte: Oxytetracycline.

while Treatments 2, 3, 4 and 5 had 100, 200, 300 and 400g Biotronic® SE inclusion while Treatment 6 had 100g Oxytetracycline. Biotronic® SE was included in diets at a level below and two levels above the manufacturer's recommended rate of 3 – 4kg/ tonne for the purpose of this feeding trial. At tfhe finisher phase, the manufacturer's recommended rate is 2 – 3kg /tonne but for the feeding trial a level lower and two levels above the recommended quantity was used.

3.15 Intestinal Microbiota Study

At four weeks of the starter phase, six birds were slaughtered per treatment having a representative average weight of each treatment were used. The birds were slaughtered and scalded and defeathered. The gastrointestinal tract from the base of the gizzard down to the rectum was dissected using a sharp knife and sections approximately 3 cm long (including digesta) were cut from the ileum and ceaca of each bird. Samples were taken into sterilized glass bottles using swabs sticks and taken to the laboratory within one and half hours for microbial test for (total aerobic plate counts, total coliform counts) and islolation and characterization of *E.coli*, *Salmonella* and *Staphyloccocci* according to procedures described by Harrigan and McMane (1976). The microbioata analysis was done at the Microbiology Laboratory of the Department of Public Health, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. The serial dilutions (10^{-3} to 10^{-7}) of these samples were prepared and cultured on the selective media of plate count agar, De Man Rogosa Sharpe Agar (MRS) and MacConkey agar for enumerating total aerobics; lactic acid bacteria and coliforms, respectively. The total aerobics and coliforms population were counted after aerobic incubation at 37 ˚C for 24 hours and lactic acid bacteria after aerobic incubation at 37 ˚C for 48 hours (Witkamp, 1963). The colony counter machine was used to count the coliforms.

Table 3.4: Ingredient composition of experimental broiler finisher diets supplemented with varing inclusion levels of Biotronic® SE (5 – 8 weeks)

	Levels of Inclusion (g/100kg) Biotronic® SE					
Ingredients	0	100 Bio	200 Bio	300 Bio	400 Bio	100g Oxytr
Maize	58.00	58.00	58.00	58.00	58.00	58.00
Soya bean cake	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00	13.00
Maize offal	4.70	4.70	4.70	4.70	4.70	4.70
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME Kcal/kg	2929	2929	2929	2929	2929	2929
Crudeprotein (%)	20.54	20.54	20.54	20.54	20.54	20.54
Crude fibre (%)	4.11	4.11	4.11	4.11	4.11	4.11
Ether extract (%)	3.35	3.35	3.35	3.35	3.35	3.35
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.85	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.27	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.50	0.50	0.50	0.50	0.50	0.50
Cost/ kg diet(₦)	79.44	79.50	79.56	79.62	79.68	86.90

[A]Biomix Broiler Finisher premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium, Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg’ Zinc, 30mg Bio: Biotronic, Oxyte: Oxytetracycline

The colony morphology was identified based on lactose and non lactose fermenting and expressed as colony forming unit per gram (CFU/g) of each of the samples analyzed.

3.15.1 Culture and isolation of *Salmonella, Escherichia coli and Staphylococcus*

The homogenized samples were incubated at 37 ^{0}C for 24 hours for enrichment. A loop full of overnight culture was inoculated on the Eosin Methylene Blue (EMB), Salmonella Shigella Agar (SSA) and Mannitol (MSA) and then incubated at 37 ^{0}C for 24 hours. The colonies from each plate was identified using the colonial morphology, gram stained and inoculated on nutrient agar slant incubated for 37 ^{0}C for 24 hours and then stored at 4 ^{0}C for 24 hours for further analysis and identification.

3.15.2 Gram reaction

Smear was made on free grease slides and passed over the Bunsen burner flame three times in order to fix. It was then covered with crystal violet solution for 30 seconds and washed with water, then covered with iodine solution for 30 seconds. It was washed with water and decolourized with alcohol, then covered with safranin for 10 seconds, washed and air dried. Adrop of immersion oil was added to the smear and viewed under a microscope using x 100 objectives (Cheesbrough, 2002).

3.15.3 Microbact analysis kit (12E)

The positive isolates base on sugar fermentation and convention biochemical was purified using selective media and used for the Microbact 12E kit. The microbact analysis was carried out according to the manufacturer's instruction (Oxoid Ltd). Four colonies were picked using a sterile wire loop and emulsified in 3ml of listeria suspension media, 100ml of the bacteria suspension was added to all wells of each strip. The overlay of mineral oil was added to wells 1, 2 and 3 then incubated at 37^{0}C for 24 hours in a water bath.

3.16 Drug Residue Test

3.16.1 Sample preparation

At the end of the 8th week, meat samples from the breast muscle were cut from six chickens per treatment from the chickens used for carcass evaluation, for drug residue detection using the Delvotest® SP test Kit from DSM. Five grams of each meat sample was weighed, macerated using sterile mortar and pestle and emulsified with equal 10mls of sterile distilled water, centrifuged at 5000 rpm for 10 minutes and the supernatant decanted as the extract, and used for analysis.

3.16.2 Antibiotic residue detection

The antibiotic residues test was carried out using Delvotest® SP test Kit from DSM. Delvotest® SP test Kit is a commercially available agar diffusion test, based on the principle of inhibition of microorganism like other microbiological tests. It contained ampoules of agar imbedded with standardized number of spores of *Bacillus stearothermophilus* as test organism and Bromocrescol purple colour indicator. The extract from each of the meat sample was transfered into the coresponding labell ed ampoules with solid agar medium which contained *Bacillus stearothermophilius* var. *calidolactis* together with nutrients for growth purposes and bromocresol purple. The ampoules were placed in a preheated water bath premi test incubator, set at 64 ^{0}C for three hours. After incubation for 3 hours at 64 ^{0}C the samples were withdrawn from the water bath and readings of the test results were taken according to the specified colour guide of the Delvotest® SP test Kit manual. The test was used to detect the presence of drug at three levels; No drugs yellow colour, low concentration yellow/purple colour and high concentarion deep purple.

3.17 Data Collection

The measurements of weights, haematological parameters, serum biochemical parameters, digestibility studies and carcass evaluation were carried out as described in experiment 1.

3.18 Statistical Analysis

All the data collected from the experiment were subjected to analysis of variance (ANOVA) using general linear model of Statistiscal Analysis System (SAS, 2003) Software package. Significant differences between means were compared using Dunnetts Test.

The Linear Model used for the experiment was:

$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{t}_i + \mathbf{e}_{ij}$

Where:

$\mathbf{Y}_{ij}$= Dependent variable

$\boldsymbol{\mu}$= Overall mean

$\mathbf{t}_i$= i^{th} Effect of treatment

$\mathbf{e}_{ij}$ = Random error

3.19 Experiment 3: Effect of Single and Combined Inclusion Levels Mycofix® and Biotronic® SE in Diets of Broiler Chickens on Performance

3.20 Experimental Treatments

A total of 396 unsexed Ross day-old broiler chicks were used for the study. The chicks were randomly allocated to six dietary treatments with three replicates containing 22 birds each in a completely randomized design. Diets were formulated to meet the NRC (1994) requirements for broiler chickens. Experimental starter and finisher diets are presented on Tables 3.5 and 3.6. Treatment one was the control diet without Mycofix®, Biotronic® SE and Oxytetracycline, the best inclusion level of Mycofix® (400g) obtained from experiment one, and the best inclusion level of Biotronic® SE (500g) from experiment 2, were used as treatments two and three. Treatment four was a combination of 200g Mycofix® and 250g Biotronic® SE. Treatment 5 was a combination of 400g of Mycofix® and 400g Biotronic® SE. Treatment 6 consisted of 100g Oxytetracycline.

T1 - Basal diet (Positive control)

T2 - Basal diet +Best treatment from expeiment1 (400g Mycofix®)

T3 - Basal diet + Best treatment from experiment 2 (500g Biotronic® SE)

T4 - Basal diet + combination of 200g Mycofix® + 250g Biotronic® SE

T5 - Basal diet + combination of 400g Mycofix® + 500g Biotronic® SE

T6 - Basal diet + 100g Oxytetracycline (Negative control)

N.B Biotronic® SE inclusion for starter phase is 200, 300, 400 and 500g / 100kg of feed
Biotronic® SE inclusion for finisher phase is 100 200, 300 and 400g / 100kg of feed

3.21 Data Collection

The data collected for measurements of weights, haematological parameters, serum biochemical indices, digestibility studies and carcass evaluation was done at the 7th week of the experiment. The procedures of data collection were the same as described in experiment 2 above.

3.22 Quantitative Assessememt of Gut Morphology

A portion of the jejenum approximately 6cm long was cut from the intestines of six birds per treatment used for carcass analysis.

Table 3.5: Ingredient composition of experimental broiler starter diets supplemented with single and combined levels of Mycofix® and Biotronic® SE (0 – 4 weeks)

Ingredients	0g	400g Myco	500g Bio	200g Myco + 250g Bio	400g Myco + 500g Bio	100g Oxyt
Maize	56.00	56.00	56.00	56.00	56.00	56.00
Soya bean cake	29.70	29.70	29.70	29.70	29.70	29.70
Groundnut cake	10.00	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[*A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME Kcal/kg	2902	2902	2902	2902	2902	2902
Crude protein (%)	23.19	23.19	23.19	23.19	23.19	23.19
Crude fibre (%)	3.59	3.59	3.59	3.59	3.59	3.59
Ether extract (%)	3.16	3.16	3.16	3.16	3.16	3.16
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.87	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.46	1.46	1.46	1.46	1.46	1.46
Methionine (%)	0.56	0.56	0.56	0.56	0.56	0.56
Cost/ kg diet(₦)	83.82	84.06	84.13	84.24	84.36	91.32

[A]Biomix Broiler starter premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium, Pantothenate,7.5mgnB12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40m g; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 50mg
Myco.: Mycofix, Bio: Biotronic, Oxyte: Oxytetracycline

Table 3.6: Ingredient composition of experimental broiler diets supplemented with single and combined levels of Mycofix® and Biotronic® SE diets (5 – 7 weeks)

	Levels of Mycofix® and Biotronic SE®					
Ingredients	0	400g Myco	400g Bio	200g Myco + 200g Bio	400gMyco + 400g Bio	100g Oxytr
Maize	58.00	58.00	58.00	58.00	58.00	58.00
Soya bean cake	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00	13.00
Maize offal	4.70	4.70	4.70	4.70	4.70	4.70
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
ME Kcal/kg	2929	2929	2929	2929	2929	2929
Crude protein (%)	20.54	20.54	20.54	20.54	20.54	20.54
Crude fibre (%)	4.11	4.11	4.11	4.11	4.11	4.11
Ether extract (%)	3.35	3.35	3.35	3.35	3.35	3.35
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.85	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.27	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.50	0.50	0.50	0.50	0.50	0.50
Cost/ kg diet(₦)	79.44	79.68	79.68	76.68	79.92	86.90

[A]Biomix Broiler Finisher premix provide per kg f diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 30mg Myco.: Mycofix, Bio: Biotronic, Oxyte: Oxytetracycline

Each sample was fixed in 10% formal saline for 24 hours. Grossed sections of the jejunum were processed with the aid of an automated tissue processor at the Histology Laboratory, Faculty of Human Medicine, Ahmadu Bello University, Zaria for Histo- morphological assessment. Sections of the processed tissues were cut using a rotator microtome at 8µ and each sample was prepared on a slide.The photomicrographs were taken at a magnification of x40 using a MD9000 Amscope digital camera. Ten readings per sample were taken for villi area, villi height, villi width, villi perimeter, villi roundness and villi crypts were measured using digimizer image analyzer software. Villi height was measured from the basal region, (starts at a higher portion of crypts until villi tip), perimeter was measured around the border where the microvilli are located as described by Uni *et al*. (1995)

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Feed Ingredient Survey for Aflatoxin B1

Prevalence of Aflatoxin B1 (AfB1) in some selected ingredients used in poultry feed is presented in Table 4.1. Results obtained from feed mill A showed, 0.1 ppb in Maize (MZ) and 1.8 ppb in Soya bean cake (SBC) which were less than 20 ppb AfB1 permissible level in feed ingredients and categorised as low, while 736.5 ppb in Groundnut cake (GNC), 129.3 ppb in Brewers dried grain (BDG) and 61.7 ppb in Maize offal (M/O) were above 20pp and categorized as high concentration. In feed mill B, 5.0 ppb MZ, 3.3 ppb SBC, 7.8 ppb in M/O, the permissible level of were low in concentration, while 15320.9 ppb in GNC and 160 ppb in BDG were above the permissible level of 20ppb. In feedmill C, 1.7ppb in MZ, 3.8 ppb in SBC, 0.1 ppb in M/O and 1.2 ppb BDG were below 20 ppb while 6539.9 ppb in GNC was above the permissible level of 20ppb. In feed mill D, 0.1 ppb in MZ, 2.2 ppb in SBC, 3.4 ppb in GNC, 0.1 in M/O and 0.8 ppb in BDG which were all below 20 ppb and the Giwa grain market sample E, had 0.1 ppb in MZ, 0.3 ppb in SBC, 6.0 ppb in BDG were below 20ppb and 80ppb in M/O and 111.5 ppb in GNC were above the permissible level 20 ppb.

Contamination of feed ingredients with aflatoxins is not an unusual phenomenon. Several workers (Okoli *et al*., 2007; Shareef, 2010) in different parts of the world have reported incidence of aflatoxins. The result of the present study showed the presence of AfB1 in the sampled ingredients. The general guidelines of United States Food and Drug Administration (USFDA) recommended the permissible limits of 20ppb contamination level of aflatoxins in poultry ingredients and feed (Ghahri, 2010).

Table 4.1: Mycotoxin content of some feed ingredient samples

Ingredients	Aflatoxin B1 (ppb)				
	A	B	C	D	E
Maize (MZ)	0.1	5.0	1.7	0.1	0.1
Soya bean cake (SBC)	1.8	3.3	3.8	2.2	0.3
Groundnut cake (GNC)	736.5	15,320.9	6,539.9	3.4	111.5
Brewers dried grain (BDG)	129.3	160.0	1.2	0.8	6.0
Maize offal (M/O)	61.7	7.8	0.1	0.1	80.0

A, B, C, D = feed mills E = Grain market sample, ppb = parts per billion

From the study, there was an alarming high level of AfB1 contamination in GNC across four out of the five study areas constituting 80 %. This high level of AfB1 contamination in GNC fom feed mill B could be as a result of long duration of storage which encouraged the growth of mould. It could also be an indication of high moisture content before storage and a possibility of poor storage facility in the feedmill. This is not good for the poultry feed industry as GNC is one of the major protein sources used in poultry finished feed.This high incidence of AfB1 in GNC is not only hazardous to poultry alone because of direct exposure, but also suggests a high risk to human health with the possibility of indirect exposure through contaminated meat, eggs and other poultry products and by products (Maqbool *et al*., 2004; Bintvihok and Kositcharoenkul, 2006).

