AMELIORATING THE EFFECTS OF DIETARY AFLATOXINS IN BROILER CHICKEN USING YEAST BETA-GLUCANS, ANTIOXIDANTS AND VITAMIN K

BY

Jubril BayonleADEOGUN
Matriculation No.: 67148

B.Sc. Animal Science (Ibadan)
M.Sc. Agric. Biochemistry and Nutrition (Ibadan)

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CERTIFICATION

We certify that this study was carried out by Jubril Bayonle ADEOGUN in Agricultural Biochemistry and Nutrition, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

i) …… Date: ……

Dr O. A. ABU,
SUPERVISOR,
B.Sc. Animal Science, M.Sc., Ph.D. (Ibadan), RAS,
Reader, Agricultural Biochemistry and Nutrition,
Department of Animal Science,
University of Ibadan, Ibadan, Nigeria.

ii) …… Date: ……

Dr E. O. EWUOLA,
SUPERVISOR,
B. Agric. Tech. (FUTA), M.Sc., Ph.D. (Ibadan), RAS,
Snr. Lecturer, Animal Physiology and Bioclimatology,
Department of Animal Science,
University of Ibadan, Ibadan, Nigeria.

DEDICATION

I dedicate this to my Sustainer, the Almighty God, with absolute submission to Your Oneness and Greatness and in deep appreciation of Your love and favours.

And

To the memory of my dear late father (May Allah grant him "*Al-Jannah Firdaous*", Amin)

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ABSTRACT

Aflatoxins are toxic secondary metabolites of fungi that may retard growth and cause upsurge in mortality of animals when ingested. Existing toxin binders do not mitigate the inevitably absorbed fraction of the toxins, which may be sufficient to elicit deleterious effects. The use of yeast cell wall beta-glucans with established *in vitro* aflatoxins binding activity, antioxidants such as selenium, vitamins E and C, and an anti-haemorrhagic agent (vitamin K), in mitigating the impact of aflatoxins in chickens is less explored. Therefore, effects of beta-glucans, antioxidants and vitamin K in reducing cellular oxidative activities that may result from absorbed aflatoxins in broiler chicken were investigated.

Aspergillus flavus 3228 inoculated maize was used to formulate a Basal Diet-BD containing 270ppb aflatoxins. Seven diets comprising Negative Control (NC- aflatoxin-free diet), BD and BD containing beta-glucans at: 125ppm (BD125), 250ppm (BD250), 375ppm (BD375), 500ppm (BD500) and 625ppm (BD625) were fed to 210 one-day-old Arbor Acres Chicks (AAC), replicated thrice with 10 birds each for 42 days. Absorbed Aflatoxins-AA was determined by balance trial method. In another trial, NC, BD and BD containing vitamins: E (VE), C (VC), K (VK) and selenium at 200mg, 250mg, 3mg and 0.3mg/kg diet, respectively in four combinations: R1 (BD+VE+VC), R2 (BD+VE+VC+Se), R3 (BD+VE+VC+VK) and R4 (BD+VE+VC+VK+Se), were fed to 180 AAC, replicated thrice with 10 birds/replicate for 42 days. Serum malondialdehyde-MDA (nmol/mL) and reduced:oxidised glutathione (GSH:GSSG) were measured. Also, NC, BD and combinations of two best treatments each from previous studies: TD1 (BD250+R3), TD2 (BD250+R4), TD3 (BD375+R3) and TD4 (BD375+R4) were fed to 180 AAC for 49 days in augmented (2x2)+2 factorial arrangement. Experimental design was completely randomised. Body Weight Changes-BWC (g/bird) was measured and mortality recorded. At day 49, two birds/replicate were sacrificed for Residual Aflatoxin-RA (µg/kg) determination in liver and Breast Meat-BM by ELISA method.Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Increased levels of beta-glucans resulted in decreased AA from 98.9±0.5% (BD) to 20.7±8.5% (BD375). Beyond 375 ppm, AA increased to 64.4±2.0% (BD500) and 75.4±2.2% (BD625). Serum MDA ranged between 12.4±10.5 (NC) and 128.2±31.1 (BD). This was significantly reduced by combinations of antioxidants and VK from 128.2±31.1 to 42.0±10.4 (R4), while 79.2±38.6 (R1), 80.1±12.5 (R2) and 72.5±24.9 (R3) were similar and lower than in BD. The GSH:GSSG was highest in NC (3.64±1.44), inclusion of antioxidants with or without selenium and/or VK elevated GSH:GSSG to 1.98±0.89 (R1), 1.94±0.78 (R2), 1.88±0.72 (R3) and 2.19±0.92 (R4) from 0.83±0.58 (BD), which indicated effective mitigation. Combinations of beta-glucans and selenium improved BWC up to 99%, from 956.27±19.34 (BD) to 1,903.98±32.56 (TD4). Mortality was reduced from 39.4±5.3% (BD) to 9.1±5.3% (TD3), 12.1±5.3% (TD4), 21.2±5.3% (TD1) and 24.2±5.3% (TD2). The RA decreased 10-fold, from 2.56±0.34 (BM) and 3.46±0.43 (liver) for BD to 0.23±0.02 in BM (TD3) and 0.35±0.11 in liver (TD4).

Yeast beta-glucans inclusion in aflatoxin-contaminated diet up to 375 ppm reduced aflatoxins absorption and combinations of beta-glucans, vitamins E, C, K and selenium ameliorated theeffects of inevitably absorbed aflatoxins in broiler chicken.

Keywords: Toxin binders, Absorbed aflatoxins, Oxidative activities, Residual aflatoxins

Word count: 494

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LIST OF ABBREVIATIONS

AA:	-	Absorbed Aflatoxins
ADAI:	-	Average Daily Aflatoxins Intake
ADFI:	-	Average Daily Feed Intake
ADFO:	-	Average Daily Faecal Output
ADVA:	-	Average Daily Voided Aflatoxins
AFB1:	-	Aflatoxin B1
AFB2:	-	Aflatoxin B2
AFBW:	-	Average Final Body Weight
AFC:	-	Average Feed Cost
AFG1:	-	Aflatoxin G1
AFG2:	-	Aflatoxin G2
AFM:	-	Aflasafe Maize
ALB:	-	Albumin
ALP:	-	Alkaline Phosphatase
ALT:	-	Alanine Amino-transferase
ALWV:	-	Average Live Weight Value
AMR:	-	Average Marginal Return
ANOVA:	-	Analysis of Variance
AOAC:	-	Association of Official and Analytical Chemistry
AR:	-	Aflatoxins Retention
ASAN:	-	Animal Science Association of Nigeria
AST:	-	Aspartate Amino-transferase
ATP:	-	Adenosine Tri-phosphate
ATRC:	-	Average Total Raising Cost
BD:	-	Basal Diet
BHA:	-	Butylated Hydroxy Anisole
BHT:	-	Butylated Hydroxy Toluene
BV:	-	Biological Value
BWG:	-	Body Weight Gain

cAMP:	-	Cyclic Adenosine Mono-phosphate
CAST:	-	Council for Agricultural Science and Technology
CBWG:	-	Cummulative Body Weight Gain
CFI:	-	Cummulative Feed Intake
CP:	-	Crude Protein
CRD:	-	Chronic Respiratory Disease
CVu:	-	Uniformity Coefficient of Variability
CYP 450:	-	Cytochrome P450
dAI:	-	Daily Aflatoxins Intake
dFAO:	-	Daily Faecal Aflatoxins Output
DFI:	-	Daily Feed Intake
DHAA:	-	Dehydroascorbic Acid
DMRT:	-	Duncan Multiple Range Test
DNA:	-	Deoxyribonucleic Acid
DTAC:	-	Digesta Total Aflatoxins Concentration
DTAI:	-	Daily Total Aflatoxins Intake
EC:	-	European Commission
EDTA:	-	Ethylene Diaminetetracetic Acid
EFSA:	-	European Foof and Safety Agency
ELISA:	-	Enzyme Linked Immuno-sorbent Assay
ER:	-	Endoplasmic Reticulum
EU:	-	European Union
FAE:	-	Follicle Associated Epithelium
FAO:	-	Food and Agriculture Organisation
FCR:	-	Feed Conversion Ratio
FDA:	-	Feed and Drug Administration
FI:	-	Feed Intake
FR:	-	Free Radicals
G:	-	Guanine
G3PDH:	-	Glyceraldehyde-3-phosphate dehydrogenase

G6P:	-	Glucose-6-phosphate
G6PDH:	-	Glucose-6-phosphate dehydrogenase
GIT:	-	Gastrointestinal tract
GLB:	-	Globulin
GLO:	-	Gulonolactone oxidase
GPx:	-	Glutathione peroxidase
GSH:	-	Reduced glutathione
GSSG:	-	Oxidised glutathione
GSR:	-	Glutathione-S-reductase
GST:	-	Glutathione-S-transferase
4-HNE:	-	4-Hydroxynonenal
H:L:	-	Heterophil to Lymphocyte ratio
Hb:	-	Haemoglobin
HPTLC:	-	High Performance Thin Layer Chromatography
HSCAS:	-	Hydrated Sodium Calcium Aluminosilicate
IARC:	-	International Agency for Research on Cancer
IB:	-	Infectious Bronchitis
IBD:	-	Infectious Bursal Disease
iBWG:	-	Improvement in Body Weight Gain
IgA:	-	Immunoglobulin A
IgG:	-	Immunoglobulin G
IgM:	-	Immunoglobulin M
IITA:	-	International Institute of Tropical Agriculture
IM:	-	Inoculated Maize
LAB:	-	Lactic Acid Bacteria
ME:	-	Metabolisable Energy
MDA:	-	Malondialdehyde
NADH:	-	Reduced Nicotinamide Adenine dinucleotide
NADPH:	-	Reduced Nicotinamide Adenine dinucleotide phosphate
NC:	-	Negative Control

ND:	-	New Castle Disease
NIAS:	-	Nigerian Institute of Animal Science
NRC:	-	National Research Council
NSPRI:	-	Nigerian Stored Products Research Institute
8-OHdG:	-	8-Hydroxy- 2'- deoxyguanosine
OD:	-	Optical Density
PL-OOH:	-	Phospholipids hydroperoxide
ppb:	-	Parts per billion
ppm:	-	Parts per million
PUFA:	-	Polyunsaturated Fatty Acid
PCV:	-	Packed Cell Volume
PVP:	-	Polyvinylpyrrolidone
PVPP:	-	Polyvinylpolypyrrolidone
Qty:	-	Quantity
R:	-	Ration
RBC:	-	Red Blood Cell
rBWG:	-	Reduction in Body Weight Gain
Rel.:	-	Relative
RLA:	-	Residual Liver Aflatoxins
ROS:	-	Reactive Oxygen Species
ROOH:	-	Lipid hydroperoxide
SEM:	-	Standard Error of Mean
SOD:	-	Superoxide Dismutase
T:	-	Thymine
TA:	-	Total Aflatoxins
TAAb:	-	Total Aflatoxins Absorbed
TAC:	-	Total Antioxidant Capacity
TAID:	-	Total Aflatoxins in Ileal Digesta
TD:	-	Treatment Diet
T-GSH:	-	Total Glutathione

TP:	-	Total Protein
TTC:	-	Twin Trough Chamber
UDP:	-	Uridine diphosphate
UGT:	-	Uridine diphosphate glucoronosyltransferase
UNU:	-	United Nations University
USAID:	-	United States Agency for International Development
UV:	-	Ultra Violet
VC:	-	Vitamin C
VE:	-	Vitamin E
VK:	-	Vitamin K
WBC:	-	White Blood Cell
WHO:	-	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background to the current study

Aflatoxins are very toxic substances produced by toxigenicspecies of fungi. Fungi spores are air borne and therefore, fungi growth on agricultural crops and produce is difficult to control. The growth of toxigenic fungi such as *Aspergillus flavus*, on agricultural products results in the production of aflatoxins, which are invisible and hard to remove from the contaminated products. The presence of dietary aflatoxins therefore, in broiler chickens'nutrition is a serious health concern, as it is hepatotoxic, mutagenic, immunotoxic, carcinogenic and teratogenic. Aflatoxins ingestion results in a disease condition known as aflatoxicosis and verifiable ways of preventing this disease condition is never by medication but by the elimination of the toxin in poultry diet. Since fungi are ubiquitous and the complete elimination of aflatoxins in feed materials is difficult and expensive to achieve or the affected material may be economically huge to be destroyed, perhaps during scarcity.A second way therefore, of preventing the negative effects of aflatoxins ingestion is to mitigate the induction of aflatoxicosis, a goal set out in the current study.

The average daily total protein requirement for an adult human was estimated to be between 0.66 to 0.91g protein/kg bodyweight/day (FAO, 2013). The European Food Safety Authority (EFSA) suggests the average recommended protein intake of 92.4g/day for an adult weighing 70kg (EFSA, 2012). If the average body weight of an adult is assumed to be 70kg, then the average daily protein requirement will range from 52.5 for female and 58.1g/day for male3 (WHO/FAO/UNU, 2007) to 92.4g/day (EFSA, 2012), whilethe updated FAO Expert Consultation on Protein Quality EvaluationReport will give a protein requirement range of46.2to 63.7g/day (FAO, 2013). Revised recommended daily protein intake by the nutrition societies of Germany, Austria, and Switzerland (D-A-CH) in 2017 for adult men and women was 0.83g protein/kg body

weight (Richter *et al*.,2019). This corresponds to a recommended daily protein intake of 55–57g protein/day for men and 47–48g protein/day for women, respectively. D-A-CH is the acronym formed from member countries identification initial letter(s): (Germany [D], Austria [A]and Switzerland [CH]).

Speaking scientifically, there is no specified requirement *per se* for protein, but for nitrogen and the nine indispensable amino acids (WHO/FAO/UNU, 2007; Richter *et al*., 2019). The Protein Biological Value (BV) of a food is the quantity of absorbed amino acids from the protein contained in the ingested food, that is retained in the body and is therefore available for incorporation into the proteins within the body of the organism that consumed it. It is a critical factor to be considered in determining the protein source or quality (Li *et al*., 2014). Biological value of protein essentially refers to how quick and how effective the body can incorporate the amino acids from the consumed protein into body protein (Moore *et al.,* 2014). As the requirement for protein is usually the requirement for nitrogen (protein nitrogen,) and the indispensable amino acids, there is usually no distinction in literature, for plant protein or animal protein requirement. Therefore, the requirement for protein in human diet may be assumed to be the requirement for animal protein basically, for the reasons given below:

a) Nitrogen derivable from plant proteins could be non-protein nitrogen, which cannot be incorporated into tissue protein (Leeson and Summers, 2005)

b) Human protein requirement is difficult to estimate with desirable accuracy because of endogenous protein or amino acids turnover which may be 3-4 times more than the average daily intake (Waterlow, 1995). Therefore, only proteins with closely related biological amino acids to humans can be of reliable availability.

c) The body does not have store for excess amino acids compared to other nutrients such as fat and carbohydrates, therefore, the ease and rate of incorporation of amino acids into tissue proteins is crucial to prevent dietary protein wastage (Richter *et al*., 2019).

Also, the biological value of protein can be evaluated by determining the most limiting amino acid(s) of the whole protein (Food-info.net, 2017). Plant proteins are usually

limiting mostly in methionine and lysine to some extent (Souci *et al*., 2016), while animal proteins are rich sources of these two plant proteins limiting amino acids (Hoffman *et al*., 2004). Animal proteins therefore are of higher biological value than plant proteins. Protein sources having a biological value (BV) of 70% and above are the most desirable protein sources in human nutrition to provide the best proteins and amino acids (Lemieux *et al*., 2014).

Meat protein content including that of chicken is usually between 15 and 35% (Marangoni *et al*., 2015), depending on the fat and water content and with a BV of between 79 and 83% (Food-info.net, 2017). To have between 58.1 and 92.4g average daily protein intake therefore, between 193.67 – 308g of meat must be consumed per day per individual (assuming the protein content to be 30% on the average) to meet up with the recommended average daily protein requirement. For a large population like Nigeria to cope with this huge demand for daily protein intake, the broiler sector of the poultry industry is one area that can produce enough meat to meet up with the estimate of about 32.92 – 52.36 thousand tonnes of meat daily, in a population of about 200 million people (with the assumption that 70% of this population are adults, that are 18 years and above and that the requirement of the remaining 30% is half that of the adults).

Poultry meat is regarded to be the second most preferred and most consumed meat in the world, second only to pork (aldelis.com, 2018), and it can ensure an average dietary protein intake of 90g/day (minimum) per individual. Chicken meat has high acceptance as source of quality and nutritive protein with reduced fat and minimal cholesterol compared to meat from ruminants (Zdanowska-Sasiadek *et al*., 2018). Poultry meat has a good overall nutrients profile, as represented bygood biological value protein, vitamins and minerals content, and it is also associated with a low unsaturated fat content, and this improves the utilisation of poultry meat in the diets of humans at all ages (Marangoni *et al*., 2015).

The productivity and sustainability of the broiler chicken enterprise to meet up with this challenge is highly dependent on high quality feed. High quality feed is far moreimportant than just having a balanced ration. The quality of a balanced ration can be compromised by the presence of antinutrients in the feed. Antinutrients could be plant derived e.g. saponins, tannins, alkaloids, coumarins, or it could be introduced by

man (e.g. dioxins) or from micro-organisms.Feed-derived antinutrients from micro-organisms such as fungi are referred to as mycotoxins. The presence of fungi in feed or feed materials is usually unavoidable, as fungi are ubiquitous (Oyegunwa,2015; Iqbal, *et al*., 2015; Alshannaq and Yu, 2017). Hence, mycotoxins could be regarded as inevitable contaminant in feed and feed materials and a serious food safety issue of global concern (Medina *et al*., 2014; Salvo *et al*., 2018; Nazhand *et al*., 2020). Mycotoxins are fungi harmful secondary metabolites expressed by toxigenic strains (Alshannaq and Yu, 2017; Hassan and Zhou, 2018), and may or may not be required for the fungi survival. Brakchage *et al*. (2013), reported that fungi use secondary metabolites to protect themselves from predators and competitors and also to channel information.

Mycotoxins are also low molecular weight compounds (Solis-Cruz *et al*., 2018), though, they may exhibit some degree of antigenicity (that is, their ability to react with antibody and one of the reasons for the applicability of ELISA in aflatoxins detection and quantification) but they usually devoid immunogenicity (that is, their presence cannot elicit antibody production in animal's body) and are therefore perceived to be "elusive" to the immune system within the circulatory system. Multiple mycotoxins are produced by fungi, but the most economically important ones that naturally contaminate food, feed or feed materials are aflatoxins, ochratoxins, fumonisins, deoxynivalenol(the trichothecenes) and zearalenone (Mgbeahuruike, *et al*., 2018). Aflatoxins are the most toxic, the most encountered (because the *Aspergillus* species that produce them are ubiquitous) and also the most researched of all the mycotoxins (Pitt and Miller, 2016). This is due to their possible carcinogenic ability to induce liver cancer in man (De Ruyck *et al*., 2015) and their residual in edible animal food products (such as milk, cheese, eggs and tissues), which could serve as an indirect source of exposure of humans to aflatoxins (Kaushik, 2015; Manafi, 2018).

One major factor, which predisposes animals to disease apart from poor or sub-optimal nutrition, extremes of temperature and humidity, dehydration, starvation, unclean or polluted environment and excessive physical stress, is the contamination of animal feeds and feedstuffs (especially the monogastric) with mycotoxins.Mycotoxins had been verified and adjudged as potent predisposing factor to diseases (Yang *et al*., 2012; Fouad *et al*., 2019). They are also regarded to be one of the crucial stress factors

in animal feed (Surai and Dvorska, 2005; Monson *et al*., 2015). Conditions within (such as mycotoxins ingestion) and around an animal (such as heat) which allow invading micro-organisms to multiply or proliferate rapidly are of equal, and in some cases of more, importance than the actual presence of pathogens. Aflatoxin ingestion results in high economic losses in animal production and also require huge prevention costs (Feddern *et al*., 2013). Agricultural commodities that are highly susceptible to the invasion of *Aspergillus flavus* and *A. parasiticus* include wheat, maize, rice, peanut (Oyegunwa*et al*., 2017; Temba *et al*., 2017), cotton seed, dried pepper, spices and others (Varga *et al*., 2015; Akpo-Djènontin *et al*., 2018).

When consumed, aflatoxininduces a disease condition known as aflatoxicosis. Aflatoxicosis is either acute or chronic. Acute aflatoxicosis will result in death within hours or few days after ingestion without obvious clinical signs. Chronic aflatoxicosis is the most common way in which animals usually suffered from under field situation (CAST, 2003; Kaya, 2014, Nazhand*et al*., 2020). The effect is usually very subtle and may goon for a long time before being noticed (Lizarraga-Paulin *et al*., 2011;Peles *et al*., 2019). Aflatoxin ingestion in broiler chickens and poultry in general is characterised mainly by decreased feed consumption, poor efficiency of feed utilisation, there is marked decrease in liveweight gain, increased mortality (Monson *et al*., 2015; Oyegunwa*et al*., 2017), stimulation of lipid peroxidation (Muhammad *et al*., 2018), induction of hepatic oxidation stress (Omar, 2013; Ma *et al*., 2015), bile duct proliferation or hyperplasia (Chen *et al*, 2014a; Peles *et al*., 2019), enhancement of haemorrhage in tissues (Klein *et al*., 2002) and increases in serum level of hepatic enzymes (Lizarraga-Paulin *et al*., 2011).

To improve livestock productivity since it is difficult to avoid fungal growth in feed and or feed materials and prevent the deposition of aflatoxins (Atanda *et al*., 2013, Oyegunwa, 2015), different prevention methods had been employed to ameliorate the negative effects of aflatoxins on livestock and poultry in particular. Among the methods currently in use and that has received general acceptance among poultry producers is the use of organic or inorganic adsorbents or toxin binders. These products are clay based such as Hydrated Sodium Calcium Aluminosilicate (HSCAS), Na/Ca Bentonite, Zeolite, montmorillonite, including activated charcoal or activated carbon (Chen *et al*., 2014b; Bhatti *et al*., 2018).

These products are mixed together with livestock feeds, either singly or concurrently with others to trap or bind aflatoxins or mycotoxins in general,thereby reducing the amount of the toxin that will be absorbed from the gastrointestinal tracts (GIT) of the animal into the circulatory system. Hence, minimising the adverse effects of the toxin on the animal andits performance.

1.2 Statement of the problem

The use of mycotoxin adsorbents in poultry feeds to prevent the induction of aflatoxicosis has been greatly successful to a large extent, in improving the performance of poultry exposed to aflatoxin-contaminated diets (Mgbeahuruike, *et al*., 2018). A major shortcoming in the use of mycotoxin binders is that major nutrients such as vitamins and minerals which are vital components of the feed, compete with the toxins for their binding sites (Abad *et al*., 2002). This results in reduction in the amount of the toxin that would be trapped, and also a reduction in the amount of valuable nutrients available to the animal. Hence, toxin adsorption by mycotoxin binders may never be 100 percent effective. Also, high level of residual antibiotics has been reported in the tissues of chickens feeding onmycotoxins sequestering agent or binder (Osselaere *et al*., 2011). Clay-based mycotoxins binders could also be contaminated with dioxins (Abad *et al*., 2002),another very potent carcinogens that is usually of soil origin (Rathoure, 2018).

The binding potential of toxin binders might be easy to quantify in an *in vitro* assessment but the environment surrounding a toxin binder in an *in vivo* study is quite unique and different from test apparatus environment (Kolosova and Stroka, 2011.). During*in vivo* study, factors that could influence toxins adsorption are: (a) there is a limit to the time the digesta will stay within each segment of the GIT; (b) there could be fluctuation in gut pH; (c) the state of the structural integrity of the intestinal lining; (d) changes in the concentration of the mycotoxin and (e) the influence of the gut microbes are all crucial to influencing adsorption and absorption of the toxin and these may greatly affect the efficacy of the toxin binder *in vivo*. These were noted by Colovic *et al*. (2019), in their review on strategies to decontaminate mycotoxins in feed and feed materials. Also, since mycotoxins generally are low molecular weight substance (Solis-Cruz *et al*., 2018), the implication of this is that mycotoxins or aflatoxins absorption in the GIT occur by passive diffusion (Gratz *et al*., 2006; Di

Gregorio *et al*., 2014). This therefore implied that absorption is taking place as the feed is being ingested and digested in the GIT but mycotoxins binding by probiotics or microorganisms (Vinderola and Ritieni, 2015), and adsorption by organic or inorganic adsorbent (Goncalves *et al*., 2017; Mgbeahuruike, *et al*., 2018) require some time for the binding process to take place, as it is seen during *in vitro* mycotoxins binding evaluations (Kolawole *et al*., 2019).

Therefore, aflatoxins absorption into the circulatory system from the GIT is expected to be taking place simultaneously as the binding process is occurring, because aflatoxins absorption is passive and this will eventually affect overall binding efficiency *in vivo*.The inevitably absorbed portion of the toxin may be sufficient to induce toxicological effects on the animal, depending on the original concentration of the toxin in the diet and the duration of time the animal is exposed to the contaminated diet. Also, it is expected that as the concentration of the toxin in the feed increases, the fraction that is inevitably absorbed will also be increasing. However, there is scanty information by mycotoxins binders' producers about the fate of this fraction of the toxin that is inevitably absorbed into the animal's circulatory system.

1.3 Aim and objectives

This research work is primarily aimed at improving productivity in boiler chicken fed aflatoxin-contaminated poultry feed, through mitigation. This was achieved by the execution of the specific objectives below:

a. To prevent or decrease the absorption of aflatoxin from the gastro-intestinal tract and also, to determine the effective inclusion dose of beta-glucans that is required to reduce or minimise aflatoxin absorption.
b. To determine the best supplemental dietary antioxidants combination that willprevent the expression of the adverse effects of aflatoxin in broiler chickens.
c. To minimise aflatoxins residue and assess aflatoxin carry-over rate from feed into liver and breast meat of broiler birds offered aflatoxin-contaminated poultry feed.

1.4 Justification for the current study

Aflatoxins presence in agricultural produce is a world-wide problem, and appeared to be inevitable contaminant of poultry feeds/or feed-stuff. Hence, there is the need for an array of mitigation strategies to prevent its adverse effects in humans and animals. The current study was envisaged and proposed because:

i. There is paucity of information on the "*in vivo*" application of yeastbeta-glucans in animal nutrition as a toxin adsorbent unlike the use of yeast cell wall, which contains other components of the cell wall other than beta-glucans.
ii. Modulating the inevitably absorbed dietary aflatoxins metabolism with nutritional antioxidants, to ameliorate its deleterious effects and facilitate aflatoxins and its metabolites excretion from the body has not been adequately reported.
iii. Aflatoxin contamination of poultry feeds usually lead to huge economic losses to the farmers, due to marked reduction in total birds' performance and in increased mortality.
iv. In addition, there is also the need to minimise aflatoxins carry-over into broiler chicken meat and therefore, prevent possible health risk in humans through exposure to residual aflatoxins in broiler chickens' meat.

1.5 Scope of study

To address this knowledge gap, attempts were made in a series of three experimental studies to assess the detrimental effects of this unadsorbed fraction of the toxin and to counteract the adverse effects that may arise from it, using aflatoxin-contaminated poultry feed in broiler chicken. Three different but related approaches were adopted for the current research work. The first is a primary prevention trial, whose goal was to prevent the absorption of aflatoxins (that have been unavoidably ingested in the feed), from the GIT. To achieve this, beta-glucans, homopolysaccharide of D-glucose from bakers' yeast (*Saccharomyces cerevisia*e) cell wall (Kwiatkowski and Kwiatkowski, 2012), was chosen as an organic adsorbent for aflatoxins due to its reported ability to adsorb aflatoxin-B_1*in vitro* through intermolecular hydrogen bond and Van der Waals force (Yiannikouris *et al*., 2006; Pizzolitto *et al*., 2012; Bovo *et al*., 2015), and for its very low inclusion level (which allows more space for the inclusion of other nutrients contributing ingredients to the feed formulation).

The secondary intervention or mitigation trial was aimed at modulating the metabolism of the fraction of the ingested aflatoxins that could not be adsorbed by beta-glucans, through the inclusion of supplemental dietary antioxidants such as selenium, vitamins E and C, and vitamin K. Vitamin K was included to counteract the haemorrhagic or coagulation disorder potential of aflatoxins (Bababunmi and Bassir, 1982; Gomez-Espinosa *et al*., 2017; Vijayalingam *et al*., 2017). The secondary trial, using dietary antioxidants was also intended to: i) facilitate the elimination of the metabolised absorbed aflatoxin from the body of the broiler chicken (Diaz and Murcia, 2011; Heidtmann-Bemvenuti *et al*., 2011); ii) minimise aflatoxin carry-over or residue in edible tissues (Herzallah *et al*., 2014; Frazzoli *et al*., 2017) and to also iii) ameliorate the damages that could be caused by the generated free-radicals (FR) and reactive oxygen species (ROS) (Neeff *et al*., 2016; Adhikari *et al*., 2017; Colovic *et al*., 2019)during the metabolism or biotransformation of the inevitably absorbed aflatoxins. The tertiary or last trial combined the benefits or potentials inherent in the first two trials to synergise and harness the benefits of their additive effects.

1.6 Significance of the current study to farmers

This investigation will add to the existing mitigation strategies in preventing the induction of aflatoxicosis in broiler chickens consuming rations contaminated with aflatoxins. This will ultimately remove or minimise negative effects that usually characterised aflatoxins poisoning in animals, such as:

a) Severe growth retardation
b) Relapse of previously treated diseases due to immune suppression
c) Unusual upsurge in mortality
d) Deposition of aflatoxins as residue in edible tissues
e) Gross loss of investment to farmers due to reduction in flock size, arising from high mortality rate and very poor body weight of birds at slaughter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mycotoxins contamination of agricultural produce

Mycotoxins are chemically potent fungi secondary metabolites produced by toxigenic strains of fungi, which elicit some complicated toxicological manifestations when ingested (Bennett and Klich, 2003; Alshannaq and Yu, 2017, Hassan and Zhou, 2018). Generally, mycotoxins are low molecular weight organic compounds (Kim *et al*., 2017; Solis-Cruz*et al*., 2018), their molecules are very stableat high temperature and are very difficult to separate or isolate their toxic potential from contaminated agricultural produce (EFSA, 2009).

The associated economic losses arising from mycotoxins contamination are difficult to preciselyestimate (Wu *et al*., 2008), but the United States Food and Drug Administration (FDA) reportedthat in the US alone,an average annual economic crop lossdue to mycotoxins contaminationwas $932m USD (about ₦149b) (CAST, 2003). Huge industrial and agricultural losses accounting for about 25% of the world's crop produce are annually contaminated with mycotoxins, leading to losses in billions of dollars (Marin *et al*., 2013).Mitchell *et al*., (2016) reported that annually in the US, losses in the maize industry associated with aflatoxins contamination was valued between $52.1mand $1.68b USD.Mycotoxin contamination is regarded to be an unpredictable and unavoidable agricultural problem which is an ongoing global concern (Alshannaq and Yu, 2017; Salvo *et al*., 2018) and the Food and Agriculture Organisation (FAO, 1997) and theCouncil for Agricultural Science and Technologythat up to 25% of the world's agricultural produceand animal feed-stuff are grosslyaffectedwith mycotoxins (CAST, 2003; Marin *et al*., 2013). For the developing countries, the resultant losses are basically market losses, arising fromborder rejection of crops (Marin *et al*., 2013). However, health related issues are the main challenges of the third world countries consequent upon mycotoxins contamination (Wu, 2004; Hassan and Zhou, 2018).

The group of toxic secondary metabolites produced by the *Aspergillus* species of fungi– *Aspergillus flavus, Aspergillus parasiticus*, mainly and *Aspergillus nomius* to a lesserextent (Mughal *et al*., 2017; Kumar, 2018), are referred to as aflatoxins. Aflatoxin produces thehighest losses and control costs to crop and livestock farmers, due to itsextreme toxicity, stringent regulations and possibly due to its potential carcinogenic effect in humans (Feddern *etal*., 2013; Pereira *et al*., 2014). Aflatoxin contamination of food/or animal feed is regarded as a serious health risk to consumers(Santini and Ritieni, 2013; Ketney *et al*., 2017).

Basically, four types of aflatoxins: B_1, B_2, G_1 and G_2, exists naturally. Even though the four types are common in the same feed materials, aflatoxin B_1 (AFB_1) predominates – it is about 60- 80% of the total aflatoxins content (Dhanasekaran *et al*., 2011) and above 80% in a mixture of AFB_1 and aflatoxin B_2 ($AFB_{2)}$ (Yunus *et al*., 2011). Naturally, no aflatoxin producing fungi produces AFB_2, AFG_1 and AFG_2 in the absence of AFB_1 and generally, AFG_1 concentration is usually more than AFB_2 and AFG_2, while AFB_2 concentration is also higher than AFG_2(Weidenborner, 2001; Rawal *et al*., 2010).

The vinyl-ether double bond on the $8^{th}$and 9^{th} position of the terminal furan ring (Figure 2.1) is responsible for the highbiological potency of aflatoxin B_1 and AFG_1 to a little extent(Diaz and Murcia, 2011; Lizarraga-Paulin *et al*., 2011). Aflatoxin B_1 is adjudged the most toxic, the most prevalent and is probably also the highly researched of the aflatoxins (Yunus *et al*., 2011; Pitt and Miller, 2016). Squire (1981), reported that toxigenic strains of *Aspergillus* species produces mainlyAFB_1 and little of the remaining B aflatoxins. Therefore, in a large collection of papers published on aflatoxins, AFB_1 is usually assumed to refer to total aflatoxins and vice-versa.

2.2 Aflatoxins occurrence and prevalence in Nigeria

Aflatoxins producing fungi are mostly found in Latitudes 40^oN and 40^oS of the equator (Falade, 2018). However, developing countries in tropical regions of the world, which depend heavily on aflatoxins susceptible agricultural produce for their staple food and animal feed-stuff faces the greatest health risk (Strosnider *et al*., 2006; Nazhand *et al*., 2020). Figure 2.1 on page 13 is the chemical structures of the four major aflatoxins. The tropical climate as it exists in Nigeria is very conducive for toxigenic *Aspergillus* species growth and aflatoxins deposition (Atanda *et al*., 2013).

Environmental conditions conducive for optimum growth and aflatoxins production include temperature range of 24-32^{o}C, moisture content above 12-16% with relative humidity of 62% or higher (Coppock *et al*., 2018; Mwakinyali *et al*., 2019).

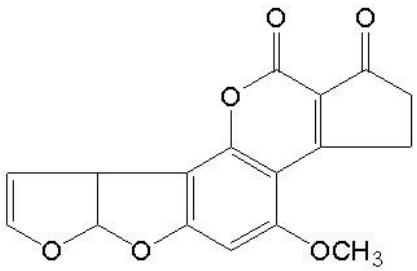
Maize has been reported to favour the colonization of aflatoxins producing fungi, relative to others (Abbasi *et al*., 2018), while groundnut and cottonseed were reported to be preferred substrate for aflatoxin production (Bankole and Adebanjo, 2003; Guerre, 2016). Maize and groundnut have been noted to be the main sources of animal and human exposure of aflatoxins (Lizarraga-Paulin *et al*., 2011). Perhaps this may be due to their high inclusion rate in animals' feed and as human staples.

The huge economic resources that exist in the developed grain-producing countries enabledthem to enforce market regulations to minimise aflatoxins exposure in feed/or food. Thus, the consequence of these stringent regulations and enforcements in the developed world is that people in the third world countries will be exposed to concentration above the permissible aflatoxins level in their diets and animal feeds (Groopman *et al*., 2008), because they will be the consumers of rejected grains by the developed world (Lizarraga-Paulin *et al*., 2011).

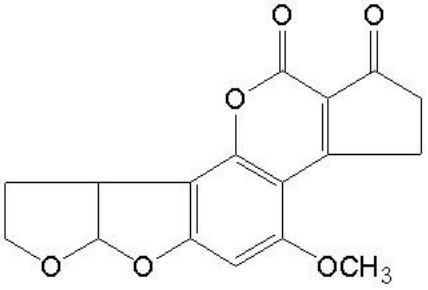
Documented cases of aflatoxins contamination of agricultural produce in Nigeria dates back as far as 1967. Scientists from Nigerian Stored Products Research Institute (NSPRI) in their routine assessments of groundnut delivered to Kano Oil Mills, and also productsfrom the mills, during the years 1962-1968, found the levels of aflatoxins to below, averaging 250µg/kg, and ranged from 50-1000µg/kg (Halliday and Kazaure, 1967 and McDonald, 1976). In 1988, groundnut cake sampled from Bodija, Oje, Dugbe and Shasha markets in Ibadan, Oyo State, had aflatoxin B_1 level that ranged from 20-455µg/kg (Akano and Atanda, 1990). Ezekiel *et al*. (2012a), reported that levels of aflatoxins above the 20µg/kg permissible level were recorded in poultry feed in the Nigerian markets.

Also, high concentration of toxigenic strains of *Aspergillus* species were isolated in Nigerian poultry feed, an indication of unsafe aflatoxin level existence (Ezekiel *et al*., 2014). Akinmusire*et al*. (2019), reported 23 different mycotoxins in 30 samples of poultry feed and 72 different feed ingredient samples from 12 states in Nigeria and observed high levels of aflatoxins above the 20µg/kg recommended safe level.A

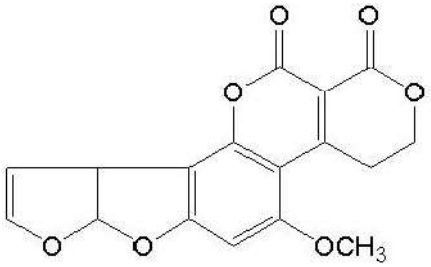
summary of establishment of aflatoxins occurrence in Nigerian foods and feedstuffis presented in Table 2.1.



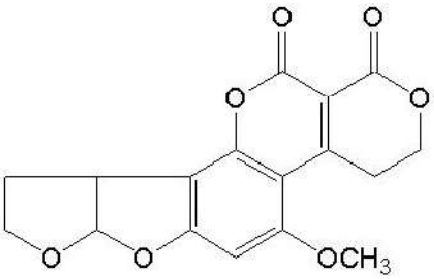
Aflatoxin B_1



Aflatoxin B_2



Aflatoxin G_1



Aflatoxin G_2

Figure 2.1 The Natural Aflatoxin Structures

(Adapted from: Maurice, 2002)

Table 2.1 Aflatoxins Contamination of Nigeria Feeds/Feedstuff

No	Aflatoxin	Crop Contaminated	Location	Frequency of Contamination	Percentage Frequency of Contamination (%)	Conc. Range (mg/kg)	Mean Conc. (mg/kg)	Author
1.	AFB_1	Maize	Plateau State	27/64	42.18	0-960	372	Gbodi, 1986
2.	AFB_1	Cottonseed	Plateau State	3/8	37.50	0-271	52.25	Gbodi, 1986
3.	AFB_1	Poultry feed	Southern Nigeria	69/120	57.50	0.57-2.55	N.A.	Oyejide *et al*., 1987
4.	AFB_1	Groundnut Cake	Ibadan	18/20	90.00	20 – 455	236.69	Akano and Atanda, 1990
5.	AFB_1	Maize	Lagos	81/281	28.83	0 – 1250	248	Opadokun, 1992
		Groundnut		44/634	6.94	0 – 8000	767	Opadokun, 1992
6.	AFB_1	Maize	Western Nigeria	N.D.	-	25 – 777	200	Adebajo *et al*., 1994
7.	Total AF	Maize	Western Nigeria	20/103	19.42	3 – 138	N.A.	Bankole and Mabekoje, 2004
8.	AFB1	Maize	Niger State	144/288	50.00	234 – 908	N.A.	Tijani, 2005
9.	AFB_1	Poultry feed	Nigeria	44/58	75.86	6 – 1067	198	Ezekiel *et al*., 2012a
10.	AFB_1	Groundnut Cake	Nigeria	29/29	100.00	13 – 2824	N.A.	Ezekiel *et al*., 2012b
11	AFB_1	Maize	Nigeria	33/72	45.83	6.1 – 567	176	Akinmusire *et al*., 2019
12	AFB_1	Poultry feed	Nigeria	25/30	83.33	0.5 – 760	74	Akinmusire *et al*., 2019

N.D: Not Determined

Sources:Atanda *et al*., 2013; Ezekiel *et al*., 2012a&b; Akinmusire *et al*., 2019

2.3 Aflatoxins and aflatoxicosis

Aflatoxins, especially AFB_1 is regarded by nutritional toxicologists as the most potent hepatocarcinogen not invented by the activities of man but by living microorganisms (Abrar *et al*., 2013; Tian and Chun, 2017). The International Agency for Research on Cancer (IARC) in 1987 classified aflatoxin B_1 as a carcinogen and it was further re-classified in 1993 as a group 1 carcinogenic agent due to its additive effect in the presence of hepatitis B virus (IARC, 1993; Ostry *et al*., 2017).

Aflatoxin toxicity is determined not only on the amount or level of the toxin consumed but also the length of time (Yunus *et al*., 2011; Peles *et al*., 2019). Other factors that affect the susceptibility to aflatoxicosis include: age, sex, species, nutritional status, and physiological state of the animal and health conditions (Mottet and Tempio, 2017). Aflatoxicosis (aflatoxins poisoning) is the disease condition that arises from the ingestion of aflatoxins contaminated foods or feeds (Wogan *et al*., 2012). Aflatoxicosis may result from acute or chronic exposure. Under normal field situation, acute toxicity is less encountered while chronic toxicity (the most common), is marked by depression, anorexia, weight loss and severe hepatic damage (Wu and Guclu, 2012; Filazi *etal*., 2017).Acute injury is characterized by coagulopathy, increased capillary fragility, haemorrhages, icterus (jaundice), fat infiltration of the hepatocytes, resulting in fatty liver and death may occur within hours or few days (Manafi *et al*., 2014, Asiki *et al*., 2014).

It has been estimated that more than five million people world-wide are at risk of chronic exposure to aflatoxins, particularly in the third world countries, through contaminated foods (Williams *et al*. 2004; Van Egmond *et al*., 2007). Smela *et al*. (2001), reported thatchronic exposure for extended periods leads to tumour induction in several animal species. Hepatic damage is also the most noticeable in chronic aflatoxicosis but on a milder scale. Chronic toxicity is usually characterized by enlarged gall bladder, bile duct proliferation, prolonged blood clotting time, elevation of serum glutamic oxaloacetic transaminase, ornithine carbamoyl transferase and cholic acid levels. Usually, the signs of chronic aflatoxicosis aresubtle that the situation may persistunnoticed fora very long time (Lizarraga-Paulin *etal*., 2011).

2.4 Effects of aflatoxins in poultry

The natural unavoidable toxic contaminant of feed and feed materials known as aflatoxin, came to limelight in 1960 in the United Kingdom, following the death of about 100,000 turkey poults that were fed ration produced with mouldy Brazilian groundnut cake, in what was characterised as the mysterious Turkey – X disease (Agag, 2004). Poultry species is the first animal species to have documented evidence of the toxicological effects of aflatoxins (Agag, 2004).Comparative analysison the toxicity of aflatoxin in avian species revealed that the ducks and turkey are the most susceptible species of poultry to aflatoxins (Dhanasekaran *et al.,* 2011). Bintvihok, (2011)noted that gooslings, quails and pheasants displaymoderate sensitivity to aflatoxin, as compared to turkey and ducks, while chickens appearedless affected. The susceptibility range as given by (Chen *et al*., 2014c) is as indicated: ducklings > turkey poults > gooslings > pheasant chicks > chickens.

In poultry generally, production parameters that aflatoxin ingestion affects include, reduced feed consumption, reduction in weight gain, reduction in feed conversion into edible products, poor processing yield, pigmentation impairment, immune suppression, reduction in egg production and also reduction in reproductive performance (Yunus *et al*., 2011; Streit *et al*., 2013). Mckenzie *et al*. (1998), reported that diet containing 560 µg/kg of aflatoxins B_1 fed to turkey poults for three weeks led to 23% decrease in body weight gain. A 16 percent reduction in body weight gain in a diet containing 500 µg/kg and 39 percent reduction in body weight when the diet had 1000 µg/kg of total aflatoxins were reported in turkey poults at the starter stage (Rauber *et al*., 2007). Verma *et al*. (2007), observed that at 1-2mg/kg of aflatoxins in laying hens, led to decreased egg production, increased mortality and poor egg quality. Crude protein apparent digestibility was adversely affected in ducks by 8-13 percent when the diet contained 20 and 40 µg/kg aflatoxin B_1 (Grenier and Applegate, 2013). Chen *et al*. (2013), reported thatusuallybody weight gain decreased by 11 percent, when the diet of poultry is contaminated with about 950 µg/kg of total aflatoxins. This reduction was attributed in part to the reduction in feed intake, GIT damage and metabolic inadequacies or disruptions in the liver. A reduction of about 230g and 163g/bird in feed consumption and liveweight was recorded in broilers in just 14 dayswhen fed a diet containing between 110-200 µg/kg of aflatoxin B_1(Chen *et al*.,2014a).

Ingestion of aflatoxins in broiler chickens and poultry generally result in reduced feed intake (Chen *et al*., 2014b) and also reduce the efficiency of feed utilization (Verma *et al*., 2004). The implication of this is that feed conversion ratio will rise, leading to more feed intake by broilers to produce edible muscle (Chen *et al*., 2014b). This will increase production cost and result in economic losses to producers (Sarma *et al*., 2017)In natural aflatoxicosis outbreak, ruffled feathers, paleness, legs and wings paralysis, marked decrease in feed and water consumption, stunting, emaciation, trembling, gasping and possibly death are the usual symptoms. These are equally observed in artificially induced aflatoxicosis (Sarma *et al*., 2017). Gross hepatic injury, including coagulopathy and high capillary fragility are noticed during aflatoxins poisoning (Peles *et al*., 2019).

Gall bladder and bile duct enlargements, biliary hyperplasia(Chen *et al*., 2014d) and blood in the intestine (as seen during coccidiosis), hepatic haemorrhages and coagulation disorders, leading to prolonged blood clotting time (Bababunmi and Bassir, 1982; Vijayalingan *et al*., 2017) were observedduring aflatoxicosis.Chen *et al*. (2014d), also reported enlarged liver, kidney and spleen with a reduction in bursa of fabricius and thymus.In chronic aflatoxicosis in broilers, the liver appeared regressed, nodular and distention of the gall bladder(Ortatatli*et al*., 2005). Kidneys were reported to be enlarged and congested with mottled enlarged spleen (Quezada *et al*., 2000).Liver histology report showed congestion of hepatic sinusoids,haemorrhages in faeces necrosis, biliary hyperplasia and infiltration of nodular lymphoid, with the epithelial cells vacuolation of the kidney tubules (Manafi *et al*., 2014).

Effects of aflatoxins on broiler chicken's performance had been extensively reviewed. A decrease of 21 percent in body weight was recorded in broilers offered a diet having up to 300µg aflatoxin B_1/kg of feed after 35 daysfeeding period (Raju and Gevegowda, 2000).Other researchers however recorded lower reduction rate in body weight of broiler chickens even at higher aflatoxin B_1 ingestion than 300µg/kg. A 15 percent reduction in body weight after 42 days period of feeding was reported by Denli *et al*. (2009), while Miazzo *et al.* (2000) and Valdivia *et al.* (2001) reported 11 percent body weight gain reduction when 2.5mg aflatoxin B_1/kg of feed and 3mg aflatoxin B_1/kg diet were fed for 21 days respectively. Reduction of 10 percent in body weight gain after feeding 800µg aflatoxin B_1/kg diet for 28 days was observed by

Tedesco *et al*. (2004), while Zhao *et al*. (2010) fed 1mg (1000µg) aflatoxin B_1/kg of feed for 21 days and also recorded 10% reduction in body weight gain. At 500µg of aflatoxins/kg diet, there was significant decrease in body weight and increased carcass bruising in broilers (Dersjant *et al*., 2003) Even at 20µg of aflatoxin B_1/kg diet, the permissible level by the FDA in finished feed for poultry, up to 5 percent reduction was recorded in body weight gain of broiler chickens fed for 21 days (Kana *et al*., 2010). Andretta *et al*. (2011), fed aflatoxins contaminated feed at 950µg/kg to broiler chickens and recorded 11 percent decrease in body weight gain and 6 percent rise in feed conversion ratio.

Aflatoxin ingestion result in elevated serum AST level in broilers (Kaki *et al*., 2012). Raju and Devegowda (2000) reported that 300ng/g of aflatoxins B_1 decreases serum cholesterol and total protein and led to a rise in serum Aspartate aminotransferase (AST). Yunus *et al*. (2011), noted that alteration in serum enzymes concentration was noticeable at 1mg of aflatoxin B_1/kg diet. Kermanshashi *et al*. (2007), reported elevation in the serum levels of both alanine aminotransferase (ALT) and AST at 1.2mg aflatoxin B_1/kg diet. Tessari *et al*. (2010) also reported elevated serum AST level and reduction in total protein concentration in broilers at 200µg of aflatoxin B1/kg diet after 42 days feeding period. On the contrary, Manegar *et al*. (2010) observed no difference in ALT serum level while they reported a decrease in serum AST concentration.