4.2 Fungi Isolation and Identification

The overall frequency of isolation of the different fungi species from feed raw materials sold in feed mills in Zaria are presented in Table 4.2a. Six fungal species were identified with *Aspergillus spp* having the highest prevalence rate (30.77%), followed by *Rhizopus spp* (23.08%) and *Fusarium spp* (20.0%) while *Mucor, Penicilliun* and *Curvularia* spp were 12.31, 7.69 and 6.15% respectively. Table 4.2b shows the total of each fungi isolates and their frequency in the individual sampled feed ingredients. Six fungi species with a total number of *Aspergillus spp* (18), *Fusarium spp* (15), *Penicilliun* (5s), *Rhizopus spp* (15), *Mucor* (8) and *Curvularia spp* (3) were found in the sampled ingredients. The results of individual contamination levels for each sampled ingredients is shown on Table 4.2b. The plates of the fungi isolates are in the appendices.

Table 4.2a: Overall frequency of isolation of different fungal species from feed ingredients materials

Fungi species	Frequency	Percentage
Aspergillus	18	30.77
Rhizopus	15	23.08
Fusarium	13	20.0
Mucor	8	12.31
Penicillium	5	7.69
Curvularia	4	6.15
Total	63	100

Table 4.2b: Frequency of isolation of fungal species from 5 different feed ingredients

	Frequency of each fungi isolates in ingredients					
Fungi spp	Total	MZ	SBC	GNC	BDG	M/O
Aspergillus	18	4.17	16.67	62.50	8.30	3.33
Fusarium	15	38.46	15.38	30.77	7.69	7.89
Penicillium	5	66.67	0.00	0.00	0.00	0.00
Rhizopus	15	0.00	40,00	33.33	13.33	20.00
Mucor	8	0.00	50.00	37.50	25.00	0.00
Curvularia	3	35.33	0.00	0.00	0.00	0.00

MZ: Maize, SBC: Soyabean cake, GNC: Groundnut cake, BDG: Brewers dried grain and M/O: Maize Offal

The present study revealed that *Aspergillus spp*, *Rhizopus spp* and *Fusarium spp* were the common moulds growing in poultry commercial feed raw materials sold in the feed mills sampled in Zaria.

The high prevalence rate of fungal species observed in this study is in agreement with the findings of Bastianelli and Le Bas (2002), Cheesbroughi (2002) and Atanda *et al.* (2013), that tropical climates as exists in Nigeria were more conducive for fungal and microbial growth and subsequently contaminations of poultry feed raw materials. Certain agricultural produce have been observed to permit the growth of some moulds over others. For example, maize allowed the growth of aflatoxins and fumonisins producing moulds above others, while groundnuts have been found to be excellent substrate for aflatoxin contamination (Bankole and Adebanjo, 2003). Several factors such as climatic conditions (Dersjant-Li *et al.*, 2003) and storage conditions (Moss, 2002), like high temperature and humidity were optimal for the growth of moulds that affect fungal colonization in grains and compound feeds (Rawal *et al*., 2010).

4.3 Experiment 1: Effect of Varying Inclusion Levels of Mycofix® in the Diets of Broiler Chickens

4.3.1 Growth performance of broiler starter chicks fed diets containing varying levels of Mycofix®

The result of the growth performance of broiler chicks fed different levels of Mycofix® is presented in Table 4.3. Final body weight, weight gain, feed intake, feed conversion ratio and feed cost per kilogram gain were significantly ($P < 0.05$) affected by dietary treatments.

The final body weight (902.67g), weight gain (839.67g) and daily gain (29.98g) were significantly ($P<0.05$) higher in diets containing 400g Mycofix®.

Table 4.3: Growth performance of broiler chicks fed diets containing diets with varying levels of Mycofix®

	Levels of Mycofix® inclusion (g/100 Kg diet)					
Parameters	0	100	200	300	400	SEM
Initial wt (g/bird)	63.13	63.06	63.01	63.05	63.03	0.03
Final weight (g/bird)	833.33^c	818.33^c	827.00^c	874.33^b	902.67^a	13.65
Wt gain (g/bird)	770.33^c	755.27^c	763.99^c	811.33^b	839.67^a	14.07
Av. daily Wt. gain (g/bird)	27.51^c	26.97^c	26.80^c	28.97^b	29.98^a	0.50
Total FI (g/bird)	1454.7^a	1264.33^b	1260.67^b	1469.33^a	1401.33^a	26.98
Av daily feed intake (g/bird	51.95^a	45.15^b	45.02^b	52.04^a	50.04^a	0.96
Feed conversion ratio	1.87^b	1.63^a	1.60^a	1.73^a	1.67^a	0.03
Feed cost/kg gain (₦/kg gain)	158.46^c	140.23^a	138.52^a	152.20^b	140.23^a	6.73
Mortality (%)	0.00	1.50	0.09	0.00	3.00	0.01

^{abc}, Means with different superscripts along same rows show significant differences (P < 0.05)
SEM : Standard Error Mean Av; Average, FI: Feed intake, % percentage, wt; weight, kg; kilogram

Feed intake was higher ($P<0.05$) in treatments containing 300, 400g Mycofix® and control compared to 100 and 200g Mycofix® inclusions.

All the treatments containing Mycofix® had a significantly ($P< 0.05$) lower feed conversion ratio value than than the control treatment. Feed cost per kilogram gain were significantly ($P < 0.05$) better for all the Mycofix® treatments compared to the control and T_3 (200g Mycofix®) had the least cost (₦138.52). Mycofix® at 100, 200, 300 and 400 g/100kg diet reduced the cost of production by 11.51% (₦18.23), 12.54% (₦19.94), 3.95% (₦6.26) and 11.51 % (₦18.23) respective per bird at the starter phase. The highest percentage mortality (3.0) was recorded in the 400g Mycofix® diet.

The increasing body weight gain observed as the inclusion level 300 and 400 g/kg of Mycofix® in the diets increased was indicative of the presence of mycotoxins in the feed materials. Liu *et al*. (2011) mentioned that several grain sources were the constituents of usual poultry diets which might be contaminated by different mycotoxins. It was observed that higher inclusion levels of Mycofix® gave better results. The use of Mycofix® binders in the feed decontaminated the toxins to a level that enhanced better nutrient utilization with increased body weight gain and improved growth. The result obtained showed that Mycofix® was able to mitigate to a reasonable level the problem mycotoxins in broiler feed through better availability and utilization of nutrients that manifested in enhanced growth performance of the birds. Birds fed 400g/100kg Mycofix® in their diet had 8.97% gain in body weight than those on the control feed. They also have 11.2% gain above broilers fed diets with 300g/100kg Mycofix® inclusion. There appear to be an improved gain in weight as the binder level increased in the diets. This result agreed with that of Hedayati *et al.* (2014), where birds fed toxin binder in their feed gave the best and highest body weight in all the treatments groups.

Agboola *et al*. (2015) reported in their findings that supplementation with mycotoxin binder and probiotics resulted in improved body weight gain over the control diet, an indication of the positive effect of mycotoxin binder on broiler performance through the control of the gut microbiota. The results obtained showed that Mycofix® at 300 and 400 g/100kg levels of inclusion was able to ease the problem of mycotoxins in feed by enhanced performance of the birds. In other studies conducted where toxins were introduced into the basal diet and toxin binders was used to evaluate the efficacy of handling the toxins, similar results were obtained. Shareef (2010) reported that there were 20.03, 23.29, 19.91, 18.27 and 20.60 % reduction in treatments as compared with the control group when mycofix+3 was added to contaminated diet to give significantly ($P < 0.05$) improved body weight gain by 33.26, 28.66, 17.62, 15.37 and 18.65.

The marked increase in feed intake observed at higher inclusion levels also showed the positive effect of the binder on feed consumption by the birds. This agreed with results obtained by Abdelaziz *et al.* (2015), who reported a profound increase in feed consumption during the growing period between 4-5week to be 9.9%, in treatments given different natural toxin binder such as Pepper mint *Mentha piperita* oil and biological toxin binder which includes Mycofix plus and rice hulls.

The use of Mycofix® at the started phase significantly ($P<0.05$) gave a lower feed conversion ratio, for birds on the Mycofix® treatments groups. The inclusion of Mycofix® showed the ability of the binder to effect a good measure of interaction between growth and consumption. This contradicted the findings of Kamalzadeh *et al*. (2009), who reported that feed conversion ratio decreased significantly when the concentration of Mycosorb in the diets. Inclusion of Mycosorb reduced feed conversion by 2.09, 5.24 and 5.47% in treatments with 0.5, 1.0 and 1.5 g Mycosob kg-1 diet, respectively compared with the control. These results however disagreed with the findings of Shebl *et al.* (2010), who reported that feed intake and feed conversion ratio were not affected by using Hydrated Sodium Calcium Aluminosilicate (HSCAS) as anti-mycotoxin in broilers diets.

The best feed cost per kilogram gain was observed in the treatment groups with the highest inclusion level (400g/100kg) of Mycofix®. This showed that it was profitable and cost effective to use Mycofix® in broiler starter diets at this inclusion level.

Mortality percentage recorded for birds on 400g Mycofix® diet was not attributed to the treatment effect, as post mortem results showed that the birds that died did not show signs of any disease in particular and might have just be a natural occurrence. Their hearts were however enlarged which could be attributed to the rapid growth rate observed in the group compared with the others.

4.3.2 Growth performance of broiler finisher chickens fed diets containing varying levels of Mycofix®

The growth parameters of broiler finisher chickens fed diets containing four levels of Mycofix® is presented in Table 4.4. Significant ($P < 0.05$) differences were observed across the treatments for final weight, weight gain, total feed intake, FCR and feed cost per kilogram gain. Birds on 400g/100kg Mycofix® diet had the highest ($P < 0.05$) final body weight (2350.3g), weight gain (1403.3g), average daily weight gain (50.1g) in all the treatment groups compared with the control. Total feed intake was significantly ($P < 0.05$) higher in the control, 100, 300 and 400g Mycofix® compared to 200g Mycofix®. The feed conversion ratio was better (2.10) in birds fed diets containing 400g Mycofix®. The feed cost per kilogram gain of ₦167.20 was the least in 400g/100kg Mycofix® diet. Mycofix® at 400 g/100kg inclusion level reduced the cost of production per bird by 12.31% or ₦23.47. Mortality percentage was highest (3.5%) in 200g Mycofix® diet.

Table 4.4: Growth performance of broiler finisher chickens fed diets containing varying levels of Mycofix®

	Levels of Mycofix® inclusion (g/100 Kg diet)					
Parameters	0	100	200	300	400	SEM
Initial weight (g/bird)	947.04	947.06	947.10	947.07	947.04	0.03
Final weight (g/bird)	2186.70^{b}	2060.36^{b}	1936.70^{b}	2105.27^{b}	2350.34^{a}	79.94
Weight gain (g/bird)	1239.7^{b}	1113.3^{b}	989.6^{b}	1158.2^{b}	1403.3^{a}	79.95
Av. daily weight gain (g/bird)	44.3^{ab}	39.8^{b}	35.3^{b}	41.4^{b}	50.1^{a}	2.86
Total Feed intake (g/bird)	3039.0^{a}	2844.3^{a}	2579.7^{b}	2852.3^{a}	2997.3^{a}	87.53
Av.daily feed intake (g/bird)	108.5^{a}	101.6^{a}	92.1^{b}	101.9^{a}	107.0^{a}	3.13
Feed conversion ratio	2.45^{b}	2.55^{b}	2.60^{b}	2.46^{b}	2.14^{a}	0.12
Feed cost/kg gain (₦/kg gain)	190.67^{a}	198.75^{a}	198.90^{a}	191.09^{a}	167.20^{b}	9.54
Mortality (%)	1.70	1.70	3.50	0.0	3.00	

ab: Means with different superscripts along same rows show significant differences ($P< 0.05$), SEM : Standard Error of Means , %: percentage AV: average.

The improved performance recorded for birds fed 400g Mycofix® per 100kg diet for all the parameters measured where better in all their treatments, this may be attributed to the fact that a high concentration of the specific binder (Mycofix®) maybe needed to counteract the effect of the toxins present in the diet which resulted in an improved performance. The same trend was observed at the starter phase, which may be due to early introduction of the binder in the chicks diet and been more effective as a form of protection in the gut.

At the finisher phase however, 400g /100kg inclusion clearly performed best in all the parameters measured, which is indicative of the need for a higher level at this phase of the birds to improve growth rate. Lan *et al.* (2007) reported that gut dominant contaminants becomes more complex as broilers grow older.

Ologhobo *et al.* (2015) reported that the final live weight was highest in birds fed positive control diet containing a basal diet + binder and least in birds fed diet that contained 0.2 kg SB (Sodiun Bentonite) binder + 2000 ppb aflatoxins / 100kg diet compared to others. The result showed that the binder improved the basal diet and suppressed the effect of contamination.

The diet containing 400g Mycofix® positively influenced the feed conversion ability of the birds on the treatment because they showed a better FCR particularly at 300 and 400g inclusion levels than the control. This suggested that Mycofix® at this level (400g) was able to aid conversion of feeds appropriately that resulted in production of meat and reduced the cost of production per bird. This result agreed with the findings of Hedayati *et al.* (2014) who reported that the toxin binder alone group showed a FCR of 1.89 which was significantly ($P<0.05$) better, when compared with the control group 2.20. Ogunwole *et al.* (2013) reported that Mycofix® inclusion at 0.3% gave the lowest feed conversion ratio value compared with the control and four other types of binders namely charcoal, Toxiroak, Toxynil and A- Tox E.

The mortality recorded was not as a result of the Mycofix®as post mortem results showed signs of chronic respiratory tract disease which was treated appropriately with the drugs prescribed at the Veterinary clinic of the Ahmadu Bello University, Zaria.

4.3.3 Carcass characteristics of broiler finisher chickens fed diets supplemented with varying levels Mycofix®

The results obtained from the carcass evaluation of broiler chickens fed four levels of Mycofix® are presented in Table 4.5. The result showed that treatment with 400g Mycofix® inclusion had a higher ($P<0.05$) values for live weight and carcass weight. Dressing percentage, cut parts and organ weights showed no significant ($P>0.05$) differences across all the treatments.

The better carcass weight of the birds fed with diets that contained 400g Mycofix® treatment could be attributed to the final weight compared with the other treatments. The improved final weight will afford the farmer a better market value when Mycofix® 400g /100kg of diets were fed although, the effect was not significant in the other parameters measured.The result contrast that of Abdelaziz *et al*. (2015) who reported no significant differences for carcass characteristics when Mycofix® and three other organic binders were used.

Table 4.5: Carcass characteristics of broiler finisher chickens fed diets containing varying levels of Mycofix®

Parameters	Inclusion levels of Mycofix® g/100Kg diet					SEM
	0	100	200	300	400	
Live weight g)	2138.67[b]	1928.33[c]	1949.17[c]	2072.00[b]	2240.83[a]	23.96
Carcass weight (g)	1343.83[b]	1387.50[b]	1273.33[c]	1344.67[b]	1457.50[a]	48.61
Dressing percentage (%)	62.72	64.31	63.67	64.71	65.01	1.87
Cut parts expressed as percentage of carcass weight (%)						
Back (%)	18.23	17.12	20.11	22.04	19.49	1.36
Breast muscle (%)	27.27	28.04	30.65	30.79	32.34	1.65
Thigh (%)	16.06	15.48	17.71	18.15	17.68	0.85
Drum stick (%)	13.35	14.17	15.64	16.54	16.13	1.02
Wings (%)	11.04	13.26	12.88	12.79	12.68	0.68
Organs weights expressed as percentage of live weight (%)						
Heart (%)	0.39	0.39	0.40	0.44	0.40	0.04
Full gizzard (%)	2.71	3.08	3.44	3.28	3.07	0.20
Empty gizzard (%)	2.08	2.13	2.41	2.44	2.09	0.16
Liver (%)	1.68	2.13	1.91	1.91	1.62	0.15
Lungs (%)	0.61	0.63	0.72	0.68	0.60	0.06
Kidneys (%)	0.61	0.63	0.59	0.58	0.60	0.06
Spleen (%)	0.13	0.15	0.15	0.15	0.16	0.02
Abdominal fat (%)	1.62	0.80	1.49	1.95	1.47	0.29
Intestinal weight (%)	4.55	4.99	5.07	5.37	4.40	0.39
Intestinal length cm	251.67	289.33	274.50	251.17	271.33	11.05

[abc:] Means with different superscripts along same rows show significant differences, SEM = Standard Error Means.

4.3.4 Haematological parameters of broiler finisher chickens fed diets containing varying levels of Mycofix®

The haematological parameters of broiler finisher chickens fed diets containing varying levels of Mycofix® are presented in Table 4.6. The results showed no significant (P>0.05) differences in packed cell volume (PCV), haemoglobin concentration (Hb), total white blood count (WBC), red blood cell counts (RBC) mean corpuscular volume and lymphocytes. Significant (P<0.05) differences were observed for values of mean corpuscular haemoglobin (MCH) and heterophils across the birds in the treatments groups with the control having significantly (P<0.05) lower values.

The blood contains several metabolites which provide useful information on nutritional status and clinical investigation of an individual organism hence, the use of blood parameters is recommended for medical and nutritional assessments (Egbunike *et al*., 2009). The haematological indices in this study showed that packed cell volume (24.0 - 27.0%), fell within the normal range for healthy chickens of 24.00-44.00% as reported by Mitruka and Rawnsley (1977). This result showed that inclusion of high levels of Mycofix® in broiler diets had no adverse effect on the percentage of red blood cells in chickens. This result agreed with the findings of Adebiyi *et al*. (2010) who reported PCV of 27.88 – 28.88% when they varied Diatomaceous Earth (DE) as a binder in broiler diet. PCV values according to Isaac *et al.,* (2013) involved the transport of oxygen and absorbption of nutrients.

The haemoglobin concentration of 7.97- 8.92mg/dl, also fell within the reference range values of 9.10 -13.9mg/dl as reported by Mitruka and Rawnsley (1977) for healthy chickens. The result implies that the birds had stable health status even when Myvofix® toxin binder was used.

Table 4.6: Haematological parameters of broiler finisher chickens fed diets containing varying levels of Mycofix®

Parameters	Levels of Mycofix® inclusion (g/100kg diet)					SEM	Reference Values
	0	100	200	300	400		
Packed cell volume (%)	25.83	24.00	25.50	27.00	26.83	1.32	24.00 – 44.50*
Haemoglobin (g /dl)	8.58	7.97	8.47	8.90	8.92	0.45	9.10 – 13.90**
White blood cell (x 10^{12} /L)	11.23	7.17	11.63	8.10	5.00	2.53	9.20 – 31.00*
Heterophils (%)	2.60^a	10.30^b	12.00^b	4.08^b	5.72^b	3.94	
Lymphocytes (%)	92.00	86.17	85.17	89.80	89.50	2.98	47.20 – 85.00
Red blood cell (x 10^9 /L)	4.42	3.92	4.20	4.50	4.55	0.24	1.58 – 4.10*
MCV (fl)	58.62	60.99	60.99	60.68	59.19	1.08	
MCH (pg)	9.36^a	12.64^b	9.84^b	12.61^b	21.31^b	3.33	
MCHC(g/l)	3.32	3.32	3.32	3.29	3.32	0.02	

ab Means with different superscripts along same rows show significant differences ($P < 0.05$) SEM = Standard Error of Means, MCHC = Mean corpuscular haemoglobin concentration, MCV =Mean corpuscular volume, MCH = Mean corpuscular haemoglobin *Mitruka and Rawnsley (1977),** Swenson, 1977.

Sufficient haemoglobin is used as an index of available oxygen needed for transport of digested food Haemoglobin had the physiological function of transporting oxygen to tissues of the animal for oxidation of digested food so as to release energy for the other body functions as well as transportation of carbon dioxide out of the body of animals (Soetan *et al.*, 2013).

Total white blood cell count of broiler chickens fed diets containing different inclusion levels of Mycofix® and the control all fell within the reference values of 9.20 – 31.00 x $10^{12/}$/l as reported by Swenson (1970). It was observed that 400g Mycofix® inclusion had the least white blood cells compared to the other treatments. With the performance recorded in this group it could be inferred that Mycofix® had a way of boosting immunity even with a challenge in health status since the birds in this group had the best final live weight. The red blood cells (3.92- 4.55 x $10^{9/l}$) did not fall within the reference range of (1.58-4.10 x $10^{9/l}$) for healthy chickens as reported by Mitruka and Rawnsley (1977).

There was significant increase in the heterophils values as the levels of Mycofix® increased from 100 and 200g (10.30 and 12.00%) then declined. This increase in the treatment groups could be as a result of the effect of the toxin binder's protection against harmful toxins present in the feed ingredient thereby increasing the immunity of the birds. Heterophils are a common type of white blood cell which respond to infection and attack bacteria and other foreign invaders directly. They are phagocytes that engulf the invading organisms and kill them. The values of the lymphocytes showed that the birds were not overwhelmed by any fungi viruses, bacteria, fungi and protista.

4.3.5 Serum biochemical indices of broiler finisher chickens fed diets containing varying levels of Mycofix®

The result of serum biochemical parameters of broiler finisher chickens fed varying levels of Mycofix® are presented in Table 4.7. There were no significant (P>0.05) differences in all the parameters measured.

Serum biochemistry is a reliable biochemical system which could reflect the condition of the organism and the changes happening to it under the influence of internal and external factors (Toghyani *et al*., 2010). Serum enzymes alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amainotransferase (ALT), analyses were used to assess liver function (Kwari *et al.,* 2010). There was a non significant slight increase in the serum indices values ALP, AST and ALT observed in birds on the binder groups compared with the control. This did not have adverse effects on the chickens since the differences were not significant. This was in agreement with the findings of Abdelaziz *et al*. (2015), who reported no significant differences in values of AST, ALT and ALP compared with the control in this study. They however reported that using natural anti-mycotoxin (toxin binders) increased liver activities without any adverse effect. The energy content of the diet was adequate which was evident in the non significant values of the glucose level in the blood irrespective of the treatments.

The highest glucose value was in 400g mycofix® diet an indication that birds fed that diet `probably had more available metabolizable glucose as source of energy. It implied then that mycofix® inclusion enriched the energy source of broilers. Inclusion of Mycofix® did not affect total serum protein as there was no difference in the treatment diets fed to the chickens compared with the control.

Table 4.7: Biochemical indices of broiler finisher chickens fed diets containing levels of Mycofix®

Parameters	Levels of Mycofix inclusion (g/100kg diet)					
	0	100	200	300	400	SEM
Glucose (mg/dl)	149.50	128.00	153.00	143.00	159.83	16.06
Total Protein (g/dl)	2. 03	2.80	2.17	2.70	2.13	0.46
ALT (µ/l)	19.33	24.83	28.00	25.33	33.83	7.24
ALP (µ/l)	138.50	150.17	132.17	167.83	173.67	31.20
AST (µ/l))	29.00	33.67	35.17	36.33	40.55	6.42

SEM = Standard error means, AST= aspartate aminotransferase, ALT= alanine aminotransferase. ALP = alkaline phosphatase

4.3.6 Apparent nutrient digestibility and ash content of broiler finisher chickens fed diets containing varying levels of Mycofix®

The nutrient digestibility results of broiler finisher chickens fed diets containing four levels of Mycofix®are presented in Table 4.8. There were no significant (P>0.05) differences in the digestibility of crude fibre, ether extract and nitrogen free extract by the broilers on the treatments.

The dry matter digestibility of birds fed 400g Mycofix® inclusion was significantly (P<0.05) higher than the other treatments. The crude protein was similar (P>0.05) for control, 300 and 400g Mycofix® treatments, but significantly (P<0.05) different from 100 and 200g Mycofix® which were similar. The ash rententio also followed the same trend as crude protein.

The measure of apparent digestibility reflects the net effect of all digestive and absorptive processes along the digestive tract (Grenier and Applegate, 2013). The improved nutrient digestibility of dry matter, crude protein and ash observed in broiler chickens fed 300 and 400g Mycofix® treatments, were an indication that Mycofix® positively affected digestion of nutrients. The increased apparent digestibility of nutrients could be attributed to the ability of Mycofix® to protect the gut from direct contact with the possible myotoxins present in the feed.

From literature the effect of mycotoxins on apparent nutrient and energy digestibility had been documented, because of its ability to reduce the apparent digestibility of crude protein by increasing the amino acid requirements i.e higher doses, *Aspergillus* metabolite showed similar effects on the apparent digestibility of laying hens and broiler chickens (Han *et al*., 2008; Applegate *et al*., 2009). Galvano *et al*. (2001) reported that toxin binders prevent toxic interactions with the consuming animal and prevent mycotoxin absorption across the digestive tract. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fibre, vitamins, and minerals.

Applegate *et al*., (2009) reported that the presence of mycotoxins in diets of poultry feeds have a negative effect on protein, energy and nutrients usage in the gastro intestinal tract of broilers. Mycotoxins compete for these nutrients and increase the requirements of the nutrients above normal.

Table 4.8: Effect of varying inclusion levels of Mycofix® in broiler finisher diets on apparent nutrient digestibility and ash content

Parameters	Levels of Mycofix inclusion (g/100kg) diet					SEM
	0	100	200	300	400	
Dry matter (%)	92.71^{b}	89.12^{b}	90.33^{b}	92.29^{b}	94.74^{a}	1.05
Crude protein (%)	95.60^{a}	93.80^{b}	94.12^{b}	95.36^{a}	96.35^{a}	0.49
Crude fibre (%)	95.60	93.10	94.53	95.06	95.87	0.79
Ether extract (%)	97.39	95.04	96.06	94.31	98.23	0.84
Ash content	94.88^{a}	92.01^{b}	92.51^{b}	94.38^{a}	94.47^{a}	0.95
NFE (%)	89.34	84.64	86.10	89.00	89.12	1.96

$^{abc:}$ Means with different superscripts along same rows show significant differences, SEM = Standard Error of Means, NFE= Nitrogen free extract.

4.4 Experiment 2: Effect of Varying Inclusion Levels of Biotronic® SE in Diets of Broiler Chickens

4.4.1 Growth performance of broiler starter chicks fed diets containing varying inclusion levels of Biotronic® SE

Table 4.9 shows the growth performance of broiler chicks fed diets containing varying levels of Biotronic® SE. Significant (P<0.05) differences were observed across all treatments in all the parameters measured except mortality rate. Values like final weight, weight gain and average daily weight gain, for 500g inclusion level of Biotronic® SE and 100g Oxytetracycline were similar but significantly (P<0.05) higher than the other treatments (control, 200, 300 and 400g Biotronic® SE levels). The chicks fed diets containing 500g Biotronic® SE gave higher body weight gains (1288.14g) among birds on Biotronic® SE.

Total feed intake and average daily feed intake followed the same pattern. The values obtained from the birds fed 100g Oxytetracycline were significantly (P<0.05) higher for feed intakes and lowestin the control groups. Values of feed intake recorded for birds fed 300 and 400g Biotronic® SE inclusions were similar and significantly (P<0.05) lower than those on 100g Oxytetracycline.