2.5 Aflatoxins and immunosuppression

It has been noted that chronic exposure to aflatoxin-contaminated foods and feeds resulted into immunosuppression in animals and humans (USAID, 2012). The thymus, bursa of Fabricius,and spleen are like a tripod stand upon which the avian immune system heavily relies upon (Monson *et al*., 2015). During the early developmental stage, aflatoxins consumption can result in atrophy of the immune tissue, constrict the relative size of spleen, thymus and bursa (Chen *et al*., 2014a).Aflatoxin B_1 ingestion has been reported to causeFollicle Associated Epithelium (FAE) degeneration of bursa of Fabricius and cortex of thymus (Celik *et al*., 2000). Celik *et al*. postulated that impairment in the FAE activity may result in severe inadequacies in cellular and antibody production induction in avian immune system(Celik *et al*., 2000). This is

likely to be due to the fact that the bursa FAE is vital in the presentation of antigen to the lymphoid cells (Hedayati*et al*., 2014).

Chronic aflatoxicosis impairs the immune system, reduces efficiency of the phagocytes and hypersensitivity reactions is also prolonged, making broilers to be more vulnerable to a number of viral, bacterial, fungal and parasitic infections (Oswald *et al*., 2005), leading to relapse of chronic infections, vaccinal inefficiency and reduce therapeutic efficacies (Meissonnier *et al*., 2008). Peng *et al*. (2015), observed that 300 µg of aflatoxin B_1/kg feed resulted in noticeable histopathological lesions, reduce active lymphocytes number and increase apoptosis of lymphocytes in broiler chickens.Leeson and Summers (2005), had also noted earlier that low level of aflatoxins in the feed decreases titer level following vaccination and may result in quick relapse of diseases. Aflatoxin affects the complement system in chickens by suppressing its activity (Yunus*et al*., 2011). Since the complement system is neededin engulfing pathogens, the impairment of its activity may be the reason for the reduction in phagocytotic action in chickens fed aflatoxin contaminated diet (Lakkawar *et al*., 2017). Ingestion of aflatoxin-contaminated dietdepletes the concentration of immunoglobulin IgM, IgG and IgA and also suppresses complement activity in chickens (Agag, 2004). Aflatoxins concentration of 500 to 1000µg/kg of feed is sufficient to adversely impair the activities of B- and T- lymphocytes (Lakkawar *et al*., 2017).

2.6 Detoxification of mycotoxins in feed

Under the prevailing environmental conditions in Nigeria, with favourable humidity and temperature for fungi growth, the contamination of various agricultural commodities with mycotoxins and aflatoxins in particular **is**inevitable (Falade, 2018). The quest for practical solution to totally remove mycotoxins contamination in animal's feed, has given rise to submissions that binders can sequester the mycotoxins and trap them from being taken upfrom the animal's digestive tract, thereby reducing their deleterious impact on the animals (Kolossova and Stroka, 2011; Di Gregorio *et al*. 2014and Peng *et al*. 2018).In 2009, the European Commission (EC, 2009) defines mycotoxin detoxifying agent(s) as "any substances or additives that can reduce feed contamination by mycotoxins, buffer or minimise the uptake of the toxins in the animal's digestive tract, facilitate their excretion, mitigate or counteract their adverse

effects (CFP/EFSA/FEDAP/2009). This was issued with regulation No. 386/2009 of 12th May,
2009.

Different mitigation approaches had been adopted to counteract, mitigate or detoxify mycotoxins from feed materials or compounded animal feeds when consumed. Prevention of feed materials from mycotoxins contamination on the field or pre harvest decontamination has recorded a large degree of success, particularly through biological prevention strategies (Bandyopadhyay *et al*. 2016; Udomkun, *et al*. 2017).Compound animal feed or feed materialsalready contaminated with mycotoxins (or aflatoxins) can be prevented from further contamination and can also be subjected to decontamination strategies. However, there are mixed results from decontaminationstrategies for feed and feed materials (Colovic *et al*. 2019; Elliot *et al*. 2020).

These adsorbing agents or binders' mode of action can be by direct trapping of the mycotoxins, thereby reducing their absorptionor through enzymatic process to degrade or bio-transform them into less toxic intermediate products, which can be readily disposed from the animal's body without impairing the animal's desirable performance.

2.6.1 Physical method or treatment

Separation procedures like grain cleaning,mechanical separation or sorting (Bullerman andBianchini,2007),density aggregation, floating technique, sterilizing, toasting, microwaving, Ultra Violet /or gammairradiationhave all been used to practically decontaminate mycotoxins (Jouany, 2007; Colovic *et al*., 2019). The latest in physical decontamination of mycotoxins is by using optical sorting, which uses UV radiation to sort contaminated grains by their fluorescence (Karlovsky *et al*., 2016). LumoVision is an optical sorting machine that separates aflatoxins contaminated kernel by fluorescence detection of Kojik acid, using UV radiationfrom buhlergroup.com. It can remove up to 95% aflatoxins in a contaminated batch of maize and can completely sort 15mTons of maize in one hour (LumoVision white paper, 2019).However, most of these procedures are usually expensive, high operational cost, inaccessibility of processing units on a large scale,or may also reduce, limit or transformvital nutrients

within the feed to substances not useful to the animal (Kolossova and Stroka, 2012). Therefore, practically feasible and economically viable physical decontamination methods are needed to remove aflatoxins (or mycotoxins)from animal feed and food chain (Zhu *et al*., 2016).

2.6.2 Chemical method and organic/inorganic adsorbing agents

Chemical degradation of aflatoxins has been tried and used as an approach todecontaminate affected farm produce, using substances such as calcium hydroxide,monoethylamine, ozone or ammonia (McKenzie *et al.,* 1997, Weltmann and von Woedtke, 2017) has been reported. Ozonation of compound feed and feed materials had been used with a relatively high degree of success, and this has been demonstrated over the years in food products (Colovic *et al*., 2019). Different oxidizing and reducing agents like sodium hypochlorite, sodium hydroxide,ammonium hydroxide, chlorine, hydrogen peroxide and different acids, bases and salts have been used to investigate their capability to reduce aflatoxins in farm produce (Kolossova and Stroka, 2011; Jalili *et al*., 2011). These chemicals decrease aflatoxins concentration by hydrolysis and degrade it to compound with low or no toxicity (Monson *et al*., 2015). However, an aspect of chemical detoxification of aflatoxins that should not be neglected is that it is not practicable in animals feed materials or compound feed, as these chemicals are not safe, they are hazardous, expensive,may likely decrease nutrient value and alter the physical and sensory attributes of the treated products (Scholtz *et al*., 2015).

The widely accepted practice of mixing mycotoxin binders to contaminated animal feedis generallyregarded as the most feasible dietary mitigation stepcurrently available towards ameliorating mycotoxins detrimental effects (Galvano *et al*., 2001; Kong *et al*., 2014). Essentially, adsorbing agent is meant to limit the bioavailability of the mycotoxin to the animal, leading to a decrease in the mycotoxin absorption into the blood stream and consequently limiting the amount that reaches the target organs (Huwig *et al*., 2001; Kolossova and Stroka, 2011). Therefore, adsorbents being substances of high molecular weight, has the potential to complex mycotoxins in aqueous medium of the digestive system, preventing or minimising their absorption and facilitating their faecal excretion (Tapia-Salazar *et al*., 2017).

Adsorbing agents are categorised into two main groups: the inorganic adsorbents and the organic adsorbing agents or polymers.They are usually referred to as binders,

mycotoxin enterosorbents, sequestrants, trapping agents, interceptor molecules, adsorbents, toxin binders or mycotoxin chelator and so on. Inorganic adsorbents are the mineral or clay-based binders such as bentonite, zeolite, smectite and aluminosilicates while the polymer binders are either i) natural organic polymer such as activated or pyrolysed charcoal, indigestible carbohydrates (e.g. cellulose, cell walls of fungi (yeast) and bacteria such as glucans, glucomannans, peptidoglycans) (Vila-Donat *et al*., 2018), or ii) synthetic organic polymer such as cholestrylamine and polyvinylpyrrolidone (Peng *et al*., 2018), hydoxypropyl methyl cellulose, microcrystalline cellulose and sodium carboxymethyl cellulose (Solis-Cruz *et al*., 2017).

The mode of actions of mycotoxin binders are still controversial, however, the proposed mechanisms are physical adsorption or physisorption, chemisorption, hydrogen bonding, furan ring bonding, ion interaction, electron donating (Di Gregorio *et al*., 2014; Wang *et al*, 2018). Physisorption by *Van der Waals* interaction is an adsorption phenomenon that is always almost reversible and it is mostly noted with adsorbents that have ionic configurationlike zeolite (Tapia-Salazar *et a*l., 2017).With respect to aflatoxins adsorption, hydrated aluminosilicates of Na, K, Ca and Mg and most clay binders have preference to bind aflatoxins to a high degree (De Mil *et al*., 2015; D'Ascanio *et al*., 2019),but their inclusion rate of between 2-3% (Kolawole *et al*., 2019) in feed is rather too high in broiler feed formulation.

The major benefits of mineral adsorbents are: they are less expensive, practicable, as they can be easily incorporated and they are relatively safe. However, comprehensive shortcomings of mineral-based adsorbents had been reviewed (Ditta *et al*. 2018; Elliot *et al*. 2020). Some of these are: their high ion-exchange potential may result in the adsorption of vital feed vitamins and minerals, creating a deficiency scenario of these micronutrients (Ralla *et al*., 2010), interaction that resulted in reduced bioavailability of veterinary antibiotics with bentonite had been reported (Goossens *et al*., 2012; De Mil *et al*., 2015), zeolite had been reported of capable of adsorbing nitrogen and some amino acids, decrease energy availability and can also suppress phosphorus utilization by forming an indigestible product with it (Eleroglu *et al*., 2011). Baek *et al*. (2012) noted that at just between 20-1000µg of montmorillonite/mL, it is capable of inhibiting cell proliferation, induce both oxidative stress and membrane injury with

more cytotoxic consequences if exposure period is prolonged. Zhang *et al*, (2010) had earlier observed the induction of oxidative stress with bentonite and that the induction was higher with modified bentonite than the natural one.

Another problem with the use of clay binders is the risk of being contaminated with dioxins, especially when the clay binder is natural and harvested from a burntforest or a burning site (Abad *et al*., 2002; Elliot *et al*., 2020). In farm animals, trace elements imbalance between the feed concentration and serum level had been attributed to mineral adsorbents (Yang *et al*., 2017). Zeolite and bentonite added to poultry feed at 0.5-2% had been reported to decrease serum zinc, manganese and copper levels with a significantly elevated aluminium concentration (Utlu *et al*., 2007). Inhibition of dietary absorption of iron by mineral adsorbents *in vitro* had been reported (Seim *et al*., 2013).

Pyrolysed or activated carbon is only effective in adsorbing aflatoxins but show little or no effect against other mycotoxins, however, at higher inclusion level, certain essential nutrients such as vitamins may be preferentially adsorbed relative to mycotoxins (Kolossova and Stroka, 2012). There are limited literature demonstrating the effects of synthetic polymers to adsorb aflatoxins or mycotoxins and just few of them such as cholestyramine, polyvinylpyrrolidone (PVP) and its modified form – polyvinylpolypyrrolidone (PVPP) had been evaluated *in vitro* and *in vivo*(Kolossova and Stroka, 2011). However, these synthetic polymers are very expensive and safety are the limiting factors in their practical applications (Celik *et al*., 2000).

Considering the inefficiency of inorganic or clay-based adsorbents towards other mycotoxins apart from aflatoxinsand the expensiveness and the selective binding of the synthetic ones, led to the suggestions of deploying natural organic binders in the search for new adsorbents that will be stable, effective over a wide range of pH, preserve nutritional value, that are non-toxic to the animal, prevent or reduce toxins carry-over to edible tissues, easy to use and cost effective (Jouany, 2007; Kolossova and Stroka, 2011).

2.6.3 The use of aflatoxins bio-transforming microorganisms/ aflatoxins modifiers

A novel strategy for biodegradation of aflatoxin into substances of reduced toxicity include the isolation of microorganisms (bacteria, yeasts, fungi and enzymes produced from them) capable of bio-transforming the toxinsinto relatively harmless products and also enhancing their easy disposition out of the animal's body (Santini and Ritieni, 2013).Toxigenic *Aspergillus flavus*synthesizes the highly toxic secondary metabolite, aflatoxins, to inhibit the growth of other microorganisms from its food substrate, thereby ensuring its survival in the presence of other competing microorganisms in the same ecosystem (Brakhage *et al*. 2013; Shcherbakova *et al*. 2015). The idea that some microorganisms can detoxify aflatoxins came about from observations that despite itshigh toxicity potency, some other microorganisms were able to coexist with toxigenic *Aspergillus flavus*.

Ciegler and his Co-researchers were the first set of researchers to have documented report on microbial detoxification capability on aflatoxins (Ciegler *et al*., 1966). Within the last five decades that followed, several microorganisms had been isolated from different substances like:faeces or from intestines of animalsthat fedon aflatoxin-contaminated diet, from aflatoxin-contaminated materials, from the gut of insects feeding on such materials and from the soil (Filazi *et al*., 2017). Probably the ability of the isolated microorganisms to survive within the habitats where they were isolated may be indicative of their capability to degrade aflatoxins.Biological or microbial detoxification of aflatoxins and mycotoxins in general is either by enzymatic degradation/transformation or by binding into the cell wall of the microorganisms (Colovic *et al*., 2019).

2.6.4 Enzymatic transformation or detoxification of aflatoxins

The fungus *Phoma glomerate* PG41, produces aflatoxin-degrading enzymes to coexist with toxigenic *Aspergillus flavus* (Shcherbakova *et al*. 2015). Zhang *et al*. (2014) also observed that *Aspergillus niger* was able to biodegrade aflatoxin B_1 in feed samples. Some fungi strains such as *Trametes versicolor* and *Pleurotus ostretus* produce oxidative enzymes like laccase and manganese peroxidase to detoxify aflatoxins (Alberts *et al*., 2009; da Luz *et al*., 2012). Motomura *et al*. (2003) reported that laccase

oxidase uses copper as co-factor to cleave the lactone ring of aflatoxins, eliminating or reducing its fluorescence, which is central to its toxicity. Another oxidase enzyme having manganese as its co-factor, purified from *Phanerochaete sordida* was able to detoxify up to 70% aflatoxins and that repeated addition of the enzyme removed aflatoxins up to 100 percent (Wang *et al*. 2011). Hackbart*et al*. (2014) observed that *Tricoderma reesei* and *Rhizopus oryzae* are also capable of detoxifying the B and G aflatoxins, including aflatoxin M_1.

Cao *et al*. (2011) observed that aflatoxin-oxidase enzyme has dual reaction steps in detoxifying aflatoxins by cleaving the bis-furan ring to form an epoxide followed by hydrolysis of the epoxide formed. They reported that the opening of the bis-furanring was unable to completely eliminate fluorescence of aflatoxins. Other fungi species that were reported to be capable of degrading/detoxifying aflatoxins are: *Mucor ambiguous, Dactylium dendroides, Mucor griseocyanus, Absiddia repens, Helminthosporium sativum, Mucor alternans* and *Tetrahymena pyriformis*(aprotozoa), (Karlovsky, 1999),*Saccharomycescerevisiae, Saccharomyces pastorianus* (Inoue *et al*.2013), *Rhizopus stolonifer, Rhizopus arrhizus*, and *Rhizopus oligosporus*(Kim*et al*. 2016). Kusumaningtyas *et al*. (2006) had earlier noted that *Rhizopus oligosporus* exihibited the highest detoxification potential and that its combination with *Saccharomyces cerevisiae* (aflatoxin-biotransforming yeast) gave an excellent result.

Praveen Rao and Co-researchers observed that aflatoxins oxidase or detoxifying enzymes of fungi origin are very stable than those secreted by bacteria. However, bacterial enzymes are more rapid in their activity than that of fungal enzymes (Praveen Rao *et al*., 1998).It has also been observed that most of the bacteria exhibiting aflatoxin-degrading or biotransforming activity were usually isolated from animal faeces, as they use coumarin as their carbon source (Kim *et al*., 2016). This may be the reason why aflatoxin - a bis-furano coumarin compound, is a substrate for these identified aflatoxin-detoxifying bacteria.Bacteria belonging to the genera *Norcardia,Mycobacterum* and *Rhodococcus*generally have the capability to degrade aflatoxins (Taylor *et al*., 2010). Bacterial species from:*Bacillus, Enterococcus, Bifidobacterium, Lactobacillus, Streptococcus* and *Lactococcus* had been identified and isolated for aflatoxins detoxification in livestock animals (Solis-Cruz *et al*.,

2018).Degradation of aflatoxins by means of microbial enzymes usually occur on one of the two reactive sites of aflatoxins, which gave them their potency and toxicity.

Enzymatic degradation of aflatoxins' molecule may take place at the 1,2-doublepositionof the terminal furan ring (Mishra and Das, 2003), or on the lactone ring of the coumarin moiety (Lee *et al*., 1981). Aflatoxins are usually detoxified when they lose the reactive site composition (Adebo *et al*., 2017), either by altering the coumarin structure by opening the lactone ring or breaking double bond on the terminal furan structure (Samuel *et al*., 2013). Disruption of the coumarin moiety by the lactone ring opening eliminate or decreases fluorescence, a key characteristic in aflatoxins toxicity, but the cleavage of the double bond on the terminal furan structure of the bis-furano ring does not eliminate aflatoxins fluorescence property (Cao *et al*., 2011). However, epoxidation of the bis-furan ring is pivotal to the formation of the most reactive metabolite of aflatoxin, the aflatoxin $B_1$8,9-exo-epoxide. In the absence of this toxic aflatoxin metabolicintermediate, the toxicity of aflatoxins is lost(Bedard and Massey, 2006; Diaz and Murcia, 2011).

Smiley and Draughon (2000), demonstrated that*Norcardia corynebacterioides* (formerly known as *Flavobacterium aurantiacum*) produces enzyme that was able to detoxify aflatoxin B_1 in an aqueous solution at neutal pH. A feeding trial with broiler chickens at 800 - 1200 ppb supplemented with *Norcardia corynebacterioides* gave result indicative of the bacteria ability to detoxify aflatoxin B_1 in animal compound feed (Tejada-Castaneda *et al.,* 2008). *Mycobacterium fluoranthenivorans* sp. nov., a bacterium known for its ability to metabolized polycyclic aromatic hydrocarbons in the soil has been demonstrated by Teniola *et al*. (2005), to exhibit the capability to biotransform aflatoxin B_1 into a product of much reduced toxicity. Lapalikar *et al*. (2012), noted that *Mycobacterium smegmatis* biotransforms aflatoxin $B_1$by reducing the α, and β-unsaturated lactone moiety, followed by hydrolysis into a less toxic substance. Extracellular extracts from *Rhodococcus erythropolis*uses enzymatic reaction to biotransform aflatoxins in aqueous at neutral pH (Alberts *et al*., 2006).In another study, *R. erythropolis* was reported to reduce aflatoxin B_1 in liquid cultures by 83% and 94% after 48 and 72 hours of incubation respectively (Teniola *et al*., 2005).*Myxococcus fulvus* isolated from the faeces of deer degraded aflatoxin B_1 by 80.7% after 72 hours of incubation (Guan *et al*., 2010).Other identified bacteria with

aflatoxins biotransforming capability are: *Bacillus licheniformis,Bacillus subtilis, Bacillus stearothermophilus* (Petchkongkaew *et al*., 2008; Fan *et al*., 2013).*B. subtilis*JSW-1 was reported to be capable of degrading about 70% of aflatoxin B_1 within 72 hours, by producing extracellular enzymes (Xia *et al*., 2017).

The fact that most of the bacteria isolates capable of bio-transforming aflatoxins into less- toxic compounds are taken from hind gut or animal faeces, makes them more adaptable of being sustained for a long period in the gastrointestinal tracts and they continuously detoxify aflatoxins, making them more practicable as aflatoxins detoxifying feed additive (Kim *et al*., 2016).There are however some drawbacks as to the applicability of microbial enzymatic detoxification of aflatoxins in poultry feed. Most of the studies on aflatoxins biotransformation/biodegradation were carried out in laboratory conditions and there is limited information on *in vivo* aflatoxin detoxification, especially in poultry (Solis-Cruz *et al*., 2018). There is the need for more practical and robust *in vivo*studies in poultry to evaluate the efficacy, toxicity and safety of microbial enzymatic biodetoxification of aflatoxins, as *in vitro* studies usually are not good indicator of *in vivo* response or do not reveal reliable consequences of *in vivo* exposure, because of physiological factors such as pH, differences in cell phenotypes, peristaltic movement, different protein reactions, gastric and intestinal secretions, the animals immune system, the influence of residentgut microbiome, influence of intestinal mucous, presence of bile, intracellular signaling, temperature fluctuations due to stress situation and the stability of the detoxification product (Solis-Cruz *et al*., 2018; Elliot *et al*., 2020).

Also, fungi species aflatoxins detoxification requires a long degradation time and culture pigmentation (Teniola *et al*., 2005) while bacterial enzyme production on a large scale is a real challenge, as they can be easily contaminated with other bacteria during the fermentation process and the enzyme yield is equally poor (Zhao *et al*., 2011). In addition, reports of Teniola *et al*. (2005), Guan *et al*. (2010) and Xia *et al*. (2017) were indications that microbial biodegradation of aflatoxins is never an absolute one. No microbial enzymatic decontamination of feed and feed materials has been able to fulfil the requirements for their practical and safe applications in animal nutrition, such as: i) very high decontamination potential, ii) both the microorganisms and its reaction products must be safe and non-toxic, iii) detoxification process needs

to be as fast as possible to completion within the GIT, as aflatoxins absorption is by passive diffusion, iv) must be compatible and stable in the GIT, considering the dynamics of multiple factors that exist in the GIT, v) they must be stable in feed and also during the processing stage and vi) there must not be any negative impact or impairment in the nutritive qualities of the ingredients or compound feed (Filazi *et al*., 2017).

2.6.5 Microbial cell wall binding of mycotoxins – organic binders

Different from aflatoxins degradation by enzymatic process, *Saccharomyces cerevisiae*, *Bifidobacteria* and *Lactobacilli* (Lactic acid bacteria - LAB) species when added to contaminated poultry feed has been observed to bind aflatoxins to their cell wall components without adverse effects on the animal's health (Goncalves *et al*., 2017; Damayanti *et al*., 2017). *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705 are both capable in degrading aflatoxin B_1, asup to 80% of the toxin was removed *in vitro* (Turbic *et al*., 2002). *Saccharomyces cerevisiae* has been demonstrated to bind mycotoxins stronglyto its cell wall components (Shetty and Jespersen, 2006; Jouany, 2007). The yeast strain can adsorb up to 90% of aflatoxin B_1 in aqueous medium (Goncalves *et al*., 2015). LAB is capable of inhibiting both the fungi growth and bind aflatoxins, thereby reducing the health risk associated with the toxin (Ahlberg *et al*., 2015). Both dead and living cells of LAB can bind aflatoxins. Damayanti *et al*. (2017) observed that dead LAB cells had a higher adsorbing potential than life cells while a contrary report from Liew *et al*. (2018) showed that living cells of LAB had higher binding efficiency. However, whether the bacterial cells are dead or alive, aflatoxins binding by LAB is a reversible one and the trapped toxins get dissociated gradually with progression in time (Verheecke *et al*., 2016).

Yeast (*Saccharomyces cerevisiae*) cell wall is a non-digestible substance in poultry species and has been reported to be efficient in adsorbing mycotoxins in the small intestine of the GIT when added to broiler chicken feed (Farooqui *et al*., 2019). Mycotoxins adsorption in yeast has been reported to be greater with yeast cell walls than with whole cells (Joannis-Cassan *et al*., 2011). It had been revealed that beta-glucansis the constituent of yeast cell wall that is responsible for mycotoxins binding (Jouany *et al*., 2005; Bovo *et al*., 2015). Yeast cell wall from another yeast genera: *Candida utilis* ATCC 9950 has recently been reported to be effective in mycotoxins

binding (Bzducha-Wróbel *et al*., 2018a). Mycotoxins complexation with this genus of yeast was reported to be due to the beta-glucans in the cell wall of *Candida utilis* (Bzducha-Wróbel *et al*., 2019).

2.6.6 Dietary manipulation method

According to the joint experts scientific report (CFP/EFSA/FEEDAP/2009) submitted to EFSA, bio-transforming agents could also be compounds or agents that do not react directly with mycotoxins, such as antioxidants and immunostimulatory compounds, and are not regarded as detoxifying agents but are very efficient in minimising the toxicity of mycotoxins (CFP/EFSA/FEEDAP/2009). In this regard, dietary approaches to mitigate the effects of mycotoxins include the adoption of nutrients, antioxidants and plant extracts (Galvano *et al*., 2001; Surai, 2007) in reducing or ameliorating the toxicity of mycotoxins.Adding specific amino acids like methionine above the requirement has been reported to minimise growth suppressing effects of aflatoxin B_1in chicks (Gowda *et al*., 2013). Basically, the toxicity of aflatoxin is due to free radicals (FR) /or reactive oxygen species (ROS) generationand induction of lipid peroxidation (Ma *et al*., 2015; Muhammad *et al*., 2018). Antioxidants are a group of natural or artificially produced chemical substances which in little concentrations are capable of significantly delaying, or inhibiting or preventing oxidation of bio-membranes lipids, by (FR) or (ROS) and they become oxidised derivative of themselves, which are less or non-reactive (Sorrenti *et al*., 2013). Preventing antioxidants depletion is crucial to alleviating the harmful effects of ingested aflatoxins.

Aflatoxin exerts its toxic effects during metabolism (Diaz and Murcia, 2011), to facilitate its excretion out of the body, by way of aflatoxin epoxide formation or free radicals (FR)/reactive oxygen species (ROS) generation. Both the epoxide metabolite and the FR/ROS require antioxidants (endogenous/or exogenous) to counteract the adverse effects of aflatoxins (Reuter *et al*., 2010). Cells used antioxidant enzymes (such as superoxide dismutase, catalase, glutathione peroxidase) and antioxidants molecules (such as α-tocopherol, ascorbic acid, reduced glutathione, selenium) to mitigate the toxic effects of aflatoxins (Neeff, *et al*., 2018). Disease condition characteristic of the cellular degeneration caused by FR/ROS ensue when these

oxidants are not effectively removed by cellular or supplemental dietary antioxidants (Ak and Gulcin, 2008).Artificially produced antioxidant compounds such as butylated hydroxyanisole (BHA), parahydroxybenzoic acid (Parabens) and butylated hydroxytoluene (BHT) had been added to foodto provide protection against oxidative damage. However, they have been implicated in liver damage and carcinogenesis (Ak and Gulcin, 2008).

Considering the differences in literature regarding the merits (Di Gregorio *et al*., 2014; Nazhand *et al*., 2020) and demerits (Elliot *et al*., 2020) of the available decontamination or counteracting strategies, and the inability to have an approach that will preclude aflatoxins 100 percent(Kolawole *et al*., 2019; Arif *et al*., 2020) from feed and feed materials, no single method can be adopted to be very effective in aflatoxins adverse effects mitigation in poultry (Murugesan *et al*., 2015). On this background therefore, beta-glucans(baker's yeast cell wall fraction) and dietary antioxidant with vitamin K'santihaemorrhagic potentialsto counteract production depressing effects of aflatoxins in broiler chickens were investigated in this report.

2.7 Beta-glucans as an adsorbent for aflatoxins

Beta-glucans (homopolymers of D-glucose) are the main structural materialsfound in yeasts (*Saccharomyces cerevisiae, Candida utilis* and others) cell wall components (Yiannikouris *et al*., 2004; Bzducha-Wróbel *et al*., 2018b). The cell wall helps in protectingyeast's organelles away from the adverse influence of the environment (Kwiatkowski and Kwiatkowski, 2012). The cell wall componentsare linked together by covalent bonds. The main structural compositions of yeast cell wall are β-D-glucans and mannoproteins,proteins, lipids and chitin are also present in little quantity (Klis *et al*., 2006; Yiannikouris *et al*., 2006). The relative proposition of these various components ispresented in Table 2.2.

Yeast derived products such as beta- D-glucans' mycotoxins binding efficiency is related to the polymer composition, the structure, medium pH, accessible surface area, yeast origin and strains (Magnoli *et al*., 2016; Pereyra *et al*., 2018). The adsorption mechanisms towards mycotoxins binding by these different components of the yeast cell wall include: non-covalent bond, hydrogen bond, hydrophobic interactions, ionic bond or electrostatic attraction (Huwig *et al*., 2001; Vila-Donat *et al*., 2018). Jouany

(2007) noted that weak hydrogen bonding and Van der Waals force may also be involved in mycotoxins adsorption by beta-glucans.

The beta-glucans component of the yeast cell wall is made up of β- (1,3) -D-glucan and β-(1,6) - D-glucan was reported to be largely responsible for the binding to aflatoxins (Jouany *et al*., 2005; Bzducha-Wróbel *et al*., 2019).β- (1,3)- D- glucan and β- (1,6)- D-glucan are a single unit,with β- (1,3)- D-glucan forminglinear andmain skeletonand β-(1,6)-D-glucan branchesare attached to the C- 6 of the glucopyranose ring of β-(1,3)- D-glucan (Lessage and Bussey, 2006). β-(1,3)- D-glucan function is to maintainthe cell wall shape and rigidity (Klis *et al*., 2006) and it forms a triple helix, in a tridimensional (3D) structure stabilized by inter and intra hydrogen bonds (Kogan, 2000) and it is the main component (β- 1,3- D- glucan) responsible for β-glucan's toxins adsorbing potential (Yiannikouris *et al*., 2004; Vila-Donat *et al*., 2018).

The β- (1,6)- D-glucan is the nexus between β- (1,3)- D-glucan and all the other cell wall polysaccharides (Kollar *et al*., 1997). Even though β- (1,3)- D-glucan componenthas the higher complexing potential, due to their greater affinity towards mycotoxins than the β-(1,6)- D-glucan, they are however very vulnerable to high pH, e.g. pH > 8.

Cell Wall Fraction	(% dry weight)
β- 1, 3- D- glucan	50 – 55
β - 1, 6- D- glucan	5 – 10
α- 1, 4- β- 1, 3- D- glucan	3 – 7
Mannoproteins Complex	35 – 40
Chitin	1 – 2

Source: Kath and Kulicke, (1999).

Table 2.2 Baker's Yeast (Saccharomyces Cerevisiae) Cell Wall Composition

Bzducha-Wrobel *et al*. (2019) noted that at pH 6.0, aflatoxins B_1 binding by beta-glucanswas positivelyinfluenced. Jouany *et al*.(2005), had demonstrated complexes from binders made of β- 1,3-D-glucan highly branched β- (1,6)- D-glucan units are less affected to alkaline pH than those from β- (1,3)- D-glucan alone.Bueno *et al*. (2007) observed that toxin binding may quickly get to saturation level and is reversible, as adsorbed mycotoxins are never altered chemically by the binding process. Figure 2.2is an adapted structure of β-(1-3), (1-6) - D- Glucans.

The helical structure of β-(1,3)- D-glucan is open and this facilitates easy access of AFB_1 molecule into its inner core, owing to the latter's geometry (Jouany *et al*., 2005). Two different bonds had been identified to be responsible for thecomplexing of β–glucans and AFB_1 molecule. The first is hydrogen bonds which connects the hydroxyl groups of glucose units in β-(1,3)- D-glucans and the aromatic rings, the ketone and lactone groups of AFB_1 together, leading to the formation of a complex that is less readily resorbed, facilitating excretion of the toxin through the faeces. Secondly, β-(1,3)-glucopyranose rings and coumarin moiety of AFB_1 had Van der Waals bond created between them and this also keeps the toxin attached to the β-D-glucans units (Jouany *et al*., 2005; Yiannikouris *et al.*, 2006). When the insoluble fraction of β-D-glucans following alkali treatment is fortified, it enhances the glucan's rigidity and strength towards high pH and heat. The encrustation or infiltration of Chitin into the beta-glucans structure enhances insolubility and resistance. However, the presence of chitin limits the ease of penetration by mycotoxins into the inner complexing core of the beta-glucans (Jouany *et al*., 2005).

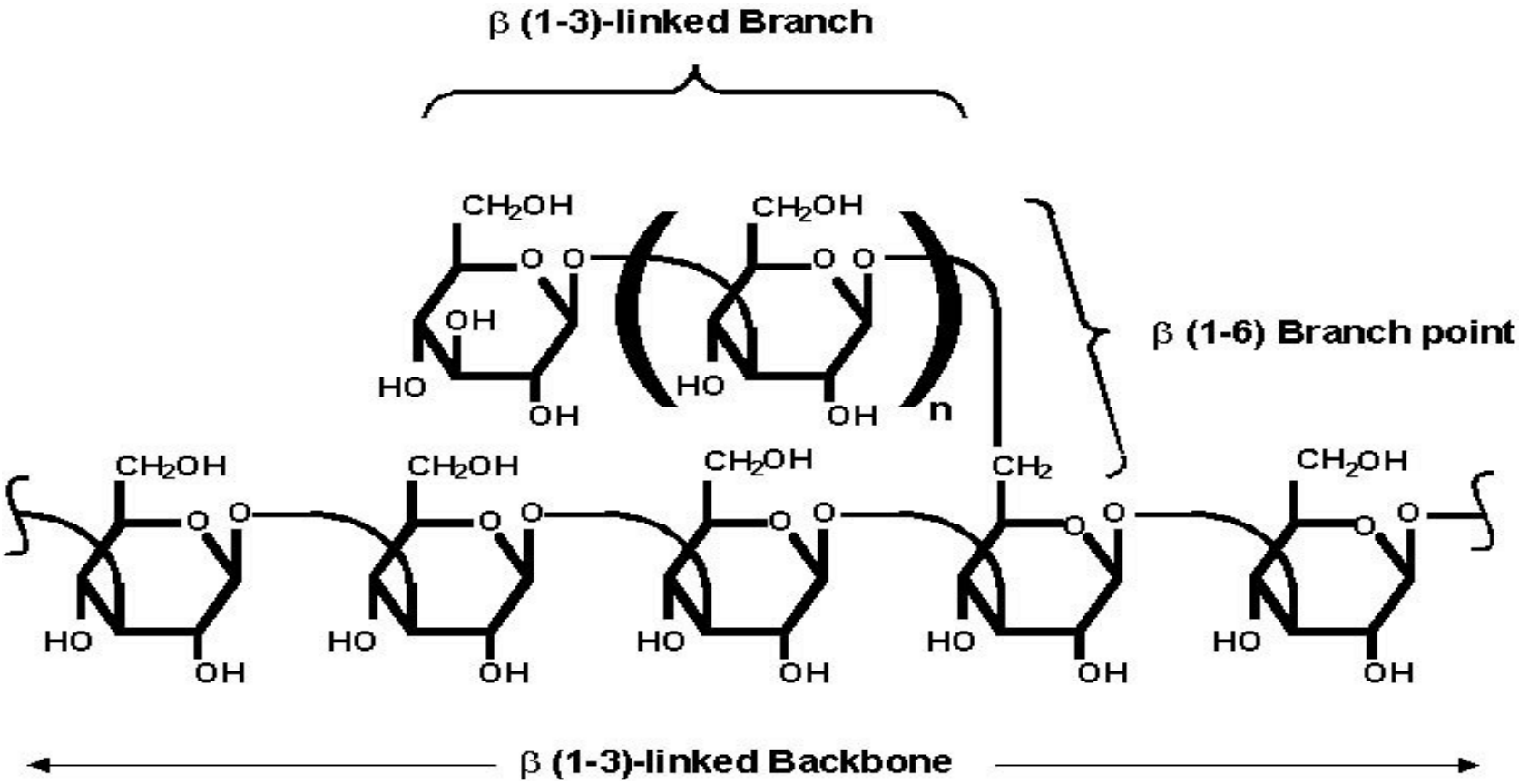

β (1-3)-linked Branch
β (1-6) Branch point
β (1-3)-linked Backbone
CH2OH
OH
HO
n
CH2

Figure 2.2 Structure of beta-(1-3), (1-6) - D- Glucans.

Adapted from: Scott and Kautzman, (2014)

2.8 Modulating aflatoxins metabolism with nutritionalantioxidants

Aflatoxin B_1 in its pure form is not so toxic (Heidtmann-Bemvenuti*et al*., 2011). However, being a lipophilic compound, it can accumulate in the body to a very dangerous level. Therefore, the biotransformation or metabolism of mycotoxins or xenobiotics such as aflatoxins, in the hepatic microsomes of vertebrates by the Cytochrome P450 (CYP450) monooxygenase enzymes is a process of making lipophilic substrates to become water-soluble products (Ewuola and Bolarinwa, 2016), to facilitate easy and speedy excretion from the body (Omiecinski *et al*, 2011), usually result in the generation of free radicals (e.g. superoxide anion radical - $O_2^{\cdot-}$) and/or reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) (Reed *et al*., 2011). Some biochemists believed that the biotransformation of xenobiotics or foreign chemicals such as aflatoxins takes place in two stages (Diaz and Murcia, 2011; Omiecinski *et al*, 2011), known as Phase I and Phase II, while others do not (Josephy *et al*., 2005).In brief, phase I metabolism is represented with reactions that involved reductions, oxidations, hydroxylation, epoxidation and O-demethylation (Omiecinski *et al*, 2011). The main CYP450 enzymes involved in the phase I reactions are: CYP1A1, CYP1A2, CYP3A4, CYP2A6 (Diaz and Murcia, 2011). Phase I is essentially a bioactivation phase.

Phase II reactions are often catalyzed by transferase enzymes, performing differentconjugation reactions, such as glucuronidation, methylation, acetylation, amino acidconjugation and glutathione conjugation (Omiecinski *et al*, 2011; Diaz and Murcia, 2011). Notable phase II metabolizing enzymes are Uridine diphosphate (UDP)- glucuronosyl-transferases(UGTs), N-acetyltransferases, sulphotransferases, methyltransferases and glutathione S- transferases (GSTs) (Holeski *et al*., 1987; Josephy and Mannervick, 2006). The products of phase II metabolism are more hydrophilic than the original compound and are more easily excreted (Diaz and Murcia, 2011).

The toxicity of aflatoxin B_1 stems from its bioactivation in the phase I metabolism (Eaton and Groopman, 1994). The epoxidation of the vinyl ether double bond on the terminal furan ring of aflatoxin B_1generates two reactive metabolites namely: Aflatoxin B_1-8,9-exo-epoxide and aflatoxin B_1-8,9-endo-epoxide (Wild and Turner, 2002). The latter is less reactive while the former is a highly reactive electrophile that

can react covalently to form adduct when it alkylates with DNA and proteins (Bedard and Massey, 2006). Aflatoxin B_1-DNA adduct results in G to T transversion and it is the basis for aflatoxin B_1 mutagenesis and carcinogenesis (Smela *et al*., 2001). As epoxidation reaction is essentially an oxidation reaction, the insertion of one oxygen atom across the terminal furan ring double bond of aflatoxin B_1 by the CPY450 enzymes leaves the other half of oxygen as a free radical, in the form of superoxide anion radical ($O_2^{\cdot-}$) (Reed *et al*., 2011), and through Fenton's and Haber-Weiss reactions, the highly reactive hydroxyl radical ($HO^{\cdot}$) and other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2)are also generated.

Fenton's reaction is given below as:

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + HO^-$$

Also, the superoxide radical participates in Haber-Weiss reaction as shownin the next equationsto generate hydroxyl radical:

$$Fe^{3+} + O_2^{\cdot-} \longrightarrow Fe^{2+} + O_2$$

$$2H_2O_2 + O_2^{\cdot-} \xrightarrow{\sout{O_2 + 2OH^{\bullet}}} + 2OH^-$$

The superoxide anion radical can also combine with the Fe^{3+}ion produced in the Fenton reaction and reduce it to Fe^{2+} ion(Liochev and Fridovich, 2002), and this is made available for another Fenton's reaction and the viscous cycle continues in the presence of consistent generation of superoxide anion radical.Although many reactive oxygen species such as hydrogen peroxide, lipid hydroperoxide and superoxide anion do not react with nucleic acids directly, rather, they are precursors for the highly reactive hydroxyl radical (Halliwell and Gutteridge, 1999).

Hydroxyl radical ($OH^{\bullet}$) is capable of attacking the guanine residue of DNA forming 8-hydroxy-2'-deoxyguanosine (8-OHdG), which can also produce a Guanine to Thymine transversion mutation (Cheng *et al*., 1992) and can also initiate the process of lipid peroxidation (Ayala *et al*., 2014).Hydroxyl radical causes oxidative damages in cells, as it indiscriminately attacks biomolecules (Halliwell and Gutteridge, 1984).Hydrogen

peroxide (H_2O_2) is membranes permeable (Halliwell and Gutteridge, 1999), andcan directly reacts with cellular components, especially biochemical enzymes. High concentration of H_2O_2 can inhibit the glycolyticenzyme, glyceraldehydes-3-phosphate dehydrogenase (Hyslop *et al*., 1988). High level of oxidantsis also capable of inactivating the activity of the enzyme glucose- 6- phosphate dehydrogenase. (Mahmoud and Nor El-Din, 2013).

Hydroxyl radical ($HO^{\cdot}$) along with hydroperoxyl radical ($HO^{\bullet}_2$) are strong oxidants that could initiate the oxidation of polyunsaturated phospholipids, leading to lipid peroxidation and the impairment of membrane functions (Schneider *et al*., 2008).Aldehyde end-products derivable from membranelipid oxidation such as malondialdehyde (MDA), propanal, hexanal and 4-hydroxunonenal (4-HNE) have been reported to be mutagenic and cytotoxic (Esterbauer *et al*., 1991).Elevated concentration of serum MDA, one of the terminal products of membrane PUFA oxidation or lipid peroxidation, that can cause functional abnormalities and pathological changes in bio-membranes (O'Brien, 1987).

Although oxidative processes are crucial tocellular activities, because they generate energy required for several cellular activities. However, these normal oxidative or metabolic functions generate highly reactive compound (Poljsak and Milisav, 2013). Substrate for the production of ROS can also be of dietary origin e.g. xenobiotics such as aflatoxins (Krishnamurthy and Wadhwani, 2012).Nature has endowed biological subjects with series of sophisticated antioxidant principles to protect their cells and organ systems from the degenerative effects of FR/ROS. Antioxidants are natural or synthetic low molecular weight chemical compounds, capable of delaying, neutralizing or inhibiting oxidative processes from occurring uncontrollably and in the process they get oxidised. (Vaya and Aviram, 2001; Dauqan *et al.,* 2011). Oxidised antioxidants are relatively stable and less reactive compared to the products of oxidation derivable from biological moleculesthat the reduced antioxidants have prevented from being oxidised (Yamauchi, 1997).Essentially, antioxidants effect their actions by donating hydrogen atoms to FR or ROS, leading to charge stability of the latter and a concomitant generation of poorly reactive radical derivative of the antioxidants (Burton and Traber, 1990; Yamauchi, 1997).

During normal physiological situation, a balance exists between endogenous antioxidants and the physiologically generated reactive oxygen species (ROS), thereby protecting cellular components from FR/ROS mediated oxidative damage or injury.Naturally occurring antioxidants can be categorized into two main groups:

i) The enzymatic endogenous antioxidants which include: (a) copper, zinc or manganese – dependent superoxide dismutase (SOD), which catalysis the transformation of superoxide anion radical into hydrogen peroxide and oxygen (McCord and Fridovich, 1969); (b) Iron-dependent catalase, which transformed the hydrogen peroxide produced by SOD into water and oxygen (Santoro and Thiele, 1997). Catalase reduce the availability of H_2O_2 required in Fenton's reaction with ferrous ion (Fe^{2+}) to produce one of the most reactive radicals – the hydroxyl radical ($OH^{\cdot}$); and (c) Selenium – dependent glutathione peroxidase, which catalysis the transformation of hydrogen peroxide (H_2O_2) into water and O_2 and also remove or reduce lipid hydroperoxides in cell membranes into their corresponding alcohol by using glutathione as the reductant (Halliwell and Gutteridge, 1999).

ii) Non-enzymatic endogenous antioxidants.These include: (a) the low molecular weight endogens such as glutathione and uric acid (the water soluble) and bilirubin, lipoic acid and quinones (the lipo-soluble). (b) The high molecular weight proteins: These are the metal binding (ligands) proteins or chelators. They are: Albumin and Ceruloplasmin, which bind copper ions, and Ferritin and Transferring, bind free iron while myoglobin and Haptoglobin bind haeme – containing proteins and thereby clear them from the circulation (Vaya and Aviram, 2001 and Krishnamurthy and Wadhwani, 2012).

Endogenous antioxidants are naturally endowed only, to be sufficient in neutralising free-radicals (FR) and/or ROS produced during normal physiological processes(Ashok and Sushil, 2005). Therefore, the presence of additional FR/or ROS arising from stress, drug or xenobiotic metabolism such as aflatoxinwill overwhelm and deplete natural antioxidants principles within the animal.Preventing antioxidant depletion is crucial to alleviating the adverse impacts of ingested aflatoxins. Both the epoxide intermediates formed and the FR/ROS generated during aflatoxin metabolism require antioxidants (endogenous/or exogenous) to facilitate their excretion or to counteract their adverse effects. Reverberi *et al*. (2010) and Neeff *et al*. (2018) noted that cells

use antioxidant enzymes and antioxidant molecules such as α-tocopherol, ascorbic acid, reduced glutathione, selenium and others to mitigate the toxic effect of aflatoxins. Disease condition characteristic of the cellular degeneration caused by FR/ROS ensue when these oxidants are not effectively removed by cellular /or supplemental dietary antioxidants, either due to their depletion or insufficiency (Ak and Gulcin, 2008).

Synthetic compounds with antioxidant activity such as butylated hydroxyanisole (BHA), parahydroxybenzoic acid (Parabens) and butylated hydroxytoluene (BHT) had been used to provide protection against oxidative damage in food. However, they have been implicated in liver damage and carcinogenesis (Ak and Gulcin, 2008; Gowda and Ledoux, 2008). Addition of selenium at 1mg/kg diet of ducklings contaminated with 100μg of aflatoxin B_1/kg of feed improved important biochemical indices and reduce hepatic malondialdehyde level (Shi *et al*., 2012). Nockels (1988a), reported the essentiality of nutrients in the development of animal's resistance to diseases, and that disease resistance involves maintaining protective barriers from pathogens and an immune system that is functioning optimally.

Hence supplementing animals' diets with natural or synthetic supplemental dietary antioxidants such as Vitamin E, vitamin C and Selenium will assist affected animals greatly in overcoming oxidative stress effects arising from endogenous antioxidants overwhelming by oxidants coming from nutrition derived xenobiotics such asaflatoxins contamination.

2.9 The use of vitamin E as dietary antioxidant supplement

Vitamin E also known as anti-sterility vitamin, is lipophilic, methylated and a phenolic compound. Vitamin E has an alcohol functional group and are broadly categorised or divided into either the tocopherols or the tocotrienols broad groups, and is one of the most efficient primary exogenous antioxidant defences in animals(Paul and Sumit, 2002). Synthesized only in plants, vitamin E is an indispensable nutrient in humans and animals' diet (Fryer, 1992). Of all the different eight types or isomers of vitamin E (tocopherol and tocotrienol), α-tocopherol is the most potent biologically.It is the vitamin E form that is most abundant in nature andhas the greatest antioxidant potential(Burton *et al*., 1985 and Yamauchi, 1997).Being fat soluble, vitamin E is permeableto cell membranes where the oxidation of membrane polyunsaturated fatty

acids (PUFA) occurs.For this reason, it is regarded as the most versatilecell membraneshield, preventing its PUFA from cellular oxidative damage (Romero *et al*., 2013).

α-Tocopherol is regarded as the most efficient lipid peroxidation chain reaction breaker (Neeff *et al*., 2018). Vitamin E is capable of quenching chain propagation process during peroxidation of membrane lipid and has high affinity towards peroxyl radical ($ROO^{\cdot}$) than the latter has towards polyunsaturated fatty acids (Burton and Traber, 1990 and Yamauchi *et al*., 1993).It has been observed that α-tocopherol rapidly reacts to neutralise peroxyl radical 200 times faster than other forms of antioxidants, such as butylated hydroxytoluene (BHT) (Burton *et al*., 1985). This makes α-tocopherol an efficient peroxidation chain breaker and excellent protector of cell membrane PUFA. In its association with peroxyl radical, vitamin E readily relinquish its phenolic hydrogen atom, thereby leading to formation ofhydroperoxide product, aless reactive product to peroxyl radical, and is easily degraded to water and carbon dioxide (Burton and Traber, 1990). The efficiency of α-tocopherol depends not only on its fast rate of trapping peroxyl radicals and on the capability of the emerging α-tocopheryl radical to intercept additional peroxyl radical, but also on the competence of the emerging α-tocopheroxyl-quinone derivative to be inactive and a non-radical (Yamauchi, 1997).