The birds on 500g Biotronic® SE treatment had a significantly (P < 0.05) lower FCR value which is the best compared with the other treatments and control groups. The cost per kilogram gain followed same trend as the FCR. The cost per kilogram gain was significantly (P < 0.05) lowest for birds on 500g Biotronic® SE Mortality record did not follow any trend and was highest in birds fed 200g Biotronic® SE. This study demonstrated the positive dietary supplemental effect of levels of Biotronic ®SE and Oxytetracycline on performance compared with a control. Although, there was no significant difference with the negative control that is the Oxytetracycline group (1384.75g), there was the risk of residual effect

Table 4.9: Growth performance of broiler starter chicks fed diets containing varying levels of Biotronic® SE

Parameters	Levels of Biotronic® SE inclusion (g/100 Kg diet)						
	0	200	300	400	500	100g Oxty	SEM
Initial weight (g/bird)	46.98	47.00	46.98	47.06	47.01	46.98	0.03
Final weight g/bird	875.74^{d}	1076.94^{c}	1187.50^{b}	1223.13^{b}	1335.15^{a}	1384.75^{a}	51.53
Weight gain (g / bird)	828.76^{d}	1029.94^{c}	1140.52^{b}	1176.07^{b}	1288.14^{a}	1337.73^{a}	51.52
Av. daily wt gain (g / bird)	29.60^{d}	36.79^{c}	40.73^{b}	42.00^{b}	46.070^{a}	47.78^{a}	1.84
Total feed intake (g / bird)	1660.40^{d}	1982.90^{c}	2178.70^{b}	2233.20^{b}	2307.10^{b}	2507.10^{a}	85.93
Av daily feed intake (g / bird)	48.51^{d}	69.83^{c}	77.81^{b}	79.76^{b}	82.40^{b}	89.54^{a}	3.07
Feed conversion ratio	2.00^{a}	1.90^{a}	1.90^{a}	1.90^{a}	1.70^{b}	1.80^{a}	0.05
Feed cost / kg gain ((₦/kg gain)	167.36^{a}	159.49^{a}	159.90^{a}	159.68^{a}	151.42^{b}	171.56^{a}	4.49
Mortality Percentage (%)	1.2	1.44	0.00	0.48	0.48	0.48	0.04

abcd Means with different superscripts along same rows show significant differences (P < 0.05) SEM = Standard Error of Means, Oxy= Oxytetracycline, %:percentage,Wt = weight. Av = average, kg = kilogram of antibiotics in humans that consume meat of chickens fed with diets containing antibiotics is of concern.

The World Health Organisation has called antibiotics resistance “a problem so serious that it threatened the achievement of modern medicine” (Grow and Huffstutter 2015).

There was a remarkable increase in body weight gain as the level of the organic acid increased in the diet. Kim *et al*. (2004) reported that the magnitude of response to acidifier was influenced by the supplementatal level employed. The range for growth response obviously varied with supplemented levels of acidifier and age of animals. The supplementation of acidifiers in young animals’ diets showed beneficial effects. Researchers have stated that feed additives may stimulate early gut development and improve overall efficiency of the chicken gastro intestinal tract. Dietary organic acids, probiotics and prebiotics directly affect development of the gut (Gilmorems and Ferrettijj, 2003; Apajalahtti *et al*., 2004). In general, the growth performance tended to depend on dose, higher levels of inclusion and increasing chain length of the acidifier. This effect could be due to proper utilization of nutrients by the chicks since organic acids aids in maintaining a balance in the population of the gut micro organism in favour of the commensals microflora thereby reducing competition for host nutrients. The use of organic acids has been reported to protect the young chicks by competitive exclusion (Mansoub *et al.*, 2011), enhancement of nutrient utilization, and growth and feed conversion efficiency (Lückstädt and Mellor, 2011). Organic acids have growth-promoting properties and can be used as alternatives to antibiotics as reported by (Fascina *et al*., 2012). Hashemi *et al*. (2014) added an acidifier mixture of formic, phosphoric, lactic, tartaric, citric and malic acids in the broiler diet at the rate of 0.15%. An increase in body weight gain of 2402 g was observed in the organic acid group when compared to 2276 g obtained for the control group at the end of 42 day of the experiment.

Ocak *et al*. (2008) noted similar increase in feed intake when lactic acid was included in broiler ration.This contrasted the earlier remarks of Cave (1984) that high levels of organic acid in broiler ration depressed feed intake. Cumulative feed intake was observed to be higher in Oxytetracycline group when compared with the experimental groups and the control. .This agrees with the report of Paul *et al.* (2007), who observed that feed intake after the 3rd and 6th weeks were significantly (P<0.05) higher in the antibiotic group when compared with the acidifier treated groups.

This result disagrees with the findings of Sultan *et al.* (2015) who reported that feed intake was not significantly affected by different levels of Acidflex which is an organic acid used during the finisher phase in broiler chickens. The feed conversion ratio of birds fed 500g Biotronic® SE diet was the best compared to the other treatment groups. Better feed conversion ratio was also reported by Vogt *et al.* (1982) and Veeramani *et al.* (2003) in broilers given diets with propionic acid. In a similar experiment conducted with different levels of organic acid treatments, Hedayati *et al.* (2014) reported FCR of broilers fed different levels of acidifier to have shown a significant decrease in FCR, when compared with their respective control groups.

Feed cost per kilogram gain was least in the different levels of organic acid groups with 500g Biotronic® SE as the best. Biotronic® SE at an inclusion level of 500g/100kg diets which reduced the cost of production by 9.52 % or ₦15.94. This indicated that the use of organic acids did not only result in better growth performance alone, but could be a strategy to improve the cost effectiveness of broiler production.

On the 28th day, mortality records showed that only birds on 300g Biotronic® SE diet had record of mortality. 100% survivability of broilers chickens were recorded in all other treatments. The mortality recorded was significant for only one treatment and maybe as a result of natural occurence and not treatment effect.

4.4.2 Growth performance of broiler finisher chickens fed varying levels of Biotronic SE diets

Table 4.10 shows growth performance of broiler finisher chickens fed diets containing varying levels Biotronic®SE. There were significant (P<0.05) differences among the different treatment groups for all the parameters measured.

The results from birds in the control group and 100g Biotronic® SE treatments were similar, but were significantly (P<0.05) lower than the remaining groups (200, 300, 400g Biotronic® SE and 100g Oxytetracycline) that were also similar in values. The feed cost per kilogram gain was least for birds on 400g Biotronic® SE treatment.

The similarities in values of the monitored parameters between the Biotronic® SE and the Oxytetracycline groups indicate the positive effect of these additives on the diets and the consequential improvement in performance of the birds over the control group. In all the performance parameters measured, final live weight, weight gain and feed conversion ratio of the organic acid groups from 200g inclusion showed similar improvements. This indicates that Biotronic® SE (organic acid) had a positive effect on nutrient utilization because of its pH lowering ability which has beneficial effect on balancing the gut microflora making nutrients available to the host. Adil *et al*. (2011) reported that lowered pH of the gut was conducive for the growth of favourable bacteria simultaneously hampering the growth of pathogenic bacteria which grow at relatively higher pH.

The direct anti-microbial and pH reducing properties of organic acids might have resulted in the inhibition of intestinal pathogenic bacteria.

Table 4.10: Growth performance of broiler finisher chickens fed diets containing varying levels of Biotronic® SE

Parameters	Levels of Biotronic® SE inclusion (g/100 Kg diet)						
	0	100	200	300	400	100 Oxty	SEM
Initial weight (g/bird)	1296.68	1300.02	1300.01	1300.03	1300.02	1300.02	1.08
Final weight (g/bird)	2385.58^{b}	2364.58^{b}	2843.75^{a}	2851.39^{a}	3062.50^{a}	2927.08^{a}	69.05
Weight gain (g/bird)	1085.42^{b}	1064.58^{b}	1543.73^{a}	1551.39^{a}	1762.50^{a}	1627.08^{a}	69.05
Av. daily wt gain (g/bird)	51.69^{b}	50.70^{b}	73.51^{a}	73.88^{a}	83.93^{a}	77.48^{a}	3.29
Total feed intake (g/bird)	2972.90^{b}	2821.92^{b}	3446.91^{a}	3540.00^{a}	3556.30^{a}	3503.10^{a}	143.65
Av daily feed intake (g/bird)	141.57^{b}	134.37^{b}	164.14^{a}	168.57^{a}	169.35^{a}	166.82^{a}	6.84
Feed conversion ratio	2.74^{b}	2.65^{b}	2.23^{a}	2.28^{a}	2.02^{a}	2.15^{a}	0.06
Feed cost /gain (₦/kg gain)	214.49^{c}	206.70^{c}	175.03^{b}	175.16^{b}	159.36^{a}	187.50^{b}	5.20
Mortality (%)	0.00	0.00	0.00	0.24	0.00	0.00	

ab Means with different superscripts along same rows show significant differences ($P < 0.05$) SEM = Standard Error of Means, Oxy = Oxytetracycline, Wt = weight. Av = average, kg = kilogram

Panda *et al*. (2009) reported that dietary supplementation of organic acids increased the body weight and feed conversion ratio in broiler chicken.

The results agreed with finding of Tabidi *et al.* (2016), who reported no significant differences in the growth performance indices namely body weight gain, feed intake and feed conversion ratio of broiler chickens fed on diets supplemented with flavomycin and diets supplemented with graded levels of Biotronic at 0.2, 0.3 and 0.4 %. Hedayati *et al.* (2014) reported that all three levels (0.25, 0.05 and 0.1%) of Totacid acidifier fed groups had shown a significant increase in body weight of broilers, when compared with control.

Vijaya *et al.* (2013) reported that the average body weights were significantly higher with the inclusion of Latic acid (LA) and citric acid (CA) in diets when compared to antibiotic (Virginiamycin) and control diets at 42 days of age.

The increasing feed intake observed as levels of Biotronic® SE increased, contrasted earlier reports of Cave (1984) that high levels of organic acid in broiler ration depresses feed intake. Adil *et al.* (2011a) reported that cumulative feed consumption was found to decrease in all the groups fed organic acids compared to the control group. The reduction they said in the feed intake might be due to the strong taste associated with the organic acids which would have decreased the palatability of the feed, thereby reducing feed intake. This result agrees with the report of Ogunwole *et al.* (2011) in which they reported higher feed intake in the acidifier group (Biotronic® SE) compared with the antibiotic group (Oxyteracycline) and control.

The feed conversion ratio were better in the acidifier fed treatments group at 200- 400g and those on the Oxytetracycline treatment which were similar and significantly ($P<0.05$) different from the control and the 100g Biotronic® SE group. The result is in agreement with Adil *et al.* (2011b) that chicks fed diet supplemented with organic acids showed significant improvement in the FCR as against the chicks fed the control diet. The improvement in the

FCR could be due to better utilization of nutrients and consequently increased body weight gain of the birds fed organic acids in their diets. Hassan *et al*. (2010) reported that organic acids mixtures and Enramycin supplementation significantly improved FCR.

The cost implication involved in the use of organic acid was significantly lower than of antibiotics (Oxytretracycline) as the feed cost per kilogram gain was lowest at ₦159.36/kg gain for birds on 400g Biotronic® SE group. This amounted to a reduction in the cost of production by 25.70% or ₦55.13 per bird.

The survivability percentage was not significant across treatments, an indication of no adverse effect from the Biotronic® SE, stable health condition, good diet, management practice and hygiene.

4.4.3 Carcass characteristics of of broiler finisher chickens fed diets containing varying levels of Biotronic® SE

Table 4.11 shows the carcass characteristics of broiler finisher chickens fed diets containing four levels of Biotronic® SE. There were significant ($P<0.05$) differences in most of the parameters measured. Live weight was significantly ($P<0.05$) higher in the Oxytetracycline fed bird than the others. Carcass weight values were similar in birds fed 400g Biotronic®SE and Oxytetracycline birds. The dressing percentage values were similar for birds on Biotronic and Oxytetracycline but significantly higher than ($P<0.05$) those in the control group. The cut parts were significantly ($P<0.05$) higher in birds fed 500g Biotronic diet for breast, drumstick and wings across the treatment groups and control. Organ weights showed no significant difference in all the parameters measured except the gizzard that recorded higher value ($P<0.05$) in the control

The dressing percentage recorded were similar for birds on Biotronic® SE and Oxytetracycline treatments but significantly ($P<0.05$) higher than those in the control group.

Table 4.11: Carcass evaluation of broiler finisher chickens fed diets containing varying levels Biotronic ® SE

	Levels of inclusion of Biotronic ® SE g/100kg						
Parameters	0	100	200	300	400	100g Oxyt	SEM
Live weight g)	2258.33^{d}	2273.33^{d}	2783.33^{c}	2762.67^{c}	3008.33^{b}	3100.00^{a}	42.85
Carcass weight (g)	1454.00^{c}	1536.83^{c}	1935.17^{b}	1987.33^{b}	2179.83^{a}	2194.00^{a}	53.47
Dressing percentage %	64.41^{b}	67.66^{a}	69.54^{a}	71.86^{a}	72.44^{a}	70.77^{a}	0.29
Cut parts expressed as percentage of carcass weight (%)							
Back	18.16	18.69	18.59	18.76	19.26	18.86	0.79
Breast	30.42^{b}	30.05^{b}	31.58^{b}	29.17^{b}	34.44^{a}	31.88^{b}	1.27
Thigh	15.25^{b}	15.13^{b}	16.03^{b}	16.28^{b}	17.11^{b}	18.78^{a}	0.92
Drum stick	8.90^{d}	8.96^{d}	10.09^{c}	9.86^{c}	13.43^{a}	12.71^{b}	0.29
Wings	908^{d}	9.70^{c}	9.70^{c}	10.05^{b}	11.38^{a}	10.22^{b}	0.25
Organs expressed as percentage of live weight (%)							
Heart	0.41	0.53	0.50	0.44	0.51	0.41	0.03
Full gizzard	3.14^{a}	2.72^{b}	2.75^{b}	2.71^{b}	2.69^{b}	2.47^{b}	0.18
Empty gizzard	2.32^{a}	2.02^{b}	2.11^{b}	2.27^{b}	1.95^{b}	2.47a	0.30
Liver	1.71	1.97	1.80	1.75	1.66	1.95	0.12
Lungs	0.48	0.62	0.66	0.43	0.55	0.59	0.08
Kidneys	0.34	0.38	0.52	0.38	0.41	0.37	0.08
Spleen	0.14	0.16	0.14	0.13	0.18	0.13	0.02
Abdominal fat	0.56	0.74	1.09	1.05	1.54	1.09	0.25
Intestinal weight	5.13	4.09	3.51	4.09	4.09	3.35	0.51
Intestinal length (cm)	85.17	76.83	87.00	90.67	100.33	102.5	7.77

abcd Means with different superscripts along same rows show significant differences ($P < 0.05$), SEM= Standard error mean, Oxyt = Oxytetracycline

This showed that organic acids have the potentials to be used as growth promoter since it improved growth rate and nutrient utilization efficiency which resulted in higher carcass yield. This result disagrees with reports of Hassan *et al.* (2010) that better dressing percentage was obtained when 0.06% galliacid was used with 0.02% enramycin in broiler diets. Brzóska *et al.* (2013) reported that organic acids (0.3 – 0.9 %) had no significant influence on carcass yield or proportion of individual carcass parts. The findings agrees with Hossain *et al.* (2016) who reported that supplementation of organic acids increased carcass, breast, thigh and drumstick meat yields when activated liquid organic acid blend composed of propionic acid, formic acid and hydroxy-4-methylthio-butanoic acid were used in diets of broilers. Saki *et al*. (2012) reported an increase in breast and thigh meat yields at 21 days whereas the effect could not be observed at 42 days. Increased dressing yield upon organic acid supplementation could be attributed to higher live weight.