Each molecule of α-tocopherol can trap two of peroxyl radicals (Yamauchi, 1997). In protecting against bio-membrane peroxidation or damage, vitamin E in its α-tocopherol form will be eventually depleted and unless it is reconverted or reactivated through the α-tocopheroxyl radical intermediate by vitamin C, which had been proven to have a strong co-antioxidant ability (Lu *et al*., 2010), α-tocopherol might be depleted in the plasma, red blood cells and tissues (Brigelius-Flohe and Traber, 1999).At high concentrations, α-tocopherol acts as pro-oxidants, by inducing the formation of lipid hydroperoxide (Terao and Matsushita, 1986). This pro-oxidant effect is synonymous to the reaction of α-tocopheroxyl radicals with other lipids (Mukai *et al*., 1993a). Addition of ubiquinol or vitamin C, which are co-antioxidants,is effective in halting the pro-oxidant function of α-tocopheroxyl radicals(Upston *et al*., 1999).

The relative bioavailability of α-tocopherol is of interest, because it is the free form naturally found in foods and is less stable in air and can be easily oxidised, whereas the ester form (α-tocopherol acetate) is relatively stable in air than the natural phenol type (α-tocopherol). The tocopherol acetate is the derivativeof vitamin E mostly used in supplementing animal feed.As an antioxidantit is not active (Burton and Traber, 1990), and has to be hydrolysed to the phenol prototype before absorption of the vitamin can occur in the gastrointestinal tract.After its absorption in the intestine, vitamin E diffuses into the circulatory system via the lymphatic system and transported by chylomicrons into the liver, andthereafter α-tocopherolpreferentially appears in the plasma (Traber and Sies, 1996). Most of the ingested β, ɣ, and δ – tocopherols are secreted into the bile and are disposed out of the body through the faeces or droppings (Drevon, 1991).

2.10 Vitamin E inclusion in broiler chickens diet

DL-α-tocopherol acetate has the highest stability and it is the form of vitamin E commonly used to augment losses of vitamin E activity in animal feed. Vitamin E has been shown to exhibit up to 98% potency in the acetate form following a six-month usage in feed but the phenol or alcohol typewas absolutelydegraded within this period. The presence of heavy metals like zinc, copper, manganese or iron facilitate the oxidative destruction of natural α-tocopherol in formulated livestock diets (Dove and Ewan, 1991).

Vitamin E requirement specified by NRC (1994) to be included in broilers diet is 10mg/
kg of the ration. High levels of supplemental vitamin E at 48mg or 178mg per kg of feed offered to broilersinfected with subclinical infectious bursal disease (IBD) gave desirable result (McLlroy *et al*., 1993). Supplementing vitamin E in feed is necessaryfollowing a stressful management practice, to quickly restore normal level of α-tocopherolin tissues(Nockels *et al*., 1996). Bollengier-Lee *et al*., (1999), recommended that 250mg vitamin E per kg of ration is effectivein mitigating the adverse consequences of heat stress in laying birds.

Producers of poultry had been reported to be supplementing vitamin E up to 10 times above the NRC recommendation, as it has been shown to give desirable response on performance and improve immunity in poultry (Ward, 1993). Fortifying vitamin E

inclusion levels up to 25 times NRC guideline have produced beneficialresult by raising antibody response in turkeys (Ferket *et al*., 1993). Nockels (1988b), reported that under field conditions, levels of nutrients regarded as beingoptimal for animal growth and development may not be adequate for best immune systems response towards diseases. To attain optimum health, best performance and desirable processing efficiency,Chung and Boren (1999) suggested adding vitamin E at 240mg/kg of broiler starter ration. Using net income as a comparative tool to analyse broilers performance indices, Kennedy *et al*. (1992), recommended a level of 180mg per kg of diet.

Sheldon *et al*., (1997), noted that when vitamin E was supplementedbetween 20 to 25 times above NRC guideline in diets,the best turkey meat was produced, having the most typically accepted flavor for both fresh and frozen samples. Feeding 200mg of α-tocopherol per kg of broiler chick diet for at least four weeks resulted in maximum muscle fraction and minimise lipid peroxidation activity (Morrissey *et al*., 1997). He *et al*. (2013) supplemented duck feed with 50IU of vitamin E and 0.2mg of selenium yeast/kg diet, improvement in feed conversion was recorded and reverted the reduction in lymphocytes proliferation.

Recommendations by three different vitamin manufactures for vitamin E in broiler chickens per kg of feed are reported as: 30-50mg, 30-50mg and 30-150mg for Roche, BASF and Rhone-Poulenc respectively, with a mean value of 30-83mg vitamin E per kg diet (Avitech Scientific Bulletin, 2001). It should be noted that these values given herein are for optimum broiler production or performance and does not include consideration for cases like that of oxidative stress arising from xenobiotics (such as aflatoxins) metabolism.

2.11 Vitamin C as an antioxidant

L-Ascorbic acid also known as Vitamin C, is a 6 – carbon ketolactone that is structurally related to glucose. It is synthesized by plants and in the liver of most mammals except in primates (including humans), guinea pigs and fruit bats (McDowell, 2000). These animals are able to express the enzyme gulonolactone oxidase (GLO), which is fundamentally required in the bio-production of vitamin C immediate precursor 2-keto-1-gulonolactone (Padayatty *et al*., 2003). Animals that are unable to produce this rate-determining enzyme will have to source for vitamin C by other means, perhaps by dietary addition. However, reptiles and the avian species are

renal synthesizers of vitamin C (Roy and Guha, 1958).It is generally assumed that poultry do not require dietary source of vitamin C, owing to their natural endowment to synthesize this important vitamin. This may explain why vitamin C is not in the list of recommendation by NRC for supplementation in poultry diet (Lohakare *et al*., 2005).

Newly hatched poultry expresses limited degree of ascorbic acid synthesis and they are regularly exposed to hot and cold temperature, starvation prior to vaccination, vaccination handling stress and stress from disease condition such as in coccidiosis. Pardue (1987) and Nockels (1988b), reported the ameliorating effect of vitamin C in birds subjected to heat stress.Scott *et al*.(1982), affirmed that modern system of poultry management makes them vulnerable to higher vitamins demand. Reasons given are that: a) poultry has limited ways of utilisingvitaminssynthesized through microbial activities in the gut, b) the desire for utmost production in modern day poultry has raised theirdemand for vitamins, and c) the high stocking density of modern intensive poultry operations imposes stress on the birds, hence, the need for increased vitamin requirements.

Vitamin C participates in collagen hydroxylation andthe addition of hydroxyl groups to lysine or proline in the collagen molecule significantly enhances the stability of the collagen molecule(Peterkofsky, 1991).It is also the preferred reducing agent in the synthesis of carnitine (Dunn *et al.,* 1984). Carnitine is crucialin the transport of long chain fatty acids from the cytoplasm into the mitochondria inner matrix for ATP generation during the beta-oxidation of fat. Vitamin C is also involved in the biosynthesis of nor-epinephrine from dopamine (Levine *et al*., 1996).

Vitamin C is readilyassimilated in the duodenum andgets into the blood by active transport and perhaps too, by diffusion (Montecinos *et al*., 2007). The mechanism of absorption is by saturation and a dose dependent process. Hence, the rate of intestinal absorption reduces as the intake of ascorbic acid increases. Vitamin C absorption is greater when several small quantities are consumed at a time, at severally spaced interval in a day, than in one lump dose (Romero *et al*., 2013). The feeding practices in broilers or poultry production generally, will encourage better uptake.

The physiological and biochemical potency of vitamin C as an antioxidantare due to its electrons donating capability. Vitamin C donates two hydrogen atoms, each from the two hydroxyl groups on the 2nd and 3rd carbon molecule of the lactone ring (Padayatty *et al*.,2003), in a two-step reaction. Following the sequential loss of two electrons by vitamins C, the first derivative of vitamin C formedfollowing thedonation of one hydrogen atom is a free radical, semi-dehydroascobic acid or ascorbyl radical. Ascorbyl radical is relatively stable and is fairly unreactive (Buettner and Moseley, 1993). Upon the donation of a second hydrogen atom, dehydroascorbic acid (DHAA) is produced and its stability is determinedby the temperature and pH of the surrounding medium (Washko *et al*., 1993).The formation of both ascorbyl radical and DHAA is influenced by the presence of biological oxidants in an animal system. These oxidants could besinglet oxygen, superoxide anion and hydroxyl radicals, hypochlorous acid, reactive nitrogen species and the heavy metals such as iron and copper (Padayatty *et al*., 2003). Once they have been generated within a biological system, ascorbyl radical and DHAA can be recycledback to vitamin Cin the presence of glutathione (Padayatty *et al*., 2003; Montecinos *et al*., 2007).

Toxicity of vitamin C is scarcely probable to occur because the vitamin is hydrophilic and excesses are metabolized and excreted as dehydroascorbic acid, 2-keto-ascorbitol, oxalic acid and 2-O-methylascorbic acid (Groff *et al*., 1995). However, excessively high dose of vitamin C can induce diarrhoea and gastrointestinal discomfort, induce abnormal intake of iron, oxalates and uric kidney stones formations (WHO, 2004).Vitamin C beinga water-soluble antioxidant effects its antioxidant activities in extracellular fluids of the cytosol (Montecinos *et al*., 2007).

2.12 Relationship between vitamins E and C as antioxidants

Vitamin E, usually found within the body lipids or PUFA, where lipid peroxidation of membrane polyunsaturated fatty acids takes place and in this medium, it is the most efficient chain-propagation quenching antioxidant (Dauqan *et al*., 2011; Neeff *et al*., 2018).

As mentioned earlier in section 2.9, Vitamin C has the capability to effectively recycle α–tocopheroxyl radical back into its active reduced form (α–tocopherol) (Lu *et al*., 2010). After the release of one hydrogen atom from the chroman head of vitamin E to neutralize peroxyl radical,resulting into α-tocopheroxyl radical, vitamin E has to be

reconverted to the reduced form (α–tocopherol),as further donation of a second hydrogen atom will generate an unrecyclable α–tocopheroxyl-quinone derivative. Herein lies the limitation of vitamin E in its huge power as membrane protector.Vitamin C on the other hand will donate two hydrogen atoms to FR or ROS and can still be reconverted back toascorbic acid from dehydroascorbic acid.

2.13 Vitamin C inclusion in broiler chickens diet

Chew (1995)reported that the requirement for vitamin C to protect tissue against oxidative injury is subject to the level of stressor confronting the animal.McKee and Harrison (1995) reported that adding150mg of vitamin C into a kilogrammeof broiler feed improved chicks'performance when exposed to various environmental stressorssimultaneously. In the hot and humid tropics, heat stress effect on broilers was minimised when the diet was fortified with 200mg of vitamin C and that body weight gain and feed efficiency also improved (Njoku, 1986).

During heat stress, serum corticosterone concentration will rise and elevated plasma corticosterone has a suppressive effect on the immune system. Adrenal corticosteroid concentration in the plasma is reduced when diet is fortified with vitamin C. Perhaps, high vitamin C concentration in the adrenal gland downregulates glucocorticoid production, thereby amelioratingmost of the negative responses observedduring thermal stress and minimise steroid hormone precursors depletion (Pardue *et al*., 1985). Being a renal synthesizer of vitamin C,the level of ascorbic acid producedby poultry might be adequate for normal physiological and metabolic processes, however, the amount so produced might not be sufficientto cushion the deleterious effects arising from environmental and oxidative stressors(Ferket, 1994).Stressful conditions deplete vitamin C quickly than the bird's innate capability to bio-synthesize it (Pardue and Thaxton, 1986). The ability of the avian species to manufacturevitamin C decreases with age, while the requirement for protection increases with age, management systems, environmental stressors, toxins and diseases (Pardue and Williams, 1990).

2.14 Antioxidant role of selenium

Selenium (Se) is an essential trace mineral, taken up by plants from the soil. It is incorporated into amino acid in plants as selenomethionine. Selenomethionine is

considered to be in the organic form, and it is the form that is easily absorbed by animals from the digestive tract (Christensen, 2006). Following its absorption from the intestine, selenium is transported to the liver where it is bio-transformed into selenocysteine. Selenocysteinethen getsincorporated with other amino acids to produce selenoproteins (Allmang *et al*., 2009).Selenium is an important component of the selenoprotein-glutathione peroxidase (Garcia, 2013). Glutathione peroxidase (GP_X) is about 10 – 30% of plasma selenium (Ashton *et al*., 2009), and the level of blood selenium is determined through the amount of this enzyme (Christensen, 2006). Selenium is an essential cofactor necessary for the enzymatic activity of glutathione peroxidase, where it protects cell membranes from oxidative damage from hydrogen peroxide (H_2O_2) and lipid hydroperoxide formed during normal metabolic processes and during xenobiotic metabolism in the liver microsomes (Garcia, 2013).

Selenium deficiency in animals impairs glutathione peroxidase formation (Christensen, 2006). Muscular dystrophy isthe nutritional disorder thatensuesas a consequence of damages of cell membranes of skeletal and heart muscles, when both selenium and vitamin E are deficient in the diet of farm animals. Inadequate selenium in the body also results in impaired immune and thyroid functions (Christensen, 2006).

2.15 Selenium supplementation in poultry production

It has been observed that when a diet is supplemented with vitamin E, itreduces requirement for selenium (He *et al*., 2013). In a vitamin E deficient diet, supplementing selenium at 1-5mg per kg of rationresulted in reduced incidence of muscular dystrophy (Bermudez *et al*., 2012). When adequate level of vitamin E was used, between 0.3 and 0.5ppm selenium reversed signs of exudative diathesis and muscular dystrophy in chicks
(Bermudez *et al*., 2012).

2.16 Vitamin K and haemorrhage prevention

Vitamin K, also known as anti-haemorrhagic vitamin, is a family of chemically related quinone compounds that are liposoluble. All forms of vitamin K are members ofa large chemical group of substances known as“naphthoquinones”. The first type, called phylloquinones or $K_1$is produced by plants. The menaquinones or K_2, the second basic type, is synthesized by bacteria and third type is synthetic and is known as menadione

or K_3 (DSM-Vitamin K Compendium).In poultry, vitamin K biosynthesis is through the actionsof the bacteria inhabiting the bird's digestive system, most importantly the hind gut.Therefore, the vitamin so produced remains within the bacteria cell and could only bebeneficial to the birdsthrough the digestion of the bacteria cell, via coprophagy.It has been reported that chicks have less hepatic vitamin K epoxide reductase, an enzyme necessary to recycle vitamin K epoxide (Will *et al*., 1992), and may be a reason for the high requirement of this vitamin in chicks.

Deficiency of Vitamin K results in reduced prothrombin levelin the blood. Plasma vitamin K level of hatchlings or newly hatched chicks is about 2% and less than 40% in adult birds (Bermudez *et al*., 2012). Hence, chicks are readily affected when vitamin K is deficient in their diet or in vitamin K malabsorption. Mild deficiency may cause little haemorrhagic blemishes. Haemorrhages can also be seen on the breast meat, the thighs, the wings, abdominal cavity and the intestinal surface of affected birds.

The bioavailability of vitamin K is affected by gallbladder or biliary disease(Ferland, 2006), as in the case of aflatoxicosis and in prolonged use of antibiotics. Antimicrobial agents reduce the microbial load of the GIT, and impair intestinal synthesis of vitamin K, thereby rendering birds to rely completely on supplemental dietaryvitamin K(NRC, 1994). Therefore, the deficiency of vitamin K in poultry could be attributed to or results from inadequate dietary levels of the vitamin, suboptimal levels in maternal diet, rate of intestinal biosynthesis(as affected by long duration of antibiotics usage), presence of sulphur containing drugs, mycotoxins presence in feed (such as aflatoxins) and the interaction between vitamin K and other feed additives in the diet (Bermudez *et al*., 2012). In a disease condition such as coccidiosis and other enteric infections which may severely damage or erode the intestinal wall, broilers that are being fed aflatoxin-contaminated feed may experience excessive bleeding and may require higher levels of vitamin k supplementation (Leeson and Summers, 2001).

Bababunmi and Bassir (1982), reported that aflatoxin is a compound with anticoagulant property, in view of the structural similarity between aflatoxin and 4-hydoxycoumarin. In an experiment with rat, they concluded that aflatoxin B1 was more effective than 4-hydroxycoumarin inprolonging blood clotting time. It has long been reported that 4-hydroxycoumarin and dicoumarol prolong blood clotting time by

competing with vitamin K for the apoenzyme required for the biosynthesis of prothrombin in the liver (Doerr and Hamilton, 1981; Vijayalingam *et al*., 2017). Bababunmi (1989), was able to demonstrate that adding vitamin K to prepared liver slices of rats treated with aflatoxin B1 was effective in reversing the prolonged blood clotting time.Vitamin K was also reported to have a free radical scavengingpotential when assayed in non-aqueous solvents (Mukai *et al*., 1993b), and that it could suppress lipid peroxidation in liver microsomal preparation (Vervoort *et al*., 1997). However, the underlying mechanism of this protective function of vitamin K remains unknown.

The dietary vitamin K requirement suggested by the NRC (1994) ranges from 0.4 to 1.75mg per kg of diet. However, Rennie *et al*. (1997), noted that requirement for vitamin K can be altered by age, sex, strain, disease conditions (e.g. coccidiosis), anti-vitamin K factors (e.g. Dicumarol, Sulfaquinozoline, aflatoxins), and any condition that affect or influence lipid absorption.

2.17 Vitamin K inclusion levels in Poultry

Bermudez *et al*. (2012), reported that vitamin K deficiency was prevented when menadione was added to animal feed at 1-4gm per ton of feed.Vitamin K inclusion recommendations by some vitamin manufactures in broiler chickens per kg of feed were given as: 2-4mg, 2-3mg, 3-4mg and 1-4 mg for Roche, BASF, Rhone-Poulenc and Merck respectively(Avitech Scientific Bulletin, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preliminary study:Aflatoxins production, extraction and quantification

3.1.1 Aflatoxinsproduction

Maize was used as the culture material for the production of aflatoxinsadapting the methodofAtehnkeng *et al*. (2008). The aflatoxigenic strain of *Aspergillus flavus* (isolate 3228) used for maize culturing was obtained from the Plant Pathology Unit, mycotoxin laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

The fungi multiplication was carried out on a 5/2 agar medium, to generate enough fungi spores for inoculum production. A 50mL of V8 vegetable juice was measured into 1L beaker and filled up to the mark with 950mL of sterile distilled water. Stirred with Fisher scientific magnetic stirrer at 7 x 100rpm, for about 5 minutes. While stirring, the pH wasregulated and maintained at 6.0, using 0.1M NaOH and 0.1M HCl, and the temperature was kept at 26ºC (Atehnkeng *et al*., 2008).A 20g Bacto agar was accurately weighed into 1L autoclavable bottle and the diluted V8 juice was added to it and filled up to the mark with little sterile distilled water to produce the 5/2 medium. It was vigorously shaken and autoclaved at 121ºC for 15 minutes at 0.11MPa, using TOMY SX, high pressure steam sterilizer.

After cooling to room temperature (about 26º to 27ºC), the sterilised 5/2 agar medium was carefully poured into petri dishes at about 20mL per plate, inside alamina flow hood. The plates were inoculated with sterilised needle tip picking sticks, using one stick per plate.The inoculated plates were incubated at 37ºC for 7days. At the end of the seventh day, the fungi spores were harvested with the aid of a spreader and 0.1% Tween 20 solution.The inoculum obtained assumed a deep green colour. The concentration

of the inoculum was standardised to 2 million spores per mL, with the aid of haemacytometer and a microscope at x400 magnification.

3.1.2 Maize grain culturing

The maize grains were manually separated from mouldy and broken grains to obtainclean whole grains.A 100kg whole, clean maize grains was soaked in clean fresh water for 10 hours, thereafter it was sieved and thoroughly washed with fresh clean water. The soaked grains were divided into 500g and placed in autoclave bags. The grains were autoclaved at 121ºC for 45 minutes.

The autoclaved grains were allowed to cool to room temperature in the fume hood, thereafter, each wrap was transferred into inoculation bag and inoculated with 50mL of the inoculum. The grain-inoculum mixture was thoroughly and vigorously shaken to ensure a uniform distribution of the inoculum within the grains. The inoculated grains were spread out in the inoculation bag and incubated for 7days at a temperature range of 28º - 38ºC, using two different maximum and minimum thermometers, hanged at two different heights in the culturing room, to monitortemperature. After day seven, the cultured grains were wrapped with autoclave bags and autoclaved to destroy the live spores of the fungi. The cultured grains were oven dried at 45ºC to a moisture level of 12%, and thereafter pooled together and separated into two batches (A and B), based on the degree of grain colonisation.

The 100kg of clean whole maize grains produced 82kg of contaminated grains after the inoculation exercisedue to partial utilisation of the nutrients contained in the maize by the fungi. The contaminated grains were thoroughly mixed and samples were taken for aflatoxin screening and quantification at the Mycotoxin Laboratory of IITA, Ibadan.

3.1.3 Aflatoxins extraction from maize and animal feed samples

Generally, mycotoxins distribution within the produce or commodity is usually uneven. To have a representative aflatoxin concentration within the material, rigorous and systematic sampling procedureas described by Coker, (1998) and by the EU, under the Commission Regulation (EC) No. 401/2006 (Shephard, 2016)were adopted. Each bulk sample of the two batches (A and B) was thoroughly mixed and divided

into ten equal parts. A 10g sub-sample was taken from each of the ten divisions and thoroughly mixed and ground with Romer mill. From this, samples

were taken from each batch for analysis.

Aflasafe® maize grains and feed samples aflatoxins content were extracted using AOAC method 968.22 (AOAC,1990). A20 or 25g of the ground contaminated feed was weighed for analysis. This was blended with 100 mL of 85% methanol for 3 minutes, using Waring blender. Ground sample was placedon a 250 mL Pyrex conical flask and sealed with parafilm tape. The thoroughly blended feed sample was mounted on orbit shaker at 4 x 100 rpm and left for 30 minutes.The content was filtered into a conical flask already rinsed with 85% methanol,with No. 1 quantitative Whatman filter paper, 185 mm. The filtrate (about 40 mL), was transferred into a separating flask. A 40 mL of 10% NaCl solution was added, and a 25 mL of hexane was also added. The mixture was shaken vigorously by hand for one to two minutes and left to separate. The extract at the bottom of the separating flask was drained into a 250 mL conical flask and what was left in the separating flask was poured away.

The filtrate was returned into the separating flask, and a 25 mL of chloroform was added. Vigorously shaken and left in the separating flask to stand (to allow mixture to differentiate into top and bottom phases). The extract at the bottom phase was again drained through a layer of 20g anhydrous sodium sulphate into a 150 mL plastic beaker (preferably white in colour). A 10 mL of chloroform was again added to the remaining mixture in the separating flask. It was shaken vigorously and left to separate.The extract which is the bottom phase was again drained through a bed of 20g anhydrous sodium sulphate into a 150 mL plastic beaker (that contains the first extract filtrate). The total extract was left to dry overnight in the fume hood.

3.1.4 Qualitative and Quantitative analysis of extracts

The dried extracted filtratewas liquefied in tri-pour beaker with 1.5mL of dichloromethane into a clean Eppendorf tube. The extract was again left to dry overnight in the fume gadget. The next day, the dried extract was again reconstituted with 1 mL dichloromethane and vortex to homogenise the extract and the dichloromethane. The High-PerformanceThin Layer Chromatographic (HPTLC) plate was calibrated at theIITAMycotoxins Laboratory to standard format. This was done by

use of ruler to measure 1.5 cm from the edge of the HPTLC plate which serves as the position of first track with the distance between tracks to be 1 cm apart, making the number of tracks to be 18 (Ramesh *et al*., 2013). The HPTLC is a chromatogram of aluminium sheets, coated with silica gel. Standard solutions of aflatoxin B1 and B2 and for the G-aflatoxins (G1 and G2) were prepared.

A clean micro-capillary tube was inserted into a bulb assembly through silicone tip and made firm. The micro-capillary tube was consciously and gently cleaned with acetone thrice. Four(4)µL of aflatoxin standard was spotted on 1st and 10th marked spots respectively. Another 4 µL of each sample extract was also carefully spotted as marked on the plate. Spotted and air-dried plate was further processed indiethyl ether, mixed with methanol and sterile distilled water in ratio 96:3:1, in a 20 x 10cm developing tank or twin-trough chamber (TTC) for the maize sample. Forfeed samples, the solutions in the TTCwere chloroform, acetone and isopropanol in ratio 90:9:1. Each isolate was qualitatively scored under ultraviolet light as: no fluorescence (negative) or with fluorescence (positive) in comparison with the standards (e.g.aflatoxigenic is scored as +, ++, +++ based on the intensity of the fluorescence). The developed plates were screened under the ultraviolet light-box (wavelength = 365 or 366nm) to determinewhich extract fluoresces or do not. Fluorescedextracts and those without were compared relative to the standard. Quantitatively, fluoresced extracts during qualitative analysis were further treated quantitatively to ascertain totalaflatoxins (B1, B2, G1 and G2) in the sample. This was done with the aid of a scanning densitometer, CAMAG TLC, Scanner 3, with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described by Ramesh *et al*. (2013),which enables quantitative evaluation of densitometric data to be generated.

3.2 Study one: Effects of yeast beta-glucans on dietary aflatoxins absorption in broiler chicken fed aflatoxin-contaminated diets

3.2.1 Hypothesis tested

3.2.1.1 Null Hypothesis

(H_O): $\mu 2 = \mu 3 = \mu 4 = \mu 5 = \mu 6 = \mu 7$

That the effect of beta-glucans on aflatoxins absorption in the gastrointestinal tract(GIT) will be the same across the dietary treatments, irrespective of beta-glucans inclusion
level.

3.2.1.2 Alternate Hypothesis (Ha): $\mu 2 \neq \mu 3 \neq \mu 4 \neq \mu 5 \neq \mu 6 \neq \mu 7$

Thatbeta-glucansinclusion in aflatoxin-contaminated feed will be effective in preventing aflatoxinsabsorption in the GIT and that the degree of uptake will vary depending on its level of inclusion.

3.2.2 Experimental site

The site of the experimentwas Poultry Unit of the Teaching and ResearchFarm, University of Ibadan, Ibadan, Nigeria.

3.2.3 Experimental materials

A 100kg whole and clean maize grains wascultured with toxigenic strain of *Aspergillus flavus*, isolate 3228, gotten from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture, IITA,Ibadan. The fungi isolate multiplication and the grain culturing to produce aflatoxin were done in the Department of Animal Science, University of Ibadan, by the method of Atehnkeng *et al*. (2008), as described **in** section **3.1.2**.

3.2.4 Experimental diets

The inoculated maize grains were blended with aflasafe® maize grains to formulate a Basal Diet (BD) containing 300ppb aflatoxin per kg of the feed. Seven experimental diets comprising a Negative Control – NC (aflatoxin-free diet), the Basal diet (beta-glucans-free diet)and BD containing beta-glucansat: 125ppm (BD125), 250ppm (BD250), 375ppm (BD375), 500ppm (BD500) and 625ppm (BD625) were produced.

The test diets were produced and designed to be isocaloric and isonitrogenous, to ensure that observed treatment differences are not ascribed to the inequality in the nutrients' levels of the experimental rations. The starter diet contained approximately 3,000kcal Metabolisable Energy (ME)/kg diet and 23% Crude Protein (CP) level, while the finisher ration contained approximately 3,100kcal ME/kg diet and 19% CP respectively, using nutrients requirements in broiler chickens byLeesonand Summers

(2005) as a guide.The gross composition and analysed composition of the starter and finisher rations are presented in Tables 3.1 and 3.2, respectively.

3.2.5 Calculating inoculated maize grains inclusion in the experimental diets

This experiment was designed to contain 300ppb or 300µg/kg aflatoxins in a kg of feed, Given the concentration of 7,911.0±199.0 ppb total aflatoxins in the contaminated maize
grains (Table 4.1), this showed that 7,911µg of aflatoxin is contained in 1kg of the inoculated maize grains.

Therefore, if 7.911µg is contained in 1g, then 300µg aflatoxinsin 1kg (1000g) feed will be provided by:

$$\left(\frac{7.911\mu g}{1g} = \frac{300\mu g}{Xkg}\right) \text{of inoculated maize grains}$$

$$Xkg = \left(\frac{300}{7.911}\right) g = 37.9219g \text{ of inoculated maize grains}$$

(approx. 38g of the inoculated maize grains at 7.911µg/g aflatoxins concentration leveladded to 962g of other feed materials will produce aflatoxins contamination level of 300µg per kg of feed.

Hence, 3.8kg of the contaminated maize grains made up the quantity of aflasafe® maize required to produce 100kg of feed, to obtain a contamination level of 300ppb aflatoxins in the experimental diets.

3.2.6 Experimental diets layout

Following the laboratory analysis of the feed samples produced at the Mycotoxin Lab. of IITA, samples fromthe contaminated feed were however found to contain on the average (after two replicates analysis) 270.0±16.0ppb total aflatoxins, as presented in Table 4.1.Beta-glucans inclusion levels were informed by adapting the inclusion levels of Yildiz *et al*., (2004) who used up to 2,000mg yeast cell wall/kg feed and Zhang *et al*., (2008) used between 50 and 150mg beta-glucans/kg diet.

Below is the dietary treatments layout for study 1:

Diet 1 (NC) = 0ppb Aflatoxin+ 0 ppm beta- D-glucans
Diet 2 (BD) = 270ppb Aflatoxins + 0 ppm beta- D-glucans

Diet 3 (BD125) = BD + 125ppm beta- D-glucans

Diet 4 (BD250) = BD + 250ppm beta- D-glucans

Diet 5 (BD375) = BD + 375ppm beta- D-glucans

Diet 6 (BD500) = BD + 500ppm beta- D-glucans

Diet 7 (BD625) = BD + 625ppm beta- D-glucans

3.2.7 Beta-Glucansinclusion rate (%)

Diet 1 (NC) = 0%

Diet 2 (BD) = 0%

Diet 3 (BD125) = 0.0125%

Diet 4 (BD250) = 0.0250%

Diet 5 (BD375) = 0.0375%

Diet 6 (BD500) = 0.0500%

Diet 7 (BD625) = 0.0625%

3.2.8 Beta-Glucansinclusion in the experimental diets

The beta-glucans product (Fibosel™) used in this experiment was produced byTrouw Nutrition, France and it contains 20% of beta-glucans.

Therefore, each 100g pack of the product contains 20g of beta-glucans or 20,000mg of beta-glucans.

For 125ppm beta-glucans inthe feed, 125ppm is equivalent to 125mg of beta-glucans inclusion in 1kg of feed.

In 1kg of feed, the total amount of Fibosel™that will contain 125ppm beta-glucansinclusionwas calculated as shown below:

If 1kg of feed is to contain 125mg of beta-glucans,

Then, if 20,000mg beta-glucansis contained in 100g of Fibosel™, 125ppm beta-glucans in 1kgof feed will be contained in

$$\left(\frac{20{,}000\text{mg}}{100\text{g}} = \frac{125\text{mg}}{\text{X g}}\right)\text{Fibosel™}$$

$$Xg = \left[\frac{(125 \text{ x } 100)}{20{,}000}\right] = 0.625\text{g } (625\text{mg}) \text{ of Fibosel™}$$

At 20% beta-glucans concentration, 625mg Fibosel™ in 1kg of feed will contain (625 x 0.2) = 125mg of beta-glucans or 125ppm.

Therefore, 625mg of the product Fibosel™ in 1kg of feed will provide a concentration of 125ppm beta-glucans in 1kg of BD125 diet.

In the same manner,
1,250mg Fibosel™/kg of feed will provide 250ppm beta-glucans in 1kg of BD250 diet
1,875mg Fibosel™/kg of feed will provide 375ppm beta-glucans in 1kg of BD500 diet
2,500mg Fibosel™/kg of feed will give 500ppm beta-glucans in 1kg of BD500 diet
3,125mg Fibosel™/kg of feed will give 625ppm beta-glucans in 1kg of BD625 diet

3.2.9 Experimental animals, feeding and management

A 210 one-day-old Arbor Acres chicks (mixed sexes) obtained from a commercial hatchery in Ibadan were utilised for this study. The hatchlings were randomly assigned into seven treatment diets. Each treatment had 30 chicks, replicated three times with 10 chicks per replication. The chicks were raised on a deep litter in an open house system. They were exposed to the experimental ration from day one and were offered feed and water *ad libitum*. Feed was offered thrice a day and water was refreshed severally in the hot afternoon every day. Weighed quantity of feed was offered to each replicate early in the morning, providing additional 5% above the average feed intake per bird recommended in the management guide provided by Arbor Acres (Arbor Acres Broiler Management Handbook, 2014). Remnant feed were usually removed in the morningand weighed before given another fresh feed. The balance between the quantities of feed offered and the remnants was used to determine the quantity of feed consumed per replicate. The figure obtained was divided by the population of birds in each replicate to estimate the average feed consumption per bird per day.

The chicks were vaccinated against infectious bursa disease (IBD) at days 10,18 and 24 respectively. The 1st dose was given via the ocular route while the other two doses were given orally. New castle disease and infectious bronchitis (ND + IB H120) combined vaccines (containing Lasota and Massachusetts strains) were given at day 7 and day 14. They were also immunised against coccidiosis at day 3, via the oral route.

Prophylactic therapy against chronic respiratory disease (CRD) was administered at weeks 3 and 6.

Biosecurity measures such as foot dipping on disinfectants and hand washing before entry into the pen house, and regular shed cleaning to remove cobwebs were done as a routine. Wet spots on the litter were promptly removed to prevent microbial proliferation. Entry into the experimental section of the house was restricted to only the experimenter and the appointed personnel. Drinkers and feeders cleaning were the first routine operations early in the morning after checking for dead birds. The strict biosecurity procedures and other hygienic practices continued until the end of the experiment.

3.2.10 Composition of the experimental diets

Table 3.1 Gross composition (g/100g) of starter ration

Ingredients	**% Inclusion**
Maize (Aflasafe®)	55.20
Inoculated Maize	3.80
Wheat offals	1.00
Soyabean Cake (48%)	36.50
Limestone	1.75
Di Calcium phosphate	1.00
Edible salt	0.25
Broiler Premix**	0.25
L- Lysine	0.10
L- Methionine	0.15
Beta-Glucans	+
Aflatoxins (Crude)	++
Total (%)	**100.00**

Proximate composition of starterration

Nutrients	**Composition (%)**
Dry Matter	89.74
Metabolisable Energy (kcal ME/kg diet)	3,000.23
Crude Protein	23.03
Crude Fibre	3.53
Ether Extract	3.71
Nitrogen Free Extract	59.44
Calcium	1.40
Non-phytatePhosphorus	0.54
L-Lysine*	1.30
L-Methionine*	0.51
Total Aflatoxin (ppb)	270.00

*_ Calculated. + - Present in all treatment diets except treatments 1 & 2; ++ - Presents in all treatment diets except treatment 1.

** Premix composition/kg: Vit. A (5 MIU); Vit. D3 (2 MIU); Vit. E (20g); Vit. K3 (1g); Vit. B1 (0.4g); Vit. B2 (2.4g); Vit. B6 (1.8g); Vit. B12 (8µg); Niacin (17.6g); Pantothenic acid (4.8g); Folic acid (0.4g); Biotin (40µg); Mn (24.8g); Zinc (20g); Fe (10g); Cu (4g); Iodine (0.52g); Se (0.1g), Co (0.2g); Antioxidant (BHT) 50g

Table 3.2 Gross composition (g/100g) of finisher ration

Ingredients	**% Inclusion**
Maize (Aflasafe®)	62.45
Inoculated Maize	3.80
Wheat Offals	1.00
Soyabean Cake (48%)	29.00
Limestone	2.00
Di-Calcium phosphate	1.00
Edible salt	0.25
Broiler Premix**	0.25
L–Lysine	0.10
L–Methionine	0.15
Beta-Glucans	+
Aflatoxins (Crude)	++
Total	**100.00**

Proximate composition of finisher ration

Nutrients	**Composition (%)**
Dry Matter	89.91
Metabolisable Energy (kcal ME/kg diet)	3,103.05
Crude Protein	19.01
Crude Fibre	3.35
Ether Extract	4.90
Nitrogen Free Extract	62.65
Calcium	1.50
Non-phytate Phosphorus	0.51
L-Lysine*	1.11
L-Methionine*	0.45
Total Aflatoxins (ppb)	270.00

*_ Calculated. + - Present in all treatment diets except treatments 1 & 2; ++ - Presents in all treatment diets except treatment 1.

**Premix composition/kg: Vit. A (5 MIU); Vit. D3 (2 MIU); Vit. E (20g); Vit. K3 (1g); Vit. B1 (0.4g); Vit. B2 (2.4g); Vit. B6 (1.8g); Vit. B12 (8µg); Niacin (17.6g); Pantothenic acid (4.8g); Folic acid (0.4g); Biotin (40µg); Mn (24.8g); Zinc (20g); Fe (10g); Cu (4g); Iodine (0.52g); Se (0.1g), Co (0.2g); Antioxidant (BHT) 50g

3.2.11 Experimental duration

Feeding of the experimental diets to the birds started from dayone and it lasted for 42 days (6 weeks). Within this period, there was no other source of feed or supplement apart from the treatment diets.

3.2.12 Parameters measured/ data collection

3.2.12.1 Performance indices:

The indices of performance measured were:

Average feed intake per bird: Feed were offered *ad libitum*. Weighed quantity of feed were given to each replicate thrice in a day, [depending on the number of birds left in the cubicle (replicate)]. The quantity offered were added together and the remnants were weighed very early the following morning and the difference gave the quantity of feed consumed per day for that replicate. The average daily feed intake per bird was obtained by dividing the quantity of daily feed intake by the number of birds per replicate.

Average bodyweight gain per bird: The deduction of the preceding week's bodyweight from the current weight gave the average weekly bodyweight gain. The average body weight gain per bird per week is the ratio of average weeklybodyweight gain per bird to the replicate population.

Feed conversion ratio (FCR): This is obtained as follows:

$$\text{FCR} = \frac{\text{Total feed consumed/bird(g)}}{\text{Bodyweight gain/bird (g)}}$$

3.2.12.2 Haematology

Two birds were randomly taken from each replicate for blood sample collection via jugular venipuncture at the end of week 6, for haematology and serum analysis. 2ml of blood was collected in EDTA (Ethylenediaminetetracetate salt) bottle for haematology, using the method of Ewuola and Egbunike (2008). Parameters of haematology assessed were:Packed Cell Volume (PVC); (b) Haemoglobin (Hb); (c)

Red Blood Cell (RBC); d) Platelets;e) White Blood Cell (WBC) comprising: (i) Heterophils, (ii) Lymphocytes, (iii) Monocytes, (iv) Eosinophils and (v) Basophils

3.2.12.3 Serum biochemical indices

The sterile collection bottle containing 3ml blood was centrifuged at 4000rpm for about 15 minutes. The separated serum was decanted with the aid of needle and syringe, into another sterile bottle and kept frozen at about -20ºC. Biochemical indices assayed were:

Aspartate aminotransferase (AST). The AST in the serum samples collected was done by the method of Yagi *et al.*(1979).

Alanine aminotransferase (ALT) was determined by the method of Hamada and Ohkura, (1976).

Alkaline phosphatase (ALP). The ALP level in the serum samplescollected was assayed by method described by Rosalki *et al.*(1993).

Serum total protein (TP) was estimated by the method of Doumas *et al.*(1981).

Serum albumin (ALB). Serum ALB was assayed by the method ofDoumas *et al.*(1972).

Serum globulin (GLB). Serum GLB was determined by difference between TP and ALB, (TP - ALB).

3.2.12.4 Ilealdigesta collection and itsaflatoxins concentrationdetermination

At the end of week 6, two birds, randomly picked from each replication unitwere sacrificed for residual aflatoxin concentration in the liver and Ileal digesta. Birds were well fed late in the evening of the day preceding slaughtering, to make sure that birds will have adequate quantity of digesta within their ileum sections. Their body weight was taken very early in the morning and the six birds from a treatment were stunned together in a close chamber, by asphyxiating them with about 70% CO_2. When observed to be unconscious, each was shackled by the legs and hoisted head down, and immediately bled by sticking knife through the neck to put them to death.The birds were immediately cut open, the ileum was sectioned out towards the distal portion, 5cm before the ileo-caecal junction. The digesta within the sectioned ileum was carefully flushed out with a gentle pressure application.The Ileal digesta aflatoxins levels were analysed using the extraction procedure of AOAC (1990), explained in section 3.1.3for the determination of aflatoxin in the feed sample,

followed by High Performance Thin Layer Chromatography (HPTLC) and quantified with a scanning densitometer, CAMAG TLC, Scanner 3, with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland) as earlier described by Ramesh *et al*. (2013) at the IITA Mycotoxin Laboratory.

3.2.12.5 Adsorbed and absorbed aflatoxin determination

Relative amount of aflatoxin adsorbed

$$= (\text{Concentration of aflatoxin in Ileal digesta} \times \text{Estimated total Ileal digesta from daily feed intake})$$

$$\text{Adsorbed aflatoxin (\%)} = \left(\frac{\text{Relative aflatoxin adsorbed}}{\text{Daily aflatoxin intake}}\right) \times 100\%$$

Relative amount of aflatoxin absorbed

$$= (\text{Quantity of aflatoxin in daily feed intake} - \text{Relative amount of adsorbed aflatoxin})$$

$$\text{Absorbed aflatoxin (\%)} = \left(\frac{\text{Relative aflatoxin absorbed}}{\text{Daily aflatoxin intake}}\right) \times 100\%$$

"**Note**: ApproximateIleal digesta from daily feed intake was calculated to be 23% of feed consumed. A metabolic cage feeding trial was conducted for five days, for faecal collection and the percentage daily faecal output after oven drying at between 55 and 60ºC was found to be 23% (on the average) of the daily feed consumed."

3.2.12.6 Residual liver aflatoxin concentration

The residual aflatoxin in liver was assayed with enzyme-linked immunosorbent assays (ELISA) method, using Romer Labs AgraQuant® Total Aflatoxin Assay 4/40. As earlier described by Anjaiah *et al*. (1989) and Mehan (1997), a5g of the fresh liver sample was weighed and crushed to a uniform consistency with a homogeniser. The homogenised sample was transferred to a conical flask containing 25mL of 70% methanol. This was left to stand for minimum of 10 minutes, and filtered with No. 1 Whatman filter paper (185mm) for not less than 15mimutes.

While the filtration was ongoing, 100µL of the Conjugate solution was measured into the dilution well, then 50µL of the filtrate was added to make (100 + 50) µL = 150µL solution in the dilution well. This was mixed gently and thoroughly. From this solution, 100µL was measured into the antibody coated well and timed for 15 minutes.After 15 minutes, the content was discarded and washed 3 times with sterilised distilled water. The dilution or sample well was well shaken to dryness. A 100µL of the substrate solution was added to the dry sample well and timed for 5 minutes. Within this period, there was a colour change and the more intense the colour, the lesser the aflatoxin concentration in the sample will be, while the lighter the colour that developed, the more will be the aflatoxin level in the sample. At the end of the 5 minutes the reaction was halted with the aid of a stop solution, and the end product was a yellow colour solution, due to the stop solution which was acidic, while if basic, the colour will come out blue.

After this, the Optical density (OD) of the solution was determined using an ELISA reader ata wavelength of 450nm. The OD obtained was compared with the OD of the Standard (control). Aflatoxin B1 standard was prepared in 0, 2, 5, 20 and 50µg/kg and the standard for total aflatoxin was prepared as 0, 4, 10, 20 and 40µg/kg.A graph of the OD (y-axis) was plotted against the concentrations of the standard on the x-axis. The OD of the sample can then be traced on the OD-axis and the corresponding concentration of aflatoxin can be obtained on the x-axis.

3.2.13 Experimental design and statistical analysis

The experiment was laid out in a one-way factor analysis of variance (ANOVA), in a completely randomised design. Data collected were processed with descriptive statisticsand ANOVA, using (SAS, 2012) software package, version 9.20. Significant means were separated with Duncan Multiple Range Test (DMRT) of the same software.

3.3 Study two: Effects of supplemental dietary antioxidantsand vitamin K in broiler chicken fed aflatoxin-contaminated diets

3.3.1 Statement of hypothesis

3.3.1.1 Null hypothesis (H_O): $\mu2 = \mu3 = \mu4 = \mu5 = \mu6$

That supplemental dietary antioxidants andvitamin K will not have any effect in preventing the adverse effects ofaflatoxins in broiler chicken, irrespective of their combinations

3.3.1.2 Alternate hypothesis (Ha): $\mu2 \neq \mu3 \neq \mu4 \neq \mu5 \neq \mu6$

That supplemental dietary antioxidants and vitamin Kwill be effective in preventing the adverse consequences of aflatoxins inbroiler chicken and that the effect will depend greatly on the different combinations of the antioxidants and vitamin K

3.3.2 Experimental Site

The experimental site was Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

3.3.3 Experimental Materials

A 100kg whole and clean maize grains were cultured with toxigenic strain of *Aspergillus flavus*, isolate 3228, gotten from the Plant Pathology Laboratory, IITA in Ibadan. The fungi isolate multiplication and the grain culturing to produce aflatoxin were done in the Department of Animal Science, University of Ibadan, adapting the method of Atehnkeng *et al*. (2008), as described in sections 3.1.1 and 3.1.2

3.3.4 Experimental Diets

The test rationswere similar as earlier described in section 3.2.4

3.3.5 Experimental Diet Layout

Following the laboratory analysis of the feed samples produced at the Mycotoxin Lab. of IITA, the contaminated feed samples were found to contain on the average (after two replicates analysis) 270.0±16.0ppb total aflatoxins, as presented in Table 4.1.

The inoculated maize was used to formulate a Basal Diet (BD) having aflatoxins concentration of 270.0±16.0ppb aflatoxin per kg of the feed. Diet one was the Negative Control-NC (aflatoxins, supplemental dietary antioxidants and vitamin K-free diet) while diet two was the BD (the diet with aflatoxins but unmitigated). Vitamins E, C and K with Se in four different combinations were added to BD separately to produce additional four test diets. Vitamin E and vitamin C were jointly included as a single unit, because of the strong co-antioxidant relationship or strong synergistic effect between them (Vaya and Aviram, 2001; Lu *et al*., 2010). Vitamins E, C and K and Se were included in the test diets at 200mg, 250mg, 3mg and 0.3mg/kg of feed, respectively.

Vitamins E, C, K and Selenium were donated by Nutrivitas (Nig.) Limited, Lagos, Nigeria. They were presented as follows:

Product	Product Name	Percentage purity (%)
Vitamin E	Vitamin E50	50.00
Vitamin C	Ascorbic acid	99.99
Vitamin K	Vitamin K3 (Menadione)	44.93
Selenium	Sodium selenate	45.00

Dietary layout of the six treatment Rations (R) of study two:

Treatment 1 (NC) = Negative Control

Treatment 2 (BD) = Basal Diet

Treatment 3 (R1) = BD + (VE + VC).

Treatment 4 (R2) = BD + (VE+VC) + Se

Treatment 5 (R3) = BD + (VE + VC) + VK

Treatment 6 (R4) = BD + (VE + VC) + Se + VK.

NB: VE- Vitamin E, VC- Vitamin C, VK- Vitamin K, Se- Selenium.

3.3.6 Inclusion Rate of Supplemental dietary antioxidants and Vitamin K in The Experimental Diets

Vitamin E = 200mg/kg of feed or 0.02%(asrecommended by EFSA, 2010)

Vitamin C = 250mg/kg of feed or 0.025% (Nowaczewski and Kontecka, 2005; Ogunwole *et al*., 2013)

Selenium = 0.3mg/kg of feed or 0.00003%(Bermudez, *et al*., 2012)

Vitamin K = 3.0mg/kg of feed or 0.0003% (Avitech Scientific Bulletin, 2001; Bermudez *et al*., 2012)

3.3.7 Vitamin E Inclusion Calculation

If 1kg of feed is to contain 200mg of Vitamin E

= 200mg Vit. E/ kg Feed. (i.e. 0.02% Vitamin E inclusion)

at 50% concentration of Vitamin E, then 1kg of feed will contain

$$\left[\left(\frac{100}{50}\right) = \left(\frac{X}{200}\right)\right] \text{mg}$$

X = 400mg of Vit. E

Therefore, at 50% Vitamin E concentration, 400mg of vitamin E in a kilogramme of feed will give a concentration of 200mg Vitamin E/kg of feed.

3.3.8 Vitamin C Inclusion Calculation

If 1 kg of feed is to contain 250mg of Vitamin C

= 250mgVitamin C/kg of feed. (i.e. 0.025% Vitamin C inclusion at 99.99% purity)

3.3.9 Selenium Inclusion Calculation

If kg of feed is to contain 0.3mg of Selenium

= 0.3mg of Se/kg of feed. i.e. (3ppm inclusion of Selenium)

Given the concentration of Se to be 45%, then 1kg of feed will contain

$$\left[\left(\frac{100}{45}\right) = \left(\frac{Y}{0.3}\right)\right] \text{mg}$$

Y = 0.6667mg of Sodium Selenate (Na_2SeO_4)

Therefore, at 45% Se concentration, 0.6667mg of Sodium Selenate (Na_2SeO_4) in 1kg of feed, will give a concentration of 0.3mg Se/kg of feed.

3.3.10 Vitamin K Inclusion Calculation

If 1 kg of feed is to contain 3mg of Vitamin K_3,that is = 3mg of Vitamin K_3/kg of feed i.e. (3ppm inclusion of Vitamin K_3)

Given that Menadione (Vitamin K_3) is 44.93% in the preparation, then 1kg of feed will contain:

$$\left[\left(\frac{100}{44.93}\right) = \left(\frac{Z}{3}\right)\right] \text{mg}$$

Z = 6.677mg of Menadione (Vitamin K_3)

Therefore, at 44.93% Menadione concentration, 6.68mg (approximately) of Menadione in 1kg of feed, will give a concentration of 3mg Vitamin K_3/kg of feed.

3.3.11 Experimental Rations Composition

The material make-up of the experimental rations for the starter and finisher phases was similar as contained in section 3.2.12

3.3.12 Experimental Animals, Feeding and Management

A 180 one-day-old Arbor Acres chicks (mixed sexes) from a reputable hatchery in Ibadan were used for this experiment. They were randomly placed into six experimental dietary groups. Each experimental group had 30 chicks, replicated three times with 10 chicks per replication. The feeding and management practices carried out were the same as in section 3.2.9.

3.3.13 Experimental Duration

The feeding of the test diets to the birds started from dayone and it lasted for 42 days (6 weeks). Within this period, there was no other source of feed or supplement offered to the birds apart from the treatment diets.

3.3.14 Parameters measured/ Data collection

3.3.14.1 Performance indices

The indices of performance measured were similar as earlier described in section3.2.12.1

3.3.14.2 Haematology

Parameters of haematology assayed were similar to section 3.2.12.2

3.3.14.3 Serum Biochemical indices

Serum biochemical parameters were similar to those assayed in section 3.2.12.3. Others were serum malondialdehyde, reduced, oxidised and total glutathione, superoxide dismutase and total antioxidant capacity.

3.3.14.4 Serum/ Plasma malondialdehyde (MDA) determination

Serum malondialdehyde was assayed using Elabscience®Malondialdehyde Colorimetric Assay kit (through the TBA method), to estimate free radical/ or lipid peroxidation activity in the body of the broiler chickens. The level of lipid peroxidation activity was determined indirectly by reacting break down products from lipid peroxidation such as malondialdehyde (MDA) with thiobarbituric acid (TBA). Serum MDA wasestimateby adapting the method of Ohkawa *et al.*(1979). The maximum absorption of the red colour compound that developed was measured at a wavelength of 532nm with spectrophotometer.

3.3.14.5 Total glutathione/ Oxidised and reduced glutathione determination

Using Elabscience®test kits, Total Glutathione (T-GSH) and oxidised glutathione (GSSG) were assayedadapting the method of Rahman *et al.*(2006) as indicated in the user guide. Since Glutathione reductase (GSR) recycled GSSG generated when glutathione peroxidase uses reduced glutathione as reductantin neutralising hydrogen peroxide and lipid hydroperoxide, back into 2GSH, the overall amount of glutathione in the sample is determinedbyadding both reduced and oxidised glutathione together. That is: ([GSH]total = [GSH] + 2 × [GSSG]).(Rahman *et al.,* 2006).

The absorbance spectra or optical density was measured with spectrophotometer at a wavelength of 412nm

3.3.14.6 Serum superoxide dismutase (SOD) determination

Superoxide dismutase (SOD) catalysis the dismutation of superoxide anion radical ($O_2^{\cdot-}$) into hydrogen peroxideand oxygen.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

The Xanthine oxidase colorimetric method was used to determine the activity of SOD, adopting the method of McCord and Fridovich, (1969). The absorbance was taken spectrophotometrically at a wavelength of 550nm.

$$\text{SOD activity (U/mL)} = \frac{\text{ODcontrol} - \text{ODsample}}{\text{ODcontrol}} \div 50\% \times \text{Dilution factor}$$

3.3.14.7 Determination of serum total antioxidant capacity

Using the colorimetric method, the TAC determination was done based on the ability to reduce Fe^{3+} to Fe^{2+} (i.e. its Ferric reducing antioxidant power or FRAP) (Nagy *et al.,* 2006). The Fe^{2+} form stable complexes with phenanthroline substance. The TAC absorbance was measured with a spectrophotometer at a wavelength of 520nm.

TAC activity (U/mL)

$$= \frac{\text{OD}control - \text{OD}sample}{0.01} \div$$

$$30(\text{min}) \times \left(\frac{\text{Total volume of reaction system (mL)}}{\text{The volume of sample (mL)}}\right) \times \text{Dilution factor of sample before the test}$$

3.3.14.8 Residual liver aflatoxin concentration determination

At the completion of week 6, two birds were picked randomly from each replication unit and sacrificed for residual aflatoxin concentration in the liver. Their body weight was taken very early in the morning and the six birds from a treatment were stunned together in a close chamber, by asphyxiating them with about 70% CO_2. When observed to be unconscious, each was shackled by the legs and hoisted head down, and immediately bled by sticking knife through the neck to put them to death. The birds were cut open and the liver harvested.The residual aflatoxin in the liver was assayed with enzyme-linked immunosorbent assays (ELISA) method, using Romer Labs AgraQuant® Total Aflatoxin Assay 4/40. Detailed description is as contained in section 3.2.12.6.

3.3.15 Experimental design and statistical analysis

The treatmentwas laid out in a one-way factor analysis of variance (ANOVA), in a completely randomised design. Data collected were analysed with descriptive statistics and ANOVA, using (SAS, 2012) software package, version 9.20. Significant means were separated with Duncan Multiple Range Test (DMRT) of the same software.

3.4 Study three: Effects of yeast beta-glucans, supplemental dietary antioxidants and vitamin K in ameliorating the impact of aflatoxins in broiler chicken

3.4.1 Statement of hypothesis

3.4.1.1 Null hypothesis (H_O): $\mu 2 = \mu 3 = \mu 4 = \mu 5 = \mu 6$

That combining yeast beta-glucans withdifferent combinations of supplemental dietary antioxidantsand vitamin K will not be effective in mitigating the deleterious effects of aflatoxins in broiler chickens

3.4.1.2 Alternate hypothesis (Ha): $\mu 2 \neq \mu 3 \neq \mu 4 \neq \mu 5 \neq \mu 6$

That the adverse effects of aflatoxins in broiler chickens can be prevented by the inclusion of yeast beta-glucans withdifferent combinations supplemental dietary antioxidantsand vitamin K in their diets, and that the effect of one treatment will be different from the other.

3.4.2 Experimental Station

Thestudy was done at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

3.4.3 Experimental Materials

The fungi isolate multiplication and the grain culturing to produce aflatoxin were done in the Department of Animal Science, University of Ibadan, adapting the method of Atehnkeng *et al*. (2008), as described insections 3.1.1 and 3.1.2.

3.4.4 Experimental Diets

The test diets nutrients composition was the same as described in section 3.2.10. The NC and the BD as earlier described in section 3.2.4 and the combinations of the best two diets each from the previous studies (BD250 and BD375 from study 1 combined with R3 and R4 from study 2), gave rise to six experimental diets used in this study.

3.4.5 Experimental Diet Layout

Below is the dietary layout of the six treatment diets used in study three:

Negative Control (NC) = Aflatoxin-free diet

Basal Diet (BD) = Unmitigated aflatoxin diet

Treatment Diet 1 (TD1) = BD250 + R3

Treatment Diet 2 (TD2) = BD250 + R4

Treatment Diet 3 (TD3) = BD375 + R3

Treatment Diet 4 (TD4) = BD375 + R4

Where R3 = [(VE + VC) + VK]and R4 = [(VE + VC) + VK + Se]. VE- Vitamin E, VC- Vitamin C, VK- Vitamin K.

As earlier described in section 3.3.6, vitamins E, C and K and Se were included in the test diets at 200mg, 250mg, 3mg and 0.3mg/kg of feed respectively.

3.4.6 Experimental Feed Composition

Test rations composition was the same as earlier described in section 3.2.10.

3.4.7 Experimental Animals, Feeding and Management

Feeding and management practices were similar as described in sections 3.2.9 and 3.3.12.

3.4.8 Experimental Duration

The feeding trial with theexperimental diets started from dayone and lasted for 49 days. Within this period, no other source of feed or supplement was given apart from the treatment diets.

3.4.9 Parameters measured/ Data collection

3.4.9.1 Performance indices

The indices of performance measured were similar to those described in sections 3.2.12.1 and 3.3.14.1. Additional indices of performance estimated were:

3.4.9.1.1 Uniformity of bodyweight: This is also referred to as flock uniformity. It is calculated by determining 10% deviation from the mean bodyweight. The total number of birds that falls within this bracket is taken and expressed as a percentage of the total flock.

3.4.9.1.2 Uniformity coefficient of variability (CVu): This is calculated by expressing the uniformity standard deviation as a percentage of the mean. Coefficient of variability in planned experiment such as this, gives an idea of the repeatability of the parameter determined. If attached to all the parameters, it will indicate greatly the repeatability of the whole experiment.

3.4.9.2 Haematology

Two birds were picked randomly from each replicate for blood sample collection via jugular vein puncture at the end of week 7, for haematology and serum analysis. 2ml of blood was collected in EDTA (Ethylenediaminetetracetate salt) bottle for haematology as earlier mentioned in sections 3.2.12.2 and 3.3.14.2.

3.4.9.3 Serum biochemical indices

Biochemical indices assayed were similar to those determined in sections 3.2.12.3 and 3.3.14.3. Additional serum biochemical indices assyed were similar to those of sections 3.3.14.4, 3.3.14.5, 3.3.14.6 and 3.3.14.7.

3.4.9.4 Aflatoxin retention determination

By the 42nd day, twobirds were picked randomlyfrom each replication unit and taken to the metabolic cage for more precise feed monitoring and faecal collection. Birds from the same replicate were housed in the same cell. They were left to adjust to the new environment for 48 hours and data collection started on the third day and feed measurement and faecal collection or droppings were done for five consecutive days. The faecal collections were pooled together, weighed and oven dried between 65 - 70ºC to prevent decomposition. However, the substance of interest, aflatoxin, is not a volatile substance and cannot be affected at this temperature.

After 48 hours, the faecal samples were removedfrom the oven and left to coolto room temperature (27ºC±3ºC) and weighed. Samples for aflatoxin detection and quantification were done on air dry basis and not on dry matter basis, hence, the

reason for bringing it to room temperature moisture level.Samples were taking for aflatoxin screening and quantification, to estimate total aflatoxin concentration in the faecal samples, using the extraction procedures described in section 3.1.3 coupled with HPTLC for the quantification as described in section 3.1.4. This analysis was carried out at the Mycotoxin Laboratory, Plant Pathology Unit, IITA, Ibadan.

Aflatoxin retention was estimated byintake-excretion balance method, adapting the procedure of Kolawole *et al.* (2019) and was calculated thus:

$$= \left[\frac{\text{Average dAI} - \text{Average dFAO}}{\text{Average dAI}}\right] \times 100\%$$

Where:
dAI- Daily aflatoxin intake = (Average daily feed intake x Aflatoxin concentration in feed).

dFAO- Daily faecal aflatoxin output = (Average daily faecal output x Aflatoxinconcentrationin faecal samples).

3.4.9.6 Residual aflatoxin concentration in liver, breast meat and blood

At the end of week 7,twobirds,randomly taken from each replicationwere used for determination of residual aflatoxin concentration in liver and breast meat. Their body weights were taken by 6.00amin the morning, followed by blood samples collection, and then the sixbirds selected from a treatment were stunned together in a closed chamber, by asphyxiating them with about 70% CO_2. When observed to be unconscious, each was shackled by the legs and hoisted head down, and immediately bled by sticking knife through the neck to put them to death. The birds were cut open, an average of 25g of breast meat was sectioned and the liver harvested.

Theresidual aflatoxin in the liver, breast meat and blood were assayed with enzyme-linked immunosorbent assays (ELISA) method, using Romer Labs AgraQuant® Total Aflatoxin Assay 4/40. Five (5) grameach of liver and breast meat samples wereweighed and crushed to a uniform consistency with a homogeniser whilefive(5) mL of blood sample was collected in heparinized bottle and homogenised.Detailed description of residual aflatoxins extraction and quantification was described in section 3.2.12.6.

3.4.9.7 Cost benefit analysis and variables determinations

Variables of cost analysis determined arelisted as follows:

a) Average Final Bodyweight- AFBW. This is average bodyweight of each treatment at the 49th day of the experiment.

b) Average Feed Cost- AFC. The AFC per bird was determined by multiplying the quantity of feed consumed in each treatment by the feed cost per kg of that treatment. The costs of betaglucans, selenium, vitamins C, E and K were also added to the AFC, based on their inclusion rate in each treatment. Dividing this by the numberof birds left in the treatment gave rise to AFC/bird

c) Average Total Raising Cost- ATRC. This is the addition of AFC plus other costs incurred in raising the birds. These other costs include: i) cost of day-old chicks; ii) cost of vaccines and medications; iii) cost of sealing the pen house; iv) costs of charcoal, parking wood shavings, water; v) miscellaneous expensis such as transportation cost on feed, cost of disinfectants, repair expensis on pen house partitioning.

d) Average Liveweight Value_ ALWV. This was based on the average market liveweight value per kg of bodyweight. The ALWV/birdis a product of AFBW and liveweight value per kg body weight, expressed in naira (₦).

e) Average Marginal Returns- AMR. This is the difference between the ALWV and ATRC, expressed in naira (₦).

$$AMR/bird = ALWV/bird - ATRC/bird$$

3.4.9.8 Histopathology processing and procedures fortissuesections

The organs harvested in section 3.4.9.6(liver, kidney and ileum)wereappropriately labelled with codes and taken to the Department of Veterinary Pathology, University of Ibadan for histological examinations. At the point of collection, samples were preserved in 10% formalin solution (neutral buffered). Samples were further processed in automated tissue processor and embedded in paraffin wax. With the aid of a rotary microtome mounted on glass slides, sectioning was done at 4-5 microns. Reviewed detailed procedure for the automated tissue processor for histopathology of slides as described by Winsor (1994) and Hopwood (1996), and as revised by Slaoul and Fiette, (2011) was used. In brief, the laboratory procedure is as follows:

Fixation: The first stage in a successful preparation of tissues for histological examination is the fixation process. This is necessary to immobile the cell constituents, such that they were fixed in 10% formaldehyde to be able to withstand subsequent treatments with different reagents.

Dehydration: As the name implies, this**is** basically the removal of constituent moisture of tissue specimens in a gradual process, putting osmotic dynamics into consideration. Dehydrationwas usually carried out by the automated tissue processor (Shandon-Elliot®), using ethanol at different concentrations from 70 – 100% for 1 hour.

Clearing: This process removes the ethanol used during the dehydration process. This was the process that makes the cell and its constituents to be transparent during microscopy is initiated and completed. The removal of alcohol (clearing) prepares the specimen for infiltration with a molten paraffin wax.

Infiltration: Infiltration of the tissue with molten paraffin wax gave additional support to the tissue for subsequent sectioning. Paraffin wax permeates the tissues to fill up vacuoles created in the cells following dehydration. This process was completed in a wax oven for 2 hours, at about 60 ºC before embedding the specimen.

Embedding: This involves the poisoning of the infiltrated tissue in molten paraffin wax within a confined container called mould. Tissue specimen was meticulously positioned in an orientation in which the tissue was intended to be further sectioned. Embedded tissue was left till the wax solidifies.

Sectioning: The main objective of this step is to slice out 4-5µm thick specimen. This was achieved on a quintessential piece of precision knives known as microtome. The solidified tissue and paraffin were mounted on the microtome holder and the thin slice cut from the tissue in the solidified paraffin is called a section. The sectioning was done serially like a ribbon. The sections were floated out on a water bath at 45 ºC, to stretch out the paraffin sections. Satisfactory sections were picked up with frosted edge microscope glass slides. Specimen labelling was done on slides containing the sections with a pencil. Labelled specimen containing slides were arranged on a slide carrier, in readiness for staining. They were dewaxed in an oven at 40 ºC for 30 minutes to make the sections to stick to the glass slides.

Staining: Haematoxylin and Eosin (H & E) is the routine staining used in histopathology to assess changes in animal tissues and organs in toxicity examinations. Haematoxylin is basic in nature and has affinity for acidic components of the cell, mostly nucleic acids in the nucleus, while eosin is acidic and binds to cytoplasmic constituents. Therefore, with H & E, the nucleus was stained blue and the cytoplasm appeared in orange-red colour. The stained slides were left in xylene until they were mounted, using D.P.X., which is a good transparent mount, which has a refractive index comparable to glass.

Microscopy: Slides observation started with the naked eye, then further examination with Olympus CX21 microscope with attached digital camera. Low and medium power objective lens (x10) was first used and then structures that appeared doubtful were further observed under higher (x40) objective lens. Normal tissues were identified and the pathological alterations observed during microscopic examination were appropriately described based on histological structure of the tissue under investigation. With the aid of an attached computerised digital camera, photomicrographs shots were taken.

3.4.10 Feed Aflatoxin to Tissue Aflatoxin Carry-Over Ratio

Feed aflatoxin to tissue aflatoxin carry over ratio was estimated by adapting themethod of Park and Pohland, (1986).

Feed to tissue ratio was estimated as: $=\left(\frac{\text{Concentration of aflatoxin in feed consumed}}{\text{Concentration of aflatoxin in the specified tissue}}\right)$

3.4.11 Experimental design and statistical analysis

The experimental design was a (2x2)+2 augmented factorial arrangement, in a completely randomised design. Data collected were analysed with descriptive statistics and ANOVA, using SAS, (2012) software package, version 9.20 and descriptive statistics. Significant means were separated with Duncan Multiple Range Test (DMRT) of the same software.

CHAPTER FOUR

RESULTS

4.1 Preliminary study: Chemical analysis result of cultured maize grains and contaminated feed for aflatoxins level

Results in this section gave a confirmatory level of aflatoxins concentrations in the inoculated maize and the contaminated feeds.The results of the B group aflatoxins and the total aflatoxins concentration in the inoculated maize grains,contaminated feed and in aflasafe® maize grainswere presented in Table 4.1. Up to 90% of the total aflatoxins in the inoculated maize grains was aflatoxin B_1 while aflatoxin B_2 made up approximately 10%. The mean total aflatoxins concentration was 7,911.0±199.0 ppb. The proportion of aflatoxin B_1in the contaminated feed was similar as in the inoculated maize grains, being up to 96% of the total while aflatoxin B_2 made up of approximately 4% of the total aflatoxins. The feed produced with aflasafe® maize contained 0ppb aflatoxins. The mean total aflatoxins concentration in the contaminated feed samples was 270.0±16.0 ppb

Table 4.1 Aflatoxins B_1 and B_2 levels in the inoculated, aflasafe maize and contaminated feed obtained using HPTLC with scanning densitometer

Sample Name	Sample ID	Aflatoxins (ppb)		Total Aflatoxinslevel (ppb)
		B1	B2	
Inoculated maize grains	IM	7,137.0±243.0	774.0±44.0	7,911.0±199.0
Aflasafe® maize grains	AFM	5.0±1.0	0±0.0	5.0±1.0
Feed with Aflasafe® maize	S1	0	0	0
Contaminated feeds	S2	260.0±15.0	10.0±1.0	270.0±16.0

Note:

a. Recovery of toxin = >85%,

b. Zero means the aflatoxin level is below detection limit of the analytical method (1ppb),

c. Values are means of two subsamples of each sample,

AFM- Aflasafe® maize grains, IM- Inoculated maize grains, S- Sample, HPTLC- High Performance Thin Layer Chromatography

4.2 Study one: Effects of yeast beta-glucans on dietary aflatoxin absorption in broiler chicken offered aflatoxin-contaminated feed

Stated objectives a and b in section 1.3 were carried out in study one (see section 3.2) and the results presented in section 4.2.

4.2.1 Assessment of performance at starter phase of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans

The effect of varied levels of beta-glucansinclusion on performance at starter phase of broiler chickens fed aflatoxin-contaminated diet is presented in Table 4.2. Feed intake (g/bird) of birds fed the NC diet (0ppb aflatoxins; 0ppm beta-glucans), 135.81±20.4 increased significantly ($P<0.05$), relative to that of birds fed BD125ppm (112.59±7.08), BD250 (112.91±15.20) and BD375 (109.94±2.79) diets, but did not show any significant variation ($P>0.05$) in feed consumption from that of birds fed other treatment diets (BD, BD500 and BD625). Varying the levels of beta-glucans did not show any significant effect ($P>0.05$) on BWG (g/bird) of broiler chicks at starter phase, and it ranged from 197.84±22.29 in BD250 to 225.49±26.11 in BD (0ppmbeta-glucans). The FCR at starter phase was also not significantly ($P>0.05$) affected by varied levels of beta-glucans inclusion and it ranged from 0.50±0.01 in BD125 to 0.65±0.14 in NC diet.

Table 4.2 Effect of variedinclusion levels of beta-glucans on performance at starter phaseof broiler chickenoffered aflatoxin-contaminateddiet(0-3 weeks).

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
FI(g/bird)	135.81±20.41[a]	115.40±13.02[ab]	112.59±7.08[b]	112.91±15.20[b]	109.94±2.79[b]	131.77±1.42[ab]	123.41±3.35[ab]	6.51	0.08
BWG (g/bird)	215.34±48.48	225.49±26.11	224.71±10.87	197.84±22.29	216.22±34.26	218.93±16.68	210.54±22.14	16.32	0.91
FCR	0.65±0.14	0.52±0.12	0.50±0.01	0.58±0.12	0.52±0.08	0.60±0.04	0.59±0.05	0.05	0.45

[ab]Treatment means withinthe same row with unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level,FI- Feed Intake, BWG-body weight gain, FCR- feed conversion ratio,NC-Negative control, BD- Basal Diet (0ppmbeta-glucans)

4.2.2 Assessment of performance at finisher phase of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans

The effect of varied levels of beta-glucans inclusion on performance at finisher phase of broiler chickens fed aflatoxin-contaminated diets is presented in Table 4.3. Feed intake (g/bird) of birds did not show any variation by varied levels of beta-glucans inclusion. It ranged from 246.03±10.06g/bird in BD375 to 311.18±94.74g/bird in NC diet. Higher and significant ($P<0.05$) BWG (g/bird) of 420.94±19.63 was recorded in birds fed NC diet relative to birds fed BD (260.73±176.1) but was similar ($P>0.05$) to the BWG of birds fed the other treatment diets (BD125 - BD625). The FCR of birds fed varied levels of beta-glucans showed no statistical difference ($P>0.05$) and it ranged from 0.66±0.02 (BD375) to 1.15±0.44 (BD).

Table 4.3 Effect of variedinclusion levels of beta-glucans on performance at finisher phase of broiler chicken offered aflatoxin-contaminated diet(4-6 weeks)

Parameters	NC	BD	Beta-glucansInclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
FI (g/bird)	311.18±94.74	248.26±49.16	287.01±21.54	256.14±60.00	246.03±10.06	294.10±22.50	279.13±67.51	31.34	0.70
BWG (g/bird)	420.94±19.63[a]	260.73±176.1[b]	352.50±45.16[ab]	393.66±63.36[ab]	370.48±19.26[ab]	350.77±35.68[ab]	274.39±69.00[ab]	45.69	0.20
FCR	0.75±0.24	1.15±0.44	0.83±0.18	0.67±0.21	0.66±0.02	0.85±0.12	1.06±0.32	0.15	0.22

[ab]Treatment means withinthe same row with unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level, FI- Feed Intake, BWG-body weight gain, FCR- feed conversion ratio, NC-Negative control, BD- Basal Diet (0ppm beta-glucans)

4.2.3 Assessment of performance of broiler chickenoffered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans from 0 to 42days

The effect of varied inclusion levels of beta-glucans on performance indices is presented in Table 4.4. Varied levels of beta-glucans addition did not have any effect of significance (P>0.05) on cumulative feed intake (CFI- g/bird), CBWG (g/bird) and FCR of birds fed aflatoxin-contaminated diets from 0 to 42 days feeding duration.

4.2.4 Haematology at starter phase of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans

The effect of varied levels of beta-glucans inclusion on haematology at starter phase of broilers fed aflatoxin-contaminated feed is shown in Table 4.5. The PCV (%), Hb (g/dL), WBC ($x10^9$/L), heterophils (%), lymphocytes (%) and eosinophils (%) were not affected (P>0.05) significantly by varying the levels of beta-glucans addition and their values ranged from 35.50±4.17 to 40.93±5.97; 11.40±1.35 to 13.51±1.69; 11.58±0.82 to 12.98±0.79; 29.60±7.93 to 32.40±6.17; 64.00±10.97 to 66.60±9.26 and 0.53±0.29 to 1.41±0.31, respectively. However, RBC ($x10^{12}$/L) value (3.41±0.59) recorded in birds fed BD was significantly (P<0.05)elevated in comparison to that of birds fed NC (2.72±0.43) diet but did not show any significant variations (P>0.05) in RBC values of birds fedother treatment diets. Higher (P<0.05) basophils (%) value (1.47±0.28) was recorded in birds fed BD250 as against (0.67±1.01) in birds fed BD625. The monocytes (%) value (1.79±0.32) in birds fed NC diet and (1.93±0.40) in birds fed BD375 were significantly (P<0.05)elevated, above that of birds fed other treatment rations. Lower (P<0.05) monocytes value (0.80±0.28) was noticed in birds fed BD500 diet, but similar to values of BD and BD625. Significantly increased (P<0.05) platelets count was recorded in birds fed NC diet (19.14±4.79), BD (18.51±3.21) and BD625 (18.85±3.06) as against (15.27±2.55) obtained in birds fed BD500 at starter phase.

Table 4.4 Effect of varied inclusion levels of beta-glucans on performance of broiler chicken offered aflatoxin-contaminated feed from 0to 42 days

Parameters	Beta-glucans Inclusion Rate (ppm)							SEM	P-value
	NC	BD	BD125	BD250	BD375	BD500	BD625		
CFI (g/bird)	1341.00±325.42	1091.00±158.38	1198.80±73.65	1107.20±151.04	1067.90±36.13	1277.60±71.79	1207.60±212.55	100.21	0.44
CBWG (g/bird)	636.29±29.09	486.22±114.60	577.21±48.20	591.50±53.68	586.70±24.12	569.70±23.30	484.93±68.60	49.42	0.32
FCR	2.09±0.44	2.41±0.68	2.09±0.30	1.88±0.32	1.82± 0.08	2.25±0.16	2.50±0.38	0.22	0.32

[ab]Treatment means withinthe same row with unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level,CFI- Cummulative Feed Intake, CBWG-Cumulativebody weight gain, FCR- feed conversion ratio, NC-Negative control, BD- Basal Diet (0ppm beta-glucans).

Table 4.5 Effect of variedinclusion levels of beta-glucans on haematology at starter phase of broiler chickenoffered aflatoxin-contaminated feed

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
PCV (%)	35.50±4.17	40.93±5.97	38.40±3.56	39.53±3.99	38.80±4.44	37.80±3.56	38.67±6.20	2.32	0.81
Hb (g/dL)	11.40±1.35	13.51±1.69	12.33±0.97	13.11±1.29	12.49±1.26	12.27±1.14	12.23±1.77	0.76	0.60
RBC ($x10^{12}$/L)	2.72±0.43^{b}	3.41±0.59^{a}	2.96±0.45^{ab}	3.08±0.41^{ab}	3.04±0.41^{ab}	2.93±0.48^{ab}	2.87± 0.61^{ab}	0.19	0.35
WBC ($x10^{9}$/L)	12.98±0.79	12.57±1.39	11.58±0.82	12.19±0.85	11.85±1.53	12.90±1.04	12.01±1.38	0.49	0.34
Platelets ($x10^{3}$/L)	19.14±4.79^{a}	18.51±3.21^{a}	16.74±2.26^{ab}	16.54±3.83^{ab}	17.40±1.11^{ab}	15.27±2.55^{b}	18.85±3.06^{a}	0.98	0.06
Heterophils (%)	30.43±8.46	31.21±7.55	29.60±7.93	31.53±4.97	32.13±9.27	32.40±6.17	31.33±8.37	1.64	0.91
Lymphocytes (%)	65.29±8.14	65.64±7.05	67.13±8.51	64.20±6.94	64.00±10.97	65.20±5.82	66.60±9.26	1.78	0.86
Eosinophils (%)	1.21±0.26	1.41±0.31	1.07±0.26	1.07±0.35	1.07±0.35	0.73±0.28	0.53±0.29	0.22	0.28
Basophils (%)	1.29±0.33^{ab}	1.14±0.35^{ab}	1.00±0.28^{ab}	1.47±0.28^{a}	0.87±0.35^{ab}	0.87±0.73^{ab}	0.67±1.01^{b}	0.25	0.28
Monocytes (%)	1.79±0.32^{a}	1.21±0.27^{bc}	1.27±0.36^{b}	1.67±0.41^{ab}	1.93±0.40^{a}	0.80±0.28^{c}	0.87±0.37^{bc}	0.27	0.02

^{abc}Treatment means withinthe same row with unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level,PCV- packed cell volume, Hb- Haemoglobin, RBC- Red blood cells, WBC- white blood cells, NC-Negative control, BD- Basal Diet (0ppm beta-glucans).

4.2.5 Haematology of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans at finisher phase

The effect of varied inclusion rate of beta-glucans on haematology at finisher phase of broilers fed aflatoxin-contaminated feed is shown in Table 4.6. The PCV (%), Hb (g/dL), RBC ($x10^{12}$/L), WBC ($x10^{9}$/L) and basophil (%) values were not statistically different ($P>0.05$) at finisher phase and their values ranged from 36.50±7.50 to 45.50±15.29; 12.02±2.46 to 14.88±5.07; 2.92±0.45 to 3.98±1.14; 11.65±1.39 to 14.67±4.34 and 0.83±0.31 to 1.33±0.82, respectively. Elevated ($P<0.05$) heterophil (%) value (34.50±1.87) was recorded in birds fed BD375diet compared with those birds fed the other treatment diets. However, birds fed BD250 (29.33±2.58) and BD500 (29.17±2.86)diets had lower and similar ($P<0.05$) heterophil values at finisher phase. Birds fed BD250 (66.50±4.09) and BD500 (68.33±2.07)diets had significantly elevated ($P<0.05$) lymphocytes (%) as against 61.00±2.28 recorded in birds fedBD375diet. Elevated ($P<0.05$) values of eosinophil (%) were also observed in birds fedBD125 (1.33±0.82) and BD375 (1.33±0.82) diets compared to BD500 (0.33±0.21)diet. Monocytes (%) of birds fed BD375 (2.33±0.42) was significantly higher ($P<0.05$) above that of birds fedBD500 (0.83±0.31) and BD625 (0.67±0.21) diets, but did not show significant variations ($P>0.05$) in birds fed other treatment diets. Increased and significant ($P<0.05$)platelets count ($x10^{3}$/L)of 21.73±4.31 was obtained in birds fedBD625 diet as against that of birds fed NC (19.20±7.47), BD250 (15.77±2.40), BD375 (14.83±2.56) and BD500 (14.33±2.54)diets but did not show any statistical differences ($P>0.05$) from the values obtained in birds fedother treatment diets (BD and BD125).

Table 4.6 Effect of varied inclusion levels of beta-glucans on haematology at finisher phase of broiler chicken offered aflatoxin-contaminated diet

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
PCV (%)	36.50±7.50	44.17±15.51	44.67±11.72	41.50±11.95	45.50±15.29	40.83±8.61	44.67±10.17	4.85	0.85
Hb (g/dL)	12.02±2.46	14.88±5.07	14.40±3.78	13.75±3.99	14.83±5.03	13.32±2.87	14.30±3.49	1.60	0.87
RBC ($x10^{12}$/L)	2.92±0.45	3.98±1.14	3.60±0.75	3.28±0.88	3.70±0.98	3.35±0.67	3.52±0.80	0.34	0.45
WBC ($x10^{9}$/L)	14.67±4.34	12.22±1.34	13.37±2.71	12.85±1.97	12.15±1.44	13.20±2.26	11.65±1.39	0.99	0.42
Platelets ($x10^{3}$/L)	19.20±7.47^{b}	19.63±5.00^{ab}	18.28±2.66^{ab}	15.77±2.40^{b}	14.83±2.56^{b}	14.33±2.54^{b}	21.73±4.31^{a}	1.73	0.04
Heterophils (%)	30.83±4.02^{b}	32.67±3.44^{ab}	30.17±4.07^{b}	29.33±2.58^{c}	34.50±1.87^{a}	29.17±2.86^{c}	33.50±2.17^{ab}	1.27	0.03
Lymphocytes (%)	65.17±5.12^{ab}	64.50±2.59^{ab}	65.33±4.72^{ab}	66.50±4.09^{a}	61.00±2.28^{b}	68.33±2.07^{a}	64.50±2.66^{ab}	1.45	0.05
Eosinophils (%)	1.00±0.63^{ab}	1.00±0.89^{ab}	1.33±0.82^{a}	1.00±0.89^{ab}	1.33±0.82^{a}	0.33±0.21^{b}	0.50±0.34^{ab}	0.31	0.18
Basophils (%)	1.00±0.63	1.17±0.75	1.33±0.82	1.17±0.60	0.83±0.31	1.33±0.82	0.83±0.31	0.40	0.94
Monocytes (%)	2.00±1.41^{ab}	1.50±0.43^{ab}	2.00±0.37^{ab}	2.00±0.52^{ab}	2.33±0.42^{a}	0.83±0.31^{b}	0.67±0.21^{b}	0.42	0.05

^{abc} Treatment means within the same row with unidentical superscripts are distinctly different (P<0.05). SEM- Standard error of mean, P-value- probability level, PCV- packed cell volume, Hb- Haemoglobin, RBC- Red blood cells, WBC- white blood cells, NC-Negative control, BD- Basal Diet (0ppm beta-glucans)

4.2.6 Serum biochemical indices of broiler chickenoffered aflatoxin-contaminated feed with varied inclusion levels of beta-glucansat starter phase

The effect of varied inclusion level of beta-glucans on serum biochemical indices of broiler chickens offered aflatoxin-contaminated feed at starter phase is presented in Table 4.7. The AST (U/L), ALB (g/dL), TP (g/dL), GLB (g/dL) and A/G values were not affected (P>0.05) byvaried levels of beta-glucansinclusion and their values ranged from 211.08±5.18 to 232.33±22.02;0.81±0.23 to 1.15±0.45; 2.62±0.23 to 3.03±0.19; 1.80±0.14 to 2.02±0.19and 0.42±0.18 to 0.62±0.25, respectively. However, ALT (U/L) value of birds fed BD625 diet (30.83±3.39) was statistically higher (P<0.05) in comparison to that of birds fed NC (25.75±3.97), BD125 (24.60±4.07) and BD375 (27.36±3.61) diets. Birds fed NC ration had significantly reduced (P<0.05) ALP (U/L) value (145.25) below that of birds fed the other treatment diets. However, no significant variations in ALP values (P>0.05) were observed in birds fed BD (228.64±63.44 U/L) and those fed aflatoxin-contaminated diets having BD125 (224.20±43.80 U/L) and BD375 (234.27±24.42 U/L).

Table 4.7 Effect of varied inclusion levels of beta-glucans on serum biochemical indices at starter phase of broiler chicken offered aflatoxin-contaminated diet

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
AST (U/L)	211.08±5.18	229.91±21.50	212.00±31.18	210.58±54.36	226.64±20.23	232.33±22.02	223.67±20.01	7.61	0.14
ALT (U/L)	25.75±3.97^{bc}	29.09±3.78^{ab}	24.60±4.07^{c}	29.17±3.50^{ab}	27.36±3.61^{b}	29.75±3.22^{ab}	30.83±3.39^{a}	1.32	0.003
ALP (U/L)	145.25±29.24^{c}	228.64±63.44^{b}	224.20±43.80^{b}	245.92±60.81^{ab}	234.27±24.42^{b}	277.25±27.08^{a}	255.92±23.89^{ab}	13.44	<0.0001
TP (g/dL)	2.90±0.39	3.00±0.43	2.62±0.23	2.73±0.18	2.66±0.21	3.03±0.19	2.78±0.41	0.16	0.27
ALB (g/dL)	0.89±0.32	1.15±0.45	1.12±0.20	0.81±0.23	0.84±0.23	1.05±0.26	0.91±0.39	0.12	0.23
GLB (g/dL)	2.02±0.19	1.85±0.04	1.80±0.14	1.93±0.18	1.83±0.16	1.98±0.12	1.87±0.10	0.09	0.55
A/G	0.45±0.14	0.62±0.25	0.45±0.14	0.42±0.18	0.43±0.16	0.50±0.19	0.48±0.23	0.07	0.39

^{abc}Treatment means withinthe same row with unidentical superscripts are distinctly different (P<0.05). SEM- Standard error of mean, P-value- probability level, AST- Aspartate transferase, ALT- Alanine transferase, ALP- Alkaline phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, A/G- Albumin: Globulin, SEM- Standard error of mean, NC-Negative control, BD- Basal Diet (0ppm beta-glucans)

4.2.7 Serum biochemical indices of broiler offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans at finisher phase

The effects of varied inclusion rate of beta-glucans on serum biochemical parameters at finisher phase of broiler chickens fed aflatoxin-contaminated diet is presented in Table 4.8. The AST (U/L) value observed in birds fed BD500 (235.33±25.39) diet had significant elevation ($P<0.05$) above that of birds offered NC (211.17±3.71), BD (213.40±2.88), BD125 (214.80±2.28) and BD250 (212.17±2.64) diets. Elevated ($P<0.05$) ALT (U/L) value of 32.33±4.03 was recorded in birds fed BD625 diet compared to birds fed NC (24.67±4.37) and BD125 (24.60±7.76) diets. Birds fed BD500 (280.50±20.42) and BD625 (275.33±41.59) diets had significantly increased ($P<0.05$) ALP (U/L) values than in birds fed the other treatment rations. However, varying the levels of beta-glucans inclusion elicit no significant effect ($P>0.05$) on TP (g/L), ALB (g/L), GLB (g/L) and A:G. Their values ranged from 2.62±0.08 to 3.37±1.02; 0.80±0.07 to 1.30±0.43; 1.82±0.04 to 2.07±0.61 and 0.42±0.04 to 0.66±0.35, respectively.

Table 4.8 Effect of variedinclusion levels of beta-glucans on serum biochemical indices at finisher phase of broiler chicken offered aflatoxin-contaminated diet

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
AST (U/L)	211.17±3.71^{b}	213.40±2.88^{b}	214.80±2.28^{b}	212.17±2.64^{b}	220.60±19.69^{b}	235.33±25.39^{a}	220.50±18.68^{ab}	6.14	0.09
ALT (U/L)	24.67±4.37^{b}	28.40±2.88^{ab}	24.60±7.76^{b}	29.00±2.00^{ab}	27.00±3.61^{ab}	27.50±3.39^{ab}	32.33±4.03^{a}	1.81	0.06
ALP (U/L)	168.33±39.13^{c}	218.40±59.07^{b}	224.20±39.39^{b}	251.00±33.85^{ab}	224.40±5.77^{b}	280.50±20.42^{a}	275.33±41.59^{a}	15.84	0.0002
TP (g/dL)	3.00±0.65	3.12±0.42	3.18±0.66	2.73±0.43	2.62±0.08	3.37±1.02	2.87±0.50	0.26	0.43
ALB (g/dL)	1.00±0.43	1.24±0.49	1.12±0.41	0.88±0.34	0.80±0.07	1.30±0.43	1.00±0.43	0.17	0.36
GLB (g/dL)	2.00±0.71	1.88±0.31	2.06±0.33	1.87±0.19	1.82±0.04	2.07±0.61	1.87±0.14	0.18	0.91
A/G	0.55±0.35	0.66±0.35	0.46±0.15	0.48±0.13	0.42±0.04	0.57±0.10	0.50±0.20	0.09	0.68

^{abc}Treatment means withinthe same row with unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level, AST- Aspartate transferase, ALT- Alanine transferase, ALP- Alkaline phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, A/G- Albumin: Globulin, SEM- Standard error of mean, NC-Negative control, BD- Basal Diet (0ppm beta-glucans)

4.2.8 Aflatoxins concentration and residual in liver of broiler chicken offered aflatoxin-contaminated ration with varied inclusion levels of beta-glucans

The effect of varied inclusion rate of beta-glucans on aflatoxins concentration and residual in liver of broiler chickens is shown in Table 4.9. Birds fed BD had significantly higher ($P<0.05$) aflatoxins concentration (µg/kg) in their liver samples (2.57±0.06µg/kg) than in birds fed other treatment diets. Birds offered NC (0.23±0.06µg/kg) and BD250 (0.50±0.44µg/kg) diets had comparable ($P>0.05$) liver aflatoxin concentrations and both were significantly lower ($P<0.05$) compared to birds offered other treatment diets. However, mean liver weight of birds did not show any significant differences ($P>0.05$) by varying the levels of beta-glucans inclusion. However, expressing the liver weight relative to the average body weight showed that birds fed BD (4.11±0.02%) had significantly higher relative liver weight than the other dietary treatments while all the mitigated diets produced significantly reduced relative liver weight compared to birds fed on BD.Liver residual aflatoxin level reduction was significant ($P<0.05$) in the liver of birds offered NC (0.01±0.004µg), BD125 (0.02±0.01µg), BD250 (0.01±0.01µg), and BD375 (0.01±0.01µg) diets, than in birds offered other beta-glucans containing diets. However, birds fed BD had 0.06±0.01µg liver residual aflatoxins, significantly higher ($P<0.05$) above those of birds offered the other treatment rations.

Table 4.9 Effect of varied inclusion levels of beta-glucans on aflatoxins concentration and residual in the liver of broiler chicken offered aflatoxin-contaminated feeds

Parameters	NC	BD	Beta-glucans Inclusion Rate (ppm)					SEM	P-value
			BD125	BD250	BD375	BD500	BD625		
Aflatoxins conc. (µg/kg)	0.23±0.06^{e}	2.57±0.06^{a}	1.20±0.17^{c}	0.50±0.44^{de}	0.73±0.40^{d}	1.83±0.75^{ab}	1.43±0.59^{b}	0.25	<0.0001
Ave. Liver wt./bird (g)	25.92±6.29	21.82±2.33	19.08±2.10	20.90±2.67	18.42±1.45	23.03±6.09	18.00±6.22	3.44	<0.0001
Liver weight rel. to BW (%)	3.82±0.22^{b}	4.11±0.02^{a}	3.07±0.04^{d}	3.30±0.05^{c}	2.88±0.06^{e}	3.75±0.26^{b}	3.40±0.09^{c}	0.07	<0.0001
Residual aflatoxins (µg)	0.01±0.004^{c}	0.06±0.01^{a}	0.02±0.01^{c}	0.01±0.01^{c}	0.01±0.01^{c}	0.04±0.02^{ab}	0.02±0.01^{bc}	0.006	<0.0001

^{abcde} Treatment means within the same row with unidentical superscripts are distinctly different (P<0.05), SEM- Standard error of mean, P-value- probability level, conc.- concentration;rel.- relative; BW- Body Weight; NC-Negative control; BD- Basal Diet (0ppm beta-glucans), P-value- probability level, conc.- concentration, Ave.- Average, wt.- weight

4.2.9 Ileal digesta aflatoxins concentration of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans

The response of broilers to varied inclusion levels of beta-glucans on ileal digesta aflatoxins concentration is presented in Table 4.10. Birds fedBD375 (854.33±109.75ppb) diet had higher ($P<0.05$) aflatoxin B_1 level in their ileal digesta, significantly above those of birds offered other treatment diets. However, birds offered NC (0.00±0.00ppb) and BD (13.33±5.77ppb) diets had significantly reduced ($P<0.05$) aflatoxin B_1 concentration in their ileal digesta.Total aflatoxins concentration in the ileal digesta of birds fedBD375(929.00±122.29ppb) diet was distinctly higher ($P<0.05$) above those of birds offered other treatment rations. The least total aflatoxins in ileal digesta of birds offered all the treatment diets containing aflatoxins was obtained in BD (13.33±5.77ppb), which was different distinctly ($P<0.05$) from those of birdsoffered other treatment diets, indicating that higher level of aflatoxins had been absorbed.

Table 4.10 Effect of varied inclusion levels of beta-glucans on ileal digesta aflatoxin-concentration in broiler chicken

Parameters	Beta-glucans Inclusion Rate (ppm)							SEM	P-value
	NC	BD	BD125	BD250	BD375	BD500	BD625		
Aflatoxin B1 (ppb)	0.00±0.00^{f}	13.33±5.77^{f}	145.00±47.32^{e}	586.00±90.44^{b}	854.33±109.75^{a}	382.00±16.00^{c}	262.33±22.37^{d}	33.17	<0.0001
Aflatoxin B2 (ppb)	0.00±0.00^{e}	0.00±0.00^{e}	14.33±3.21^{de}	45.67±12.58^{b}	74.67±15.63^{a}	34.67±8.14^{bc}	27.00±6.56^{c}	4.99	<0.0001
Total (ppb)	0.00±0.00^{f}	13.33±5.77^{f}	159.33±49.24^{e}	631.67±102.65^{b}	929.00±122.29^{a}	416.67±23.71^{c}	289.33±26.03^{d}	37.28	<0.0001

^{abc}Treatment means within the same row with unidentical superscripts differed greatly (P<0.05), SEM- Standard error of mean, P-value- probability level, NC-Negative control, BD- Basal Diet (0ppm beta-glucans)

4.2.10 Varied inclusion levels of beta-glucans effect on total aflatoxins absorption within the digestive tract of broiler chickenoffered aflatoxin-contaminated feed

The effects of varied inclusion levels of beta-glucans on aflatoxins absorption in broiler chickens fed aflatoxin-contaminated ration is presented in Table 4.11. It was noted that variedinclusion rates of beta-glucans had no marked effect on average daily feedconsumption (g/b/d). However, birds fed NC diet had significantly lower ($P<0.05$) total daily aflatoxins intake average (0.00±0.00µg/b/d) below those of birds offered the other treatment rations. No statistical differences were observed in estimated ileal digesta (g/b/d) in all the treatment rationss. Significantly elevated ($P<0.05$) concentration of Total Aflatoxins in Ileal Digesta (TAID) was recorded in birds fed BD375 (5.44±0.54µg/kg) diet than in birds offered the other treatment diets. Birds offered NC (0.00±0.00µg/kg) and BD (0.08±0.02µg/kg) rations had comparable ($P>0.05$) reduction in quantity of total aflatoxins in their ileal digesta. Significantly higher percentage ($P<0.05$) of adsorbed aflatoxins was obtained in birds fed BD375 (79.30±10.38%) diet compared to values obtained in birds fed the other treatment diets. Birds fed NC (0.00±0.00%) and BD (1.14±0.48%) rations had similar ($P>0.05$) level of adsorbed total aflatoxins, and their values were reduced distinctly ($P<0.05$), compared to those of birds offered other treatment rations. However, it was observed that birds offered BD250 (53.86±8.81%) diet had higher level ($P<0.05$) of adsorbed or unabsorbed total aflatoxins significantly above those of birds fed BD500 (35.56±2.01%) and BD625 (24.61±2.21%) diets. It wasalso observed that birds fed BD (6.93±1.03µg/b/d) and BD125 (6.65±0.41µg/b/d) diets had significantly higher ($P<0.05$) quantity of absorbed total aflatoxins relative to those of birds fed the other treatment diets. Distinctly lower values of relative quantity of Total Aflatoxins Absorbed (TAAb) were obtained in birds fed BD250 (3.28±0.96µg/b/d) and BD375 (1.42±0.77µg/b/d) diets, and both had significant ($P<0.05$) reduction compared to birds offered the other contaminated diets. Birds fed BD375 had the least ($P<0.05$) quantity of relative TAAb. Higher percentage of absorbed aflatoxins ($P<0.05$) was obtained in birds fed BD (98.86±0.48%) ration compared to those of birds offered the other treatment rations. However, lower and the least percentage of absorbed aflatoxins was recorded in birds fed BD375 (20.69±8.47%) diet, significantly reduced ($P<0.05$) than in birds offered the other contaminated rations containing beta-glucans.