4.4.4 Haematological parameters of broiler finisher chickens fed diets containing varying levels Biotronic ® SE

The result of haematological parameters of broiler finisher chickens fed diets containing varying levels of Biotronic ®SE is presented in Table 4.12. There were no significant (P>0.05) differences in all the parameters measured except the red blood cell (RBC).

Comparative assessments of haematological indices and differentials in the investigated birds showed characteristic features of normal profile of broiler chickens, indicating a good tolerance of levels of Biotronic® SE, Oxytracycline compared to the control group, thus the lack of possible side effects.

Table 4.12: Effect varying inclusion levels of Biotronic® SE in the diet of broiler finisher chickens on haematological parameters

Parameters	Levels of Biotronic® SE (g/100kg diet)							
	0	100	200	300	400	100g Oxyt.	SEM	Reference Values
Packed cell volume (%)	30.67	25.00	29.33	29.67	28.33	30.69	2.05	24.00 – 44.00*
Haemoglobin (mg / dl)	10.20	8.27	9.77	9.87	9.43	10.20	0.69	9.10 – 13.90*
White blood cell (x 10^{12} /L)	19.53	15.43	16.97	14.27	13.90	9.43	5.31	9.20 – 31.00*
Heterophils (%)	84.00	89.00	88.00	88.67	86.67	91.67	3.23	
Lymphocytes (%)	30.67	25.00	29.33	29.67	28.33	30.69	4.03	47.20 – 85.00
Red blood cell (x 10^9 /L)	5.20^a	4.13^b	4.90^a	5.07^a	4.87^a	5.37^a	0.32	1.58 – 4.10*
MCV(fl)	19.62	19.96	19.96	19.49	19.29	19.05	1.02	
MCH (pg)	33.26	33.06	32.29	33.26	33.28	33.26	0.34	
MCHC (g/l)	15.00	10.33	10.33	10.33	8.00	8.33	3.23	

ab Means with different superscripts along same rows show significant differences ($P < 0.05$), SEM = Standard Error of Means, MCHC = Mean corpuscular haemoglobin concentration, MCH = Mean corpuscular haemoglobin, MCV = Mean corpuscular volume* Mitrika and Rawnsley (1977)

The measured parameters were all within the normal reference range as reported by Mitruka and Rawnsley (1977) which was an indication that the experimental animals were tolerant to the diets fed. The red blood cell value for birds on 100g Biotronic SE (4.13) was statistically lower than the other treatment groups, though within reference values reported by Mitruka and Rawnsley (1977). This shows that the Biotronic® SE offered some kind of immune protection to the birds. This report disagrees with reports of Al-Saad *et al.* (2014) who reported that no significant differences were observed in the RBC count of birds fed prebiotics, organic acid and control except for a non significant (P>0.05) increase in the red blood cells number in antibiotic and probiotic treatments and not in the organic acid group. The white blood counts were within the reference range for birds although the control and antibiotic groups had higher numerical values but were not statistically different from the other treatments. This finding agreed with Shareef and Al-Dabbagh (2009) who recorded a decrease in white blood cells when yeast was used as a probiotic, but in contrast with Al-Saad *et al.* (2014) documentation of significant (P < 0.05) increase in the white blood cells (WBC) count. The blood haemoglobin levels in all treatments were not significantly different (P>0.05) inspite of levels of organic acids inclusion in the diets which is contrary to the report of Al-Mayah and Al-Ahmed (2005).

The most numerous of the white blood cells are lymphocytes and heterophils. The heterophils are instrumental in body defense with incredibly large numbers available in the avian specie, as well as their ability to phagocytize bacteria and foreign bodies. The heterophlis value 91.67% recorded by birds fed the Oxytetracycline treatment could be a response to infection or stress related conditions. Heterophils are the primary phagcoytic leucocyte, and proliferate in circulation in response to infection, inflammation and stress (Thrall, 2004). The lymphocytes values obtained across treatments (25.00 – 30.69 %) were low compared with the reference range (47.20 – 85 %) as reported by Mitruka and Rawnsley (1977). This may be due to the lowering of the gut pH through the organic acid thereby making the environment to be harmful to the pathogenic microbes. Furthermore, the biochemical attacks of the antibiotics Oxytetracycline on the bacteria provide additional blockages to the harmful effect of the pathogenic microbes in the GIT and the blood.

4.4.5 Serum indices of broiler finisher chickens fed diets containing varying levels of Biotronic® SE.

Table 4.13 shows biochemical indices of broiler finisher chickens fed varying levels of Biotronic® SE. There were significant differences (P<0.05) in all the parameters measured except for total protein (TP) and glucose. Birds on the control, 100, 200 and 300g Biotronic® SE treatments had similar aspartate aminotransferase (AST) values but were significantly (P<0.05) different from those obtained among 400g Biotronic® SE and Oxytetracycline treatments which were similar. The values for alanine aminotransferase (ALT) of birds on Oxytetracycline were significantly (P<0.05) higher compared to the remaining treatments that were similar. Alkaline phosphatase (ALP) values were similar for birds fed diets containing 100 and 200g Biotronic® SE treatments, but differ significantly (P < 0.05) from those on 400g Biotronic® SE and Oxytetracycline treatments, while the control group had the lowest (66.67 μ/l). Albumin content had no specific trend but the values differed significantly (P<0.05) lower compared the treatments with birds on 200g/100kg Biotronic ® SE recording the highest value (43.67g/dl).

A non significant increase in glucose was observed as the levels of Biotronic ® SE increased though it did not follow a particular trend. The antibiotic group also recorded a numerically higher value among the treatments.

Table 4.13: Effect of varying levels of Biotronic® SE in the diet of broilers on serum parameters of broiler finisher chickens

Parameters	Levels of Inclusion (g/100kg diet) Biotronic® SE						
	0	100	200	300	400	100 (Oxyt)	SEM
Glucose (mg/l)	214.67	230.33	249.67	237.33	242.00	260.33	14.04
TP (g/dl)	3.50	3.30	3.42	2.87	4.03	3.50	0.28
Albumin (g/dl)	16.33[d]	35.67[b]	43.67[a]	25.00[c]	27.33[c]	37.33[b]	2.02
AST (μ/l)	13.33[b]	13.67[b]	20.67[b]	40.67[b]	17.33[a]	54.67[a]	7.94
ALT (μ/l)	14.67[b]	7.00[b]	13.33[b]	18.67[b]	14.33[b]	61.67[a]	5.11
ALP (μ/l))	66.67[c]	159.33[b]	246.67[b]	151.67[a]	246.67[a]	254.67[a]	18.86

[abcd,] Means with different superscripts along the same rows show significant difference (P<0.05), TP : Total protein NR Normal range values, Oxyt: Oxytetracycline SEM : Standard error means, AST : aspartate aminotransferase, ALT: alanine aminotransferase. ALP : alkaline phosphatase TP; Total protein

This result agrees with the findings of Al-Saad *et al.* (2014) who reported that no significant differences were found in the blood glucose levels in all treatments control, probiotic, prebiotic and organic acid groups' inspite of an increase in the antibiotic treatment.

This result corroborated that of Al-Mayah and Al-Ahmed (2005) who reported that it may be due to the type of antibiotic and its mechanism of action or place of impact. This finding disagreed with the observation of Adil *et al*. (2010) who reported a non significant decrease in blood glucose level of organic acid**s** treatments.

The levels of some important biochemical parameters in serum are used as diagnostic markers of hepatic injury. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as aspartate aminotransferase, and serum alkaline phosphatase. The elevated activities of these enzymes were indicative of cellular leakage and the loss of the functional integrity of the cell membranes in liver (Naik and Panda, 2008). The significant ($P<0.05$) AST values (13.33-54.67 μ/l) across treatments were however within normal range reported for healthy birds (10-98μ/l) and ALP and ALT values were also significantly different. These high values could be attributed to Oxytetracycline and levels of Biotronic® SE causing leakage of the enzymes out of the liver into the blood. This suggests that there might have been slight distortion of kidney and liver, especially at the higher doses. It is speculated that the negative consequences of high dietary citric acid may result from acidosis of blood. Acidosis occurs when blood pH drops below 7.4. Dietary changes were critical in the occurrence of acidosis (Remer, 2000). This may be related to the fact that a high level of citric acid (at 60g/ kg diet) may act as a stressor, causing dysfunction of internal organs e.g. kidney, liver, heart and skeletal muscle **(**Nourmohammadi and Khosravinia, 2015).

Total protein is made up of albumin and globulin which is a biochemical test for measuring total amount of protein in blood plasma or serum. The non significant values in total protein indicated that the protein content of the diets were sufficient.

4.4.6 Apparent nutrient digestibility and ash content of broiler finisher chickens fed diets containing varying levels Biotronic® SE

Table 4.14 shows apparent nutrient digestibility and ash content of broiler finisher chickens fed diets containing four levels Biotronic® SE. There were no significant (P>0.05) differences were observed across treatments for dry matter, ash and nitrogen free extract. However, significant (P<0.05) differences were in crude protein, crude fibre and ether extract values. The birds on 200, 300 and 400g Biotronic® SE had significantly (P<0.05) higher values for crude protein apparent nutrient digestibility when compared with control,100g Biotronic® SE and Oxytetracycline groups. Birds fed the control diet, 100, 200, 300g /100kg had similar apparent nutrient digestibilities values for crude crude fibre and ether extract but significantly (P<0.05) higher than values for Oxytetracycline.

The pH lowering ability of organic acid in the gut may have enhanced this effect and the reduced competition between host animal with pathogen organisms. Organic acids normally used as an acidifier in poultry feeds have been considered to be attractive alternatives for improving nutrient digestibility. Ghazalah *et al*. (2011) reported that dietary level of 0.5% of either fumaric or formic acid and 0.75% of acetic or 2% citric acid improved both ME and apparent nutrient digestibility, that is, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) of broiler diets. Samanta *et al*. (2010) reported that organic acids improve gastric proteolysis as well as the digestibility of proteins and amino acids in broilers.

Table 4.14: Apparent nutrient digestibility and ash content of broiler finisher chickens fed diets containing varying levels of Biotronic® SE diets

	Levels of Biotronic® SE inclusion (g/100kg) diet						
Parameters	0	100	200	300	400	100 Oxyt	SEM
Dry matter (%)	84.77	85.34	82.38	86.81	90.54	76.50	3.86
Crude protein (%)	87.40^{b}	87.67^{b}	86.54^{a}	89.31^{a}	94.50^{a}	80.55^{b}	2.90
Crude fibre (%)	85.01^{a}	87.97^{a}	86.51^{a}	87.19^{a}	90.65^{a}	76.43^{b}	3.49
Ether extract (%)	93.62^{a}	93.98^{a}	93.54^{a}	94.64^{a}	96.27^{a}	89.82^{b}	1.52
Ash content	86.01	86.30	85.05	85.42	91.36	79.96	3.57
NFE (%)	76.80	80.26	76.62	84.33	87.16	67.69	4.87

^{ab}: Means with different superscripts along same rows show significant differences (P<0.05), SEM= Standard Error of Means, NFE= Nitrogen free extract, Oxyt= Oxytetracycline

Dibner and Butin (2002) suggested that organic acids improve protein and energy digestibility by reducing microbial competition with the host for nutrients, decreasing endogenous nitrogen loss, lowering the incidence of sub-clinical infections and secretion of immune mediators, and reducing production of ammonia and other growth depressing microbial metabolites.

Acidic environment favoured the production and secretion of pepsin, gastrin and cholecystokinin, which play a significant role in the nutrient utilization and subsequent growth performance and feed efficiency (Hayat *et al*., 2014).

4.4.7 Drug residue detection in meat of broiler finisher chickens fed diets containing varying levels Biotronic® SE

The results of the test conducted to detect antibiotics residues in meat tissues taken from of broiler finisher chickens fed diets containing varying levels Biotronic® SE are presented on Table 4.15. The results showed that meat samples from birds on the control treatment showed no drug residue and those from the four levels of Biotronic® SE were low in concentration within the detection limits. However, drug was detected at a very high concentration in the Oxytetracycline treatment group and above the test detection limits. Although Delvotest® test kit is not a quantitative method it detected most antibiotics at or above their Maximum Residues Limits (MRL) or Tolerance Level as set by World Health Organisation (WHO) that can detect antibiotic residues. The Delvostest® SP interpretation, showed that the control (yellow colouration) is due to the growth of the spores at 64˚C that initiated an acidification process which caused the turning of a pH indicator from purple to yellow. The presence of antibiotic substances in the Oxytetracycline treatment on the other hand, caused a delay or inhibition of the spores, depending on the concentration of the residues. In the presence of residues therefore, the spores would not multiply and the pH indicator would remain purple.

Table 4.15: Drug residue in meat samples of broiler finisher chickens fed diets containing varying levels Biotronic® SE levels of Biotronic®SE

	Levels of Biotronic® SE nclusion in (g/ 100kg					
Indicators	0	100	200	300	400	100g Oxtry
Yellow	NR	LL	LL	LL	LL	AL
Yellow/Purple	NR	LL	LL	LL	LL	AL
Purple	NR	LL	LL	LL	LL	AL

Key: NR Yellow colour indicates the absence of antibacterial substances at a concentration at or above the test detection limit.

LL Yellow/purple colour indicates the presences of antibacterial substances in the related samples to the test detectiolimit.

AL Purple colour indicates the presences of antibacterial substance above the test detection

The prevalence of antibiotic residues in the samples in this study was a clear indication that consumers may be exposed to violative levels of antimicrobial residues which maybe consequent upon abuse and misuse of antimicrobials and most importantly, non-observance of withdrawal period.

However, the Biotronic® SE treatments were undecided between yellow /purple gearing towards light blue coloration. This may be associated with low concentrations of residues in those samples showing incomplete acidification of the medium due to partial inhibition.

4.4.8: Effect of varying levels of Biotronic® SE diets on ileal and ceacal bacteria of broiler starter chicks

The effect of varying levels of Biotronic® SE diets on ileal and ceacal bacteria in broiler starter chicks is presented in Figures 4.1 and 4.2. Figure 4.1 shows the effect of levels of Biotronic ®SE on total aerobic plate counts (APC) of the ileum and ceaca of the broiler chicks.