Table 4.11 Effect of varied inclusion levels of beta-glucans on total aflatoxins absorption in the GIT of broiler chicken offered aflatoxin-contaminated feeds

			Beta-glucans Inclusion Rate (ppm)						
Parameters	NC	BD	BD125	BD250	BD375	BD500	BD625	SEM	P-value
Ave. DFI (g/b/d)	31.93±7.74	25.98±3.76	28.54±1.75	26.36±3.59	25.43±0.86	30.42±1.71	28.75±5.06	2.39	0.44
Ave. DTAI (µg/b/d)	0.00±0.00^{b}	7.01±1.01^{a}	7.70±0.47^{a}	7.11±0.97^{a}	6.86±0.23^{a}	8.21±0.46^{a}	7.76±1.37^{a}	0.45	<0.0001
Est. Ileal digesta (g/b/d)	7.34±1.78	5.96±0.87	6.57±0.40	6.06±0.83	5.85±0.19	6.99±0.39	6.61±1.17	0.55	0.45
Ileal DTAC (µg/kg)	0.00±0.00^{f}	13.33±5.77^{f}	159.33±49.24^{e}	632.00±102.72^{b}	929.00±122.29^{a}	416.67±23.71^{c}	289.33±26.03^{d}	37.29	<0.0001
TAID/Bird (µg/b/d)	0.00±0.00^{f}	0.08±0.02^{f}	1.05±0.36^{e}	3.83±0.47^{b}	5.44±0.54^{a}	2.92±0.33^{c}	1.91±0.41^{d}	0.21	<0.0001
Adsorbed TA (%)	0.00±0.00^{f}	1.14±0.48^{f}	13.64±4.15^{e}	53.86±8.81^{b}	79.30±10.38^{a}	35.56±2.01^{c}	24.61±2.21^{d}	3.18	<0.0001
Rel. TAAb (µg/b/d)	0.00±0.00^{e}	6.93±1.03^{a}	6.65±0.41^{a}	3.28±0.96^{c}	1.42±0.77^{d}	5.29±0.16^{b}	5.85±0.99^{ab}	0.42	<0.0001
Absorbed TA (%)	0.00±0.00^{g}	98.86±0.48^{a}	86.36±4.14^{b}	46.13±8.81^{e}	20.69±8.47^{f}	64.43±2.02^{d}	75.39±2.21^{c}	2.89	<0.0001

^{abcdefg}Treatment means within the same row with unidentical superscripts are distinctly different (P<0.05), Ave.- Average, DFI- Daily Feed Intake, DTAI- Daily Total Aflatoxins Intake, Est.- Estimated, DTAC- Digesta Total Aflatoxins Concentration, TAID- Total Aflatoxins in Ileal digesta, TA- Total Aflatoxins, Rel. TAAb- Relative Total Aflatoxins Absorbed, SEM- Standard error of mean, NC-Negative control, BD- Basal Diet (0ppm beta-glucans), P-value- probability level

4.2.11 Relationship between varied inclusion levels of beta-glucans and the percentage of absorbed aflatoxins in the gastrointestinal tract (GIT) of broiler chicken

The correlation graph between varied inclusion levels ofbeta-glucans and the percentage of absorbed aflatoxins in the gastrointestinal tract (GIT) of broiler chicken offered aflatoxin-contaminated feeds is presented in Figure 4.1. It was observed that the lowest percentage of absorbed total aflatoxins (20.69±8.47%) was recorded at 375ppm beta-glucans inclusionIt was also observed that further increase in the level of beta-glucans inclusion beyond 375ppm produced an increase in the quantity of absorbed aflatoxins. At 500ppm of beta-glucans inclusion, absorbed total aflatoxins was (64.43±2.02%) while at 625ppm beta-glucans addition, absorbed total aflatoxins was (75.39±2.21%). Birds feddiets containing 250 and 375ppmbeta-glucans inclusion had the lowest levels of absorbed total aflatoxins of 46.13±8.81% and 20.69±8.47%, respectively. The R^2 value of 0.42 showed that aflatoxins absorption from the GIT in the current study is dependent on the presence of beta-glucans inclusion up to 375ppm before rising up again at 500 and 625ppm inclusion levels.

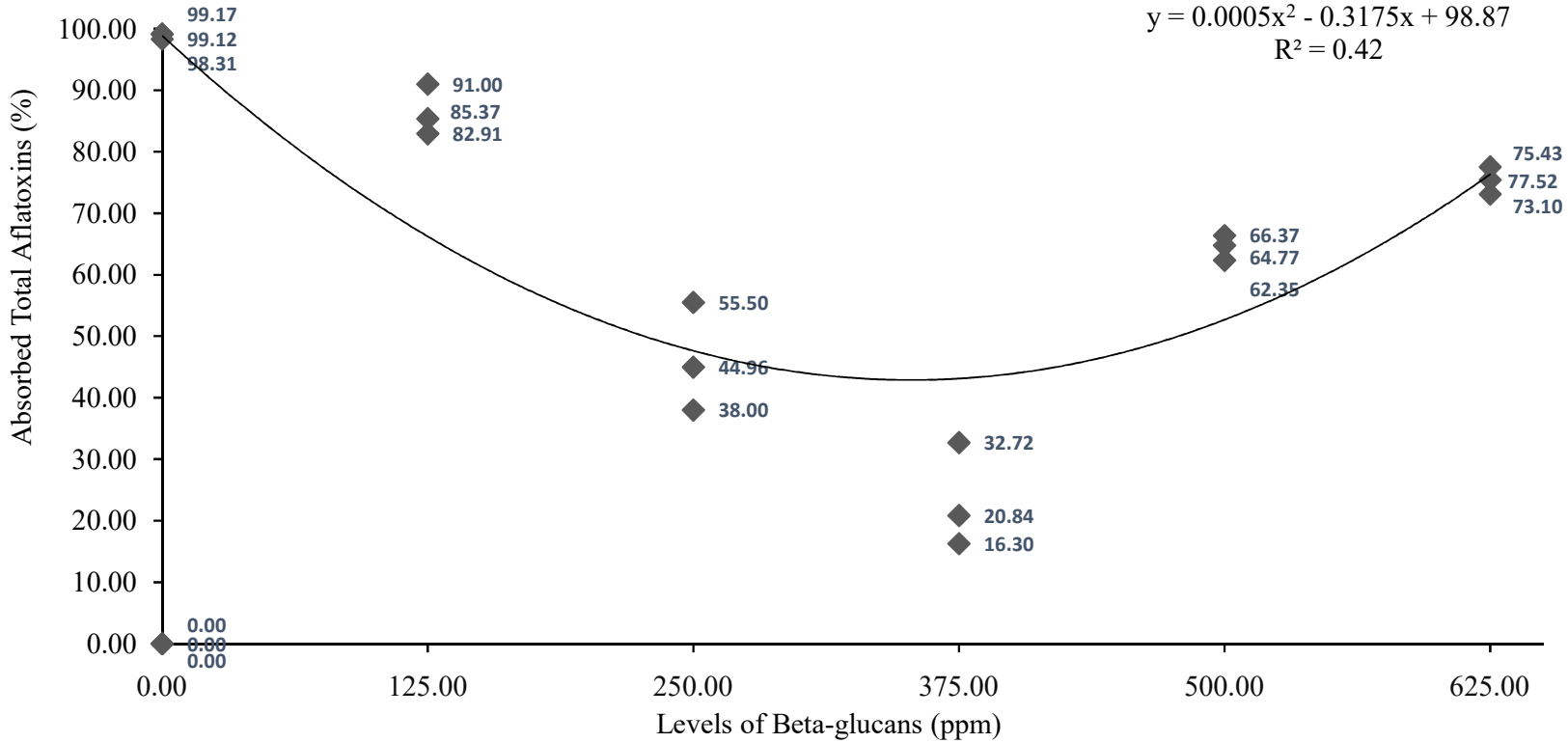


Figure 4.1 Relationship between varied levels of beta-glucans inclusion and absorbed aflatoxins in the GIT of broiler chicken offered aflatoxin-contaminated poultry feeds

4.3 Study two: Effects of supplemental antioxidants and vitamin K in broiler chicken offered aflatoxin-contaminated rations

Specific objectives b and c in section 1.3 were achieved with study two (see section 3.3) and the results obtained were presented in section 4.3.

4.3.1 Performance assessment of broiler chicken offered aflatoxin-contaminated rations with different combinations of supplemental dietary antioxidantsand vitamin K at starter phase

Effect of different combinations of supplemental dietary antioxidants and vitamin K on performance of broiler chicken offered aflatoxin-contaminated feeds at starter phase is presented in Table 4.12. Feed intakeof birds offeredNC diet (550.43±24.96g/bird) was higher ($P<0.05$) significantly above those of birds fed the other treatment diets. However, there were no significant variations ($P>0.05$) in feed intake of birds fedBD (343.96±12.41g/bird), R1 (356.22±22.49g/bird), R2 (372.26±23.95g/bird), R3 (374.65±25.76g/bird) and R4 (389.32±53.23g/bird) diets respectively. The BWG of birds fedNC (301.73±4.71g/bird) was distinctly higher ($P<0.05$) than in birds offered the other treatment diets. However, reduced BWG recorded in birds fed BD (141.43±12.16g/bird), R1 (150.57±42.99g/bird) and R2 (148.10±15.41g/bird) were significantlyreduced($P<0.05$) those of birds offeredNC (301.73±4.71g/bird), R3 (237.06±20.12g/bird) and R4 (249.55±29.90g/bird)rations. Birds fed diets R3 (1.58±0.04) and R4 (1.59±0.42) had similar FCR, and both treatments also had significantly ($P<0.05$) lower and better FCR, compared to birds offered BD (2.44±0.13), R1 (2.49±0.70), and R2 (2.53±0.31), but did not show any significant variations ($P>0.05$) when compared to birds offered R1 (1.82±0.09).

Table 4.12 Performance assessment of broiler chicken offered aflatoxin-contaminated diets with different combinations of supplemental dietary antioxidants and vitamin K at starter phase

	Dietary treatments							
Parameters	NC	BD	R1	R2	R3	R4	SEM	P-value
FI (g/bird)	550.43±24.96[a]	343.96±12.41[b]	356.22±22.49[b]	372.26±23.95[b]	374.65±25.76[b]	389.32±53.23[b]	17.25	<0.0001
BWG (g/bird)	301.73±4.71[a]	141.43±12.16[c]	150.57±42.99[c]	148.10±15.41[c]	237.06±20.12[b]	249.55±29.90[b]	14.05	<0.0001
FCR	1.82±0.09[bc]	2.44±0.13[ab]	2.49±0.70[ab]	2.53±0.31[a]	1.58±0.04[c]	1.59±0.42[c]	0.21	0.01

[abc] Treatment means within the same row having unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level, FI-feed intake, BWG-body weight gain, FCR- feed conversion ratio, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.3.2 Performance assessment of broiler chicken offered aflatoxin-contaminated rations with different combinations ofsupplementaldietary antioxidants and vitamin Kat finisher phase

The effect of different combinations of supplemental dietary antioxidants and vitamin K on performance of broiler chicken offered aflatoxin-contaminated feedsat finisher phase is shown inTable 4.13. Feed intake of birds fed R1 (1,424.92±30.91g/bird) increased distinctly ($P<0.05$) than in birds offered other treatment rations. Birds fed BD (1,008.44g/bird) had reduced ($P<0.05$) feed intake significantly below those of birds fedNC (1,424.92±30.91g/bird), R2 (1,184.87±102.64g/bird), R3 (1,194.38±70.89g/bird) and R4 (1,116.75±52.65g/bird). Higher BWG was observed in birds fed NC diet (798.10±50.76g/bird) distinctly ($P<0.05$) above those of birds offered the other treatment rations. The least and significantly reduced ($P<0.05$) BWG was obtained in birds fed BD (473.94±106.12g/bird), but was comparable ($P>0.05$) to those of birds fed R1 (532.89±36.76g/bird), R2 (572.07±154.74g/bird) and R3 (632.40±47.98g/bird). However, the BWG of birds fed R4 (648.32±11.83g/bird) increasedgreatly ($P<0.05$) compared to birds offeredBD. The FCR of birds at finisher phase did not show any variations of significance among birds offered the different treatment diets, and its values ranged from 1.72±0.06 in R4to 2.18±0.35 in BD.

Table 4.13 Performance assessment of broiler chicken offered aflatoxin-contaminated diets with different combinations of supplemental dietary antioxidants and vitamin K at finisher phase

	Dietary treatments							
Parameters	NC	BD	R1	R2	R3	R4	SEM	P- value
FI (g/bird)	1424.92±30.91[a]	1008.44±62.01[c]	1081.25±61.98[bc]	1184.87±102.64[b]	1194.38±70.89[b]	1116.75±52.65[b]	38.71	<0.0001
BWG (g/bird)	798.10±50.76[a]	473.94±106.12[c]	532.89±36.76[bc]	572.07±154.74[bc]	632.40±47.98[bc]	648.32±11.83[b]	48.06	0.01
FCR	1.79±0.11	2.18±0.35	2.03±0.13	2.15±0.46	1.89±0.03	1.72±0.06	0.14	0.19

[abc]Treatment means within the same row having unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level, FI- feed intake, BWG-body weight gain, FCR- feed conversion ratio, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.3.3 Performance assessment of broiler chicken offered aflatoxin-contaminated rations with different combinations of supplemental dietary antioxidants and vitamin K from 0 to 42 days

Effect of different combinations of supplemental dietary antioxidants and vitamin K on performance of broiler chickens offered aflatoxin-contaminated diets from 0 to 42 days is shown in Table 4.14. Higher and significant ($P<0.05$) feed intake of (1,975.35±55.87g/bird) was recorded in birds fed NC relative to birds fed the other treatment diets. Birds fed BD (1,352.40±61.39g/bird) had the least and significantly ($P<0.05$) reduced feed intake compared to birds fed other treatment diets but was not different ($P>0.05$) significantly from birds offered R1 (1,437.47±81.12g/bird). No statistical differences ($P>0.05$) were recorded in feed intake of birds fed R1 (1,437.47±81.12g/bird),R2 (1,557.13±102.61g/bird), R3 (1,569.04±54.28g/bird) and R4 (1,506.06±93.76g/bird). Average BWG was observed to be significantly higher ($P<0.05$) in birds offered NC ration (1,099.83±46.08g/bird) contrary to those of birds offered the other treatment rations. Birds offered BD (615.37±107.21g/bird) and R1 (683.46±79.58g/bird) had distinctly ($P<0.05$) reduced BWG below those of birds offered other treatment rations.The FCR of birds fedBD (2.33±0.29) and R2 (2.23±0.42) were higher greatly ($P<0.05$) above those of birds offered R4 (1.68±0.14) which was the least value and the preferred. However, even though the FCR of birds offered R4 (1.68±0.14) was the least, it did not show any significant variation ($P>0.05$) in comparison to birds offered R3 (1.81±0.02), NC (1.79±0.08) and R1 (2.12±0.0.21).Mortality recorded in birds fedNC (6.67±5.77%) was reduced significantly ($P<0.05$)below those birds offered other treatment diets. Birds fed R2 (53.33±28.87%) and R4 (50.00±15.00%) rations had remarkably lower($P<0.05$) mortality compared to R1 (70.00±10.00%), but R1 is comparable ($P>0.05$) to mortality figure of 63.33±11.55% recorded in birds offered BD and R3 respectively.

Table 4.14 Performance assessment of broiler chicken offered aflatoxin-contaminated diets with different combinations of supplemental dietary antioxidants and vitamin K from 0 to 42 days

Parameters	Dietary treatments						SEM	P- value
	NC	BD	R1	R2	R3	R4		
FI (g/bird)	1975.35±55.87^{a}	1352.40±61.39^{c}	1437.47±81.12^{bc}	1557.13±102.61^{b}	1569.04±54.28^{b}	1506.06±93.76^{b}	44.56	<0.0001
BWG (g/bird)	1099.83±46.08^{a}	615.37±107.21^{d}	683.46±79.58^{d}	720.17±169.56^{cd}	869.47±38.71^{bc}	897.87±35.95^{b}	53.48	0.0004
FCR	1.79±0.08^{ab}	2.23±0.29^{a}	2.12±0.21^{ab}	2.23±0.42^{a}	1.81±0.02^{ab}	1.68+0.14^{b}	0.14	0.04
Mortality (%)	6.67±5.77^{c}	63.33±11.55^{ab}	70.00±10.00^{a}	53.33±28.87^{b}	63.33±11.55^{ab}	50.00±15.00^{b}	7.93	0.001

^{abc}Treatment means within the same row having unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P-value- Probability level, FI- feed intake, BWG-body weight gain, FCR- feed conversion ratio, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.3.4 Parameters of haematology of broiler chicken offered aflatoxin-contaminated poultry feed with different combinations of supplemental dietary antioxidants and vitamin K at starter phase

Effect of different combinations of supplemental dietary antioxidants and vitamin K on haematology of broiler chicken offered aflatoxin-contaminated rations at starter phase is presented in Table 4.15. The PVC value was higher ($P<0.05$) significantly in birds fed BD (42.38±5.15%) in comparison to birds fed R3 (34.56±5.77%), but did not differ statistically ($P>0.05$) from those recorded in bird fed other treatment diets.Birds offered BD had higher Hb (14.13±1.72g/dL) value distinctly ($P<0.05$) above that of birds offered R3 (11.53±2.09g/dL). However, no statistical variations ($P>0.05$) were seen in Hb concentrations of birds fed NC (12.37±2.34g/dL), R1 (13.11±2.76g/dL), R2 (12.47±1.56g/dL), and R4 (13.04±1.82g/dL). The RBC ($x10^{12}$/L) of birds offered BD (3.65±0.14) was markedly higher ($P<0.05$) contrary to that of birds fed R3 (3.43±0.19).The WBC ($x10^{9}$/L), platelets ($x10^{3}$/L), lymphocytes (%), heterophils (%), monocytes (%), eosinophils (%), and basophils (%) values did not reveal any differences of significance ($P>0.05$) from one another across the dietary treatments and their values ranged from 15.44±2.78 (NC) to 17.16±2.08 (BD); 14.42±2.29 (R3) to 19.79±6.50 (R4); 64.44±5.15 (R3) to 70.38±4.14 (BD); 22.63±3.58 (BD) to 27.56±5.25 (R3); 2.67±0.87 (R4) to 3.44±1.234 (R3); 3.63±1.85 (BD) to 4.78±1.20 (R4) and 0.00±0.00 (R4) to 0.25±0.46 (BD), respectively.

Table 4.15 Haematology of broilers offered aflatoxin-contaminated poultry feed with different combinations of supplemental dietary antioxidants and vitamin K at starter phase

Parameters	Dietary treatments						SEM	P-value
	NC	BD	R1	R2	R3	R4		
PCV (%)	37.44±7.25^{ab}	42.38±5.15^{a}	39.44±8.06^{ab}	37.67±4.41^{ab}	34.56±5.77^{b}	39.22±5.38^{ab}	2.19	0.22
Hb (g/dL)	12.37±2.34^{ab}	14.13±1.72^{a}	13.11±2.76^{ab}	12.47±1.56^{ab}	11.53±2.09^{b}	13.04±1.82^{ab}	0.75	0.24
RBC (x10^{12}/L)	3.49±0.18^{ab}	3.65±0.14^{a}	3.53±0.31^{ab}	3.57±0.12^{ab}	3.43±0.19^{b}	3.61±0.13^{ab}	0.07	0.21
WBC (x10^{9}/L)	15.44±2.78	17.16±2.08	15.69±3.60	15.58±1.48	15.87±3.38	17.13±1.85	0.95	0.63
Platelets (x10^{3}/L)	16.27±6.04	17.34±3.24	18.28±5.03	16.45±6.57	14.42±2.29	19.79±6.50	1.80	0.35
Lymphocytes (%)	66.33±6.22	70.38±4.14	68.00±6.76	66.00±3.79	64.44±5.15	67.22±5.65	1.93	0.37
Heterophils (%)	26.33±6.96	22.63±3.58	24.89±7.57	26.83±3.76	27.56±5.25	25.33±5.94	2.05	0.61
Monocytes (%)	2.89±0.78	3.13±1.25	2.78±1.48	3.00±0.89	3.44±1.24	2.67±0.87	0.39	0.74
Eosinophils (%)	4.22±1.39	3.63±1.85	4.11±1.27	4.00±1.41	4.33±2.06	4.78±1.20	0.55	0.77
Basophils (%)	0.22±0.44	0.25±0.46	0.22±0.44	0.17±0.41	0.22±0.44	0.00±0.00	0.14	0.79
H:L	0.41±0.15	0.32±0.07	0.38±0.17	0.41±0.08	0.44±0.12	0.41±0.11	0.04	0.56

[abc]Treatment means within the same row with unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P-value- Probability level, PCV- Packed Cell Volume, Hb- Haemoglobin, RBC- Red Blood Cell, WBC- White Blood Cell, H:L- Heterophils to Lymphocytes ratio, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.3.5 Parameters of haematology of broiler chicken offered aflatoxin-contaminated poultry feed with different combinations of supplemental dietary antioxidants and vitamin K at finisher phase

The effect of different combinations of supplemental dietary antioxidants and vitamin K on haematology of broiler chickens fed aflatoxin-contaminated feed at finisher phase is presented in Table 4.16. The PCV (%), Hb (g/dL), RBC ($x10^{12}$/L), WBC ($x10^{9}$/L), platelets ($x10^{3}$/L), lymphocytes (%), heterophils (%) and basophils (%) of birds fed different combinations of supplemental dietary antioxidants and vitamin K did not show any variations of significance ($P>0.05$). Their values ranged from 29.67±6.78 (R4) to 35.00±7.71 (BD); 9.61±2.29 (R4) to 11.58± (BD); 3.13±0.93 (R4) to 3.48±0.32 (NC); 13.96±0.81 (R2) to 15.53±1.99 (BD); 17.69±7.08 (BD) to 22.24±6.59 (R3); 60.67±6.28 (R4) to 66.25±4.98 (BD); 29.33±6.28 (R3) to 32.56±6.69 (R4) and 0.00±0.00 (R3) to 0.33±0.50 (R4), respectively among the dietary treatments. However, significantly elevated ($P<0.05$) monocyte value was observed in birds offered R3 (3.89±0.93%) in comparison to that of birds offered NC ration (2.56±0.88%), but was not differed statistically ($P>0.05$) from other dietary treatments. Eosinophils of birds offered R4 (4.63±1.41%) was increased distinctly ($P<0.05$) above that of birds offered NC ration (2.78±1.39%). However, birds offered BD (3.50±12.07%), R1 (3.63±1.77%), R3 (3.22±1.39%) and R4 (3.33±1.32%) showed no significant variations ($P>0.05$) in their eosinophils' values at finisher phase.

Table 4.16 Haematology indices of broiler chicken offered aflatoxin-contaminated poultry feed with different combinations of supplemental dietary antioxidants and vitamin K at finisher phase

Parameters	Dietary treatments						SEM	P-value
	NC	BD	R1	R2	R3	R4		
PCV (%)	32.33±7.45	35.00±7.71	31.00±6.82	32.38±5.13	31.44±8.14	29.67±6.78	2.44	0.75
Hb (g/dL)	10.67±2.61	11.58±2.45	10.14±2.12	10.41±1.98	10.51±2.71	9.61±2.29	0.82	0.68
RBC ($x10^{12}$/L)	3.48±0.32	3.39±0.43	3.27±0.75	3.33±0.32	3.18±0.72	3.13±0.93	0.22	0.86
WBC ($x10^{9}$/L)	14.64±1.38	15.53±1.99	14.69±2.13	13.96±0.81	15.40±1.73	14.42±2.29	0.62	0.48
Platelets ($x10^{3}$/L)	19.47±6.92	17.69±7.08	19.04±4.39	19.33±7.30	22.24±6.59	18.03±4.25	2.13	0.71
Lymphocytes (%)	63.89±6.68	66.25±4.98	61.25±7.15	62.75±7.85	63.56±5.52	60.67±6.28	2.22	0.55
Heterophils (%)	31.67±6.16	29.38±8.98	31.88±6.59	29.88±8.36	29.33±6.28	32.56±6.69	2.48	0.89
Monocytes (%)	2.56±0.88^{b}	3.25±1.39^{ab}	3.00±1.31^{ab}	2.88±0.64^{ab}	3.89±0.93^{a}	3.11±1.54^{ab}	0.39	0.27
Eosinophils (%)	2.78±1.39^{b}	3.50±1.07^{ab}	3.63±1.77^{ab}	4.63±1.41^{a}	3.22±1.39^{ab}	3.33±1.32^{ab}	0.48	0.18
Basophils (%)	0.22±0.44	0.13±0.35	0.25±0.46	0.25±0.46	0.00±0.00	0.33±0.50	0.14	0.59
H:L	0.51±0.14	0.41±0.12	0.54±0.17	0.49±0.19	0.49±0.13	0.55±0.16	0.05	0.54

^{abc}Treatment means within the same row having unidentical superscripts are distinctly different ($P<0.05$). SEM- standard error of means, P-value- Probability level, PCV- Packed Cell Volume, Hb- Haemoglobin, RBC- Red Blood Cell, WBC- White Blood Cell, H:L- Heterophils to Lymphocytes ratio, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium.

4.3.6 Serum biochemical indices, lipid peroxidation and antioxidants profile of broiler chicken offered aflatoxin-contaminated feed with different combinations of supplemental dietary antioxidants and vitamin K

Table 4.17 presented the effect of different combinations of supplemental dietary antioxidants and vitamin K on serum biochemical indices, lipid peroxidation and antioxidant profile of broiler chicken offered aflatoxin-contaminated ration from 0 to 42 days. Aspartateaminotransferase (AST) values of birds were not affected statistically ($P>0.05$) among the different treatment diets, and its values ranged from 98.97±43.79U/L (R3) to 116.81±28.78U/L (BD). Alanine aminotransferase (ALT) value was elevated in birds offeredBD (14.95±11.11U/L) markedly ($P<0.05$) above that of birds offeredR4 (5.80±3.36U/L).Alkaline phosphatase (ALP) value of birds offered BD(215.59±25.01U/L) was elevated remarkably ($P<0.05$) in comparison to the other treatment rations. However, birds offered NC (76.88±26.08U/L) andR4 (77.56±22.10U/L) rations had reduced ALP values, greatly ($P<0.05$) below that of the other test rations except for diets R2 and R3, after 42 days feeding on aflatoxin-contaminated diets. Birds offered R3 (3.23±1.02g/dL) had elevated TP level, markedly ($P<0.05$) above that of birds offered R2 (2.49±0.45U/L), but did not show any variation of significance ($P>0.05$) compared to other dietary treatments. The ALB (g/dL) and GLB (g/dL) values observed in birds offered different combinations of supplemental dietary antioxidants and vitamin K did not revealany statistical variations ($P>0.05$) in comparison to one another, among birds offered the different treatmentrations and their values ranged from 2.21±0.78 (R2) to 2.68±0.77 (R4) and 0.13±1.14 (BD) to 0.44±1.17 (NC), respectively among the dietary treatments. It was noticed that birds offered BD had significantly elevated ($P<0.05$)MDA level (128.29±31.16 nmol/mL)compared to those offeredother treatment rations. Birds offered NC diet (12.41±10.56nmol/mL) had the least MDA value significantly below ($P<0.05$) those of the other treatment rations over 42 days period. However,comparable ($P>0.05$) MDA values were recorded in birds offered R1 (79.22±38.65nmol/mL), R2 (80.18±12.58nmol/mL), and R3 (72.54±24.99nmol/mL).

The MDA value obtained in birds offered R4 (42.00±10.40nmol/mL) was significantly reduced ($P<0.05$) than in the other treatment rations except for birds offered NC diet (12.41±10.56nmol/mL).A three-fold reduction in the MDA value of birds offered R4

compared with birds offered BD was noticed. The SOD (U/mL) values in all birds offered NC and the contaminated test rations were not affected by the treatment effect and the values ranged between 36.74±21.31 (BD) and 57.66±23.63 (R2) at day 42. Significantly elevated (P<0.05) TAC (U/mL) was recorded in birds offered NC diet(157.95±83.34), above those of other treatment rations were noticed except that of birds offered R3 (109.24±46.05). However, birds offered BD (59.93±53.28), R1 (65.25±34.71), R2 (64.24±30.52) and R4 (73.13±35.62) were similar (P>0.05) in their TAC values. Serum T-GSH (µmol/mL)level was not affected (P>0.05) by different combinations of supplemental dietary antioxidants and vitamin K and their values ranged from 4.51±1.45 (BD) to 10.95±8.91 (R4). Birds offered BD had reduced GSHvalue (2.19±1.16µmol/mL)distinctly (P<0.05)below those of birds offered other treatment rations.The GSSG(µmol/mL) level recorded in birds offeredNC ration (1.53±0.51) was significantly reduced(P<0.05) contrary to the other dietary treatments. Higher and better GSH:GSSG was obtained from birds fed NC diet (3.64±1.44), which differed greatly (P<0.05) from the values observed in birds on other treatment rations. However, the least and significantly reduced (P<0.05) ratio was recorded in birds offered BD (0.83±0.58), while the values obtained from birds offered R3 (1.88±0.72), R2 (1.94±0.78), R1 (1.98±0.89) and R4 (2.19±0.92) showed no significant variations (P>0.05) from one another.

Table 4.17 Serum biochemical indices, lipid peroxidation and antioxidant profile of broiler chicken offered aflatoxin-contaminated poultry feed with different combinations of supplemental dietary antioxidants and vitamin K

	Dietary treatments							
Parameters	NC	BD	R1	R2	R3	R4	SEM	P-value
AST (U/L)	113.61±25.73	116.81±28.78	100.93±24.13	107.62±38.88	98.97±43.79	110.87±31.29	11.62	0.87
ALT (U/L)	6.97±5.68^{ab}	14.95±11.11^{a}	10.88±7.23^{ab}	10.37±6.07^{ab}	9.49±7.11^{ab}	5.80±3.36^{b}	2.53	0.17
ALP (U/L)	76.88±26.08^{c}	215.59±25.01^{a}	144.21±74.33^{b}	120.03±61.48^{bc}	116.36±49.88^{bc}	77.56±22.10^{c}	16.82	<0.0001
TP (g/dL)	3.00±0.67^{ab}	2.78±0.47^{ab}	2.85±0.36^{ab}	2.49±0.45^{b}	3.23±1.02^{a}	2.91±0.62^{ab}	0.23	0.34
ALB (g/dL)	2.57±1.11	2.65±1.09	2.48±0.76	2.21±0.78	3.05±0.98	2.68±0.77	0.33	0.63
GLB (g/dL)	0.44±1.17	0.13±1.14	0.38±0.93	0.28±0.89	0.18±1.37	0.23±1.16	0.39	0.99
ALB:GLB	5.84±0.45^{c}	20.38±2.42^{a}	6.53±0.73^{c}	7.89±0.92^{c}	16.94±1.67^{a}	11.65±0.86^{b}	1.85	0.03
MDA (nmol/mL)	12.41±10.56^{d}	128.29±31.16^{a}	79.22±38.65^{b}	80.18±12.58^{b}	72.54±24.99^{b}	42.00±10.40^{c}	8.49	<0.0001
SOD (U/mL)	53.45±14.01	36.74±21.31	53.67±21.28	57.66±23.63	51.13±19.34	54.34±19.30	7.08	0.38
TAC (U/mL)	157.95±83.34^{a}	59.93±53.28^{b}	65.25±34.71^{b}	64.24±30.52^{b}	109.24±46.05^{ab}	73.13±35.62^{b}	17.85	0.002
T-GSH (µmol/mL)	10.06±6.83	4.51±1.45	8.97±6.00	8.85±6.63	10.07±6.63	10.95±8.91	2.29	0.43
GSH (µmol/mL)	5.03±0.97^{a}	2.19±1.16^{b}	5.38±1.36^{a}	5.13±1.59^{a}	4.75±1.22^{a}	5.24±1.59^{a}	0.47	0.0001
GSSG (µmol/mL)	1.53±0.51^{b}	3.08±1.05^{a}	2.97±0.99^{a}	2.74±0.68^{a}	2.81±0.97^{a}	2.59±0.73^{a}	0.30	0.01
GSH:GSSG	3.64±1.44^{a}	0.83±0.58^{c}	1.98±0.89^{b}	1.94±0.78^{b}	1.88±0.72^{b}	2.19±0.92^{b}	0.33	<0.0001

^{abcd} Treatment means within the same row having unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P-value- Probability level, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline Phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, MDA- Malondialdehyde, SOD- Superoxide dismutase, TAC- Total antioxidant capacity, T-GSH- Total glutathione, GSH- Reduced glutathione, GSSG- Oxidised glutathione, GSH:GSSH- Oxidative stress index, R– Ration, NC- Negative control, BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.3.7 Residual aflatoxin in liver of broiler chicken offered aflatoxin-contaminated poultry feed mitigated with different combinations of supplemental dietary antioxidants and vitamin K

The effect of different combinations of supplemental dietary antioxidants and vitamin K on liver weight, aflatoxins concentration in liver and liver residual aflatoxins in broiler chicken offered aflatoxin-contaminated feed is presented in Table 4.18. Birds offered BD had higher ($P<0.05$) liver aflatoxins concentration (2.71±0.28µg/kg), significantly above those of other dietary treatments.The least aflatoxins concentration in the liver ($P<0.05$) was recorded in birds offered NCration (0.00±0.00µg/kg) which was significantly reduced in comparison to those of birds offered other treatment diets. However, liver aflatoxins concentration recorded from birds fed R1 (1.72±0.08µg/kg), R2 (1.83±0.23µg/kg) and R3 (1.54±0.07µg/kg) did not show any distinct variations ($P>0.05$) from one another. Liver weight of birds offered all the different treatment rations revealed no differences and their values ranged from 23.67±2.03g/bird (NC) to 28.90±3.08g/bird (BD). However, expressing the liver weight relative to the body weight revealed that birds fed BD (4.38±0.03%)had the highest relative liver weight distinctly different for the other treatments while birds fed R2 (3.60±0.06%) and R3 (3.53±0.02%) were similar.Birds fed R3 (2.92±0.05%) and R4 (2.93±0.03%) were also similar in their relative liver weight and birds fed R1 (2.07±0.04) had significantly the least liver weight relative to the bodyweight.Higher residual aflatoxins obtained in the liver of birds offered BD (0.08±0.02µg/bird) was distinctly ($P<0.05$) above those of birds offeredthe other treatment rations. However, birds offered R1 (0.04±0.01µg/bird), R2 (0.05±0.04µg/bird), R3 (0.04±0.01µg/bird) and R4 (0.04±0.01µg/bird) had similar residual aflatoxins in their liver samples. The least and distinctly different ($P<0.05$) liver residual aflatoxins was recorded in birds offered NCration (0.00±0.00µg/bird) compared to the other experimental rations.

Table 4.18 Liver residual aflatoxin in broiler chicken offered aflatoxin-contaminated poultry feed with differentcombinations of supplemental dietary antioxidants and vitamin K

	Dietary treatments							
Parameter	NC	BD	R1	R2	R3	R4	SEM	P- value
Aflatoxins concentration (µg/kg)	0.00±0.00^{d}	2.71±0.28^{a}	1.72±0.08^{b}	1.83±0.23^{b}	1.54±0.07^{bc}	1.37±0.08^{c}	0.09	<0.0001
Liver weight (g/bird)	23.67±2.03	28.90±3.08	26.17±4.41	27.00±2.49	26.68±1.92	27.58±0.97	1.56	0.3500
Liver weight rel. to bodyweight (%)	2.07±0.04^{d}	4.38±0.03^{a}	3.60±0.06^{b}	3.53±0.02^{b}	2.92±0.05^{c}	2.93±0.03^{c}	0.72	0.0420
Residual aflatoxin (µg/bird)	0.00±0.00^{c}	0.08±0.02^{a}	0.04±0.01^{b}	0.05±0.04^{b}	0.04±0.01^{b}	0.04±0.01^{b}	0.04	<0.0001

^{abcd}Treatment means within the same row havingunidentical superscripts differed greatly (P<0.05). SEM- standard error of means, P-value- Probability level, R– Ration, rel.- relative; NC- Negative control,BD- Basal Diet, R1- BD+Vitamins (E+C), R2- BD+Vitamins (E+C)+Se, R3- BD+Vitamins [(E+C)+K], R4- BD+Vitamins [(E+C)+K]+Se, Se - Selenium

4.4 Study three: Effects of yeast beta-glucans, supplemental antioxidantsand vitamin K in ameliorating the impact of dietary aflatoxins in broiler chicken

The primary aim stated in section 1.3, which was achieved throughexecution of objectives a, b and c (see section 3.4) and the results were presented in section 4.4.

4.4.1 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chickenoffered aflatoxin-contaminated poultry feed at starter phase

Table 4.19 shows main effect of varied inclusion levels of beta-glucans and supplemental selenium on performance of broiler chickenoffered aflatoxin-contaminated poultry feedat starter phase. The feed intake (FI) of birds fed the diets having 375ppm beta-glucans (559.27±30.19g/bird) was higher andremarkably ($P<0.05$) above that of birds offered 250ppm beta-glucans (501.90±34.39g/bird) diets at starter phase. However, varying the levels of selenium did not show any statistical variation on FI at starter phase and their values ranged between 514.80±49.29g/bird and 546.37±32.26g/bird. Birds offered diets containing 375ppm beta-glucans had higher BWG (337.31±37.40g/bird), distinctly above ($P<0.05$) that of birds offered 250ppm beta-glucans (208.77±32.37g/bird) diets. Birds feddiet with supplemental selenium had higher BWG of 296.03±84.48g/bird significantlyabove that of birds fed diets withoutselenium supplementation (250.05±62.90g/bird). Birds offered diets containing 375ppm beta-glucans had greatly reduced ($P<0.05$) and lower and preferred FCR(1.67±0.17)compared to that of birds offered 250ppm beta-glucans (2.44±0.33) diets. However, supplemental selenium did not elicit significant changes ($P>0.05$) in FCR of broilers at starter phase and the values ranged between 1.97±0.56 and 2.14±0.40.

Table 4.19 Main effect of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed at starter phase

	Beta-glucans (ppm)		Selenium (mg/kg)			P- values	
Parameters	250	375	0.00	0.30	SEM	Beta-glucans	Selenium
FI (g/bird)	501.90±34.39[b]	559.27±30.19[a]	514.80±49.29	546.37±32.26	18.35	0.01	0.10
BWG (g/bird)	208.77±32.37[b]	337.31±37.40[a]	250.05±62.90[b]	296.03±84.48[a]	13.44	<0.0001	0.01
FCR	2.44±0.33[a]	1.67±0.17[b]	2.14±0.40	1.97±0.56	0.47	0.001	0.33

[ab]Treatments means within the same row having unidentical superscripts are distinctly different (P<0.05). FI- feed intake, BWG-body weight gain, FCR- feed conversion ratio, SEM- standard error of means, P- value- probability level.

4.4.2 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed at starter phase

Table 4.20presented the interaction effect of beta-glucans inclusion and supplemental selenium on performance of broiler chickenoffered aflatoxin-contaminated poultry rationat starter phase. Birds offeredNCration had distinctly ($P<0.05$) higher FI (678.67±17.92g/bird) in comparison to other dietary treatments. The least and greatly reduced FI ($P<0.05$) was obtained in birds offered diets BD (478.25±46.61g/bird) and TD3 (480.77±30.61g/bird). Higher BWG ($P<0.05$) recorded in birds offered NC diet (436.61±20.09g/bird) was significantly abovethose of other treatment rations. The least ($P<0.05$) BWG was in birds offered BD (67.26±14.02g/bird), and it was significantly reduced compared to other dietary treatments. The BWG recorded in birds offered TD1 (192.79±6.59g/bird) and TD2 (224.74±42.55g/bird) were comparable ($P>0.05$) statistically. Higher ($P<0.05$) and less desirable FCR value was obtained in birds offered BD (7.35±1.92) as against those of the other experimental rations. However, no distinct variations were seen in the FCR of birds offered NC (1.56±0.03), TD1 (2.49±0.09), TD2 (2.39±0.51), TD3 (1.79±0.13) and TD4 (1.56±0.14) at starter phase.

Table 4.20 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feedat starter phase

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	FI (g/bird)	BWG (g/bird)	FCR
NC	0	0	0.00	678.67±17.92^{a}	436.61±20.09^{a}	1.56±0.03^{b}
BD	270	0	0.00	478.25±46.61^{c}	67.26±14.02^{e}	7.35±1.92^{a}
TD 1	270	250	0.00	480.77±30.61^{c}	192.79±6.59^{d}	2.49±0.09^{b}
TD 2	270	250	0.30	523.04±26.07^{bc}	224.74±42.55^{d}	2.39±0.51^{b}
TD 3	270	375	0.00	548.83±40.79^{b}	307.32±3.43^{c}	1.79±0.13^{b}
TD 4	270	375	0.30	569.70±16.98^{b}	367.31±28.04^{b}	1.56±0.14^{b}
SEM				18.35	13.44	0.47
P-value				<0.0001	<0.0001	0.0002

^{abcde}Treatments means within the same column having unidentical superscripts differed distinctly (P<0.05). SEM- standard error of means, P- value- probability level, FI- feed intake, BWG- Body weight gain, FCR- feed conversion ratio, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.3 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

Main effect of varied inclusion levels of beta-glucans and supplemental selenium on performance of broiler chickenoffered aflatoxin-contaminated ration at finisher phase is shown in Table 4.21. Higher ($P<0.05$) FI recorded in birds fed diets containing 375ppm beta-glucans (2,974.58±66.36g/bird) was significantly above that of birds fed diets containing 250ppm beta-glucans (2,188.43±101.64g/bird). Selenium (Se) supplementation resulted in higher FI (2,648.33±406.46g/bird) distinctly ($P<0.05$) above that of birds offered diet without supplemental Se (2,514.68±457.85g/bird). The BWG of birds fed diets having 375ppm beta-glucans (1,438.63±126.79g/bird) was higher significantly ($P<0.05$) when compared to birds fed diets containing 250ppm beta-glucans (1,096.71±76.20g/bird). Supplemental selenium gave significant rise ($P<0.05$) in BWG (1,346.24±209.09g/bird) of birds in comparison to the BWG of birds fed diet without selenium supplementation (1,189.11±183.01g/bird). Different levels of beta-glucans inclusion did not alter FCR in broiler chicken offered the contaminated feed at finisher phase and the FCR values ranged between 1.99±0.08 in 250ppm and 2.08±0.17 in 375ppm beta-glucans diets. However, better and greatly ($P<0.05$) reduced FCR was obtained in birds offered supplemental selenium (1.97±0.01) diet compared to observations in birds offered diets without selenium supplementation (2.11±0.16) at finisher stage.

Table 4.21 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

	Beta-glucans (ppm)		Selenium (mg/kg)			P-value	
Parameters	250	375	0.00	0.30	SEM	Beta-glucans	Selenium
FI (g/bird)	2188.43±101.64[b]	2974.58±66.36[a]	2514.68±457.85[b]	2648.33±406.46[a]	30.77	<0.0001	0.001
BWG (g/bird)	1096.71±76.20[b]	1438.63±126.79[a]	1189.11±183.01[b]	1346.24±209.09[a]	31.49	<0.0001	0.002
FCR	1.99±0.08	2.08±0.17	2.11±0.16[a]	1.97±0.01[b]	0.05	0.21	0.04

[ab] Treatments means within the same row having unidentical superscripts differed significantly (P<0.05). FI- feed intake, BWG-body weight gain, FCR- feed conversion ratio, SEM- standard error of means, P-value- probability level

4.4.4 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

The interaction effect of beta-glucans addition and supplemental selenium on performance of broiler chicken offered aflatoxin-contaminated poultry ration at finisher phase is shown in Table 4.22. Birds fed NC diet had higher FI (3,246.16±86.57g/bird)distinctly ($P<0.05$) above those of the other dietary treatments. The least ($P<0.05$) FI(g/bird) was recorded in birds offered BD (1,801.40±51.70). The FI of birds offered diets TD3 (2,930.73±68.27) and TD4 (3,018.43±24.09) did not vary ($P>0.05$) statistically from one another. The BWG (g/bird) of birds offered NC diet (1,529.64±43.81) and TD4 (1,536.67±4.55) were comparable ($P>0.05$) but remarkably greater ($P<0.05$) than the BWG of birds offered other treatment diets. However, birds offered BD had greatly reduced ($P<0.05$) BWG (889.01±23.28) at finisher phase significantly below that of other dietary treatments. The FCR obtained in birds offered TD3 (2.19±0.17) was higher greatly ($P<0.05$) and undesirable compared to that of birds offered TD4 (1.96±0.02), but the former was similar to the FCR of birds offered NC (2.12±0.07), BD (2.03±0.07), and TD1 (2.03±0.11). Birds offered diets TD2 (1.97±0.01) and TD4 (1.96±0.02) both had desirable and comparable ($P<0.05$) FCR at finisher phase.

Table 4.22 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on performance at finisher phase of broiler chicken offered aflatoxin-contaminated poultry feed

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	FI (g/bird)	BWG (g/bird)	FCR
NC	0	0	0.00	3246.16±86.57^{a}	1529.64±43.81^{a}	2.12±0.07^{ab}
BD	270	0	0.00	1801.40±51.70^{e}	889.01±23.28^{e}	2.03±0.07^{ab}
TD 1	270	250	0.00	2098.63±10.43^{d}	1037.61±59.52^{d}	2.03±0.11^{ab}
TD 2	270	250	0.30	2278.23±39.04^{c}	1155.81±22.29^{c}	1.97±0.01^{b}
TD 3	270	375	0.00	2930.73±68.27^{b}	1340.60±106.48^{b}	2.19±0.17^{a}
TD 4	270	375	0.30	3018.43±24.09^{b}	1536.67±4.55^{a}	1.96±0.02^{b}
SEM				30.77	31.49	0.05
P-value				<0.0001	<0.0001	0.06

^{abcde}Treatments means within the same column having unidentical superscripts differed greatly (P<0.05). SEM- standard error of means, P- value- probability level, FI- feed intake, BWG- Body weight gain, FCR- feed conversion ratio, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.5 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

Table 4.23 shows the main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance of broiler chickenoffered aflatoxin-contaminated ration from 0 to 49 days. Birds offered diets containing 375ppm beta-glucans (3,533.85±93.77) had greater ($P<0.05$) FI (g/bird) in comparison to birds offered diet having 250ppm beta-glucans (2,690.34±127.52). Supplemental selenium increased FI (3,194.70±432.86) greatly ($P<0.05$) above that of birds offered diets with no selenium supplementation (3,029.48±498.21). Birds offered 375ppm beta-glucans inclusion diet had increased BWG (1,775.95±157.82g/bird) distinctly ($P<0.05$) above that of birds offered diet having 250 beta-glucans (1,305.48±93.14g/bird). Birds offered selenium supplemented diet had significant increase($P<0.05$) in BWG (1,642.26±287.94g/bird) in comparison to birds fed diets without selenium (1,439.16±242.33g/bird). The FCR of birds did not show differences ($P>0.05$) by varying the levels of beta-glucans inclusion and it ranged between 2.00±0.16 and 2.06±0.08. However, birds offered diets with supplemental selenium had desirable and significantly lower ($P<0.05$) FCR (1.96±0.09) below that of birds offered diet without selenium (2.11±0.16).

Table 4.23 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

	Beta-glucans (ppm)		Selenium (mg/kg)			P- value	
Parameters	250	375	0.00	0.30	SEM	Beta-glucans	Selenium
FI (g/bird)	2690.34±127.52[b]	3533.85±93.77[a]	3029.48±498.21[b]	3194.70±432.86[a]	42.11	<0.0001	0.002
BWG (g/bird)	1305.48±93.14[b]	1775.95±157.82[a]	1439.16±242.33[b]	1642.26±287.94[a]	32.59	<0.0001	0.001
FCR	2.06±0.08	2.00±0.16	2.11±0.16[a]	1.96±0.09[b]	0.05	0.29	0.03

[ab] Treatments means within the same row having unidentical superscripts differed distinctly (P<0.05). FI- feed intake, BWG-body weight gain, FCR- feed conversion ratio, SEM- standard error of means, P- value-probability level.

4.4.6 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on performance of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

The interaction effect of beta-glucans addition and supplemental selenium onperformance assessment of broiler chicken offered aflatoxin-contaminated ration from 0 to 49 days is presented in Table 4.24. Higher FI (g/bird) was observed in birds fed NC diet (3,924.83±91.67), distinctly ($P<0.05$) above FI of birds offered other treatment rations from 0 to 49 days. However, birds fed BD had the least($P<0.05$) (2,279.65±81.44) and significantly reduced FI. The FI of birds offered diets TD3 (3,479.57±108.09) and TD4 (3,588.13±38.16) were comparable ($P>0.05$) to one another. The BWG (g/bird) of birds fed NC (1,966.24±29.77) and TD4 (1,903.98±32.56) were comparable but significantly greater ($P<0.05$) than those offered other dietary treatments. The BWG of birds offered BD (956.27±19.34) was lower ($P<0.05$)and significantly below those of birds offered other treatment diets. The FCR obtained in birds offered BD (2.38±0.04) was distinctly ($P<0.05$) higher compared to birds offered other treatment diets from 0 to 49 days. However, birds offered NC (1.99±0.04) and TD4 (1.88±0.04) diets had significantly reduced ($P<0.05$) and desirable FCR, however, birds fed TD2 (2.03±0.06) had similar FCR with birds offered NC (1.99±0.04), TD1 (2.09±0.10), TD3 (2.12±0.15) and TD4 (1.88±0.04), respectively.

Table 4.24 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on performance assessment of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	FI (g/bird)	BWG (g/bird)	FCR
NC	0	0	0.00	3924.83±91.67^{a}	1966.24±29.77^{a}	1.99±0.04^{bc}
BD	270	0	0.00	2279.65±81.44^{e}	956.27±19.34^{e}	2.38±0.04^{a}
TD 1	270	250	0.00	2579.40±33.63^{d}	1230.40±63.58^{d}	2.09±0.10^{b}
TD 2	270	250	0.30	2801.27±51.03^{c}	1380.55±27.12^{c}	2.03±0.06^{bc}
TD 3	270	375	0.00	3479.57±108.09^{b}	1647.92±109.68^{b}	2.12±0.15^{b}
TD 4	270	375	0.30	3588.13±38.16^{b}	1903.98±32.56^{a}	1.88±0.04^{c}
SEM				42.11	32.59	0.05
P-value				<0.0001	<0.0001	0.0002

^{abcde} Treatments means within the same column having unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P- value- probability level, FI- feed intake, BWG- Body weight gain, FCR- feed conversion ratio, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se,Se- Selenium.

4.4.7 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on mortality and uniformity of bodyweight of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

Table 4.25 shows the main effect of varied inclusion levels of beta-glucans and supplemental selenium on mortality, uniformity of bodyweights, and uniformity coefficient of variability of broiler chicken fed aflatoxin-contaminated diets for 49 days. The final bodyweight (FBW) (g/bird) of birds offered diet containing 375ppm beta-glucans(1820.90±156.92), was higher distinctly($P<0.05$) above that of birds offered diet containing 250ppm beta-glucans (1350.30±93.20). Dietary supplementation with selenium significantly resulted in increased ($P<0.05$)final body weight of birds (1686.72±287.59) in comparison to those offered diets without selenium (1484.48±242.77). Uniformity of bodyweight observed in birds fed diets having 375ppm beta-glucans (74.83±17.44%) was higher ($P<0.05$) markedly when compared to birds offered diets having 250ppm beta-glucans (51.33±22.44). Supplemental seleniumwas observed to improve uniformity of bodyweight up to 77.17±14.99%, and this was remarkably ($P<0.05$) higher compared to birds offered diets without selenium (49.00±20.94%). Varing the levels of beta-glucans did not produce any differences ($P>0.05$) in bodyweight uniformity coefficient of variability (CVu). However, supplemental seleniumgreatly ($P<0.05$) reducedand improved CVu (8.04±2.43%) compared to birds offered diets without selenium (16.82±8.32). Mortality was reduced in birds fed 375ppm beta-glucans (10.61±3.71%) diet greatly ($P<0.05$) below that of birds offered 250ppm beta-glucans (22.73±4.98%) diet. However, mortality was not affected with supplemental selenium.

Table 4.25 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on uniformity of body weights, mortality, and uniformity coefficient of variability of broiler chicken offered aflatoxin-contaminated poultry feed

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- values	
	250	375	0.00	0.30		Beta-glucans	Selenium
FBW (g/bird)	1350.30±93.20[b]	1820.90±156.92[a]	1484.48±242.77	1686.72±287.59	32.49	<0.0001	0.001
Uniformity (%)	51.33±22.44[b]	74.83±17.44[a]	49.00±20.94[b]	77.17±14.99[a]	7.86	0.02	0.01
CVu (%)	15.49±8.55	9.36±5.07	16.82±8.32[a]	8.04±2.43[b]	3.36	0.09	0.03
Mortality (%)	22.73±4.98[a]	10.61±3.71[b]	15.15±7.42	18.18±8.13	2.77	0.002	0.28

[ab]Treatments means within the same row having unidentical superscripts differed greatly (P<0.05). FBW- Final bodyweight, CVu- Uniformity Coefficient of Variability, SEM- standard error of means, P- value- probability level.

4.4.8 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on mortality and uniformity of bodyweight of broiler chicken offered aflatoxin-contaminated poultry feed from 0 to 49 days

Table 4.26 shows the interaction effect of beta-glucans inclusion and supplemental selenium on uniformity of bodyweights, mortality, and uniformity coefficient of variability of broiler chicken offered aflatoxin-contaminated feed for 49 days. The FBW (g/bird) of birds fed NC (2011.82±28.79) and TD4 (1948.07±33.84) were higher distinctly ($P<0.05$) above those of birds offered other treatment rations. Birds offered BD (1001.49±17.89) had the least FBW, which was greatly reduced ($P<0.05$) in comparison to other dietary treatments. Uniformity of bodyweight (%)in birds offered NC (96.67±5.77) and TD4 (89.67±0.58) were comparable and increased ($P<0.05$) above those of birds offered other treatment diets. Birds offered BD (24.67±15.95) had the least ($P<0.05$) uniformity and did not show marked variation ($P<0.05$) from birds offered TD1 (38.00±25.16). Higher CVu (%) obtained in birds fed BD (33.77±8.84), was distinctly different ($P<0.05$) from the other dietary treatments. The CVu of birds offered NC (4.63±1.29) was highly desirable ($P<0.05$), but similar to those of birds offered TD2 (9.91±0.88), TD3 (12.56±5.49), and TD4 (6.16±1.87). Highermortality (%) in birds offered BD (39.39±5.25) varied greatly ($P<0.05$) when compared to other dietary treatments. Birds offered NC (3.03±5.25) and TD3 (9.09±5.25) had the least and markedly ($P<0.05$) reduced mortality. However, percentage mortality of birds offered TD3 did not reveal any differences ($P>0.05$) compared to that of birds offered diet TD4 (12.12±5.25).

Table 4.26 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on uniformity of bodyweight, mortality, and uniformity coefficient of variability of broilers offered aflatoxin-contaminated poultry feed

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	FBW (g/bird)	Uniformity (%)	CVu (%)	Mortality (%)
NC	0	0	0.00	2011.82±28.79^{a}	96.67±5.77^{a}	4.63±1.29^{c}	3.03±5.25^{d}
BD	270	0	0.00	1001.49±17.89^{e}	24.67±15.95^{d}	33.77±8.84^{a}	39.39±5.25^{a}
TD 1	270	250	0.00	1275.22±63.90^{d}	38.00±25.16^{cd}	21.08±9.40^{b}	21.21±5.25^{b}
TD 2	270	250	0.30	1425.37±26.93^{c}	64.67±9.61^{b}	9.91±0.88^{c}	24.24±5.25^{b}
TD 3	270	375	0.00	1693.73±109.09^{b}	60.00±10.00^{bc}	12.56±5.49^{bc}	9.09±5.25^{cd}
TD 4	270	375	0.30	1948.07±33.84^{a}	89.67±0.58^{a}	6.16±1.87^{c}	12.12±5.25^{c}
SEM				32.49	7.86	3.36	2.77
P-value				<0.0001	0.0002	0.0004	<0.0001

^{abcde}Treatments means within the same column having unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P- value-probability level, FBW- Final bodyweight, CVu- Uniformity Coefficient of variability, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se,Se- Selenium.

4.4.9 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology at starter phase of broiler chicken offered aflatoxin-contaminated poultry feed

The main effect of varied levels of beta-glucans addition and supplemental selenium on haematology at starter phase of broiler chicken offered aflatoxin-contaminated feed is shown in Table 4.27. Birds offered diet containing 375ppm beta-glucans had higher($P<0.05$) PCV (32.06±2.71%) significantly elevated above those birds offered 250ppmbeta-glucans (29.89±2.61%) diet. However, supplemental selenium did not alter PCV values and its values ranged between 30.89±3.03% and 31.06±2.73%. Birds offered diets having 375ppm beta-glucans had higher Hb (g/dL) value of 10.57±0.94 distinctly ($P<0.05$) above that of birds offered diets containing 250ppm beta-glucans (9.83±1.01). However, supplemental selenium did not affect Hb concentration($P>0.05$), and it ranged between 10.15±1.21 and 10.25±0.85. The RBC ($x10^{12}$/L), WBC ($x10^{9}$/L), platelets ($x10^{3}$/L), monocytes (%), eosinophils (%), and basophils (%) values did not reveal any differences by varying the levels of beta-glucans, and their values ranged between 3.25±0.39 and 3.44±0.15; 13.42±2.35 and 14.41±1.26; 13.21±1.96 and 13.32±3.71; 2.83±1.04 and 3.22±1.00; 3.94±1.43 and 4.06±1.35; and 0.17±0.38 and 0.22±0.43. Also, selenium supplementation also did not show any marked effect on RBC, WBC, platelets, lymphocytes, heterophils, monocytes, eosinophils and basophils values and their values ranged between 3.29±0.42 and 3.39±0.15; 13.84±1.85 and 13.99±2.05; 13.06±3.72 and 13.48±1.93; 63.33±5.64 and 63.67±5.08; 29.17±4.79 and 29.39±5.64; 2.94±0.94 and 3.11±1.13; 3.94±1.43 and 4.06±1.35; and 0.11±0.32 and 0.28±0.46, respectively.

Table 4.27 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology at starter phase of broiler chicken offered aflatoxin-contaminated poultry feed

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- values	
	250	375	0.00	0.30		Beta-glucans	Selenium
PCV (%)	29.89±2.61[b]	32.06±2.71[a]	30.89±3.03	31.06±2.73	0.92	0.02	0.86
Hb (g/dL)	9.83±1.01[b]	10.57±0.94[a]	10.15±1.21	10.25±0.85	0.33	0.04	0.77
RBC ($x10^{12}$/L)	3.25±0.39	3.44±0.15	3.29±0.42	3.39±0.15	0.09	0.06	0.33
WBC ($x10^{9}$/L)	14.41±1.26	13.42±2.35	13.84±1.85	13.99±2.05	0.66	0.13	0.83
Platelets ($x10^{3}$/L)	13.32±3.71	13.21±1.96	13.06±3.72	3.48±1.93	0.98	0.91	0.67
Lymphocytes (%)	61.72±6.19[b]	65.28±3.54[a]	63.33±5.64	63.67±5.08	1.79	0.05	0.85
Heterophils (%)	31.33±5.88[a]	27.22±3.35[b]	29.39±5.64	29.17±4.79	1.81	0.02	0.89
Monocytes (%)	2.83±1.04	3.22±1.00	3.11±1.13	2.94±0.94	0.34	0.28	0.64
Eosinophils (%)	3.94±1.43	4.06±1.35	4.06±1.35	3.94±1.43	0.46	0.82	0.82
Basophils (%)	0.17±0.38	0.22±0.43	0.11±0.32	0.28±0.46	0.15	0.69	0.23
H:L	0.52±0.16[a]	0.42±0.07[b]	0.48±0.14	0.47±0.12	0.05	0.02	0.83

[ab]Treatments means within the same row having unidentical superscripts differed greatly ($P<0.05$). PCV- Packed cell volume, RBC-red blood cells, WBC- white blood cells, Hb- Haemoglobin, H:L- Heterophils: lymphocytes ratio, SEM- standard error of means, P-value-probability.

4.4.10 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology at starter phase of broiler chicken offered aflatoxin-contaminated poultry feed

The interaction or additive effect of varied levels of beta-glucans inclusion and supplemental selenium on haematology of broiler chickenoffered aflatoxin-contaminated feed at starter phase ispresented in Table 4.28. The PCV values had no significant variations across the different dietary treatments and its values ranged from 29.67 (TD1) to 32.11 (TD3). Varied levels of beta-glucans and supplemental selenium also did not produce significant differences in Hb (g/dL) and its values ranged from 9.70 (TD1) to 10.61(NC). However, increased RBC ($x10^{12}$/L) was obtained in birds offered TD4 (3.46) which was distinctly ($P<0.05$) above that of birds fed diet TD1 (3.16), but was comparable ($P>0.05$) to those of birds offered NC (3.33), BD (3.35), TD2 (3.33) and TD3 (3.42). The WBC ($x10^{9}$/L) of birds also did not vary among the dietary treatments at starter phase and its values ranged from 13.22 (TD3) to 14.82 (NC). Platelets ($x10^{3}$/L) count was equally not affected and its values ranged from 11.57 (NC) to 14.20 (TD4). The additive effect of beta-glucans and supplemental selenium did not reveal any differences in lymphocytes, heterophils, monocytes, eosinophils, basophils and heterophils to lymphocytes (H:L) ratio, and their values ranged from 61.11 (TD1) to 65.56 (TD3); 26.78 (TD3) to 32.00 (TD1); 2.78 (TD2) to 3.33 (TD3); 3.78 (NC) to 4.33 (BD); 0.11 (TD1 and TD3) to 0.33 (BD and TD4) and 0.41 (TD3) to 0.54 (TD1),respectively from the least to the highest values(3.16), but was comparable ($P>0.05$) to those of birds offered NC (3.33), BD (3.35), TD2 (3.33) and TD3 (3.42). The WBC ($x10^{9}$/L) of birds also did not vary among the dietary treatments at starter phase and its values ranged from 13.22 (TD3) to 14.82 (NC). Platelets ($x10^{3}$/L) count was equally not affected and its values ranged from 11.57 (NC) to 14.20 (TD4). The additive effect of beta-glucans and supplemental selenium did not reveal any differences in lymphocytes, heterophils, monocytes, eosinophils, basophils and heterophils to lymphocytes (H:L) ratio, and their values ranged from 61.11 (TD1) to 65.56 (TD3); 26.78 (TD3) to 32.00 (TD1); 2.78 (TD2) to 3.33 (TD3); 3.78 (NC) to 4.33 (BD); 0.11 (TD1 and TD3) to 0.33 (BD and TD4) and 0.41 (TD3) to 0.54 (TD1), respectively from the least to the highest values.

Table 4.28 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chickens offered aflatoxin-contaminated poultry feed at starter phase

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	PCV (%)	Hb (g/dL)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)	Platelets ($x10^{3}$/L)	Lym. (%)	Het. (%)	Mon. (%)	Eos. (%)	Bas. (%)	Het: Lym
NC	0	0	0.00	31.78	10.61	3.33^{ab}	14.82	11.57	63.11	30.00	2.89	3.78	0.22	0.49
BD	270	0	0.00	31.33	10.21	3.35^{ab}	14.72	13.30	63.89	28.67	2.89	4.33	0.33	0.46
TD 1	270	250	0.00	29.67	9.70	3.16^{b}	14.47	13.89	61.11	32.00	2.89	3.89	0.11	0.54
TD 2	270	250	0.30	30.11	9.97	3.33^{ab}	14.36	12.76	62.33	30.67	2.78	4.00	0.22	0.51
TD 3	270	375	0.00	32.11	10.60	3.42^{ab}	13.22	12.22	65.56	26.78	3.33	4.22	0.11	0.41
TD 4	270	375	0.30	32.00	10.53	3.46^{a}	13.62	14.20	65.00	27.67	3.11	3.89	0.33	0.43
SEM				0.92	0.33	0.09	0.66	0.98	1.79	1.81	0.34	0.46	0.15	0.05
P-value				0.29	0.29	0.27	0.47	0.39	0.52	0.34	0.87	0.95	0.79	0.33

[ab] Treatments along the same column having unidentical superscripts differed distinctly ($P<0.05$). SEM- standard error of means, P- value- probability level, PCV- Packed cell volume, RBC-red blood cells, WBC- white blood cells, Hb- haemoglobin, Het.- Heterophils, Lym.- lymphocytes, Mon.- Monocytes, Eos.- Eosinophils, Bas.- Basophils, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.11 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

Main effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chicken offered aflatoxin-contaminated ration at finisher phase is presented in Table 4.29. Effect of beta-glucans inclusion on haematological parameters of broiler chickenoffered aflatoxin-contaminated feed did not reveal any differences of significance at finisher phase. The PCV (%) values ranged between 27.58±3.86 in 250ppm and 27.78±1.99 in 375ppm beta-glucans diets. The Hb (g/dL) values ranged between 9.04±0.71 and 9.05±1.15. The RBC ($x10^{12}$/L), WBC ($x10^{9}$/L), platelets ($x10^{3}$/L), lymphocytes (%), heterophils (%), monocytes (%), eosinophils (%), basophils (%), and H:L values ranged between 2.98±0.67 and 3.18±0.49; 14.42±1.45 and 14.55±1.65; 14.41±2.62 and 15.03±2.86; 59.78±5.87 and 60.58±6.23; 32.21±6.27 and 32.89±6.05; 2.67±0.97 and 2.89±0.99; 4.16±1.83 and 4.39±1.42;0.16±0.37 and 0.22±0.43, and 0.55±0.16 and 0.56±0.15, respectively.However, supplemental selenium had significant ($P<0.05$) influence in increasing RBC and plateletsvalues above that of birds fed diets without selenium. It was observed that selenium supplementation (2.87±0.73) greatly ($P<0.05$) resulted in reduced RBC level below that of birds fed diets without supplemental selenium (3.29±0.29). Higher platelets count (16.09±2.88)recorded in birds offeredselenium supplemented diet showed markeddifference ($P<0.05$) when comparedto that of birds offered diet without selenium (13.28±1.63). However,thePCV, Hb, WBC, lymphocytes, heterophils,monocytes, basophils, andH:L were not affected by supplemental selenium, and theirvalues ranged between 27.37±3.83 and 28.00±2.00; 8.94±1.20 and 9.17±0.59; 14.15±1.78 and 14.84±1.18; 59.56±4.62 and 60.79±7.12; 31.95±6.96 and 33.17±5.14; 2.58±0.90 and 3.00±1.03; 4.06±1.79 and 4.47±1.47; 0.17±0.38 and 0.21±0.42; and 0.55±0.18 and 0.57±0.12, respectively.

Table 4.29 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chickens offered aflatoxin-contaminated poultry feed at finisher phase

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
PCV (%)	27.58±3.86	27.78±1.99	28.00±2.00	27.37±3.83	1.08	0.89	0.56
Hb (g/dL)	9.05±1.15	9.04±0.71	9.17±0.59	8.94±1.20	0.34	0.95	0.49
RBC ($x10^{12}$/L)	2.98±0.67	3.18±0.49	3.29±0.29[a]	2.87±0.73[b]	0.18	0.31	0.03
WBC ($x10^{9}$/L)	14.55±1.65	14.42±1.45	14.84±1.18	14.15±1.78	0.50	0.76	0.19
Platelets ($x10^{3}$/L)	15.03±2.86	14.41±2.62	13.28±1.63[b]	16.09±2.88[a]	0.91	0.49	0.001
Lymphocytes (%)	60.58±6.23	59.78±5.87	59.56±4.62	60.79±7.12	1.94	0.69	0.54
Heterophils (%)	32.21±6.27	32.89±6.05	33.17±5.14	31.95±6.96	2.01	0.75	0.55
Monocytes (%)	2.89±0.99	2.67±0.97	3.00±1.03	2.58±0.90	0.31	0.45	0.19
Eosinophils (%)	4.16±1.83	4.39±1.42	4.06±1.79	4.47±1.47	0.49	0.62	0.45
Basophils (%)	0.16±0.37	0.22±0.43	0.17±0.38	0.21±0.42	0.14	0.65	0.71
H:L	0.55±0.16	0.56±0.15	0.57±0.12	0.55±0.18	0.05	0.74	0.69

[ab] Treatments means within the same row having unidentical superscripts differed greatly ($P<0.05$). PCV- Packed cell volume, RBC-red blood cells, WBC-white blood cells, Hb- haemoglobin, H:L- Heterophils to lymphocytes ratio, SEM- standard error of means, P-value- probability level.

4.4.12 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

Table 4.30presented the interaction effect of varied levels of beta-glucans and supplemental selenium on haematology of broiler chickenoffered aflatoxin-contaminated rationat finisher phase. The PCV (%) was not affected by varying the levels of beta-glucans with supplemental selenium and its values ranged from 27.00 (TD2) to 28.88 (BD). The Hb (g/dL) concentration among birds offered the different treatment diets did not produce variations of significanceby beta-glucans and selenium interaction and its values ranged from 8.73 (NC) to 9.50 (BD). The RBC ($x10^{12}$/L) of birds fed BD (3.16), TD1 (3.33), and TD3 (3.35) were higher distinctly ($P<0.05$) above that of birds offered TD2 (2.58). However, WBC ($x10^{9}$/L) in all the dietary treatments was noticed not to be affected by beta-glucans and selenium interaction and its values ranged from 14.02 (NC) to 15.07 (TD1). Higher ($P<0.05$)platelets ($x10^{3}$/L) countrecorded in birds offered NC (16.30) and BD (16.11) were significantly more than that of birds offered TD3 (13.22), but wascomparable ($P>0.05$) from those of birds offered TD1 (13.81), TD2 (15.76), and TD4 (15.98). The lymphocytes (%), heterophils (%), monocytes (%), eosinophils (%), basophils (%) and H:L did not showany variations of significance as a consequenceof beta-glucans and supplemental selenium addition and their values ranged from 58.44 (TD3) to 61.38 (BD); 30.63 (BD) to 33.89(TD3); 2.60 (TD4) to 3.44 (NC); 3.00 (NC) to 4.78 (TD2 and TD3); 0.11 (NC, TD2 andTD3) to 0.38 (BD); and 0.51 (BD) to 0.59 (TD3), respectively.

Table 4.30 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on haematology of broiler chicken offered aflatoxin-contaminated poultry feed at finisher phase

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	PCV (%)	Hb (g/dL)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)	Platelets ($x10^{3}$/L)	Lym. (%)	Het. (%)	Mon. (%)	Eos. (%)	Bas. (%)	Het:Lym
NC	0	0	0.00	27.22	8.73	2.82^{ab}	14.02	16.30^{a}	60.89	32.33	3.44	3.00^{b}	0.11	0.54
BD	270	0	0.00	28.88	9.50	3.16^{a}	14.59	16.11^{a}	61.38	30.63	3.13	4.13^{ab}	0.38	0.51
TD 1	270	250	0.00	28.11	9.32	3.33^{a}	15.07	13.81^{ab}	60.89	32.22	3.22	3.44^{ab}	0.22	0.54
TD 2	270	250	0.30	27.00	8.74	2.58^{b}	14.22	15.76^{ab}	60.33	32.11	2.67	4.78^{a}	0.11	0.56
TD 3	270	375	0.00	28.22	9.13	3.35^{a}	14.40	13.22^{b}	58.44	33.89	2.67	4.78^{a}	0.11	0.59
TD 4	270	375	0.30	27.40	9.00	3.05^{ab}	14.27	15.98^{ab}	61.00	32.00	2.60	4.10^{ab}	0.30	0.54
SEM				1.08	0.34	0.18	0.50	0.91	1.94	2.01	0.31	0.49	0.14	0.05
P-value				0.83	0.55	0.03	0.75	0.08	0.91	0.94	0.26	0.07	0.66	0.93

[ab]Treatments means within the same column having unidentical superscripts are distinctly different (P<0.05). SEM- standard error of means, P- value- probability level, PCV- Packed cell volume, RBC-red blood cells, WBC- white blood cells, Hb- haemoglobin, Het.- Heterophils, Lym.- lymphocytes, Het.:Lym.- Heterophis to Lymphocytes ratio, Mon.- Monocytes, Eos.- Eosinophils, Bas.- Basophils, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.13 Maineffects of varied inclusion levels of beta-glucans and supplemental selenium on serum enzymes and biochemical indices of broiler chicken offered aflatoxin-contaminated poultry feed from 0 - 49 days

Table 4.31 shows the main effect of varied inclusion levels of beta-glucans and supplemental selenium on serum biochemical indices, lipid peroxidation and antioxidants profile of broiler chickenoffered aflatoxin-contaminated feed for 49 days. Varyingthe levels of beta-glucans inclusion had no effect on AST (U/L), ALT (U/L), ALP (U/L), TP (g/dL), ALB (g/dL), GLB (g/dL), SOD (U/mL), TAC (U/mL), T-GSH (µM/mL), and GSH (µM/mL) concentrations of birds fed the aflatoxin-contaminated rations andtheir values ranged between 111.79±18.65 and 114.95±12.91; 7.50±2.64 and 7.77±3.27; 165.82±45.24 and 176.41±54.62; 2.30±0.24 and 2.53±0.38; 1.80±0.23 and 1.95±0.48; 0.35±0.59 and 0.72±0.42; 28.57±15.31 and 32.70±33.96; 188.70±59.35 and 209.93±116.49; 8.70±3.68 and 9.84±4.61; and 5.38±1.23 and 5.75±1.63, respectively. However, birds offered diets containing 375ppm beta-glucans (79.23±24.05) had greattly ($P<0.05$) reduced MDA level below those of birds offered diets having 250ppm beta-glucans (99.17±22.25). Elevated ($P<0.05$) GSSG serum level (2.65±0.64)obtained in birds offered diet having 250ppm beta-glucans inclusion was significantly above those of birds offered diet with 375ppm beta-glucans (2.08±0.63). It was noticed also that birds offered 375ppm beta-glucans diet had higher ($P<0.05$) GSH:GSSG (2.98±1.37) significantly above those of birds offered diet having 250ppm beta-glucans (2.11±0.53). Main effect on serum biochemical indices due to supplemental selenium on broiler chickenoffered aflatoxin-contaminated ration at finisher phase revealed no variations in AST, ALT, ALP, TP, ALB, GLB, MDA, SOD, TAC, T.GSH, GSSG and GSH:GSSG. However, birds offered selenium supplemented diet had better and higher ($P<0.05$) GSH (6.28±1.56) level, significantly above those of birds fed diet without selenium supplementation (4.84±0.85).

Table 4.31 Main effects of varied inclusion levels of β-glucan and supplemental selenium on serum biochemistry of broiler chicken offered aflatoxin-contaminated poultry feed

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
AST (U/L)	114.95±12.91	111.79±18.65	115.66±13.53	111.09±18.05	6.21	0.59	0.44
ALT (U/L)	7.50±2.64	7.77±3.27	7.10±3.19	8.17±2.63	1.33	0.80	0.32
ALP (U/L)	176.41±54.62	165.82±45.24	184.51±49.37	157.72±47.64	20.79	0.55	0.14
TP (g/dL)	2.53±0.38	2.30±0.24	2.37±0.37	2.46±0.29	0.13	0.06	0.41
ALB (g/dL)	1.80±0.23	1.95±0.48	1.86±0.20	1.89±0.50	0.12	0.29	0.75
GLB (g/dL)	0.72±0.42	0.35±0.59	0.51±0.43	0.56±0.65	0.19	0.06	0.78
ALB:GLB	2.50±0.95	1.18±0.74	1.27±0.81	1.30±0.71	0.81	0.18	0.74
MDA (nmol/mL)	99.17±22.25[a]	79.23±24.05[b]	96.41±24.13	81.99±24.34	7.65	0.02	0.08
SOD (U/mL)	28.57±15.31	32.70±33.96	27.13±31.30	34.14±19.77	8.46	0.02	0.08
TAC (U/mL)	188.70±59.35	209.93±116.49	179.21±55.78	219.41±115.59	29.31	0.67	0.47
T-GSH (µmol/mL)	9.84±4.61	8.70±3.68	8.70±3.68	9.91±6.85	2.06	0.52	0.23
GSH (µmol/mL)	5.38±1.23	5.75±1.63	4.84±0.85[b]	6.28±1.56[a]	0.76	0.59	0.55
GSSG (µmol/mL)	2.65±0.64[a]	2.08±0.63[b]	2.28±0.61	2.44±0.78	0.78	0.39	0.002
GSH:GSSG	2.11±0.53[b]	2.98±1.37[a]	2.19±0.45	2.89±1.45	0.40	0.02	0.49

[ab]Treatments means within the same row with unidentical superscripts differed greatly (P<0.05). SEM- standard error of means, P- value-probability level, AST- Aspartate transferase, ALT- Alanine transferase, ALP- Alkaline phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, MDA- Malondialdehyde, SOD- Superoxide dismutase, TAC- Total antioxidant capacity, T-GSH- Total glutathione, GSH- Reduced glutathione, GSSG- Oxidised glutathione, GSH:GSSG- Oxidative stress stress/stability index.

4.4.14 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on serum enzymes and biochemical indices of broiler chicken offered aflatoxin-contaminated poultry feed from 0 - 49 days

The interaction effect of varied levels of beta-glucans addition and supplemental selenium on serum biochemical indices, lipid peroxidation and antioxidants profile of broiler chickens fed aflatoxin-contaminated feed from 0 to 49 days is shown in Table 4.32. The AST (U/L) of birds fed all the treatment diets did not show any variationsby beta-glucans and supplemental selenium addition, and obtained values ranged from104.64 (NC) to118.58 (TD1). Birds offered BD (11.03) had greatly ($P<0.05$) elevated ALT (U/L) in comparison to birds offered NC diet (5.00), but did not differ from the other dietary treatments. The ALP (U/L) of birds offered BD (249.60) was elevated ($P<0.05$) distinctly($P<0.05$) above those of birds offeredthe other treatment diets, but was comparable ($P>0.05$) to that of birds offered TD1 (196.66). The TP (g/dL), ALB (g/dL), and GLB (g/dL) were not affected by the addition of beta-glucans and supplemental selenium, even at both levels of beta-glucans inclusion, and their values ranged from 2.20 to 2.58; 1.74 to 2.01; and 0.34 to 0.77, respectively. It was observed that birds offered BD had highly ($P<0.05$) raised MDA level (159.41nmol/mL)above the other dietarytreatments. The least ($P<0.05$) MDA value was from birds offered NCdiet (46.77).Comparable MDA values ($P>0.05$) were seen in birds offered TD1 (104.05),TD2 (94.30) and TD3 (88.78). The SOD (U/mL) values were also not affected by beta-glucans and supplemental selenium addition and its values ranged from 11.47 (BD) to 34.64 (TD4). The TAC (U/mL) of birds offered TD4 (243.23) increasedgreatly ($P<0.05$) above that of birds offered BD (124.76). Beta-glucans inclusion and supplemental selenium did not alter T-GSH (µmol/mL) concentration of all birds offered the different contaminated rations and its values ranged from 7.69 (TD3) to 10.46 (NC). The GSH (µmol/mL) obtained in birds offered NC diet (7.19) was elevated ($P<0.05$) distinctly above GSH of birds offered BD (3.50), TD1 (5.06), and TD3 (4.63) after 49 days feeding period. Birds offered BD (5.96) had highly ($P<0.05$) raised GSSG (µmol/mL) level above all the other dietary treatments. Higher ($P<0.05$) and desirable GSH to GSSH ratio (GSH:GSSG) were obtained in birds offered NC (3.66) and TD4 (3.58) significantly above other dietary

treatments. The least ($P<0.05$) and undesirable GSH:GSSH, significantly below that of all the other dietary treatments was obtained in birds offered BD (1.06).

Table 4.32 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on serum biochemistry of broiler chicken offered aflatoxin-contaminated poultry feed

Treatment	AF level (ppb)	Beta-glucans (ppm)	Se (mg/kg	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)	ALB (g/dL)	GLB (g/dL)	ALB : GLB	MDA (nM/mL)	SOD (U/mL)	TAC (U/mL)	T-GSH (µM/mL)	GSH (µM/mL)	GSSG (µM/mL)	GSH: GSSG
NC	0	0	0.00	104.64	5.00^{b}	161.21^{b}	2.58	1.96	0.62	3.16	46.77^{d}	29.55	200.59^{ab}	10.46	7.19^{a}	2.03^{b}	3.66^{a}
BD	270	0	0.00	107.78	11.0^{a}	249.60^{a}	2.20	1.74	0.46	3.78	159.41^{a}	11.47	124.76^{b}	9.64	3.50^{c}	5.96^{a}	1.06^{c}
TD 1	270	250	0.00	118.58	7.33^{ab}	196.66^{ab}	2.49	1.82	0.68	2.68	104.05^{b}	23.49	181.80^{ab}	9.70	5.06^{b}	2.61^{b}	2.01^{bc}
TD 2	270	250	0.30	111.32	7.68^{ab}	156.16^{b}	2.56	1.79	0.77	2.32	94.30^{b}	33.65	195.60^{ab}	9.97	5.68^{ab}	2.68^{b}	2.21^{bc}
TD 3	270	375	0.00	112.74	6.88^{ab}	172.35^{b}	2.24	1.89	0.34	5.56	88.78^{bc}	30.77	176.63^{ab}	7.69	4.63^{bc}	1.96^{b}	2.38^{b}
TD 4	270	375	0.30	110.86	8.66^{ab}	159.28^{b}	2.37	2.01	0.36	5.58	69.68^{c}	34.64	243.23^{a}	9.85	6.88^{ab}	2.21^{b}	3.58^{a}
SEM				6.21	1.33	20.79	0.13	0.12	0.19	1.47	7.65	8.46	29.31	2.06	0.76	0.78	0.0003
P-value				0.72	0.06	0.02	0.15	0.60	0.48	0.65	<0.0001	0.40	0.15	0.95	0.01	0.01	0.40

[abc]Treatments within the same column having unidentical superscripts differed greatly (P<0.05). SEM- Standard error of means, P- value- probability level, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, MDA- Malondialdehyde, SOD- Superoxide dismutase, TAC- Total antioxidant capacity, T-GSH- Total glutathione, GSH- Reduced glutathione, GSSG- Oxidised glutathione, GSH:GSSG- Oxidative stress/stability index, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C), TD2- BD+(E+C+K)+Se, TD3- BD+(E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.15 Maineffects of varied inclusion levels of beta-glucans and supplemental selenium on relative aflatoxins retention within broiler chicken offered aflatoxin-contaminated poultry feed

Table 4.33presented the main effect of varied levels of beta-glucans inclusion and supplemental selenium on relative aflatoxins retention within broiler chicken offered contaminated rations. Higher ADFI (g/b/d) obtained in birds offered diet containing 375ppm beta-glucans(72.23±1.97) wasremarkably (P<0.05) greater thanADFI of birds offered 250ppm beta-glucans (54.91±2.60) diet. Supplemental seleniumincreased ADFI (65.31±8.95) greatly(P<0.05)above that of birds offered diets without selenium (61.82±10.17). ADAI (µg/b/d) was distinctly (P<0.05) reduced in birds offered 250ppm beta-glucans (14.83±0.70) diet below that of birds offered 375ppm beta-glucans (19.50±0.53) diets. ADAI increased greatly (P<0.05) with supplemental selenium (17.64±2.42) compared to birds offered diet without supplemental selenium (16.69±2.74). Higher quantity (P<0.05) of ADFO (g/bird) was obtained in birds offered 375ppm beta-glucans (14.27±0.49) diet in comparison to that of birds offered 250ppm beta-glucans (11.79±1.13) diet.Results revealed that birds offered diets with supplemental selenium (13.59±1.21) had distinctly (P<0.05) greater ADFO above that of birds offered diets without supplemental selenium (12.48±1.73). Birds fed 375ppm beta-glucans (16.41±1.19) diets had highly (P<0.05) increased ADVA (µg/bird) above the value obtained in birds offered 250ppm beta-glucans diets (11.32±1.54). Selenium supplementationgreatly (P<0.05) increased ADVA (15.01±2.72) compared to diets without supplemental selenium (12.72±2.96). Approximately 84.06±4.46% of aflatoxins were expelled in birds offered 375ppm beta-glucans diets and this was highermarkedly (P<0.05) in comparison to that of birds offered 250ppm beta-glucans diets (76.14±7.72%). Supplemental selenium in broilers' diet greatly (P<0.05) resulted in higher percentage of expelled aflatoxins (84.75±5.75%) above birds offered diets without supplemental selenium (75.45±5.97%). Varied inclusion level of beta-glucans did not affect relative quantity of Aflatoxins Retention (AR) and it ranged between (3.09±0.83µg/bird) and (3.51±1.03µg/bird). However, supplemental selenium greatly reduced (P<0.05) the relative quantity of AR (2.63±0.74µg/bird), significantly below that of birds offered diets without supplemental selenium (3.98±0.46µg/bird). Significantly reduced (P<0.05) AR was obtained in birds offered 365ppm beta-glucans diets (15.94±4.46%) significantly below that of birds offered 250ppm beta-glucans

diets(23.86±7.72%). Birds offered selenium supplemented diet (15.25±5.44%) had greatly ($P<0.05$) reduced percentage of aflatoxin retention in comparison to birds offered diets without supplemental selenium (24.55±5.97%).

Table 4.33 Main effect of varied inclusion levels of beta-glucans and supplemental selenium on relative aflatoxins retention within broiler chicken offered aflatoxin-contaminated poultry feed

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
ADFI (g/b/d)	54.91±2.60[b]	72.23±1.97[a]	61.82±10.17[b]	65.31±8.95[a]	0.85	<0.0001	0.002
ADAI (µg/b/d)	14.83±0.70[b]	19.50±0.53[a]	16.69±2.74[b]	17.64±2.42[a]	0.19	<0.0001	0.002
ADFO (g/b/d)	11.79±1.13[b]	14.27±0.49[a]	12.48±1.73[b]	13.59±1.21[a]	0.43	<0.0001	0.01
ADVA (µg/b/d)	11.32±1.54[b]	16.41±1.19[a]	12.72±2.96	15.01±2.72	0.31	<0.0001	0.0002
Expelled aflatoxins (%)	76.14±7.72[b]	84.06±4.46[a]	75.45±5.97[b]	84.75±5.44[a]	2.13	0.01	0.004
Rel. Qty. of AR (µg/bird)	3.51±1.03	3.09±0.83	3.98±0.46[a]	2.63±0.74[b]	0.34	0.29	0.01
Aflatoxin retention (%)	23.86±7.72[a]	15.94±4.46[b]	24.55±5.97[a]	15.25±5.44[b]	2.13	0.01	0.004

[ab]Treatments means within the same row having unidentical superscripts differed distinctly (P<0.05). SEM- standard error of means, P-value-probability level, ADFI- Average Daily Feed Intake, ADAI- Average Daily Aflatoxins Intake, ADFO- Average Daily Faecal Output, ADVA- Average Daily Voided Aflatoxins, Rel.- Relative, Qty.- Quantity, AR- Aflatoxins Retained

4.4.16 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on relative aflatoxins retention within broiler chicken offered aflatoxin-contaminated poultry feed

The synergistic or additive effect of varied levels of beta-glucans inclusion and supplemental selenium on relative aflatoxins retention within broiler chickens is shown in Table 4.34. The ADFI (g/b/d) increased ($P<0.05$) highly in birds fed NC diet (80.10) above the ADFI of birds offered other treatment diets. The least ($P<0.05$) ADFI recorded in birds offered BD (46.52) was significantly below that of all the other dietary treatments. The ADAI (µg/b/d) greatly ($P<0.05$) increased in birds offered TD4 (19.83) above the aflatoxins intake of birds offered other treatment diets. Distinctly higher ($P<0.05$) ADFO (g/b/d) was obtainedin birds offeredNC (14.46), TD3 (14.02) and TD4 (14.52) in comparison to birds offered other treatment diets. The least ($P<0.05$) ADFO obtained in birds offered TD1 (10.94) was similar to result obtained in birds offered BD (11.81). The ADVA (µg/b/d) obtained in birds offeredTD4 (17.44) was higher andvaried greatly ($P<0.05$) compared to values obtained in birds offered other treatmentdiets. Birds offered BD (5.53) had the leastquantity ($P<0.05$) of ADVA, significantly below the values recorded in the other dietary treatments except for birds offered NC diet (0.00), which had no aflatoxins contamination. Higher ($P<0.05$) percentage of expelled aflatoxins was recorded inbirds offered TD4 (87.91%) in comparison to values obtained from other dietarytreatments but similar to that of birds offered TD2 (81.59%). However, birds offered NCdiet (0.00%) had noexpelled aflatoxins. The relative quantity of aflatoxins retained (µg/bird) was greater distinctly ($P<0.05$) in birds offered BD (7.03) above the results obtained in birds offeredother treatment diets. Birds offered TD1 (4.16) had ($P<0.05$) greater relative quantity of aflatoxins retained significantly above aflatoxins retained in birds offered TD2 (2.85) and TD4 (2.39). Birds offered NC diet (0.00) had no ($P<0.05$) retained aflatoxins. Higher ($P<0.05$) percentage of aflatoxin retention was obtained in birds offered BD (55.91%) significantly above birds offered other treatment diets. Aflatoxin retention in birds offered TD2 (18.41%) and TD3(19.79%) were similar while TD2 and TD4 (12.09%) were also comparable, and the least retention being from birds offered TD4. A comparative bar chart of aflatoxins consumed, voided and retained is presented in appendix I.

Table 4.34 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on relative aflatoxins retention within the broiler chicken offered aflatoxin-contaminated poultry feed

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	ADFI (g/b/d)	ADAI (µg/b/d)	ADFO (g/b/d)	ADVA (µg/b)	Expelled Aflatoxins (%)	Rel. Qty. Aflatoxins Retained (µg/b)	Aflatoxin Retention (%)
NC	0	0	0.00	80.10^{a}	0.00^{f}	14.46^{a}	0.00^{f}	0.00^{e}	0.00^{e}	0.00^{e}
BD	270	0	0.00	46.52^{e}	12.56^{e}	11.81^{bc}	5.53^{e}	44.09^{d}	7.03^{a}	55.91^{a}
TD 1	270	250	0.00	52.64^{d}	14.21^{d}	10.94^{c}	10.05^{d}	70.69^{c}	4.16^{b}	29.31^{b}
TD 2	270	250	0.30	57.17^{c}	15.44^{c}	12.65^{b}	12.58^{c}	81.59^{ab}	2.85^{cd}	18.41^{cd}
TD 3	270	375	0.00	71.01^{b}	19.17^{b}	14.02^{a}	15.38^{b}	80.21^{b}	3.79^{bc}	19.79^{c}
TD 4	270	375	0.30	73.46^{b}	19.33^{a}	14.52^{a}	17.44^{a}	87.91^{a}	2.39^{d}	12.09^{d}
SEM				0.85	0.19	0.43	0.31	2.13	0.34	2.13
P-value				<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001

^{abcdef} Treatment means within the same column having unidentical superscripts are distinctly different (P<0.05). SEM- Standard error of mean, P-value- probability level, ADFI- Average Daily Feed Intake, ADAI- Average Daily Aflatoxins Intake, ADFO- Average Daily Faecal Output, ADVA- Average Daily Voided Aflatoxins, Rel.- Relative, Qty.- Quantity, AR- Aflatoxins Retained, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.17 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on tissue residual aflatoxins and feed to tissue carry-over ratio in broiler chicken offered aflatoxin-contaminated poultry feed

Main effect of varied levels of beta-glucans inclusion and supplemental selenium on aflatoxins concentration in blood, breast muscle, and liver of broiler chickenoffered aflatoxin-contaminated feed is presented in Table 4.35. Aflatoxins concentration in blood (ng/mL) of birds offered 250ppm beta-glucansdiet (3.88±0.48) was raised($P<0.05$) significantlyabove that of birds offered 375ppm beta-glucans diet (2.83±0.27). However, supplemental selenium did not alter blood aflatoxins level to any significant extent and the concentration ranged between 3.34±0.79 and 3.37±0.57. Aflatoxins concentration in breast meat (µg/kg) did not show any variation by varying beta-glucans inclusion levels and it ranged between 0.25±0.04 and 0.41±0.22. Supplemental selenium also did not produce any variations in breast meat residual aflatoxins and ranged between 0.28±0.07 and 0.37±0.24. Feed:breastmuscle aflatoxins deposition ratio was not affected by either varied levels of beta-glucansaddition or with supplemental seleniumand values ranged between 822.50±398.25 in 250ppm β-glucans diet and 1117.50±211.69 in 375ppm beta-glucans diet; and 910.00±375.61without Se and 1030.00±299.82 with supplemental Se, respectively. Feed to residualliver aflatoxin ratio also did not reveal any differences of significanceby beta-glucans inclusion and supplemental selenium, and it ranged between 467.50±218.92 (250ppm beta-glucans) and 717.50±210.14 (375ppm beta-glucans); and 482.50±180.91 without Se and 702.50±260.42with Se, respectively. Residual aflatoxins in liver (µg/kg) of birds offered250ppm beta-glucans diet (0.65±0.20) was elevated significantly ($P<0.05$) above that of birds offered 375ppm beta-glucans diet (0.41±0.12). Supplemental selenium had no effect on liver aflatoxins and it ranged between 0.430.17 and 0.62±0.21. Higher ($P<0.05$) liver weight (g) recorded in birds fed375ppm beta-glucans diet (44.51±7.43) increased significantly above liver weights of birds offered 250ppm beta-glucans diet (34.59±6.45). However, liver weight of birds was not affected with selenium supplementation andit ranged between 36.89±6.76 and 42.21±9.64. Also, relative liver weight to that of the bodyweight was not affected by main effects of levels of beta-glucans and also that of selenium supplementation. The actual quantity of residual aflatoxins (µg) in liver of

birds was also not altered by varied inclusion levels of beta-glucans and supplemental selenium, and weresimilar, to one another at 0.02 µg each.

Table 4.35 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on aflatoxins concentration in blood, breast muscle and liver of broiler chicken offered aflatoxin-contaminated poultry feed

Aflatoxins concentration	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
Blood (ng/mL)	3.88±0.48[a]	2.83±0.27[b]	3.37±0.57	3.34±0.79	0.24	0.003	0.92
Breast muscle (µg/kg)	0.41±0.22	0.25±0.04	0.37±0.24	0.28±0.07	0.11	0.10	0.31
Liver (µg/kg)	0.65±0.20[a]	0.41±0.12[b]	0.62±0.21	0.43±0.17	0.12	0.02	0.05
Feed:Breast muscle	822.50±398.25	1117.50±211.69	910.00±375.61	1030.00±299.82	136.14	0.11	0.49
Feed:liver	467.50±218.92	717.50±210.14	482.50±180.91	702.50±260.42	93.51	0.06	0.09
Liver weight (g)	34.59±6.45[b]	44.51±7.43[a]	36.89±6.76	42.21±9.64	3.59	0.04	0.23
Liver weight rel. to BW (%)	2.56±0.07	2.44±0.07	2.48±0.03	2.50±0.03	0.20	0.12	0.26
RLA (µg)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.01	0.26	0.23

[ab]Treatment means within the same row having unidentical superscripts differed greatly ($P<0.05$). RLA- Residual liver aflatoxins, SEM- standard error of means, P-value- Probability level

4.4.18 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on tissue residual aflatoxins and feed to tissue carry-over ratio in broiler chicken offered aflatoxin-contaminated poultry feed

Table 4.36 shows the interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on aflatoxins concentration in blood, breast muscle, and liver of broiler chickenoffered aflatoxin-contaminated ration. Blood aflatoxins (ng/mL) concentration was raised ($P<0.05$) greatly in birds offeredBD (9.17±0.60) significantly above the level in birds offered other treatment diets. Blood aflatoxins concentration in birds offered TD3 (2.89±0.21)and TD4 (2.77±0.35) were similar. Residual aflatoxins in breast muscle (µg/kg) was greatly ($P<0.05$) increased in birds offered BD (2.56±0.34) significantly above thoseof birds offered other treatment diets. The interaction or additive effect of varied beta-glucans inclusion and supplemental selenium had distinct ($P<0.05$) effect on feed to breast muscle aflatoxins deposition ratio and feed to liver aflatoxins ratio. Higher ($P<0.05$) and preferred feed to breast muscle aflatoxins ratio was obtained in birds offered TD3 (1195.00±81.85) significantly above the ratio obtained in birds offered BD (105.00±13.23) and TD1 (625.00±319.88). Differences in feed aflatoxins to breast muscle ratios recorded in birds offered TD3 (1,195.00±81.85), TD4 (1,040.00±217.43) and TD2 (1,020.00±420.89) were not distinct ($P<0.05$). Higher ($P<0.05$) and preferred feed to liver aflatoxins ratio recorded in birds offered TD4 (820.00±230.65) varied significantly above the ratios of birds offered BD (80.00±10.89) and TD1 (350.00±58.95). However, birds offered NC oraflatoxin-free diet had no residual aflatoxins in liver and breast muscle. Liver aflatoxins concentration (µg/kg) in birds offered BD (3.46±0.43) increased greatly ($P<0.05$) above the levelseen in birds offered other treatment diets.Comparable ($P>0.05$)liver aflatoxins concentrations were obtained in birds offered TD1 (0.78±0.13), TD2 (0.52±0.19) and TD3 (0.46±0.13) but were distinctly ($P<0.05$) below the aflatoxins concentration in liver of birds offered BD. Liver weight (g) obtained in birds offered TD4 (47.03±10.12) was similar ($P<0.05$) statistically to liver weights of birds offeredNC (37.18±4.27), TD2 (37.39±7.76) and TD3 (41.99±4.03). Birds offered BD (24.00±3.93) had highly ($P<0.05$) reduced liver weight but was comparableto that of birds offered TD1 (31.81±4.52). The liver weight relative to that of the body weight in birds fed NC

(1.85±0.25%) and TD4 (2.42±0.33%) were similar. Birds fed BD, TD1, TD2 and TD3 all had similar relative liver weight that ranged between 2.40±0.22% and 2.62±0.29%.Actual quantity of residual liver aflatoxins (µg/bird) was higher remarkably($P<0.05$) in birds offered BD (0.08±0.02) compared with birds offered other treatment diets. Similar residual liver aflatoxins were obtained in the liver of birds offered TD2 (0.02±0.01), TD3 (0.02±0.01) TD4 (0.02±0.01) and NC (0.00±0.00) diets.

Table 4.36 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on aflatoxins concentration in blood, breast muscle and liver of broiler chicken offered aflatoxin-contaminated poultry feed

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	Tissue aflatoxins concentration							
				Blood (ng/mL)	Breast muscle (µg/kg)	Feed:breast muscle	Liver (µg/kg)	Feed:liver	Liver weight (g)	Liver weight rel. to Bodyweight (%)	Aflatoxin Residues in liver (µg/bird)
NC	0	0	0.00	0.03±0.01^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	37.18±4.27^{ab}	1.85±0.25^{b}	0.00±0.00^{c}
BD	270	0	0.00	9.17±0.60^{a}	2.56±0.34^{a}	105.00±13.23^{c}	3.46±0.43^{a}	80.00±10.89^{c}	24.00±3.93^{c}	2.40±0.22^{a}	0.08±0.02^{a}
TD 1	270	250	0.00	3.85±0.26^{b}	0.52±0.27^{b}	625.00±319.88^{b}	0.78±0.13^{b}	350.00±58.95^{b}	31.81±4.52^{bc}	2.50±0.07^{a}	0.03±0.01^{b}
TD 2	270	250	0.30	3.91±0.71^{b}	0.29±0.10^{bc}	1020.00±420.89^{ab}	0.52±0.19^{bc}	585.00±273.72^{ab}	37.39±7.76^{ab}	2.62±0.29^{a}	0.02±0.01^{bc}
TD 3	270	375	0.00	2.89±0.21^{c}	0.23±0.02^{bc}	1195.00±81.85^{a}	0.46±0.13^{bc}	615.00±160.23^{ab}	41.99±4.03^{ab}	2.48±0.04^{a}	0.02±0.01^{bc}
TD 4	270	375	0.30	2.77±0.35^{c}	0.27±0.05^{bc}	1040.00±217.43^{ab}	0.35±0.11^{c}	820.00±230.65^{a}	47.03±10.12^{a}	2.42±0.33^{b}	0.02±0.01^{bc}
SEM				0.24	0.11	136.14	0.12	93.51	3.59	0.23	0.01
P-value				<0.0001	<0.0001	0.0001	<0.0001	0.0003	0.01	<0.0001	<0.0001

^{abcd}Treatment means within the same column having unidentical superscripts differed greatly (P<0.05). SEM- Standard error of mean, P-value- probability level, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+(E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium, rel.- relative.