The APC followed an increasing trend in the ileum of birds fed diets containing 200g /100kg Biotronic® SE peaked at 300g/100kg Biotronic® SE before a decline was observed. It was observed that 300g/100kg Biotronic® SE was more effective in increasing bacteria population in the ileum of birds on that diet than the ileum of birds on other groups. Compared to the Oxytetracycline group the Biotronic® SE level was able to increase the gram positive bacteria in the ileum. The microbial loads of the intestinal samples were more influenced by the dietary inclusion of Biotronic® SE and Oxytetracycline in the broilers diets. This could be attributed to the ability of some non pathogenic bacteria to thrive well in the presence of Biotronic® SE and others unable to cope with the condition.

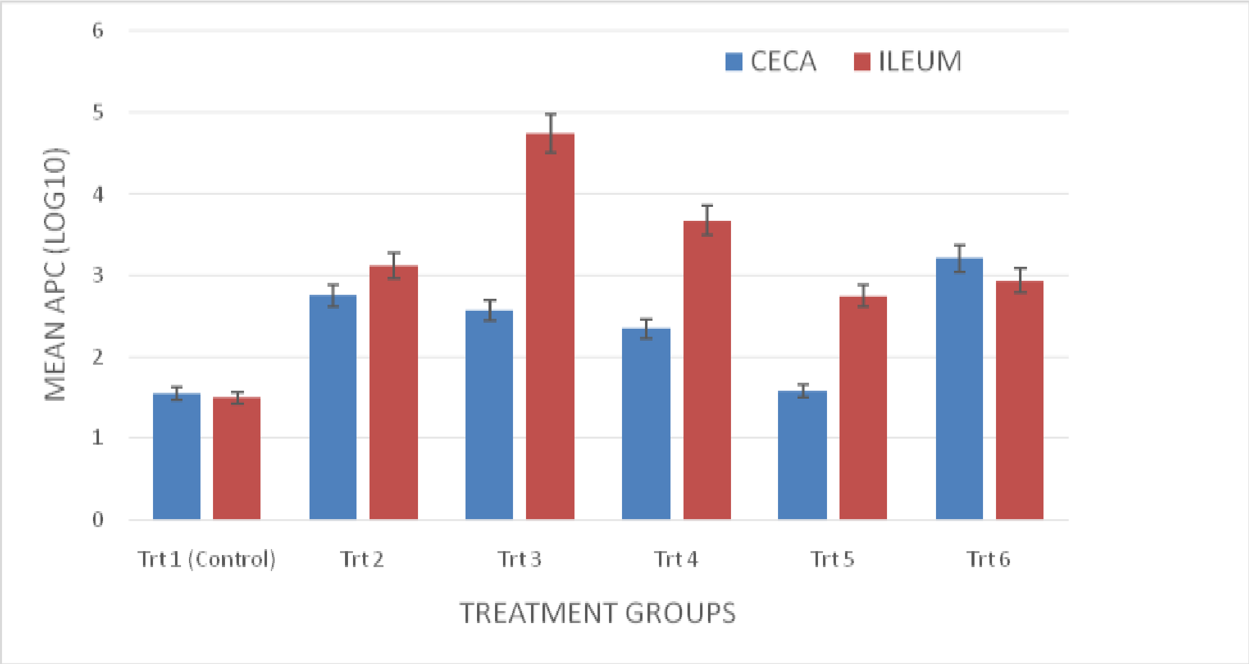

Figure 4:1 Aerobic Plate Count (APC) of broiler starter chicks fed diets varying levels of Biotronic® SE diets

This showed that Biotronic® SE is made of different constituents with anti-bacterial property that have direct or indirect effects on the intestinal micro flora population and control of gram negative bacteria such as *Salmonella sp.* and *E. coli.*

It was also observed that broiler chicks on levels varying levels of Biotronic® SE treatment recorded an increase microbial load. The profile of intestinal microflora played an important role in gut health. The gut microflora comprised both commensals (Gram-positive) and entheropathogens (Gram-negative). In healthy birds, there is a balance between the gram-positive and gram-negative populations of microflora at an ideal pH. A healthy gut has a pre dominance of Gram-positive bacteria. The balance gets disturbed when there is a change in the pH due to ingestion of toxic chemicals or chemotherapeutic agents or a change in feed composition

Figure 4.2 shows the effect of levels of Biotronic ®SE on Total Coliform Counts (TCC). The increase in coliform counts observed in this study in the ceaca and ileum of treatment groups was similar to the findings of Wahe (2016), when varying levels of *Saccharomyces cerevisae* (yeast) was fed to broiler chickens. Similarly, Moses (2016) reported that there was an increase in coliform counts while varying the levels of Biostrong® 510 a phytogenic eubiotic, was fed to broiler diet. These findings contrast those reported by Ramarao *et al.* (2004), who found that the addition of gut acidifier in broiler diets at the rate of 300 g/100 kg feed showed a reduction in coliform count. Fumaric and sorbic acids lowered the numbers of coliforms in the ileum and caeca (Pirgozliev *et al.*, 2008).

However, acidification of diets with weak organic acids such as formic, fumaric, propionic, lactic and sorbic had been reported to decrease the colonization of pathogens and production of toxic metabolites (Kirchgessner and Roth, 1988).

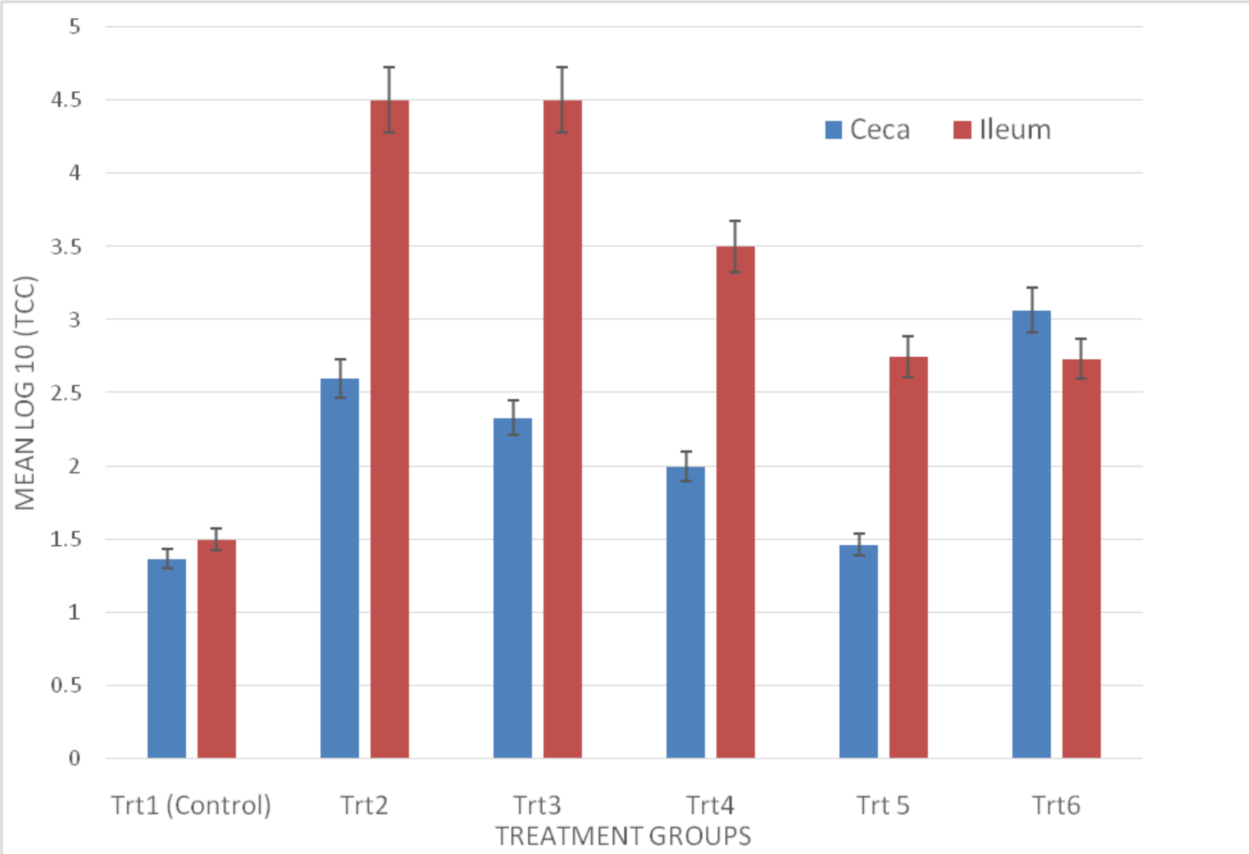

Figure 4:2 Total Coliform Count (TCC) of broiler starter chicks fed diets varying levels of Biotronic® SE diets

These findings show that organic acids can safely replace antibacterial compounds in broiler chicken diets with beneficial effects on the intestinal bacterial colonization. The result was similar to the findings of Thirumeignanam *et al.* (2006), who reported a decrease in total bacterial load with concomitant increase in lactobacilli load because of dietary acidification. Alp *et al.* (1999) reported that the inclusion of an antibiotic and an organic acid mixture that contains lactic, fumaric, propionic, citric and formic acid separately or combined reduced the Enterobacteriaceae count in the ileum of broilers. Thompson and Hinton (1997) reported that an organic acid mixture of formic and propionic acid treatment decreased Salmonella in the crops of hens.

4.5 Experiment 3: Effect of Single and Combined Inclusion Levels of Mycofix® and Biotronic® SE in Diets of Broiler Chickens on Performance

4.5.1 Growth performance of broiler starter chicks fed diets containing varying levels of single and combined Mycofix® and Biotronic® SE

Table 4.16 shows the growth performance of broiler chicks fed diets containing levels of single and combined Mycofix® and Biotronic® SE. There were no statistical ($P>0.05$) differences in all the parameters measured except total feed intake and averge daily feed intake. The feed intake for birds fed Oxytetracycline diets was significantly ($P<0.05$) higher than the other groups and the control.

The similarities in results observed in most the growth performance parameters indicated that the use of Mycofix®, Biotronics® SE either singly or in combination at the various levels were effective in promoting growth performance and were comparable to the antibiotic group but better than the control. Researchers have stated that feed additives may stimulate early gut development and improve overall efficiency of the chicken gastro intestinal tract. Dietary organic acids, probiotics and prebiotics directly affect development of the gut (Donoghue, 2003; Apajalahttijj *et al*., 2004).

Table 4.16: Growth performance of broiler starter chicks fed diet containing levels of single and combined Mycofix® and Biotronic ® SE

Parameters	Levels of Mycofix® and Biotronic® SE inclusion (g/100 Kg diet) 0	400 Myco	500 Bio	200Myco + 250 Bio	400Myco + 500 Bio	100 Oxyt	SEM
Initial weight (g/bird)	43.12	43.11	43.11	43.12	43.10	43.12	0.01
Final weight (g/bird)	1096.67	1158.33	1133.33	1100.0	1191.67	1241.67	36.36
Weight gain (g/bird)	1053.55	1115.23	1090.22	1056.88	1148.56	1198.55	36.36
Av. daily wt gain (g/bird)	37.63	39.83	38.94	37.75	41.02	42.81	1.30
Total feed intake (g/bird)	1775.01[b]	1801.08[b]	1799.43[b]	1800.92[b]	1813.18[b]	1920.44[a]	24.27
Av daily feed intake (g/bird)	63.39[b]	64.32[b]	64.27[b]	64.32[b]	64.76[b]	68.59[a]	0.87
Feed conversion ratio	1.62	1.60	1.68	1.67	1.57	1.59	0.04
Feed cost /gain (₦/kg gain)	135.84	134.81	140.97	139.56	132.26	145.31	3.18
Mortality (%)	0.2	0.00	0.00	0.00	0.00	0.00	0.02

[ab] Means with different superscripts along the same rows show significant difference SEM = Standard error mean; Myco= Mycofix, Bio.= Biotronic, Oxyt = Oxytetracycline, Av= average, wt.weight

The results of final live weight, bobdy weight gain and the average daily weight gain did not follow a particular trend. However, there were no significant (P<0.05) differences amongthe values these respective parameters. The FCR was better in the combined treatment of 400g Mycofix® and 500g of Biotronic® SE though not statistically different.

From literature reviews, there have been combinations of organic acid, probiotics and antibiotics but there is a paucity of information on toxin binders, organic acid singly and in combination with antibiotics. However, the use of toxin binder in ameliorating the effect of toxins present in feed are well documented (Diaz *et al.*, 2005; Manafi *et al.*, 2012; Ani *et al.*, 2014). In the present study, the observed non significant effect in the single and combined use of Mycofix® and Biotronic® SE as feed additives may be associated with other factors. With a good environmental condition, well-nourished, healthy chicks did not positively respond to growth promoters when they were housed under clean conditions at moderate stocking density. The two additives used singly and in combination at different levels with the antibiotic group did not improve growth performance above the control diet. Several researchers reported that when chicks were housed in a clean environment, growth promoters such as probiotic, organic acid or antibiotic may not have a pronounced effect on performance (Miller, 1987; Lyons, 1987 and Anderson *et al*., 1999).

4.5.2 Growth performance of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

The result of growth performance of broiler finisher chickens fed levels of single and combined Mycofix® and Biotronic® SE is presented in Table 4.17. Dietary treatments significantly (P<0.05) affected final live weight, body weight gain, average daily weight gain and feed conversion ratio. Birds fed Oxytetracycline were significantly (P<0.05) higher in final live weight, weight gain, average daily weight gain and had the best feed conversion ration.

The superior performance of the Oxytetracycline group at the finisher phase compared with all the other treatments showed that antibiotic increased feed efficiency and growth rate which could be be attributed to its anti-microbial effect. This result is in line with the findings of Yakhkeshi *et al*. (2011) who reported that the highest weight gain were achieved by virginiamycin ($P < 0.05$) when Sangrovit® a herbal extract was used at 35g/ton of diet, Primalac® a probiotic was used at 0.1 % of diet, Termin-8® an organic acid was added at 0.2 % of diet and Virginiamycin an antibiotic was used at 15ppm of diet were used as additives in broiler diets. The feed conversion ratio result is similar to the report of Odefemi (2016), who reported a significantly lower feed conversion ratio (1.64) at the finisher phase when 0.01%antibiotics, 0.06% probiotics 1 (Bio grow promoter), 0.1% probiotics 2 (Grow up) and 0.2% prebiotic (Manna oligosaccharide) were used in broiler finisher diets. The improved feed conversion ratio observed in this investigation may be explained that antibiotic growth promoters reduced the level of competition between substances and the host.

They also increase absorption and consumption of nutritional compounds byn thinning of the intestinal wall (Amaechi and Annueyiagu, 2012). Moreover, the expulsion of pathogenic organisms from the gut by the beneficial ones confers a better absorption of nutrients on the birds and hence enhances the bird’s ability to convert feed.

Table 4.17: Growth performance of broiler finisher chickens fed diets containing varying levels of single and combined Mycofix® and Biotronic ® SE

Parameters	Levels of Mycofix® and Biotronic® SE inclusion (g/100 Kg diet)						
	0	400 Myco	400 Bio	200 Myco + 200 Bio	400 Myco + 400 Bio	100g Oxyt	SEM
Initial weight(g/bird)	1179.33	1179.67	1179.67	1179.00	1179.55	1179.00	0.54
Final weight (g/bird)	2568.63^{b}	2529.41^{b}	2637.25^{b}	2637.25^{b}	2589.46^{b}	2990.20^{a}	58.17
Weight gain (g/bird)	1389.29^{b}	1349.75^{b}	1457.59^{b}	1458.24^{b}	1410.13^{b}	1811.20^{a}	58.31
Av. daily wt gain (g/bird)	49.21^{b}	48.21^{b}	52.06^{b}	52.08	50.36^{b}	64.89^{a}	2.08
Total feed intake (g/bird)	2841.18	2644.12	3005.88	2832.35	2750.25	2955.88	67.22
Av daily feed intake (g/bird)	135.29	125.91	143.14	134.87	130.96	140.76	3.20
Feed conversion ratio	2.04^{a}	1.96^{a}	2.07^{a}	1.95^{a}	1.98^{a}	1.63^{b}	0.06
Feed cost /gain ((₦/kg gain)	162.41	156.51	164.62	155.93	155.88	141.91	4.93
Mortality Percentage	0.05	0.00	0.00	0.00	0.00	0.00	0.02

^{ab} Means with different superscripts along the same rows show significant difference SEM = Standard error mean; Myco= Mycofix, Bio.= Biotronic, Oxyt = Oxytetracycline, Av = average, wt = weight.

4.5.3 Carcass characteristics of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

Table 4.18 shows the carcass characteristics of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE. Birds on Oxytetracycline were significantly ($P<0.05$) higher across the treatment groups in terms of live weight and carcass weight. There were no significant ($P>0.05$) differences observed across for dressing percentage. Results of cut parts showed no effect of dietary treatments except for drum stick of birds on 400g Biotronic® SE single inclusion. No statistical differences were observed across treatments for organ weights.

The better carcass yield observed was the Oxytetracycline fed group as a result of the higher weight of birds in the group compared with the other treatments and control. The use of antibiotics as a growth promoter has the ability to improve weight gain as well as carcass yield of birds have been reported (Yang *et al*. 2009).

Table 4. 18: Carcass characteristics of broiler chickens fed single and combined levels of Mycofix® and Biotronic® SE diets

Parameters	Levels of Mycofix® and Biotronic® SE inclusion (g/100 Kg diet						
	0	400g Myco	400g Bio	200 Myco + 200g Bio	400g Myco + 400g Bio	100 g Oxytr	SEM
Live weight (g/bird)	2561.67^{b}	2519.62^{b}	2601.83^{b}	2681.64^{b}	2752.83^{b}	3004.83^{a}	72.75
Carcass weight (g)	1921.67^{b}	1817.33^{b}	1851.17^{b}	1890.83^{b}	1931.83^{b}	2158.17^{a}	76.23
Dressing percentage %	71.83	70.45	71.12	70.12	72.01	75.11	1.71
Cut parts expressed as percentage of carcass weight (%)							
Back	11.70	12.91	12.72	12.41	11.77	12.54	0.62
Breast	22.76^{b}	22.83^{b}	23.46^{b}	23.23^{b}	25.30^{b}	28.73^{a}	0.94
Thigh	10.46	11.95	12.64	11.28	13.05	13.01	0.74
Drum stick	9.00^{b}	9.20^{b}	10.75^{a}	9.31^{b}	8.48^{b}	8.84^{b}	0.47
Wings	6.53	6.80	7.06	6.97	6.47	7.13	0.42
Organs expressed as percentage of live weight (%)							
Heart	0.66	0.62	0.62	0.56	0.55	0.66	0.06
Full gizzard	3.90	4.29	4.12	4.26	3.88	3.77	0.29
Empty gizzard	2.60	2.82	2.74	2.83	2.64	2.47	0.20
Liver	2.56	3.13	2.95	2.78	3.05	2.43	0.31
Lungs	1.20	0.87	0.79	0.69	0.73	0.63	0.71
Kidneys	0.77	0.85	0.83	0.86	0.82	0.71	0.06
Spleen	0.12	0.15	0.19	0.13	0.18	0.12	0.06
Abdominal fat	1.57	1.78	1.93	1.74	1.96	2.45	0.30

ab Means with different superscripts along the same rows show significant difference (P<0.05), SEM = Standard error mean, Bio = Biotronic® SE, Oxyt = Oxytetracycline,Myco = Mycofix

4.5.4 Haematological parameters of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

Table 4.19 shows the haematological parameters of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE The diets did not significantly (P>0.05) affect all the parameters measured.

The non significant results obtained showed that the levels as well as the combinations of the additives with the Oxytetracycline group and the control were not detrimental to the birds normal blood profile hence indicating that the birds were adequately nourished and mitigate anaemia nor show any sign of disease infection or parasitic problems The blood is often time used as a physiological index or indicator for disease diagnosis, nutritional status and health condition of the birds.

Olafedehan *et al.* (2010) reported that blood acts as a pathological reflector of the status of exposed animals to disease and other conditions. Isaac *et al.* (2013) reported that animals with good blood composition were likely to show good performance.

The values of PCV, RBC, WBC, platelets, MCV and MCHC obtained in this study agree with the findings of Mitruka and Rawnsley (1977) which particularly showed that the bone marrows of the birds were functioning normally. It revealed the absence of macrocytic and hypochronic anaemia. This confirmed that haematological indices, such PCV and Hb were correlated with the nutritional status of the animal (Adejumo, 2004) and agreed with Oyawoye and Ogunkunle (1998) who stated that PCV was an index of toxicity in the blood and high levels usually suggests the presence of toxic factors which had adverse effect on blood formation. The normal values of MCV, MCHC and MCH obtained in this study indicated that there were no negative interactions between the energy and protein levels in the diets.

Table 4.19: Haematological profile of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

	Single and combined levels of Mycofix® and Biotronic® SE							
Parameters	0	400g Myco	400g Bio	200g Myco + 200g Bio	400g Myco + 400g Bio	100gOxyt	SEM	Reference values
PCV (%)	32.40	36.80	31.13	37.83	30.55	36.70	3.06	24.00– 44.00*
Hgb (mg/dl)	10.27	11.55	11.69	12.00	9.70	10.27	1.46	9.10 – 13.90*
RBCx10^{12}/L	2.33	2.61	2.16	2.64	2.20	2.66	0.23	1.58 – 4.10*
MCV (fl)	140.48	139.53	144.08	142.67	138.87	138.85	2.97	90 – 140**
MCH (pg)	44.53	43.80	46.70	45.48	44.10	45.65	0.95	33 - 47**
MCHC g/dl	317.00	314.00	324.33	319.00	318.33	328.83	5.97	26 – 35**
WBCx10^9/L	77.98	87.77	73.17	84.23	68.80	85.17	9.71	
Lymphocytes (%)	66.27	75.65	62.95	74.03	60.77	75.18	7.97	40 – 70**
Platelets ($10^6/mm^3$)	4.50	7.00	11.33	8.50	8.50	7.83	3.22	

SEM: Standard Error Mean Myco; Mycofix, Bio.; Biotronic, Oxyt; Oxytetracycline PCV = Packed Cell Volume, WBC = White Blood Cell, RBC = Red Blood Cell HB = Haemoglobin, MCHC; Mean corpuscular haemoglobin concentration, MCH = Mean corpuscular haemoglobin *Mitruka and Rawnsley, 1977, **Jain, 1993

The white blood cell counts (leucocytes differential counts) which did not show any significant difference across the treatments indicated that the birds were not stressed during the experiment by nutritional or environmental factors, since leukocyte responses are considered as better indicators of chronic stress (Siegel, 1995).

4.5.5 Serum indices of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

Table 4.20 shows the effect of dietary inclusion of single and combined levels of Mycofix® and Biotronic® SE on serum indices of broiler finisher chickens. The diets showed no influence on all the parameters measured namely, liver function enzymes AST, ALT, AST: ALT, ALP, serum cholesterol, glucose, total protein, albumin and globulin.

The AST, ALT, AST: ALT and ALP are indicators to show the liver function conditions which could be adjudged as normal and not damaged based on the result obtained in this study. The results indicated that the birds could tolerate the levels of the additives without any deleterious effects on liver functions. These results also showed that the birds were in normal physiological state as there were no differences between the broiler chickens fed the test diets and the control. This further confirmed the nutritional adequacy of the experimental diets. Malik *et al*. (2013) reported that the serum biochemistry was routinely used for detection of organ diseases in domestic mammals and has also been documented to positively correlate with the quality of the diet (Adeyemi *et al.*, 2000). In general, activity of AST in birds greater than 230u/l was considered abnormal. Abnormal activities have been linked with vitamin E, selenium or methionine deficiencies (Gylstorff and Grimm, 1987), liver damage (Rosskopt and Woerpel, 1984). ALT is more specific to the liver and a better indicator for detecting liver injury. The mean values for ALT across treatments (6.62 – 10.50 u/l) were within the normal range (9.50 – 37.20 u/l) reported by Mirtuka and Rawnsley (1977). This is in line with the finding of Eggum (1970) who stated that the values of AST and ALT were normally low in

Table 4.20: Serum indices of broiler chickens finisher chickens fed single and combined levels of Mycofix® and Biotronic® SE diets

Parameters	Single and combined levels of Mycofix® and Biotronic® SE						
	0	Myco 400g	Bio 400g	200g Myco + 200g Bio	400gMyco + 400gBio	100g Oxyt	SEM
AST (µ/l)	149.17	129.50	119.17	133.00	181.67	194.50	13.84
ALT (µ/l)	6.62	7.85	8.53	7.25	10.50	9.38	1.31
AST:ALT	0.04	0.06	0.08	0.05	0.06	0.05	0.01
ALP ((µ/l))	213.67	168.17	165.83	198.00	116.17	119.17	29.46
Cholesterol mg/dl)	113.25	131.63	121.97	115.95	130.98	121.78	10.60
Glucose (mg/dl)	216.00	188.33	189.17	200.00	194.67	211.83	14.54
Total protein (g/dl)	6.83	6.27	5.13	6.22	5.58	5.05	0.74
Albumin (g/dl)	4.68	4.95	3.78	4.52	4.15	3.72	0.51
Globulin (g/dl)	2.15	1.32	1.33	1.70	1.43	1.33	0.32

SEM = Standard Error Mean, Myco = Mycofix, Bio = Biotronic, Oxyt = Oxytetracycline, ALT = alanine amino transferase, AST =.aspartate amino transferase, ALP = Alkaline Phosphatatease blood but became high when there was occurrence of liver damage by toxic substances. When the ratio of AST: ALT is greater than one (>1) compared to a control value of less than 1, it implies that the internal organs of the chickens might have been slightly distorted as Adeyemo *et al.* (2013). The AST: ALT ratio from this study showed no distortion of the internal ogans as the ratio was less than 1.

This could further suggest that the additives had an ameliorating effect on any adverse effect of diets and microorganisms in their guts. Manfred (2013) reported that ALP activity unlike most enzyme assays was induced by cellular activity (increased synthesis) rather than cell damage. The lower and non–significant cholesterol levels observed in this study were an indicator of one the health benefits of incorporating the additives in broiler chicken diet.

The similarity in serum albumin and total protein implies that there was normal protein metabolism. This showed that the diets had adequate nutritional quality, good amino acid balance (Eggum, 1989).

There was also no significant (P>0.05) difference in the ALT, AST and ALP levels between the broiler chickens fed diets supplemented with organic acid and the control group confirming the earlier finding of Abdel-Fattah *et al.* (2008) who concluded that dietary supplementation of organic acids could be done up to the level of 3% in the diet of broiler chicken without causing any adverse effect on the kidney and liver functions.

4.5.6 Kidney function test of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic SE ®

Table 4.21 shows the effect of dietary inclusion of single and combined Levels of Mycofix® and Biotronic SE ® on kidney function test of broiler finisher chickens.

Table 4:21 Kidney function test of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

	Single and combined levels of Mycofix® and Biotronic® SE						
Parameters	0	400g Myco	400g Bio	200g Myco + 200g Bio	400g Myco + 400g Bio	100g Oxyt	SEM
Urea (mg/dl)	4.08	3.97	3.12	3.70	3.12	4.75	0.42
Creatinine (mmol/l)	0.85	0.79	0.84	0.83	0.94	0.85	0.06
Na^+ (mmol/l)	148.17	143.67	141.67	141.17	149.17	151.50	5.76
K^+ (mmol/l)	4.12	4.87	4.93	4.85	4.67	4.63	0.46
Cl^- (mmol/l)	114.17	114.50	111.83	120.33	116.83	104.00	4.82
HCO_3^- (mmol/l)	74.33	84.00	47.83	48.67	50.83	54.00	13.06

SEM = Standard Error Mean, Myco = Mycofix, Bio = Biotronic, Oxyt = Oxytetracycline, Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Choride ion, HCO_3^- = Hydrogen carbonate ion SEM=

4.5.7 Apparent nutrient digestibility and ash content of broiler finisher chickens fed diets containing single and combined levels of Mycofix® and Biotronic® SE

Table 4.22 shows the effect of single and combined levels of Biotronic® SE and Mycofix® diets on nutrient digestibility of broiler finisher chickens. The dry matter, crude protein, crude fibre, ether extract and ash differed significantly (P<0.05) among various treatment groups. Dry matter results for treatment 2 (400g Mycofix) and the control were significantly (P<0.05) different from treatments 3, 4, 5 and 6 which were similar in values i.e 84.92, 85.00, 84.66and 84.66 respectively. Digestibility values for crude protein were similar for birds on control, 400g Mycofix®, 400g Biotronic® SE, 200g Mycofix and 200g Biotronic® SE and 400g Mycofix® and 400g Biotronic® SE but significantly (P<0.05) different from 100g Oxytetracycline. Control had a better digestibility value for ether extract which was significantly (P<0.05) different from other treatment groups. Ash values were better in control, 400 Mycofix®, 400g Biotronic® SE and T6 compared with the rest.