4.4.19 Main effects of varied inclusion levels of beta-glucansand supplemental selenium on improvement or loss in bodyweight gain of broiler chicken offered aflatoxin-contaminated rations

The main effect of varied inclusion levels of beta-glucans and supplemental selenium on improvement or loss in bodyweight gain (BWG) of birds offered aflatoxin-contaminated feed relative to the BWG of birds fed BD and NC, respectively is presented in Table 4.37. The BWG (g/bird) of birds offered 375ppm beta-glucans diet (1,775.96±157.79) was higher greatly ($P<0.05$) above that of birds offered 250ppm beta-glucans diet (1,305.48±93.14). Birds offered selenium supplemented diets (1,642.26±287.94) also had increased BWG distinctly ($P<0.05$) higher compared to the BWG of birds offered diet without supplemental selenium (1,439.17±242.34). Relative to the BWG of birds offered aflatoxin-free diet (NC), reduction in BWG (rBWG) recorded in birds offered 250ppm beta-glucans diet (33.62±4.47%) was higher greatly ($P<0.05$), above the reduction recorded in birds offered 375ppm beta-glucans diet (9.63±8.54%). However, relative to the BWG of birds fed NC diet, supplementalselenium (16.46±14.73%) remarkably ($P<0.05$) decreased the level of reduction in BWGin comparison to that of birds offered diet without supplemental selenium(26.78±12.54%).Improvement in BWG (iBWG), relative tothe BWG of birds offered BD, was higher distinctly ($P<0.05$) inbirds offered 375ppm beta-glucans diet (85.75±16.00%) above that of birds offered 250ppm beta-glucans diet (36.56±10.11%). It was also noticed that relative to the BWG of birds offered BD, supplemental selenium resulted in iBWG (71.77±30.25%),distinctly ($P<0.05$) higher when compared with that of birds offered diet without selenium (50.53±25.38%) supplementation.

Table 4.37 Main effect of varied inclusion levels of beta-glucans and supplemental selenium on improvement or loss in bodyweight gain of broiler chicken offered aflatoxin-contaminated poultry feed

Parameters	Beta-glucans (ppm)		Selenium (ppm)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
BWG (g/bird)	1305.48±93.14[b]	1775.96±157.79[a]	1439.17±242.34[b]	1642.26±287.94[a]	32.59	<0.0001	0.001
rBWG Rel. to NC (%)	33.62±4.47[a]	9.63±8.54[b]	26.78±12.54[a]	16.46±14.73[b]	1.87	<0.0001	0.002
iBWG Rel. to BD (%)	36.56±10.11[b]	85.75±16.00[a]	50.53±25.38[b]	71.77±30.25[a]	3.51	<0.0001	0.001

[ab]Treatment means within the same row having unidentical superscripts differed distinctly (P<0.05), SEM- standard error of means, P-value- probability level, BWG- body weight gain, rBWG- reduction in BWG relative to NC, iBWG- improvement in BWG relative to BD, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free)

4.4.20 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on improvement or loss in bodyweight gain of broiler chicken offered aflatoxin-contaminated rations

Table 4.38presented the interaction effect of varied levels of beta-glucansinclusion and supplemental selenium on improvement or loss in bodyweight gain of birds offered aflatoxin-contaminated ration relative to the BWG of birds offered BD and NC, respectively. Birds offered NC (1,966.24±29.77) and TD4 (1,903.98±32.56) had higher BWG (g/bird) greatly ($P<0.05$)above those of birds offered other treatment rations. The least andhighly ($P<0.05$) reduced BWG was obtained in birds offered BD (956.27±19.34). Birds offered all four contaminated diets treated with varied levels of beta-glucans inclusion and combination with supplemental dietary antioxidants with or without supplemental selenium had greater BWG, distinctly($P<0.05$)above that of birds offered BD. The BWG of birds offered TD1, TD2, TD3 and TD4had significantly different and greater ($P<0.05$) BWG in the order of TD4> TD3> TD2> TD1, with TD4 having the highest BWG (1,903.98±32.56), which compared favourably to NC BWG (1,966.24±29.77). Reduction in BWG (rBWG)relative to the BWG of birds fed NC diet, was distinctly ($P<0.05$) greater in birds offered BD (51.36±1.12%)compared with the rBWG of birds offeredother treatment diets. Birds offered TD4 (3.14±3.11) had no significant ($P<0.05$) difference in their BWG reduction relative to NC (0.00±0.00). The order of reduction in BWG was TD4< TD3< TD2< TD1. Birds fed TD4 had(99.15±5.08%) improvement in BWG (iBWG) similar to NC (105.62±2.10) butdistinctly ($P<0.05$) higher than iBWG of birds offered other treatment diets, relative to the BWG of birds fed BD. The iBWG of birds offered TD1 (28.72±7.62%), TD2 (44.39±3.63%), TD3 (72.35±11.15%) and TD4 (99.15±5.08%)markedly varied ($P<0.05$) from one another.The trend of improvement in BWG beingNC = TD4> TD3> TD2> TD1 over that of birds that consumed BD. A chart showing the comparison of rBWG and iBWG relative to the BWG of the NC is presented in appendix II.

Table 4.38 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on improvement or loss in bodyweight gain of broilers due to aflatoxins contamination of poultry feed

Treatments	Aflatoxins level (ppb)	Beta-glucans (ppm)	Selenium (mg/kg)	BWG (g/bird)	rBWG Relative to NC (%)	iBWG Relative to BD (%)
NC	0	0	0.00	1966.24±29.77^{a}	0.00±0.00^{e}	105.62±2.10^{a}
BD	270	0	0.00	956.27±19.34^{e}	51.36±1.12^{a}	0.00±0.00^{e}
TD 1	270	250	0.00	1230.40±63.58^{d}	37.44±2.41^{b}	28.72±7.62^{d}
TD 2	270	250	0.30	1380.55±27.12^{c}	29.79±0.32^{c}	44.39±3.63^{c}
TD 3	270	375	0.00	1647.95±109.65^{b}	16.12±6.80^{d}	72.35±11.15^{b}
TD 4	270	375	0.30	1903.98±32.56^{a}	3.14±3.11^{e}	99.15±5.08^{a}
SEM				32.59	1.87	3.51
P-value				<0.0001	<0.0001	<0.0001

^{abcde} Treatment means within the same column having unidentical superscripts differed distinctly (P<0.05). SEM- Standard error of mean, P-value- probability level, BWG- Body weight gain, rBWG- Reduction in BWG relative to NC, iBWG- Improvement in BWG relative to BD, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free),TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium.

4.4.21 Main effects of varied inclusion levels of beta-glucans and supplemental selenium on cost benefit analysis of broiler chicken offered aflatoxin-contaminated poultry feed

Table 4.39 shows the main effect of varied inclusion levels of beta-glucans and supplemental selenium on cost benefit analysis of broiler chickens that consumed aflatoxin-contaminated ration. The Average final body weight (AFBW - g/bird) at 49th day was higher in birds fed 375ppm beta-glucans diet (1,820.90±156.92) and varied (P<0.05) remarkably from the body weights of birds offered 250ppm beta-glucans diet (1,350.30±93.20). Supplemental selenium greatly (P<0.05) increased AFBW ofbirds (1,686.72±287.59) above the weights of birds that consumed diet without supplemental selenium (1,484.48±242.77). Birds offered 375ppm beta-glucans diet had increased (P<0.05).Average Feed Cost per bird (AFC/bird) (₦574.27±15.46),significantlyabove thatof birds offered 250ppm beta-glucans diet (₦420.39±20.19). Supplemental selenium (₦510.70±79.92) resulted in distinct(P<0.05) rise in AFC/bird compared to that of birds offered diet not supplementedwith selenium (₦483.96±89.66).Average Total Raising Cost (ATRC) obtained from birds that consumed 375ppm beta-glucans diet (₦953.28±15.46) was raised distinctly (P<0.05) above that of birds offered 250ppm beta-glucans diet (₦799.39±20.19). The ATRC recorded in birds offered selenium supplemented diet (₦889.71±79.92) was also raised greatly (P<0.05) over the cost of raising birds offered diets without supplemental selenium (₦862.97±89.66). Average LiveweightValue(ALWV)of birds that consumed 375ppm beta-glucans diet (₦1,183.59±101.99)was greater(P<0.05) significantly, more than the value obtained from birds offered 250ppm beta-glucans diet (₦877.69±60.58). The ALWV of birds fed selenium supplemented diet (₦1,096.37±186.94) was equally increased (P<0.05) significantly and higher, relative to the value obtained from birds offered diet without supplemental selenium (₦964.91±157.80). Higher and significant (P<0.05) Average Marginal Return (AMR) per bird was obtained in birds fed 375ppm beta-glucans diet (₦230.30±94.02) in comparison to birds offered 250ppm beta-glucans diet (₦78.29±44.29). The AMR per bird was also higherdistinctly (P<0.05) in birds offered selenium supplemented diet (₦206.65±108.17), than the value obtained in birds offered diet without supplemental selenium (₦101.94±79.92).

Table 4.39 Main effect of varied inclusion levels of beta-glucans and supplemental selenium on cost benefit analysis of broiler chicken, as affected by aflatoxins contamination

Parameters	Beta-glucans (ppm)		Selenium (mg/kg)		SEM	P- value	
	250	375	0.00	0.30		Beta-glucans	Selenium
AFBW (g/bird)	1350.30±93.20[b]	1820.90±156.92[a]	1484.48±242.77[b]	1686.72±287.59[b]	32.49	<0.0001	0.001
AFC/bird (₦)	420.39±20.19[b]	574.27±15.46[a]	483.96±89.66[b]	510.70±79.92[a]	6.46	<0.0001	0.002
ATRC/bird (₦)	799.39±20.19[b]	953.28±15.46[a]	862.97±89.66[b]	889.71±79.92[a]	6.46	<0.0001	0.002
ALWV/bird (₦)	877.69±60.58[b]	1183.59±101.99[a]	964.91±157.80[b]	1096.37±186.94[a]	21.12	<0.0001	0.001
AMR/bird (₦)	78.29±44.29[b]	230.30±94.02[a]	101.94±79.92[b]	206.65±108.17[a]	21.46	0.0003	0.004

[ab]Treatment means within the same row having unidentical superscripts differed greatly (P<0.05), SEM- standard error of means, P- value-probability level, AFBW- Average Final Body weight, AFC- Average Feed Cost, ATRC- Average Total raising cost, ALWV- Average live weight value, AMR- Average Marginal return.

4.4.22 Interaction effects of varied inclusion levels of beta-glucans and supplemental selenium on cost benefit analysis of broiler chicken offered aflatoxin-contaminated poultry feed

The interaction effect of varied levels of beta-glucans inclusion and supplementalselenium on cost benefit analysis of broiler chickenoffered aflatoxin-contaminated feed is presented in Table 4.40. The AFBW (g/bird) of birds offeredNC (2,011.82±28.79) and TD4 (1,948.07±33.84) were higher and greatly varied (P<0.05) compared to the weights of birds offeredother treatment diets. Birds that consumed BD (1,001.49±17.89) had the least (P<0.05)AFBW, significantly reduced below the weights of birds from other treatment diets. It was noticed that the AFC/bird inbirds offeredNC (₦580.81±), TD3 (₦565.12±) and TD4 (₦583.43±) were similar but showed higher variations distinctly (P<0.05)above AFCof birds offered other treatment diets. The least and greatly (P<0.05) reduced AFC/bird was recorded in birds offered BD (₦307.29±10.98). Average Total Raising Costs (ATRC)/birdobtained in birds offeredNC (₦959.82±13.86), TD3 (₦944.13±17.56), and TD4 (₦962.44±6.21) were higher distinctly(P<0.05), compared to the production cost of birds offeredother treatment diets.The least (P<0.05) ATRC/bird was also obtained in birds offered BD (₦686.31±10.98). The ALWVof birds offered NC (₦1,307.68±18.72) and TD4 (₦1,266.24±21.99) werecomparable but remarkably (P<0.05) greater than the value obtained in birds offered other treatment diets. The ALWV of birds offered BD (₦650.97±11.63) was the least and greatly (P<0.05) reduced below the values obtained in birds offered other treatment diets, even though the former had the least ATRC.The AMR/bird increasedremarkably (P<0.05) in birds offered NC (₦347.86±20.41) and TD4 (₦303.80±22.30), compared to the value obtainedin birds that consumed other treatment diets. The least and marked (P<0.05) deficit in AMR/bird was recorded in birds offered BD (-₦35.34±1.01). In appendix III, the area of intersection where ATRC was higher than ALWV indicated the loss recorded in birds fed BD

Table 4.40 Interaction effect of varied inclusion levels of beta-glucans and supplemental selenium on cost benefit analysis of broiler chicken offered aflatoxin-contaminated poultry feed

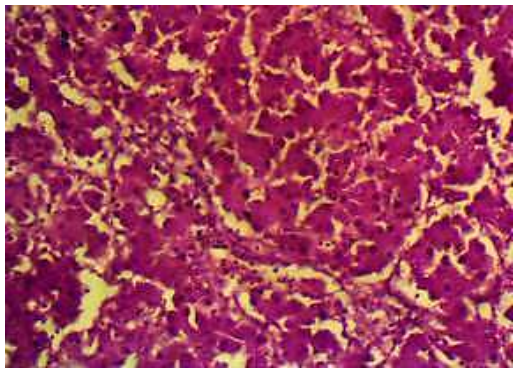
Treatments	AF level (ppb)	Beta-glucans (ppm)	Se (mg/kg)	AFBW (g/bird)	AFC/bird (₦)	ATRC/bird (₦)	ALWV/bird (₦)	AMR/bird (₦)
NC	0	0	0.00	2011.82±28.79^{a}	580.81±13.86^{a}	959.82±13.86^{a}	1307.68±18.72^{a}	347.86±20.41^{a}
BD	270	0	0.00	1001.49±17.89^{e}	307.29±10.98^{d}	686.31±10.98^{d}	650.97±11.63^{e}	-35.34±1.01^{d}
TD 1	270	250	0.00	1275.22±63.90^{d}	402.80±5.25^{c}	781.81±5.25^{c}	828.89±41.54^{d}	47.08±39.32^{c}
TD 2	270	250	0.30	1425.37±26.93^{c}	437.98±7.98^{b}	816.99±7.98^{b}	926.49±17.51^{c}	109.50±20.90^{bc}
TD 3	270	375	0.00	1693.73±109.09^{b}	565.12±17.56^{a}	944.13±17.56^{a}	1100.93±70.91^{b}	156.80±73.45^{b}
TD 4	270	375	0.30	1948.07±33.84^{a}	583.43±6.21^{a}	962.44±6.21^{a}	1266.24±21.99^{a}	303.80±22.30^{a}
SEM				32.49	9.46	9.46	21.12	21.46
P-value				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{abcde}Treatment means within the same column having unidentical superscripts are distinctly different (P<0.05). SEM- Standard error of mean, P-value- probability level, AFBW- Average Final Bodyweight, AFC- Average Feed Cost, ATRC- Average Total raising cost, ALWV- Average liveweight value, AMR- Average Marginal return, TD- Treatment Diet, NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm beta-glucans; dietary antioxidant-free), TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium

4.4.23 Report of histological section of liver of broiler chicken offered aflatoxin-contaminated feed having varied inclusion levels of beta-glucans,supplemental dietary antioxidants and vitamin K

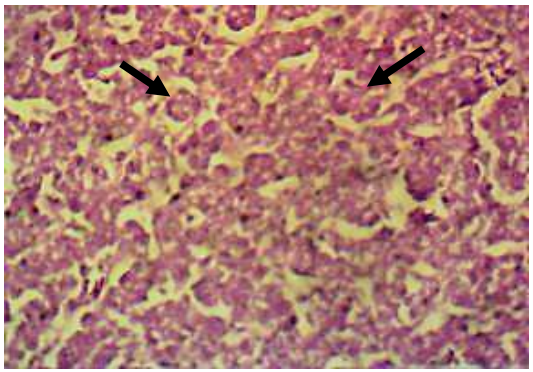
The summary of the photomicrographs showing the histological section of liverof broiler chicken offered diets contaminated with aflatoxins but treated withbeta-glucans, supplemental dietary antioxidantsand vitamin K is presented in Plate 4.1. Liver microscopic section showed that birds fed NC diet had no observable lesion while birds offered BD had gross hepatocellular coagulation and degeneration, with atrophy and sinusoids accentuation, coupled with hyperplasia of the Kupffer cells as indicated with the arrows. However, birds offered diet that had 375ppm beta-glucans with or withoutsupplemental selenium (TD4 and TD3 respectively) led to improvement in the hepatictissues histology to a level of no observable lesion as seen in birds fedNC. Addition of 250ppm of beta-glucans with selenium supplementation (TD2) or without (TD1) had moderate hepatocellular degeneration (as shown with arrows). The results of histology of liver samples from birds fed TD1 to TD4 were indicative of positive effect of beta-glucans in preventing extensive liver damage as seen in birds fed BD.

NC



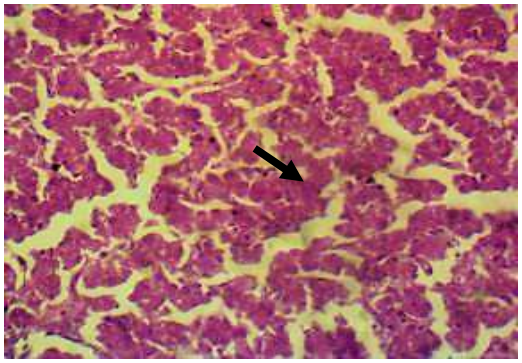
There is no observable lesion

BD



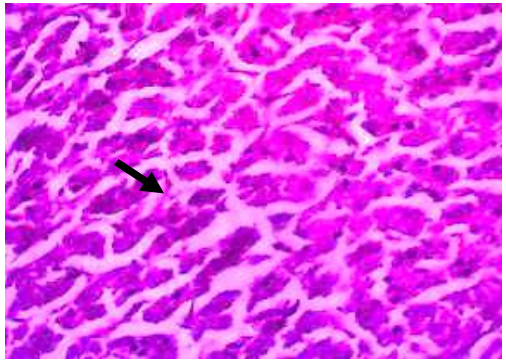
There is multifocal hepatocellular coagulation necrosis (arrows), atrophy of cords and accentuation of sinusoids with Kupffer cell hyperplasia

TD1



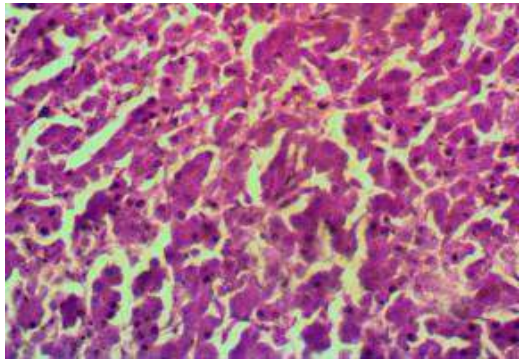
There is moderate diffuse hepatocellular atrophy (arrows)

TD2



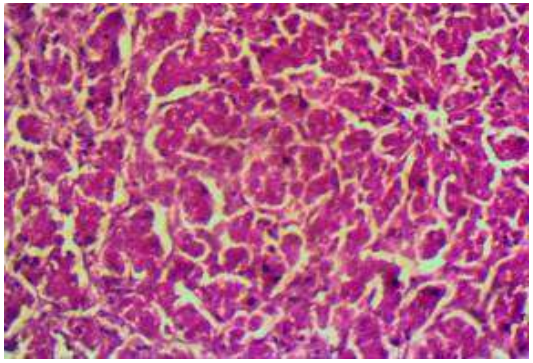
There is moderate diffuse atrophy of hepatic plates (arrow)

TD3



There is no observable lesion

TD4



There is no observable lesion

Magnification = x400 for each slide

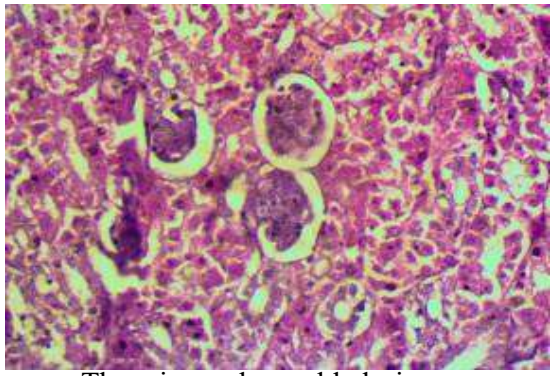
Stain = Haematoxylin and Eosin

Plate 4.1 Histological section of liver of broilers fed aflatoxin-contaminated poultry feed

4.4.24 Report ofhistological section of kidney of broiler chicken offered aflatoxin-contaminated feed having varied inclusion levels of beta-glucans, supplemental dietary antioxidants and vitamin K

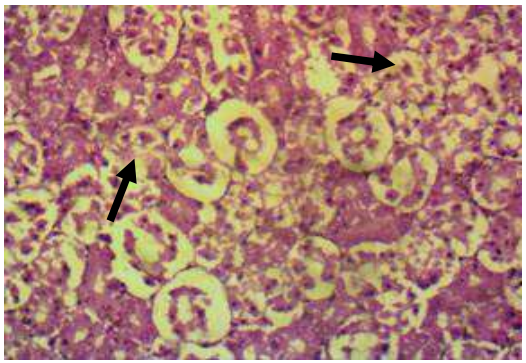
Histological section of kidney samples of broiler chicken offered aflatoxin-contaminated rations is presented in Plate 4.2. Birds offered aflatoxin-free diet (NC) had no observable lesion in their kidneys. On the contrary, birds fed aflatoxin-contaminated diet with no beta-glucans and supplemental dietary antioxidants addition (BD) had kidney samples with necrotic and multifocal tubular epithelial coagulation, coupled with defoliation and disruption of basement membranes (see arrows). Addition of 250ppm of beta-glucans with supplemental selenium (TD2) and without (TD1) were not so effective to completely counteract the adverse effects of aflatoxins on kidney cells of birds fed these two dietary treatments. However, birds fed diet with 375ppm beta-glucans inclusion with (TD4) and without (TD3) supplemental selenium had no observable lesion in their kidneys, similar to that of birds fed NC.

NC



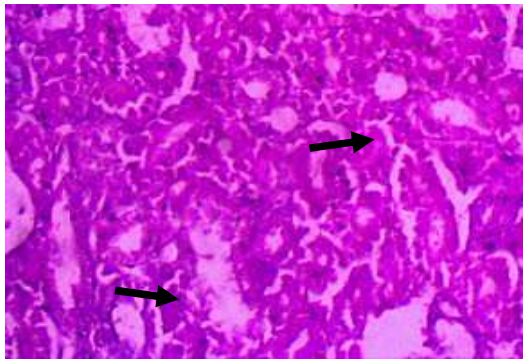
There is no observable lesion

BD



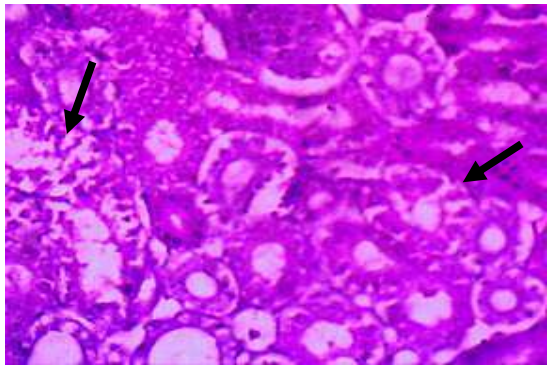
There is multifocal tubular epithelial coagulation necrosis, defoliation and disruption of basement membrane (arrows).

TD1



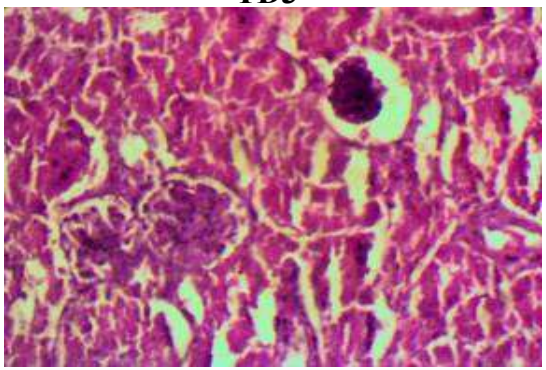
There is degeneration and necrosis of tubular epithelial cells (arrows)

TD2



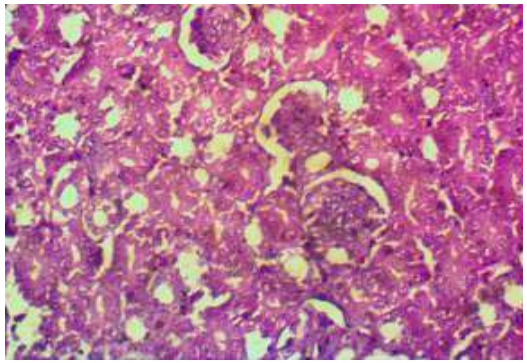
There is tubular epithelial degeneration and coagulation necrosis(arrows)

TD3



There is no observable lesion

TD4



There is no observable lesion

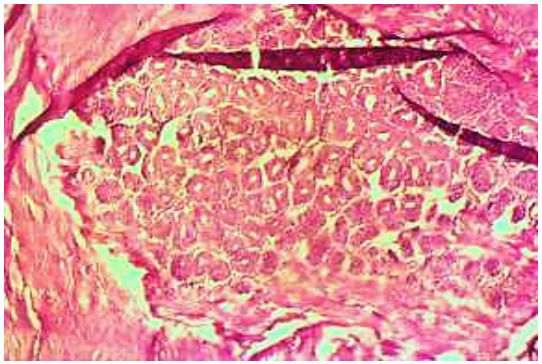
Magnification = x400 for each slide
Stain = Haematoxylin and Eosin

Plate 4.2 Histological section of kidney of broilers fed aflatoxin-contaminated poultry feed

4.4.25 Report ofhistological section of ileum of broiler chicken offered aflatoxin-contaminated feed having varied inclusion levels of beta-glucans, supplemental dietary antioxidants and vitamin K

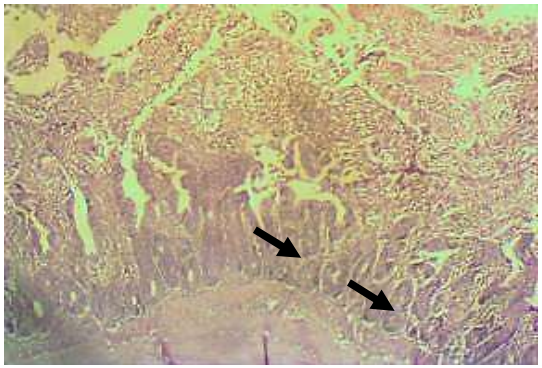
Histological section of ileum of broiler chicken offered aflatoxin-contaminated rations is shown in Plate 4.3. Birds that consumed NC diet (aflatoxin-free diet) had no observable lesion in their ileum section. Birds offered aflatoxin-contaminated diet free of beta-glucans and supplemental dietary antioxidants (BD) had necrotic enterocytes and cryptal hyperplasia and mucosa infiltrated with inflammatory cells (as indicated with arrows). Birds offered TD1 with 250ppm beta-glucans and supplemental dietary antioxidants but without supplemental selenium and vitamin K had severe diffuse denudation with atrophy of villi (see arrows). There is tubular epithelial degeneration and necrosis (black arrow) with inflammation of peri-tubular spaces (blue arrow) in the ileum of birds offered diet having supplemental selenium(TD2)addition. However, birds offered diet having 375ppm beta-glucans, supplemental dietary antioxidants, with or without selenium supplementation (TD4 and TD3, respectively) showed no observable lesion in their ileum section photomicrographs.

NC



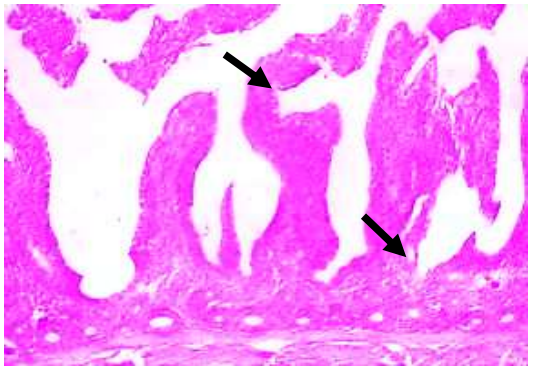
There is no observable lesion

BD



There is necrosis and loss of enterocytes, cryptal hyperplasia (arrows) with infiltration of inflammatory cells in the mucosa.

TD1

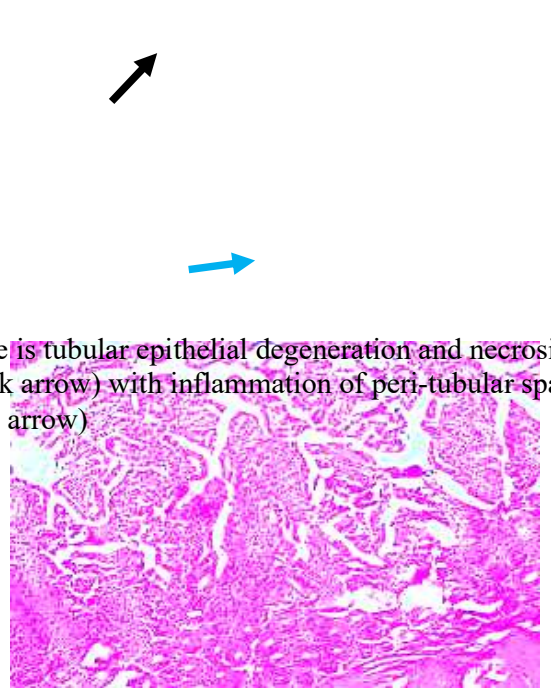


There is severe diffuse denudation and atrophy of villi (arrows).

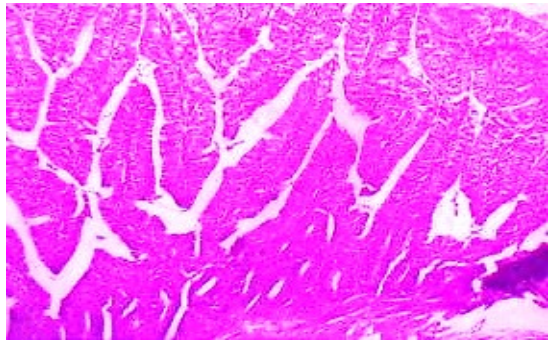
TD2

TD3

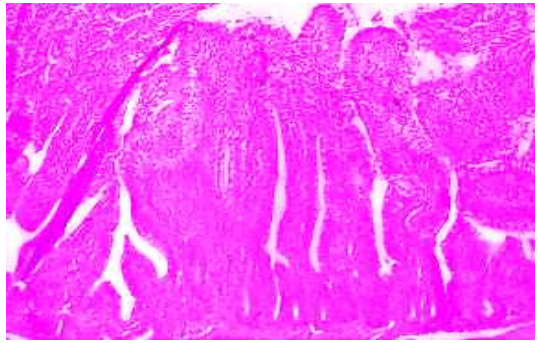
TD4



There is tubular epithelial degeneration and necrosis (black arrow) with inflammation of peri-tubular spaces (blue arrow)



There is no observable lesion



There is no observable lesion

Magnification = x400 for each slide
Stain = Haematoxylin and Eosin

Plate 4.3 Histological section of ileum of broilers fed aflatoxin-contaminated poultry feed

4.4.26 Summary of histological sections of selected tissues of broiler chicken offered aflatoxin-contaminated feed having varied inclusion levels of beta-glucans, supplemental dietary antioxidants and vitamin K

The summary of histological sections of liver, kidney and ileumof broiler chickenoffered aflatoxin-contaminated rations is presented in Table 4.41. Birds offered BD, without beta-glucans and supplemental dietary antioxidants had cellular damages in all three tissues sampled. Birds offered NC which was free of aflatoxins contamination had no observable lesionsin all three tissues sampled. Similarly, birds offered TD3 and TD4 with 375ppm beta-glucans inclusion, supplemental dietary antioxidants, with or without supplemental selenium showed no observable lesions in all the three tissues sampled. The liver, kidney and ileum sections of birds fed TD1 and TD2 with 250ppm beta-glucans inclusion had slight reduction in cellular damage compared to what was observed in birds offered BD. Protective effect observed in birds that consumed TD2 was slightly better than in birds fed TD1.

Table 4.41 Summary of Histological sections of selected tissues of broiler chicken offered aflatoxin-contaminated feed with varied inclusion levels of beta-glucans, supplemental dietary antioxidants and vitamin K

	Tissues		
Treatments	Liver	Kidney	Ileum
NC	No observable lesion	No observable lesion	No observable lesion
BD	There is multifocal hepatocellular coagulation necrosis, atrophy of cords and accentuation of sinusoids with Kupffer cell hyperplasia (see arrows)	There is multifocal tubular epithelial coagulation necrosis, defoliation and disruption of basement membrane (see arrows)	There is necrosis and loss of enterocytes, cryptal hyperplasia with infiltrate of inflammatory cells in the mucosa (see arrows)
TD 1	There is moderate diffuse hepatocellular atrophy (see arrows)	There is degeneration and necrosis of tubular epithelial cells (see arrows)	There is severe diffuse denudation and atrophy of villi (see arrows)
TD 2	There is moderate diffuse atrophy of hepatic plates (see arrow)	There is glomerular and tubular atrophy (see arrows)	There is tubular epithelialdegene-ration and necrosis (black arrow) with inflammation of peri-tubular spaces (blue arrow)
TD 3	No observable lesion	No observable lesion	No observable lesion
TD 4	No observable lesion	No observable lesion	No observable lesion

NC- Negative Control (0ppb Aflatoxins; 0ppm beta-glucans; antioxidants-free), BD- Basal Diet (270ppb Aflatoxins; 0ppm βG; antioxidants-free), TD- Treatment Diet, TD1- BD+(E+C+K), TD2- BD+(E+C+K)+Se, TD3- BD+ (E+C+K), TD4- BD+(E+C+K)+Se, Se- Selenium

CHAPTER FIVE

DISCUSSION

5.1 Preliminary study

Aflasafe® treated maize grains had 5ppb of aflatoxins when quantified but gave a feed aflatoxins level of 0ppb. This showed that the inclusion of other feed components diluted the aflatoxins level in the aflasafe® maize further to a level below the detection limit of the analytical procedure used. For High Performance Thin Layer Chromatography (HPTLC), the limit of detection is 1ng/g (1µg/kg) or 1ppb (Finley *et al*., 1992). This implied that aflatoxins level below 1ppb must be assayed with a different analytical method that is more sensitive than HPTLC.

5.2 Study one: Effects of yeast beta-glucans on dietary aflatoxin absorption in broiler chicken offered aflatoxin-contaminated feed

Diets that had 500ppm and 625ppm yeast beta-glucans inclusion alone enhanced feed intake probably by absorbed aflatoxins reduction thereby reducing the appetite suppressing potential of aflatoxins (Chen *et al*., 2013) or it might be due to the short period of exposure (21 days).Feed intake by broiler chickens over a 42 days period was not affectedby the dietary treatments including the uncontaminated feed. This implied that beta-glucans does not have marked effect on feed intake of birds fed rations containing themwhen compared to that of birds fed uncontaminated diet. Beta-glucans neither improve nor depress feed intake.This may probably be that the quantity of aflatoxins absorbedmay be within a range that could not depress feed consumption significantly, hence the similarity in feed intake between birds offered the uncontaminated and the contaminated diets.

Ileal digesta aflatoxins level analysis revealed that broiler chicken offered 375ppm beta-glucansamong the treated diets hadthe highest level of aflatoxins in their Ileal

digesta, collected at the end of 42 days of feeding. The least concentration of aflatoxins in the

Ileal digestaof birds fed contaminated ration with 0ppm beta-glucansindicated that beta-glucans can reduce aflatoxins uptake from the GIT of broiler chickens.Following the ingestion of aflatoxin-contaminated diet, aflatoxin absorption from the GIT into the circulatory system was inevitable and beta-glucans was included to the trial diets at varied levels to evaluateits effects in reducing aflatoxins absorption. Birds offered 375ppmbeta-glucans diet had thehighest quantity of adsorbed aflatoxins in their Ileal digesta (79.30±10.38%), followed by birds fed 250ppmbeta-glucans inclusion diet, which sequestered 53.86±8.81%. Kolawole *et al*. (2019) reported aflatoxin B_1 adsorption of between 56 and 58% by yeast cell wall-based binder, in a comparative assessment of multiple mycotoxins binding claims by different brands of commercial binders. Madrigal-Santillan *et al*.(2006) reported adsorption percentage between 16 and 66% of aflatoxin B_1 when the feed aflatoxin concentration was 800µg/kg and at a low level of just 150µg/kg aflatoxin contamination, percentage aflatoxin adsorption ranged from 40 to 93%. At just 100 µg/kg aflatoxin contamination level, Arif *et al*. (2020) recorded significantly improved growth performance in broiler chickens at 3.75g/kg (3,750mg/kg) of yeast (*Saccharomyces cerevisiae*)cell wall inclusion, while Yildiz *et al*. (2004) reported significant reduction in the adverse effects of aflatoxins on performance parameters at 2g/kg (2,000mg/kg) of yeast cell wall addition.Theseimplied that aflatoxins binding is probably not 100 percent efficient and that theefficiency of aflatoxins adsorption also depend both on the level of feed contamination and the binder inclusion level.Birds fed dietcontaining 375ppm beta-glucans absorbed least aflatoxins into the circulatory system, among the contaminated treatment diets. Birds fed aflatoxin-contaminated diet with no beta-glucans additionabsorbed the highest aflatoxins from the GIT. It was noticed that further addition of beta-glucansbeyond 375ppm inclusion level reduced the quantity of aflatoxins prevented from being absorbed. That is, higher beta-glucans inclusion rates above 375ppm increased thequantity of absorbed aflatoxin from the GIT into the blood circulatory system. This could probably be as a result of the fact that beta-glucans, a non-starch polysaccharide (NSP) usually increase viscosity of digesta within the gut. When the gut digesta viscosity increases, two things are likely to occur: a) there will be rise in necrotic enteritis,leading to loosening of the tight or “T” junctions within the intestinal wall. Aflatoxins absorption in the GIT is by passive absorption (Gratz *et al*., 2006; Di Gregorio *et al*., 2014) or simple diffusion across concentration gradients perhaps because they are low molecular weight compounds. As the concentration of

aflatoxinswithin the gut becomes higher than that of the blood circulatory system, and the intestinal walls had been weakened by enteritis, more aflatoxins will diffuse "freely" into the circulatory system. The more the beta-glucans level, the higher the gut viscosity will be, the more the enteritis and the morelikely will be the toxin that will find its way into the blood system. b) When the gut's viscosity is increased, digesta passage rate within the gut is reduced, allowing the aflatoxin-contaminated digesta to stay longer within the absorptive sections of the GIT and further enhancing the diffusion of the toxin into the blood stream, hence, the possible reasons for the reduced Ileal digesta aflatoxins level of birds offered 500 and 625ppmbeta-glucanscontaining diets.However, the null hypothesis that says: "the effect of beta-glucans on aflatoxins absorption in the gastrointestinal tractwill be the same across the dietary treatments irrespective of beta-glucans inclusion level" is not true and is therefore discarded. The alternate hypothesis that: "the effect of beta-glucans on aflatoxin uptake in the gastrointestinal tractwill depend on its level of inclusion".will be upheld, as this is true up to 375ppm of beta-glucans inclusion. However, because aflatoxins absorption prevention is not absolute (the highest being 79%), further research is needed to mitigate against the consequences of the portion that was inevitably absorbed.

The haematology of birds fed the contaminated diets, treated or not treated did not reveal any differences of significance. This may probably be because aflatoxins are not really recognised by blood defensive machineries owing to their low molecular weight properties. Another possible reason for the lack of marked differences in haematology may be due to the level of aflatoxins in the test diets, which might not be large enough to provoke acute changes in the parameters of haematology.Beta-glucansaddition to the contaminated diets did not elicit significant changes in serum biochemistry, perhaps due to the level **of**aflatoxins contamination, which is below the level of contamination that will invoke acute toxicity. However, Alkaline phosphatase (ALP) values of birds feduncontaminated feed significantly reducedin comparison to ALP level of birds that consumedthe othertreatment diets. The ALP is an enzyme that can be found in the bone, placenta and in the cells that lines the biliary tracts of liver to the gall bladder. Consequent upon the consumption of aflatoxin-contaminated feed, it may possibly cause obstruction in the bile duct of the birds,leading to bile duct proliferation (Chen *et al*, 2014a) or biliary hyperplasia (Peles *et al*., 2019). When this

happens, the ALP levels in the serum will rise above the normal or reference range. Birds fed uncontaminated feed, had the least ALP value compared to birds offeredunmitigated ration. Also, the fraction of theingested aflatoxins that could not be trapped by beta-glucans was significantlysufficient to elicit a rise in ALP values of birds fed aflatoxin-contaminated feed with variedinclusion levels of beta-glucans.

Addition of beta-glucansresulted in positive effect in reducing liver residual aflatoxins. Birds fed aflatoxin-contaminated feed with 0ppm beta-glucanshad highest level of residual aflatoxins in the liver(0.06±0.01µg) and the inclusion of beta-glucansat 250 and 375ppm resulted in significant decreaseof up to 83% in liver residual aflatoxins, similar to that of birds fed uncontaminated feed. The highest level of adsorbed aflatoxins in the Ileal digesta, which means reduction in the quantity of absorbed toxins, was recorded in birds feddiets that had 250ppm and 375ppm beta-glucans. Reduction in the quantity of absorbed aflatoxins from the GIT to 46.13±8.81% in BD250 and 20.69±8.47% in BD375 resulted in reduced aflatoxins flow into the liver for biotransformation and may bethe probable reason for the significant reduction in liver residual aflatoxins below that of birds fed unmitigated diet and similar to that of birds fed uncontaminated feed. All other levels of beta-glucans inclusion also led to reduction in residual liveraflatoxin except in 500ppm beta-glucans inclusion. The lack of differences in liver weights across all the treatment diets, was an indication that the intoxication level in the current study is at a chronic level and not acute. Increases in organs weight are usually characteristic of acute situation of a disease or infection (Dinev-Ivanov, 2007). The assessment of residual aflatoxin in the liver gave a positive insight into the notion that combining mycotoxin adsorbent with another detoxification strategy, especially one that can modulate the metabolism of the unavoidably absorbed fraction of the toxin (Whitlow, 2006), may produce remarkable effect in the detoxification of ingested aflatoxins in poultry species.

Performance of broiler chickens withbeta-glucansaddition did notproduce any marked improvement in body weight gain (BWG) and feed conversion ratio (FCR). Apart from age, sex, state of health, the presence of other anti-nutrients and other stress factors, two other factors which directly influence the susceptibility of animals to the detrimental effects of aflatoxinsare: aflatoxinslevel in the feed and the duration of time the toxin is being fed upon (Santini and Ritieni, 2013; Kolawole *et al*., 2019). The starter phase probably appeared to be a short exposure period to see any significant

effect of aflatoxins, given the current contamination level of 270ppb aflatoxins in the feed. This may be the reason for the lack of significant variations in the BWG and FCR at starter stage. Birds fed the contaminated diet with 0ppm beta-glucansat finisherstage had the least BWG, though it did not differ from that of birds offered variedinclusion levels of beta-glucans.Even though beta-glucans reduced the quantity of aflatoxins absorbed in all the diets it was included, BWG in the treated contaminated diets was not significantly different from that of birds fedunmitigated feed. This perhaps implied that the amount of the toxin left un-trapped by beta-glucansmay have been sufficientenough to have affected metabolic processes that would have resulted in increase in BWG between birds fed the contaminated but mitigated diets with beta-glucans and birds fedthe unmitigated or 0ppm beta-glucans diet. The R^2valuewas also an indication that beta-glucans alone cannot completely prevent aflatoxins absorption in broiler chicken. This further calls for a combined strategy to fully mitigate the effect of aflatoxins in broiler chicken beyond the use of an adsorbent alone, as reported that mycotoxins adsorbents are never absolute in their efficacy (Zavala-Franco *et al*., 2018; Kolawole *et al*., 2019; Elliot *et al*., 2020).

From 0 - 42 days period of this experiment, BWG and FCR were similar in birds offered all the treated test diets including the uncontaminated diet. Documented reports on the effects of beta-glucanson performance in farm animals are conflicting. Increase in BWG of broiler chickens was reported by Zhang *et al*. (2008) when yeast beta-glucanswas added to the diet at 50 and 75ppm. Hahn *et al.* (2006) equally reported improved performance in swine with beta-glucans addition to the diet. On the contrary, Cox *et al*. (2010) did not record any improvement in BWG of chickens fed beta-glucans supplemented diets. Cheng *et al.* (2004) and Chae *et al.* (2006) reported no marked effect of beta-glucans on growth performance and that beta-glucans do not affect performance negatively in both challenged and unchallenged birds. In the current study, the challenged birds were those on the contaminated feed and the unchallenged birds were those on uncontaminated feedand the results obtained in the current studycorrelated with the performance results obtained in the cited cases having no observable improvement in growth performance due to beta-glucans addition.

5.3 Study two: Effects of supplemental antioxidants and vitamin K in broiler chicken offered aflatoxin-contaminated rations

Haematology of birds fed the contaminated diets with supplemental dietary antioxidants and vitamin K did not reveal marked changes from one treatment to another. Birds offeredunmitigated contaminated rationand those offereduncontaminated ration did not differ in their haematological parameters. The level of aflatoxins in the diet may be low to produce significant differences on the haematology or it may be due to the low molecular weight of aflatoxins, allowing it to travel through the blood un-hindered by the blood defence parameters, as they are unable to recognise it as being foreign and this might be responsible for the lackofmarked effect observed on parameters of haematology.Gowda *et al*. (2013) reported that aflatoxins poisoning basically affects the liver and kidneythan the haematology. The blood therefore is not the target site for the manifestation of the adverse consequences of aflatoxins, especially at the contamination level in the current study.Diaz and Murcia (2011) also noted that aflatoxinsingestion primarily result in hepatic damage.

Combinations of (200mgVE+250mgVC+0.3mgSe); (200mgVE+250mgVC+3mgVK) and(200mgVE+250mgVC+0.3mgSe+3mgVK)decreased Alkaline phosphatase below that of birds onunmitigated contaminated diet. The treatment diet having (200mgVE+250mgVC+0.3mgSe+3mgVK) combination had similar ALP with birds offered the uncontaminated diet.This may be due to effective removal or neutralisation of free radicals or reactive oxygen species produced during aflatoxins bio-transformation in the liver,which may lead to bile duct hyperplasia(Diaz and Murcia, 2011; Peles *et al*., 2019), thereby preventing excessive infiltrationof ALP into the circulatory system.Supplemental dietary antioxidants, especially vitamin E (α-tocopherol), vitamin C (ascorbic acid) and selenium in glutathione peroxidase are capable of neutralising free radicals and/or reactive oxygen species arising from the metabolism of aflatoxins in the endoplasmic reticulum of the hepatocytes. Bababunmi (1989) and Gomez-Espinosa *et al*. (2017) reported that the addition of vitamin K was effective in reversing the competition between aflatoxins and vitamin K for the apoenzyme required for the formation of prothrombin in the liver, in favour of the

latter. This may be the reason for the reduction**in**serum ALP level, perhaps due to reduced biliary cells lininginjury.

Lipid peroxidation is a degradative process in biological subjects, in which the polyunsaturated fatty acid (PUFA), the structural component of cell membranes phospholipids and that of organelles are usually attacked by excessive FR and/or ROS production. This may lead to the loss of the cell or organelle structural integrity (fluidity), leading to failure of their physiological function and triggering the process of apoptosis (Ayala *et al*., 2014). Malondialdehyde, a thiobarbituric acid reacting substance is a universally accepted biomarker or index of lipid peroxidation activity(Giera *et al*., 2012). Birds offered the unmitigated contaminated diet had the highest level of serum MDA, and the addition of different combinations of supplemental dietary antioxidants and vitamin Ksignificantly resulted in a gradual reduction in MDA level from 128.29±31.16 nmol/mL in birds offeredunmitigated contaminated diet to a level three times lower, (42.00±10.40 nmol/mL), as obtained in the serum of birds offeredR4. The MDA level of birds offered the unmitigated contaminated diet was 10 times more than what was observed in the serum of birds offered the uncontaminated diet and the addition of supplemental dietary antioxidants and vitamin K to the contaminated diets as laid out inR4 (see section 3.3.5), resulted in significant malondialdehydereduction, to a level just 3.5 times higher than the level of birds offereduncontaminated ration.This implied effective free radical/or reactive oxygen speciesremoval or neutralisation by the combinations of supplemental dietary antioxidants and vitamin K in preventing attacks on hepaticmicrosomes polyunsaturated fatty acids.