The improved digestibility results obtained with birds on 400g Biotronic ®SE indicated the positive effect of organic acid on nutrient utilization. With the reduction of pH in the upper part of the GIT it leads to acidification which helps to stimulate the secretion of endogenoue enzymes like proteolytic enzymes and improves pancreatic secretion leading to effective digestion. Acidification helps activites of the beneficial micro organism which creates a conducive gut environment for digestion and absorption. With acidification there is increase passage time of nutrients which enables better absorption and digestibility, hence, nutrient utilization.

It has been documented that dietary supplementation of organic acids can improve the retention of protein and other nutrients. Broiler chickens fed diets containing various inclusion

Table 4.22: Apparent nutrient digestibility and ash content of broiler finisher chickens fed single and combined levels on Mycofix® and Biotronic® SE diets

	Inclusion Levels of Mycofix® and Biotronic® SE						
Parameters	0g	400g Myco	400g Bio	200g Myco + 200g Bio	400gMyco + 400g Bio	100g Oxyt	SEM
Dry matter (%)	70.44^{c}	75.70^{b}	84.92^{a}	85.00^{a}	84.66^{a}	82.66^{a}	1.49
Crude protein (%)	81.40^{a}	82.52^{a}	81.38^{a}	83.52^{a}	85.13^{a}	77.42^{b}	1.42
Crude fibre (%)	77.44^{a}	76.07^{a}	75.57^{a}	65.38^{b}	60.61^{b}	74.20^{a}	2.46
Ether extract (%)	93.01^{a}	85.16^{b}	88.17^{b}	82.39^{b}	86.64^{b}	85.84^{b}	1.96
Ash (%)	84.99^{a}	84.16^{a}	85.03^{a}	77.63^{b}	75.78^{b}	84.16^{a}	1.42
NFE (%)	59.16	48.74	54.41	48.09	43.41	49.17	3.86

^{abc} : Means with different superscripts along same rows show significant differences, SEM: Standard Error of Measns NFE: Nitrogen , free extract, Oxyt: Oxytetracycline, Myco: Mycofix®, Bio: Biotronic® SE levels of dietary organic acids generally had greater retention of dry matter (DM) and protein than those fed control diets (Van Der Sluis, 2002; Samanta *et. al,* 2010; Ghazala *et.al,* 2011).

Ao *et al.* (2009) reported that there was 1.3% increase in DM and 2.1% increase in crude protein over the control when citric acid 20 g/kg was included in broiler diets. Esmaeilipour *et al.* (2011) reported 4.4% and 2.9% increase respectively for DM and CP over the control when 40g/kg citric acid was included in broiler diets. According to Van Der Sluis (2002), the positive effect of organic acids on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings.

4.5.8 Villi morphometrics of sections of jejunum of broiler finisher chickens fed diets containing single and combined levels on Mycofix® and Biotronic® SE diets

Table 4.23 shows the effect of single and combined levels on Mycofix® and Biotronic® SE diets on villi morphometrics of section of the jejunum of broiler finisher chickens. The results showed significant ($P < 0.05$) differences in terms of villi roundness and crypt across treatments. No influence of the applied treatments was observed for villi area, perimeter, height and width across treatments.

The villus crypt was regarded as the villus factory, and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue (Yason *et al.,* 1987).

There was a significant increase ($P<0.05$) in the intestinal crypt of birds fed 400g Mycofix® and 400g Biotronic ® SE, compared with control, and the other treatment groups. Xu *et al.* (2003) reported that decreased crypts depth may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract and lower performance.

Table 4.23: Villi morphometrics of Jejunum sections of broiler finisher chickens fed diets containing single and combined levels on single and combined levels of Mycofix® and Biotronic® SE

Parameters	Levels of Inclusion						
	0g	400g Myco	200g Myco + 400 Bio	400Myco + 200 Bio	400 Bio	100 (Oxyt)	SEM
Villi Area (µm)	52876	41205	40379	73664	63575	36051	12372.34
Villi Perimetre (µm)	2375	2920	5944	4925	3678	3185	1670.34
Villi Height (µm)	675.50	664.80	723.30	1124.40	767.80	546.30	320.35
Villi Width (µm)	172.93	187.94	205.75	269.43	217.38	237.20	34.30
Villi crypt (µm)	643.16	627.97	696.50	643.16	895.05	555.46	0.03
Villi Roundness (µm)	0.16^{a}	0.06^{b}	0.06^{b}	0.05^{b}	0.08^{b}	0.07^{b}	0.03

ab; Means with different superscripts along same rows show significant differences, SEM = Standard error mean; Oxyt = Oxytetracycline; Myco = Mycofix; Bio = Biotronic

Conversly, Garcıá *et al.* (2007) reported that crypts of jejunum were significantly deeper in birds fed the formic acid diet (1.0%) than birds fed the antibiotic diets (266 vs. 186 µm, respectively; P <0 .05) in the same experiment. Thus, formic acid supplementation increased both the villus height and crypt depth. Short-chain fatty acids have been demonstrated to stimulate the proliferation of normal crypt cells, enhancing healthy tissue turnover and maintenance. This reduction in the muscularis thickness was helpful in improving the digestion and absorption of nutrients as reported by Teirlynck *et al.* (2009) that the thickening of mucous layer on the intestinal mucosa contributed to the reduced digestive efficiency and nutrient absorption.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Modern production strategy focuses on improved gut health and efficient feed utilization for optimum performance. The realization of the above is hindered by toxins present in feed ingredients and competition in the birds gut by pathogenic microorganisms for nutrients meant for the host. One nutritional strategy that had been used to tackle these two has been used of mycotoxin binders and organic acids in poultry feed.

This study was designed to evaluate mycotoxin binder and acidifier as feed additives on performance of broiler chickens. The three experiments were conducted at the poultry unit of the Department of Animal Science teaching and research farm, Faculty of Agriculture, Ahmadu Bello University, Samaru, Zaria.

Three separate broiler feeding trials were conducted. Experiment 1 was to determine the effect of inclusion levels of Mycofix® (mycotoxin binder) on broiler chicken performance. A total of 330 day old chicks were used for this study consisting of five treatments; control no Mycofix®, treatment 2, 100g/100kg Mycofix®, treatment 3, 200g/100kg Mycofix® treatment 4, 300g/100kg Mycofix® and treatment 5, 400g/100kg diet both starter and finisher diets. Data obtained from the study were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS. Significant differences among treatments means were compared using Dunnette’s Test in the SAS. Mycofix® inclusion at 400g/kg100 of feed gave the best performance compared with the control and other dietary treatments.

The second evaluated the effect of levels of Biotronic® SE and Oxytetacycline on performance of broiler chickens. There were six dietary treatments; control (no Biotronic® SE, antibiotics positive control), Treatment 2, 200g/100kg Biotronic® SE, treatment 3, 300g/100kg Biotronic® SE, treatment 4, 400g/100kg Biotronic® SE, treatment 5, 500g/100kg and treatment 6, 100g Oxytetracycline/100kg (negative control). Data obtained from the study was subjected to analysis of variance (ANOVA) usng the general linear model procedure of SAS. Significant differences among treatments means were compared using Dunnette's Test in the SAS.

Birds fed 500g Biotronic® SE gave the best FCR value but Oxytetracycline and 500g Biotronic® SE were statistically similar in all the performance parameters measured. At the finisher phase the trend was different as the levels of organic acid treatments from 200g were similar with the Oxtytetracycline treatment. Birds on levels of organic acids also gave better nutrient digestibility results. Results of the microbial population in the gut revealed that the population of lactose fermenting bacteria in the gut was higher in the groups fed with Biotronic® SE treatments compared to the control and Oxytetracycline treatments. The drug residue test showed no drug residue in control samples, low drug concetratiion within limits for the Biotronic® SE treatments and high drug concentration / residue in the Oxytetracycline treatment.

The third experiment was conducted to determine the single and combined effect of the best levels of Mycofix® and Biotronic® SE obtained from the two previous experiments conducted compared with Oxytetracycline treatments. There were six treatments the control had no antibiotics, Mycofix® and Biotronic® SE; treatment 2 contained 400g Mycofix®, treatment 3 contained 500g Biotronic® SE at starter and 400g for finisher treatment 4 had 200g Mycofix® + Biotronic® SE, treatment 5contained 400gMycofix® + 500g Biotronic® SE and treatment 6, 100g Oxytetracycline/100kg was the negative control).

The starter phase showed a non significant response to dietary treatments and the control although 100g Oxytetracycline/100kg feed had a significantly different feed intake. The trend changed at the finisher phase birds fed Oxtyteracycline gave a better growth performance in terms of weight gain, average daily weight gain and FCR. There were no significant (P>0.05) differences in liver function test, Kidney function test and Haematological profile.

The gut morphology proliferation result showed that the combined use of 400g Mycofix® and 400g Biotronic® SE in the diet improved the development of the villi above the control and rest treatments. The villi roundness for control was significantly (P<0.05) higher than the rest treatment groups.

5.2 Conclusion

The following conclusions were made from the results of the studies:

i. The highest inclusion level of Mycofix® (400g/100kg feed) gave the best performance at both starter and finisher phases of the experiment 1.

ii. The higher doses of Mycofix® had no adverse effect on haematological (PCV, WBC, RBC and Hb and biochemical profile of birds.

iii. Mycofix® at 100, 200, 300 and 400 g/100kg diet reduced the cost of production by 11.51% (₦18.23), 12.54% (₦19.94), 3.95% (₦6.26) and 11.51 % (₦18.23) respective per bird at the starter phase and Mycofix® at 400 g/100kg inclusion level reduced the cost of production per bird by 12.31% or ₦23.47 at the finisher phase.

iv. The effect of organic acids was dose dependent, the more active ingredients reach the site of action, the higher will be the desired effects.

v. Biotronic® SE at an inclusion level of 500g/100kg diets reduced the cost of production by 9.52 % or ₦15.94 per bird at starter phase and 25% or ₦55.13 at finisher phase.

vi. Dietary organic acids may be exploited as growth promoters for broiler chicken as in the present study it had positive outcome on the performance.

vii. The Biotronic® SE at 500g inclusion can be used as a replacement for antibiotic use since it gave better FCR and feed cost per kilogram gain over the control and Oxytetracyline group.

viii. Use of Biotronic® SE improved the activities of beneficial bacteria in the ileum and caecum of broiler chickens by improving nutrient utilization.

ix. Combined effect of Biotronic® SE and Mycofix® in broiler feed worked in synergy with each other even at higher levels.

x. No adverse effect on haematological parameter, biochemical indices, liver and kidney function tests of the chickens.

xi. Treatment 5 (400g Mycofix® + Biotronic®SE) significantly improved the villi crypt over the control and the other treatment groups.

5.3 Recommendations

i. Farmers can use either singly 400g and 500g per 100kg feed for Mycofix® and Biotronic® SE respectively in poultry feed to attain an average of 2 kilogram (table size) weight of birds on or before 6 weeks.

ii. Poultry feed millers should be encouraged to use toxin binders and organic acids as a means of alleviating the incidences of mycotoxins in feed and improving gut health.

iii Use of antibiotics as growth promoters in feed production can be stopped with good management and hygiene and can be replaced with organic acids.

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APPENDICES

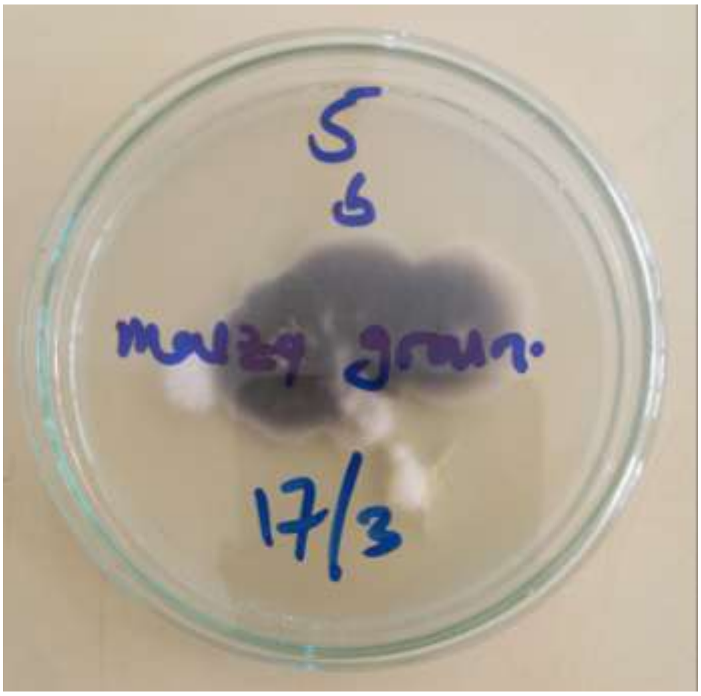
Mucor

Penicilium

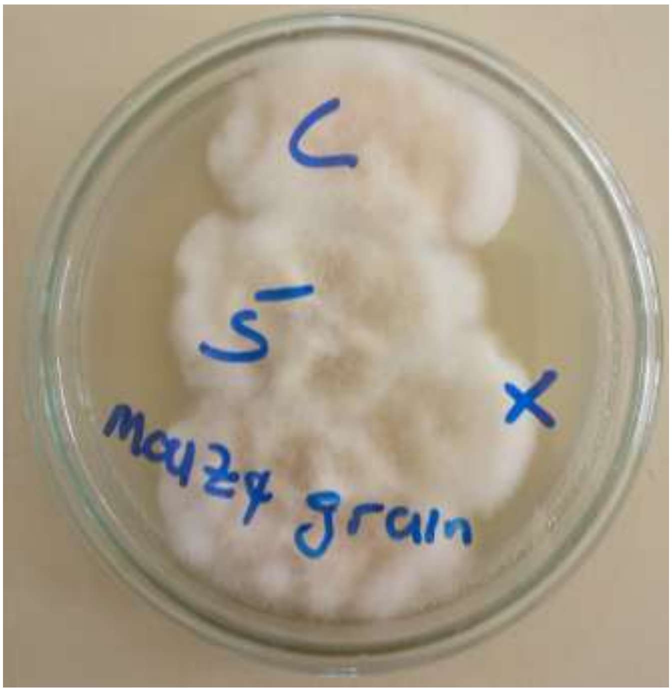
Rhizopus

Aspergilus flabus

Aspergilus niger



Curvularia



Fusarium