Total antioxidant capacitylevel in the serum of birds offered the mitigated treatment rations did not differ from that of birds offered the unmitigated contaminated diet. This implied that the level of aflatoxins absorbed from the diets wasenough not to have allowed significant improvement in the serum TAC, despite the addition of nutritional antioxidants. However, birds offered R3 had TAC value similar to that of birds offered the uncontaminated diet. This indicated that if the level of aflatoxins that was absorbed from the diet could be reduced, then the addition of supplemental dietary antioxidants will give marked improvement. Reduced glutathione is an important cytosolic antioxidant and was noticed to be depleted in birds that consumed the unmitigated

diet, to less than 50% of the GSH of birds offeredthe uncontaminated diet. Birds offered all the mitigated diets had marked improvement in their serum GSH concentration, similar to one another and to the level observed in birds offered uncontaminated diet. This implied that birds offered the mitigated diets had sufficient supplementary antioxidants to neutralise free radicals and reactive oxygen species, and spared or assisted GSH in its endowed role as free radicals and reactive oxygen species scavenging and as toxins conjugants, hence preventing its depletion. Oxidised glutathione(GSSG) value in the serum was least in birds offered NC diet. However, the GSSG of birds offered the contaminated but mitigated diets and those fed the unmitigated contaminated diet were similar. This may be due to the fact that even though birds on the mitigated diets had supplemental antioxidants in them, the rate of free radicals and/orreactive oxygen species production may be more thanthe rate of scavenging, because aflatoxins absorption was unhindered (no adsorbent added), leading toelevation of serum GSSG inall birdsoffered the treatedcontaminated diets. It appeared that the presence of an antidote may not be enough remedy for toxin consumption.

The ratio of reduced glutathioneto the oxidised (GSH:GSSG), is an index of oxidative stress or oxidative stability (Pastore *et al*., 2001), depending on the levels of these two. Higher level of GSSG above that of GSH indicates oxidative stress while higher GSH level above that of GSSG indicates oxidative stability or less oxidative stress. The GSH:GSSG result indicated positive response by birds offeredsupplemental dietary antioxidantstreated contaminated diets, with or without selenium and/or vitamin K. The GSH:GSSG in birds offered the mitigated diets had higher reduced glutathione balance relative to oxidised glutathione, while birds offered the unmitigated diet, which had GSH:GSSH of 0.83, indicated oxidative stress.The addition of supplemental dietary antioxidants in different combinations to thecontaminated treatment diets, reducedoxidative stress and increased oxidative stability of broiler chicken offered aflatoxin-contaminated diets by preventing the depletion of reduced glutathione.

Residual aflatoxinconcentration inliver increased in birds offered the unmitigated rations while birds offeredthe uncontaminated diet had no detectable residual aflatoxins in their liver. Birds offered all the mitigated treatment diets had similar

residual liver aflatoxins, which were at least 50% reductionrelative to those of birds offeredunmitigated feed.The effectiveness of supplemental dietary antioxidants in preventing reduced glutathione depletion may had facilitated the excretion of aflatoxins or its metabolic derivatives from the liver and may probably be the reason why birds offeredR3 and R4 had similar and reduced aflatoxins concentration in their liver. Mortality recordindicated that the addition of different combinations of supplemental dietary antioxidants and vitamin K alone cannot keep mortality at bay in broiler chickenoffered aflatoxin-contaminated rations up to 270ppb aflatoxins. Birds offeredthe uncontaminateddiet had the least mortality rate while birds offered all the mitigated diets had high mortality rates, the least being 50% in R4 and the highest was 70% in birds offeredunmitigated feed. The highest and the least mortality figures that arosed from all the mitigated diets did not reveal any differences of significance from the mortality rate obtained in birds offered the unmitigated diet. Perhaps this could be due to high production of free radicals/or reactive oxygen species during the metabolism of aflatoxins in the liver, which could have been more than what the ability of the differentantioxidants can neutralise, as reflected in the serummalondialdehyde and GSH:GSSG
values.

Considering some of the improvements brought about by the addition of different nutritional antioxidants combinationsand vitamin K on broiler chicken offered aflatoxin-contaminated diets, it is worth appraising the observedimpacts on the performance indices of the trial birds. Feed intake of birds offeredthe treated contaminated diets in R3 and R4 had increased feed intake above that of birds fed the unmitigated feed. This resulted in higher body weight gain in these two dietary treatments over that of birds fed the unmitigated feed, with impressive feed conversion ratio (FCR) of 1.81±0.02 and 1.68±0.14 in R3 and R4, respectively. The desirableand the least FCR in birds offeredthe mitigated treatment diets R3 and R4however, did not differ from the FCR of birds fed uncontaminated feed. The presence of vitamin K, the antihaemorrhagic compoundcommon to treatment diets R3 and R4, may be the factor responsible for possible reduction in intestinal haemorrhage, as reported by Vijayalingam *et al*. (2017), that aflatoxins had anticoagulant potential and that the addition of vitamin K in rats can halt the induced haemorrhage in rats and this might be the reason for the better BWG and FCR observed in birds offeredR3 and R4.The

performance of birds offered the unmitigated diet was in corroboration with the findings of He *et al*. (2013). However, the performance of birds offeredtreatment diets R3 and R4 gave an indication that the combination of supplemental vitamins E, C and K (R3) and supplemental vitamins E, C, K and Se (R4) hold a promise in counteracting the adverse effects of ingested aflatoxins in broiler chickens. Since supplemental dietary antioxidants and vitamin K did have effect in preventing the deleterious consequences of aflatoxins ingestion in broiler chicken, the null hypothesis which stated that: "supplemental dietary antioxidant and vitamin K will not have any effect in preventing the adverse effects of aflatoxins in broiler chicken, irrespective of their combinations" is discarded. However, the alternate hypothesis that said: "supplemental dietary antioxidants and vitamin K will be effective in preventing the adverse consequences of aflatoxins in broiler chicken and that the effect will depend greatly on the different combinations of the antioxidants and vitamin K" do not completely hold. Even though the performance of birds offered the mitigated contaminated rations was better than those offered the unmitigated contaminated feed, but it was not comparable to that of birds fed the uncontaminated rations. Therefore, due to the promise shown by the combinations of supplemental dietary antioxidants and vitamin Kin alleviating the adverse effects of aflatoxins, further strategy that will minimise or prevent the level of aflatoxins that is delivered to the liver for bio-transformation is required, to be combined with supplemental antioxidants and vitamin K in ameliorating the deleterious effects of aflatoxins in broiler chicken.

5.4 Study three: Effects of yeast beta-glucans, supplemental antioxidants and vitamin K in ameliorating the impact of dietary aflatoxins in broiler chicken

To harness the benefits observed in beta-glucans ability in reducingaflatoxins absorption in the gastrointestinal tracts and the high potentials observed in supplemental dietary antioxidants and vitamin Kin reducing excessive oxidative activities, arising from aflatoxins metabolism, two test diets each from the previous two studies that performed better were combined.

Beta-glucansinclusion at 250ppm and 375ppm did not haveeffect on red blood cell, white blood cell, platelets, monocytes, eosinophils and basophils. Birds offered 375ppmbeta-glucansdiet significantly had increased PCV, Hb and lymphocytes above

that of birds offered 250ppm beta-glucans diet. The heterophils of birds offered 250ppm beta-glucans was elevated abovewhat wasobtained in birds offered 375ppm beta-glucans diet. The rise in the lymphocytes level at starter phase could be due to the level of beta-glucans (375ppm) which is higher than 250ppm, as the body immune system usually recognisesbeta-glucans as an antigen, as it is one of the structural components of most bacterial cell wall (Kwiatkowski and Kwiatkowski, 2012). The PCV and Hb are related, such that Hb indicates the oxygen carrying capacity of RBC while the PCV measures the proportion of RBC in the blood and also an indicator as to whether the animal is anaemic or not. Heterophils and heterophil tolymphocyte ratiowere reduced in birds fed 375ppm beta-glucansdiet, probably an indication that some had been used up to attack beta-glucans presence in the body, as it is being recognised as foreign to the body. Addition of 0.3mg/kg selenium did not produce any distinctvariations on any of the parameters of haematology examined, perhaps because selenium's effect as a cofactor in glutathione peroxidase is evident in the serum than on the haematology.

Addition of variedinclusion levels of beta-glucans and supplemental selenium on serum biochemistry of broiler chickens fed aflatoxin-contaminated feed showed that the level of aflatoxins left unadsorbed at 250ppm and 375ppm of beta-glucansinclusions were not sufficient to producemarked differences in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, globulin, albumin, superoxide dismutase, total antioxidant capacity, total glutathione andreduced glutathione in the serum of birds fed 250ppm and 375ppm beta-glucans diets. Regardless of how little the level of aflatoxins that is inevitably absorbed is, it will be metabolised or bio-transformed in the liver. During this oxidative biotransformation process, free-radicals/or reactive oxygen speciesareusually generated(Reed *et al*., 2011). Not all the free-radicals/or reactive oxygen speciesso generated will be quenched or neutralised by both endogenous and exogenous antioxidants present within the body of the birds, as some will eventually attack membrane lipids or polyunsaturated fatty acids (PUFA) of cells and organelles membrane phospholipids in a process referred to as lipid peroxidation. Malondialdehyde is one of the biomarkers of lipid peroxidation activity and can be assayed in the serum. The malondialdehyde value of birds fed 375ppm beta-glucans (79.23±24.05nmol/mL) diet was reduced significantlycompared to the level

99.17±22.25nmol/mL obtained in birds fed 250ppm beta-glucans diet due to the reduction in the quantity of aflatoxins absorbed from the GIT by beta-glucansinclusion.

The neutralisation of free-radicals/or reactive oxygen speciesand the conjugation of the toxic metabolite of aflatoxins by both endogenous and exogenous antioxidants within the body of the birds will elicit a rise in the oxidised glutathione (GSSG) level, depending on the quantity of aflatoxinsabsorbed for biotransformation. Consequently, the GSH:GSSG, an index of oxidative stress, revealed that birds fed 375ppm beta-glucans diet had significantly higher GSH value relative to GSSG. As birds offered 250ppm beta-glucans diethad higher level of absorbed aflatoxinsabove that of birds fed 375ppm beta-glucans diet,addition of 375ppm beta-glucans resulted in reduction in serummalondialdehyde and oxidised glutathione levels and also produced higherand better OS ratioin birds fed aflatoxin-contaminated diets.Addition of 0.3mg/kg supplemental selenium showed no differences of significance in all the serum biochemistry parameters assayed from that of birds that consumed 0ppm supplemental selenium diet, except for reduced glutathione. Galvano *et al.* (2001),noticed a favourable response in broiler chickens fed aflatoxin-contaminated feed with supplemental selenium. It appeared that selenium facilitates the neutralisation of FR/or ROS through glutathione peroxidase activity enhancement and this was reflected in sparing effect on reduced GSH and a consequent significant rise in reduced glutathione level compared to the oxidised. Reducedlevel of oxidised glutathione led to a higher GSH:GSSG (2.98±1.37) on the main effect of birds offered375ppmbeta-glucans containing feed.

The additive or synergistic effect of beta-glucans, supplemental dietary antioxidants andvitamin K with or without supplemental selenium showed that the absence of differences in aspartate aminotransferase, total protein, albumin, globulin, albumin-globulin ratio, superoxide dismutase, total antioxidant capacity and total glutathione of birds offered the contaminated and the uncontaminated diets was at variance with the observation ofRaju and Devegowda(2000), who reported that at 300ppb of aflatoxin B_1 in the feed, serum total protein and albumin levels were observed to decrease in broiler chickens. Tedesco *et al*. (2004) report was in consonant with some of the observations in the current study. They reported that high aflatoxin B_1 dosage did not

resultin significant differencesin serum biochemical indices. The ALT levelof birds offered unmitigated was higher, and this was comparableto Dafalla *et al.* (1987) and Denli *et al*. (2009) reports.Both teams of researchers noticedelevation in serum AST and ALT levels in broilersoffered aflatoxin-contaminated diets.Birds fed the mitigated diets had ALT values that were similar to one another and to that of birds offereduncontaminatedration. Manegar *et al*. (2010), without mitigation, observed no significant variations in ALT values in their dietary treatments while AST values did not increase but decreased, with aflatoxin levels of 200, 400 and 600ppb in the treatment rations, in comparison to the control diet. With mitigation however, theabsence of differences in serum ALT and AST levelsseen in the current study was in line with earlier findingsof Edrington *et al*. (1997) and Dalvi and MacGowan (1984) who equally observed no significant alterations in serum AST level, following the feeding of diets containing 10ppm and 2.5ppm aflatoxin B_1. Manegar *et al*. (2010) and Abd El-Ghany *et al*. (2013) opined that going by the inconsistency in liver enzyme profiles, AST and ALT levels may not be a better indicator of the degree of hepatic damage.

Highalkaline phosphatasevalue recorded in birds offered the unmitigated contaminated dietwas reduced by the combinations of beta-glucans and different supplemental dietary antioxidants as presented in the mitigated diets to the extent of having similaritywhen compared with the ALP value of birds offered the uncontaminated ration. The elevated level of ALP as noticed in birds offered the unmitigated diet was an indication of biliary tract obstruction or enlarged gall bladder (Chen *et al*, 2014a), and the reduction in ALP values in birds that consumed the mitigated treatmentdietswas indicative of improvement brought about by the treatment effect while birds fed diets that had supplemental selenium, had the least values. However, the reduction in ALP valuesof birds fedzero selenium were similar to those having supplemental selenium.

Lipid peroxidation activity declined in all the mitigated treatment diets as indicated by reduced MDA values compared to birds fed the unmitigated feed. The least MDA valuewas noticed in birds offered TD4 with the mitigant combination of 375ppm beta-glucans+200mg VE+250mg VC+3mgVK+0.3mg Selenium. Elevation of serum MDA in birds fed the unmitigated diet might be due to the effects offree-radicals/or reactive

oxygen species generated during aflatoxinsmetabolism by the hepatic cytochrome P450 (CYP 450) monooxygenase enzymes. As their name implies (monooxygenase), CYP 450s inserts one oxygen atom intoaflatoxin molecule, duringthe bio-transformation process of aflatoxin from a lipophilic compound, into a compound that is hydrophilic, which could be readily eliminatedvia the bile into faeces orexcreted by the kidneys into urine or mixed with uric acid, as in the avian species. The other atom of the split oxygen becomes superoxide anion($O_2^{\cdot -}$), a free radical and through the processes of Fenton's and Haber Weiss reactions, the very reactive hydroxyl radical ($HO^{\cdot}$)will be produced.The $HO^{\cdot}$is a very reactive oxygen species (ROS), capable of removing hydrogen atom (H^+) from membrane polyunsaturated fatty acid, in three major reaction steps that involved: initiation, propagation and termination reactions (Yin *et al.,* 2011) to form a alkyoxy radical.

The lipid alkyoxy radical formed undergo series of chain propagation reactions, culminating in the addition of oxygen molecule to form peroxyl radical. The peroxyl radical is reactive and can attack other PUFA molecule, to generate more peroxyl radical and itself turning into hydroperoxide. This oxidation process ofmembrane fatty acid is referred to as lipid peroxidation. It is a chain reaction and more and more membrane PUFA will be attacked by energised lipid peroxyl radicals. Once the process of lipid peroxidation has been initiated, it can no longer be halted midway,the chain propagation reaction will proceed until the termination products are produced (Ayala *et al*., 2014). When this process overwhelms the endogenous antioxidants endowed to put this process in check, affected cellslose their structural integrity, cells are lysed and destroyed through apoptosis (Reis and Spickett, 2012). The oxidised membrane PUFA undergo series of degradation processes and produce many aldehyde derivatives such asMDA, 4-hydroxynonenal (4-HNE), propanal, hexanal and others (Esterbauer *et al.,* 1991). The MDA and 4-HNE are usually the evidence most often adduced for the role of FR/or ROS in human and animal diseases or tissue damage by toxins (Halliwell and Chirico, 1993). The 4-HNE is cytotoxic while MDA is both cytotoxic and mutagenic (Poli *et al.,* 1985). The MDA is the most accepted and reliable biomarker used in determining oxidative stress clinically (Giera *et al*., 2012). It is produced under stress condition and is capable of reacting with multiple biomolecules like proteins or DNA (Zarkovic *et al*., 2013). The MDA can move across membranes and it is more stable than ROS (Esterbauer *et al.,* 1991). Exposure of cells

to excessive MDA as seen with birds fed the unmitigated diet will result in different pathological degeneration states (Garcia *et al*., 2013) of affected tissues, and in the case of aflatoxins ingestion, the liver is the most affected organ. It is therefore unlikely, that ingested diets contaminated withtoxin such as aflatoxins could be mitigated to the extent of having reduced MDA level similar to the level seen inbirds offeredtoxin-free diet, because the little unadsorbed fraction of the toxin will be metabolised, elevating the free-radicals/or reactive oxygen species load above that of animals fed uncontaminated feed, leading to higher oxidative stress indices relative to the latter.

Under normal physiological condition, hydroperoxides, the primary product of lipid peroxidation process can be terminated or inhibited and its peroxidative damage to tissues minimised (in this situation, the hepatic cells) with the enzyme glutathione peroxidases (GPx) and Selenoproteins (Ayala *et al*., 2014). The GPx is a selenium-dependent metalloenzyme and is involved in the reduction of H_2O_2 generated from Fenton's reaction into water, in conjunction with catalase. It can also reduce oxidised lipids (ROOH) and phospholipids hydroperoxide (PL-OOH) to their corresponding alcohols respectively (Thomas *et al*., 1990), using glutathione as the reductant. Glutathione peroxidase utilisedreduced glutathione (GSH) to prevent oxidative damage in tissue by reducing lipid hydroperoxides in a typical reaction given below:

$$ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O$$

(Krishnamurthyand Wadhwani, 2012).

Reduced GSH is regarded asthe master antioxidant in the body, preventing damages to important cellular and biomolecules resultingfrom ROS, such as FR and peroxides (Pompella *et al*., 2003), and it is found primarily in the aqueous medium of cell (cytosol), nuclei and mitochondria (Brigelius-Flohe and Maiorino, 2013). Outside the normal physiological condition, in the presence of xenobiotics such as aflatoxin, GSH and GPx alone might not be sufficient and efficient in preventing oxidative damage to cellular components and tissue injury. This may perhaps be the reason for the depletion of GSH in the serum ofbirds offered the unmitigated diet, in comparison to that of birds fed the mitigated treatment diets. Also, FR/or ROS production during aflatoxinbiotransformation occur in the endoplasmic reticulum (ER) or hepatic

microsomes, andthe membrane of the ER is only permeable to low molecular weight, lipid-soluble antioxidants such as α-tocopherols (vitamin E), carotenoids, quinones and some polyphenols (Vaya and Aviram, 2001). Hence, GPx and GSH alone may not be efficient in preventing cellular damage by toxins occurring in the polyunsaturated fatty acids of the ER. In preventing FR/or ROS mediated tissue damage therefore, vitamin E a low molecular weight fat soluble antioxidant was added as part of the mitigants in the feed of birds offeredtreatment diets TD1 to TD4, to protect hepatic microsomes from oxidative injury. Vitamin E is regarded as the most efficient chain-breaking antioxidant within cell membranes (Burton and Traber, 1990; Neff *et al*., 2018) and can protect membrane fatty acids of the ER, from FR/or ROS that are inevitably generated during aflatoxin metabolism by the CYP-450 mediated biotransformation process. In this regard, vitamin E is expected to produce a synergistic effect with GPx, and produce a sparing effect on glutathione consumption hence, reducing GSH depletion. This may be the reason why birds fed all the mitigated treatment diets had similar GSH values to one another, butwere higher significantly above the value obtained from birds fed unmiti-gatedtreatment diet.

In protecting against membrane damage of the ER of the hepatocytes, vitamin E has a huge limitation in its ability to donate hydrogen atom to neutralize peroxyl radical or inhibit the initiation of chain reaction during lipid peroxidation process. This is due to the fact that following the release of one hydrogen atom by Vitamin E from the -OH group on its chroman head (Burton and Traber, 1990; Garcia, 2013),vitamin E itself becomes oxidised in the process into α-tocopheroxyl or α-tocopheryl radical, a less reactive radical (Yamauchi, 1997). Additional donation of a second hydrogen atom by tocopheryl radical will result in the formation of an irreversible α–tocopheroxyl-quinone derivative (Vaya and Aviram, 2001). To synergize the antioxidant capability of vitamin E, ascorbic acid (vitamin C) was added to the diets. Vitamin C isahydrophilic antioxidant that is regarded as very efficient in extracellular fluids or the cytosol (Krishnamurthy and Wadhwani, 2012) due to their high hydrogen atoms or electrons donating potential. Way back in 1941, the ability of vitamin C to increase the antioxidant potency of vitamin E had been observed (Hacisevki, 2009).It has been widely documented thatvitamin C has the ability to regenerate α-tocopheryl radical, formed when vitamin E neutralises lipid peroxyl radical, back to the reduced α-tocopherol form (Montecinos *et al*., 2007 and Lu *et al*., 2010). Ascorbic acid can

release two hydrogen atoms to FR or ROS,yet it can be recycled from its oxidised form(dehydroascorbic acid - DHAA), as an ascorbate radical, to the reduced ascorbic acid, in an NADH-dependent process.

The elevated values of GSH noticed in birds offered mitigated treatment diets TD2 and TD4with supplemental selenium, were similar tothat ofbirds offeredthe uncontaminated diet. Selenium is an essential co-factor of glutathione peroxidase (GPx) (Sodhi *et al*., 2008) and it is expected that birds offeredtreatment diet containing supplemental selenium will be better protected from the effects of oxidative stress.The addition of supplemental dietary antioxidants to counteract the effects of aflatoxins in birds that consumed the mitigated contaminateddiets had significant effect in reducing oxidised glutathione (GSSG), compared to birds offered unmitigated treatment diet.The reductionwas to a level similarto that of birds offered aflatoxin-freediet. Oxidative stress index (GSH:GSSG), appeared in a pattern reflective of the quantity of aflatoxins that is absorbed. Birds fed 250ppm beta-glucans diet (TD1 and TD2) had higher levels of absorbed aflatoxinsfrom the daily ingested aflatoxins, compared to birds offeredtreatment diet with 375ppm beta-glucans (TD3 and TD4). The quantity of aflatoxinsinevitably absorbed from 250ppm beta-glucanstreatment diets seemedsufficient to have resulted in higher OS index in birds fed mitigated treatment diets TD1 and TD2to a levelnot differentfromthe valuesobtained in birds offered the unmitigated contaminateddiet. While birds fedtreatment diets mitigated with 375ppmbeta-glucans (TD3 and TD4) had GSH:GSSG values that were higher above the valueobtained inbirds offeredthe unmitigateddiet. Mitigation improved OS index similar to birds fed uncontaminated feed, and resulted in reduction in mortality compared to birds fed unmitigated diet.

Once aflatoxins had been unavoidably absorbed, beta-glucans has no effect any longer on the fate of the absorbed fraction of the toxin. Supplemental selenium was observed to have markedeffect on relative quantity of aflatoxins retained within the body of broiler chickens. This may be because selenium is an essential co-factor of GPx, the endogenous antioxidant enzyme involved in neutralising H_2O_2 to water and also scavenges lipid peroxyl radical in aqueous medium. Protection of liver cells from oxidative damage, partly by GPx, will enhance the capability of the birds'hepatocytes to rid the body of the inevitably absorbed toxin.Selenium addition reduced aflatoxins

retention in broiler chickenoffered aflatoxin-contaminated poultry feed down to15.25±5.44% below 24.55±5.97% in birds offered diet without selenium. Galvano *et al*.(2001), observed that aflatoxin B_1-DNA (AFB_1-DNA) binding and adduct formation was inhibited with supplemental selenium addition. However, the underlying mechanism behind the inhibition of AFB_1-DNA adduct formation by selenium appeared unknown. Probably this may be due to selenium acting through GPx and Selenoproteins to reduceROS accumulation and also in neutralising lipid hydroperoxide radical (Lauterburg *et al*., 1984), which will also eventually result in reduced MDA production.Once produced, MDAcan either be metabolised enzymatically, probably by mitochondrial aldehyde dehydrogenase into acetaldehyde and further into CO_2 and H_2O (Siu and Draper, 1982), or react with cellular components, tissue proteins or DNA to form adducts leading to biomolecular damages and/or alterations (Ayala *et al*., 2014).Aflatoxins retention also depends on the quantum of the ingested toxin absorbed from the GIT into the animal body. Birds fed 375ppm beta-glucans mitigated treatment diets had reduced aflatoxins absorption and hence significantly reduced their aflatoxins retention below the level observed in birds offeredtreatment diets with 250ppm beta-glucans addition.

Birds that consumed mitigated treatment diet TD4 had the highest percentage of expelled aflatoxins or the least percentage of aflatoxin retention, followed by birds offeredtreatment diet TD2. Birds offered mitigated treatment diet TD3 had a level of unretained aflatoxins similar to the value obtained in birds offered dietTD4 (the diet with supplemental selenium), perhaps this might be because of the advantage of higher beta-glucansaddition (375ppm), which led to reduced aflatoxins absorption by birds offeredtreatment diet TD3 compared to birdsoffered diet TD2.Aflatoxins retention in birds that consumed mitigated treatment dietshaving supplemental selenium were the least andcomparable. However, for the same reasons given earlier, aflatoxins retention in birds offeredmitigated treatment diet TD3 was alsosimilar to birdsofferedtreatment diets with supplemental selenium. Birds offered treatment diet TD4 combined the advantages inherent in higher beta-glucans inclusion level and supplemental selenium in obtaining the least aflatoxins retention of 12.09% and it was the most efficient mitigated treatment diet in reducing aflatoxins retention within the body, among birds offered the contaminated test diets. The reason for this may be due to the possibility of vitamins E and C working in synergy in the hepatic endoplasmic reticulum and

cytosol, respectively in neutralising free-radicals arising from the extra oxygen molecule during the epoxidation of aflatoxins to the toxic aflatoxin-epoxide derivative (aflatoxin 8,9 exo-epoxide). Hence, reducing reactive oxygen species such as H_2O_2 formation. Reduction in H_2O_2formation will minimise or prevent the depletion of reduced glutathione, which is the reductantused by glutathione peroxidase to neutralise H_2O_2 into H_2O and O_2. This will ensure that there is enough reduced glutathione for the phase II detoxification of aflatoxin 8,9 exo-epoxide by the enzyme glutathione-S-transferase (GST).Conjugation of the toxic aflatoxin 8,9 exo-epoxide with reduced glutathione by GST will facilitate the excretion of aflatoxins' derivatives (as aflatoxin-mercapturate) in either the faeces or urine or both. Effective conjugation of toxic aflatoxins' metabolites and its eliminationfrom the body of birds fed the mitigated diets is the possiblereason for the reduction in aflatoxins retention compared to birds offeredthe unmitigated treatment diet which had the highest level of aflatoxins retention (55.91%),while 29.31% of the ingested aflatoxinswas retained by birds offered mitigated treatment diet TD1. This therefore implied that when aflatoxin-contaminated ration is not mitigated, as seen in birds offered the unmitigated diet or when not efficiently mitigated, as in birds offered TD1, it may enhance aflatoxins retention within the body of animals consuming such diets.

If the consumption of aflatoxin-contaminated diets resulted in the retention of the toxin within the body of broiler chickens, effort was made in tracking the major sections or areas of the animals' body where this toxin could have been retained. Blood, liver and breast meat were sampled for aflatoxinsconcentration and residualaflatoxins respectively.Blood aflatoxins level was observed to be a function of the level of the toxin absorbed from the GIT, in this regard supplemental selenium was observed not to have effect in reducing circulatory aflatoxins level.Aflatoxinsconcentrationin liver was observed to be dependent on the level of aflatoxins in the blood that is transported to the liver. Diets with 375ppm beta-glucansinclusion were effective in reducing residual aflatoxins in liver of birds. Feed aflatoxin to tissue aflatoxin ratio did not differ markedly between birds fed250 and 375ppm beta-glucans inclusion into the contaminated diets for breast meat and liver. Also, supplemental selenium did not produce any differences of significance in feed aflatoxin to tissue aflatoxin ratio in breast meat and liver of birds offered diets with and without supplemental selenium.Residual aflatoxin in liver is the quantity of

aflatoxins deposited in µg/liver weight (expressed as µg/bird).Similarlevels of residual aflatoxins in liver was obtained in birds feddiets mitigated with beta-glucans, irrespective of the two inclusion levels and with or without supplemental selenium.

Thesynergisticeffectof variedinclusion levels of beta-glucans and supplemental dietary antioxidantson broiler chickens offered feed contaminated with aflatoxinsrevealed that birds offered unmitigated treatment diet had increased level of aflatoxins in their blood, significantly higher than other treatment diets. Birds fed 250ppm beta-glucansdiets had similar levels of blood aflatoxins while birds offered treatment diets with 375ppm beta-glucans also had similar level of aflatoxins in their blood.Birds fed 375ppm beta-glucansdiet had greater reduction in their circulatory blood aflatoxins level compared to birds offered 250ppm beta-glucans diet. This clearly indicated that blood aflatoxinslevel is directly influenced by the quantity of absorbed aflatoxins from the GIT, following aflatoxins ingestion. Breast meat aflatoxins concentration was highest in birds that consumed unmitigated treatment diet, while birds offeredmitigated treatment diet TD1 had 79.7% reduction, (five-fold reduction) in breast meat residual aflatoxins, compared to birds fedunmitigated treatment diet. Birds offered mitigated treatment dietsTD2, TD3 and TD4 had up to 88.7% (ten-fold) reduction in breast meat aflatoxins concentration. However, the four mitigated treatment diets were effective in reducing breast meat aflatoxin residues, with values ranging from 0.23±0.02 to 0.52±0.27µg/kg were similarto one another and this represented 0.02 to 0.07% of the average total aflatoxins ingested.Aflatoxin residues in the liver of birdsoffered all the four mitigated treatment diets with values ranging from 0.35±0.11 to 0.78±0.13 µg/kg, (representing 0.04 to 0.11% of average aflatoxins ingested)were also significantly reduced compared to 3.46±0.43 µg/kg and 2.56µg/kg (0.56% and 0.42% of ingested aflatoxins) in liver and breast meat of birds fedunmitigated diet, respectively. Liver residual aflatoxins reduction with the mitigated diets was significant and ranged between 77.5 to 89.9%,when compared with birds fedunmitigated feed.

As at 26th February, 2010 revision of the European Union (EU) Commission regulations, the maximum levels of 2 µg/kg for aflatoxin B1 and 4 µg/kg total aflatoxin were upheld for cereals and all products derived from cereals. An exception was given, “that if maize is to be further processed by sorting or other physical

treatment before human consumption or before being used as an ingredient in animal feedstuffs, then aflatoxin B1 maximum levels of 5 µg/kg and 10 µg/kg for aflatoxin total will beallowed" (EU commission, 2010). Since maize are not subjected to further sorting in Nigeria before use in poultry feed, the EU regulation limit should be taken to be 4 µg/kg for aflatoxins total. As far back as 1969, the Food and Drug Administration (FDA) of the United States set an action level of 20 parts per billion (ppb) or 20 µg/kg aflatoxins (i.e. for aflatoxins B1, B2, G1 and G2) in all foods, including animal food or feed, with the main objective of limiting aflatoxin exposure to the least possible level (FDA, 2019).

As at the time of this reporting, there is no regulation limit by the EU Commission for animal tissue residual aflatoxin level and also there is no action level by the FDA to date on residual aflatoxin level in animal products. The only animal derived product that has EU Commission regulation limit for aflatoxin is milk. Raw milk, heat-treated milk and milk meant for production of milk-based products were regulated to have not more than 0.05 µg/kg (EU Commission, 2010).The EU noted that "*reduction of total dietary exposure to aflatoxins (in humans) could be achieved by reducing the number of heavily contaminated foods (or feedstuffs) reaching the market through effective enforcement and reducing exposure from food sources*". Going by this statement, the mitigation strategy combinations adopted in all the four mitigated treatment diets used in thecurrent study were able to produce chicken products (breast meat and liver) that had significantly reduced total aflatoxin level.

If the 4 µg/kg total aflatoxin limit set by the EU Commission in all cereals and all cereals derived products, is safe to be consumed by humans, the levels of residual aflatoxin obtained in this study, for breast meat and liver of broiler chickens fed all four mitigated diets, could therefore, be assumed to be safe enough for human consumption and the set objective of reducing residual aflatoxin in the meat of broiler chickens used in this study could also be said to be effective.Reports of aflatoxins residues in eggs were found to be within the safe limit for human consumption. Reported aflatoxin residue of 0.07 and 0.01%were observed in eggs by Hassan *et al*. (2012) and Herzallah (2013) respectively. In the current study, only birds fed the mitigated diets had levels of aflatoxin residues as low as reported by (Hassan *et al*., 2012 and Herzallah, 2013). Aflatoxins entry routes as residues through edible tissues

of pigs, poultry and cattle do not account for any marked source of aflatoxins exposure in humans (Fink-Gremmels and Van der Merwe, 2019). However, they noted that aflatoxins in poultry diet at low level may not produce hazardous aflatoxins residues in edible tissues to humans but that residual aflatoxins in poultry liver may be exceptional. Mitigation of aflatoxin-contaminated diets as presented in treatment diet TD4 appeared effective in reducing aflatoxin residues not only in edible flesh of broiler chickens but also in liver.A related result was obtained by Singh *et al.* (2017), theynoticed that the inclusion of baker's yeast (*Saccharomyces cerevisiae*) at 0.1 or 0.075% (1,000 or 750mg/kg) with 100 or 200mg/kg of vitamin E to a feed contaminated with 150µg/kg of ochratoxin reduced the associated adverse effects in broiler chicken.

Feed aflatoxin to tissue aflatoxin carry over ratio is the level of aflatoxins in animal tissues relative to the level of aflatoxins in the feed. The feed to breast meat carry-over ratio of 105:1 and feed to liver carry over ratio of 80:1 were observed in birds that consumed unmitigateddiet. These ratiosappeared worrisome when compared to 1,200:1 in broiler chickens liver reported by Park and Pohland (1986). The report obtained in the current study revealed that a feed containing up to 80 µg aflatoxins/kg feed will likely produce up to 1 µg/kg residual aflatoxins in liver and 105 µgaflatoxins/kg feed will deposit 1 µg/kg aflatoxins in breast meat. Contrary to the result by Park and Pohland (1986), the result obtained in this study showed that it will take far less than 1,200 µg aflatoxins/kg feed to obtain 1 µg aflatoxins residues/kg in liver of broiler chickens. Using the ratio of 80:1 of feed to liver aflatoxin obtained in the current study, 1,200 µg aflatoxins/kg feed reported by Park and Pohland (1986) will result in the deposition of about 15 µg aflatoxins/kg in liver of broiler chickens. To achieve a wider deposition ratio as earlier documented, mitigation of aflatoxin-contaminated diets with beta-glucans and supplemental dietary antioxidants resulted in carry-over ratios of 625, 1020, 1195, 1040 to 1 in breast meat and 350, 585, 615, and 820 to 1 in liver of birds fed treated contaminated feed, with feed aflatoxins level of 270 µg/kg.

In poultry production, especially in broiler chickens and grow-out pullets rearing, uniformity of body weight or flock uniformity is an important parameter of performance that guides the farmer and informs him what proportion of his stock will

be available for market at a specified time or how many of his growing pullets will come to lay about the same time and how soon the peak of egg production will be attained.Both 375ppm beta-glucans and 0.3mg supplemental selenium per kg of feed resulted in significant improvement in flock uniformity of birds.Addition of 0.3mg/kg supplemental selenium resulted inhigher uniformity coefficient of variability of 8.04%, meaning that 92 times out of 100, the uniformity recorded in birds fedsupplemental selenium will likely produce 77% uniformity in bodyweight. Improvements recorded with oxidative stress indices (MDA and GSH:GSSG) and the reduction in absorbed aflatoxins, leading to reduction in aflatoxins retentionmay be the reason for reduction in oxidative stress effect, resulting in the marked increase in uniformity of bodyweight recorded in birds fed 375ppm beta-glucans and 0.3mg supplemental selenium treatment diets. Mortality was also significantly reduced by in birds offered 375ppm beta-glucans inclusion diets, while supplemental selenium (with or without)had no effect on mortality.The additive effect of beta-glucansand supplemental dietary antioxidants with or without seleniumreduced mortality to 9.1 and 12.1% from 39.4%, indicating the effect of supplemental dietary antioxidants in facilitating the elimination of the inevitably absorbed fraction of the ingested toxin. This will probably result in less damage to the liver, improving livability and hence reducing death rate.

Varied levels of beta-glucansinclusion(250 and 375ppm) were observed to have effect on average feed intake compared to supplemental selenium diet. A 375ppm beta-glucans inclusion as toxin adsorbent resulted in significant increase in feed intake. Adding 375ppm beta-glucans, with supplemental dietary antioxidants and selenium produced higher body weight gain (BWG) in birds.The two different levels of beta-glucans inclusion were also observed to have significant effect on feed conversion ratio (FCR). Birds offered 375ppm beta-glucans inclusion diet had greatly reduced FCR compared to birds offered 250ppm beta-glucans inclusion. Supplemental selenium howeverdid not produce any significant effect on FCR.

The reduction infeed intakein birds offeredunmitigated feedmay be due to the presence of coumarin, the main component in aflatoxin (a compound regarded asa difurano-coumarinderivative) molecule. It isa bitter-tasting and appetite suppressant (Borges, *et al*., 2005; Chen *et al*., 2013). Some plants synthesize coumarin as a natural defence to prevent their leaves from being foraged upon by animals (Borges, *et al*., 2005).

Higherfeed intakewas invoked in birds offered diets containing 375ppm beta-glucans with supplemental dietary antioxidants than those offered the othercontaminated treatment diets. Though reduction in feed intakeor reduced nutrients intake issufficient to reduce body weight gain, however, the effect is expected to be complicated when the reduced feed consumed is contaminated with toxins. The poor performance of birds offered the unmitigated diet could be the consequences arising from aflatoxins metabolism, where metabolite that attack DNA such as aflatoxins 8,9- exo-epoxide, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and other reactive oxygen species such as hydrogen peroxide, that will elicit the degeneration of macromolecules(cell membranes, organelles and others) are produced. To facilitate theelimination of aflatoxins out of the body, ingested toxin must be processed or metabolised in the liver to products that are hydrophilic (water soluble) and can be readily excreted by the kidney into urinary products or channelled into bile through the bile duct back into the GIT and eventually into faeces.

During the process of bio-transformation, oxygen molecule is split by the cytochrome P450 monooxygenase enzymes and one atom of oxygen is attached to the aflatoxin molecule. The other half becomes a superoxide anion ($O_2^{\cdot-}$), a free-radical which undergo dismutation by Superoxide dismutase into H_2O_2 and O_2, while catalase and Glutathione peroxidase will neutralise the hydrogen peroxide so produced into $H_2O$and O_2.In the presence of xenobiotic such as aflatoxins, excessive generation of superoxide anion ($O_2^{\bullet-}$) radical will occur because of persistent supply of aflatoxinsthrough the feed. As reviewed in chapter 2, section 2.8, Fenton's and Haber-Weiss reactions will come into play and there will be further free-radicalsgeneration such as$O_2^{\bullet-}$, hydroxyl radical ($HO^{\bullet}$) and ROS such as hydrogen peroxide (H_2O_2). Excessive H_2O_2 production may overwhelm and deplete the endogenous antioxidants and the accumulation of H_2O_2 will impair glucose metabolism and protein synthesis, which will ultimately affect growth, measured in BWG. The exposure of animals to high levels of toxin will make cells to utilise more glutathione for conjugation (Krishnamurthy and Wadhwani, 2012) and also as reductant in glutathione peroxidase activity to reduce plasma level of H_2O_2. Cells react to rising levels of oxidants (ROS)by producingmore GSH to neutralise or detoxify the accumulated H_2O_2 (Bellomo *et al*., 1992). Hence, as more $H_2O_2$are produced, more glutathione will be

generated, but increased production of GSH might not happen during persistent aflatoxins ingestion.

Hydrogen peroxide as an oxidant, is membrane permeable (Halliwell and Gutteridge, 1989) and has been reported to have the capability to inactivate the glycolytic enzyme - glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The G3PDH had been observed to be inactivated within minutes of exposure to oxidants such as H_2O_2, predominantly via direct enzyme inhibition (Hyslop *et al*., 1988). In glycolysis, G3PDHcatalysis the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. When this enzyme is inactivated, glucose and intermediates of glycolysis will accumulate (Zhang, *et al*., 2000) upstream and this will result in the flux of glucose at the nexus of glucose-6-phosphate (G6P) into the pentose phosphate pathway (PPP) (Fernandez-Checa *et al*., 1997), to generate more NADPH, the required co-enzyme by glutathione reductase (GSR) in regeneratingreduced glutathione from its oxidised form.

In hyperglycaemia (consequent upon glucose accumulation), adenylate cyclase will be activated. This enzyme catalysisthe conversion of ATP into cyclic adenosine monophosphate (cAMP). Increased cellular level of cAMPwill activate the enzyme protein kinase A, a potent inhibitor of glucose-6-phosphate dehydrogenase (G6PDH) (Mahmoud and Nor El-Din, 2013). In the PPP, glucose-6-phosphate is oxidised to 6-phosphogluconate by G6PDH, leading to the production of the co-enzyme NADPH. A second molecule of NADPH is also generated by the next reaction step, which involves the oxidative decarboxylation of 6-phosphogluconate by the enzyme 6-phosphogluconate dehydrogenase, to produce a five-carbon keto sugar, ribulose-5-phosphate (Campbell and Farrell, 2008). Ribulose-5-phosphate is required to produce the deoxyribose derivative of ribose, which plays an important role in nucleic acid (nucleoside) biosynthesis.

Inhibition of G3PDH will impair glycolytic process and energy production via ATP will be reduced, NADH, the co-enzyme required to initiate the process of electron transfer to oxygen in the electron transport chain, in the mitochondria and also required during gluconeogenesis, will not be produced or its production impaired. While the inhibition of G6PDH will halt the generation of NADPH, required by GSR to regenerate GSH from its oxidised state (GSSG), hence, increasing oxidants or ROS

load in the animal's body. The production of the five-carbon sugar needed in nucleoside synthesis and hence, protein synthesis will also be altered. This may be the cause for the poor growth rate and the significantly reduced BWG of birds fed unmitigated treatment diet. The differential reduction in the oxidants load and prevention of antioxidant (GSH) depletion in birds offered the mitigated treatment diets, may be the reason for the differences observed in their BWG pattern.Inhibition of the two rate-limiting enzymes of glycolysis and PPP (G3PDH and G6PDH) will lead to excessive accumulation of H_2O_2 and this will eventually result in cell death (Molavian *et al*., 2016). This could also be the reason for the poor growth rate and the high mortality of birds that consumed unmitigated treatment diet. Also, the combination of mitigants (beta-glucans and supplemental dietary antioxidants) used in the mitigated treatment diets reduced the oxidants or ROS (H_2O_2)load within the experimental birds, as evident in the positive balance of reduced glutathione and the resultant differential improvement in BWG across the mitigated treatment diets. Mortality was reduced significantly in birds offered mitigated treatment diets, which was also a consequence of reduced ROS load, compared to birds fed unmitigated treatment diet.

Another consequence of excessive ROS (H_2O_2) production during aflatoxins metabolism is that three important enzymes that are involved in energy production in the Tri-

carboxylic acid Cycle (TCA or Krebs cycle) can be inactivated. Aconitase was reported to be inhibited at lower concentration of H_2O_2 and α-ketoglutarate dehydrogenase at higher concentration, whilesuccinate dehydrogenase is partially inhibited (Tretter and Adam-Vizi, 2000). Intermediate products or metabolites of the Krebs cycle such as isocitrate, α-ketoglutarate, succinyl-CoA, malate and oxaloacetate, though produced in the mitochondria, are capable of crossing the mitochondrial membrane into the cytosol and undergo one or more transamination reactions to produce their corresponding amino acids, the building blocks in protein synthesis (Campbell and Farrell, 2008). Inhibition of enzymes of the TCA cycle as a result of excessive or uncontrolled H_2O_2 production, such as duringpersistent aflatoxinsingestion and metabolism, will hinder the smooth flow and production of these intermediate products and impair the production of amino acids derivable from these intermediates of Krebs cycle. Ultimately, protein synthesis will be impaired and growth of the affected animal will be poor or reduced.

These are the possibilities for the reduced BWG recorded in birds offered unmitigated treatment diet and the progressive improvement in BWG recorded in birds that consumed mitigated treatment diets. Themitigated treatment diets performance improved progressively as a result of the addition of beta-glucans, which enabled the reduction in the absorption of the ingested aflatoxinsfrom the GIT and the inclusion of supplemental dietary antioxidants to quench free-radicals and/or reactive oxygen species production from the fraction of the toxin that **was**inevitably absorbed. These combined mitigation strategies resulted in reduced MDA production and also reduced GSH depletion. Reduction in these two parameters of oxidative stress was an indicator of reduced ROS or H_2O_2 production.The significant improvement observed in BWG of birds fed mitigated treatment diet TD4, which had similar BWG withbirds offeredaflatoxin-free treatment diet was another indication of ROS or H_2O_2 cellular production
reduction.

Mitigation was observed to be effective in decreasing FCR in birds offered themitigated treatment diets, to such a level similarto the FCR of birds that consumed aflatoxin-free treatment diet. Attheend of the experimental period, birds fed treatment diet TD4 containing 270ppb aflatoxins, mitigated with 375ppm beta-glucans + 200mg Vitamin E + 250mg Vitamin C + 3mg Vitamin K + 0.3mg Selenium, had BWG and FCRsimilar to that of birds offeredthe uncontaminated diet. Birds fed TD4 had an edge on performance over birds offered the uncontaminated diet, by having a significantly lower feed intake, an advantage that is of economic importance in commercial broiler chickens' production.

Improvement in Body Weight Gain (iBWG)observed in birds offered mitigated treatment dietsrelativetothe BWG of birds fed the unmitigated diet was highly significant.Also,the reduction in Body Weight Gain(rBWG) consequent upon aflatoxins ingestion by birds that consumed the mitigated dietsrelative to the BWG of birds offeredthe uncontaminatedtreatment diet, equally improved.Birds fed 375ppm beta-glucansaddition diet had lowerrBWG and theyalso had significant rise in BWG compared to birds fed 250ppm β-glucan diets. This could be attributed to higher reduction in absorbed aflatoxins at 375ppm beta-glucans inclusion in the diet, which culminated inreduction in serum MDAlevel and improvement in GSH:GSSG in favour of GSH.Supplemental selenium equally minimised rBWG compared tobirds

offeredtreatment diets without supplemental selenium. Selenium supplementation also had significant effect in improving body weight gain of birds offered diets containing it. Main effect of 375ppm beta-glucans and 0.3mg supplemental selenium was seen to effectively minimised bodyweight loss and significantly enhanced BWG in broiler chickens fed aflatoxin-contaminated feed.

The rBWG or loss in body weight gain of birdsthat consumed unmitigated treatment dietwas 51.36% less the BWG of birds offereduncontaminated feed. With mitigation, Tedesco *et al*. (2004) reported 10% reduction in bodyweight gain of broiler chickens fed diets containing 800ppb aflatoxin. Zhao *et al*. (2010) fed 1mg/kg (1000ppb) aflatoxin B_1 to broiler chicks for 21 days and reported 10% reduction in weight gain, while Denli *et al*. (2009) observed 15% decrease in bodyweight over 42 days feeding period. After 21 days feeding of broiler chickens on 3mg AFB1/kg diet (Valdivia *et al*., 2001) and after 21 to 42 days feeding on 2.5mg AFB1/kg diet (Miazzo *et al*., 2000), 11% reduction in bodyweight gain compared to the control were reported respectively.In the current study, with mitigation, rBWG gradually declined or improved from 37.44% to 3.14%. The reduction in bodyweight gain of birds offered diet TD3 (16.12%) was similar to the 15% reduction in bodyweight gain of broiler chickenoffered aflatoxin-contaminated rations reported by Denli *et al*. (2009).The rBWG of birds fed diet TD4 (3.14%) was an improvement over the results reported byMiazzo *et al*. (2000)and Denli *et al*. (2009).

The BWG of birds offered the uncontaminated diet (1966.24±29.77g/bird) was 105.65±2.1% higher, relative to the BWG of birds offered unmitigated treatment diet (956.27±19.34g/bird), as the former bodyweight was more than twice the body weight of the latter. Birds that consumed treatment diet TD1 had 28.72% iBWG, relative to the BWG of birds fed the unmitigated diet. While birds offeredmitigated treatment diets TD2, TD3 and TD4 had 44.39, 72.35 and 99.15% improvements in their BWG respectively, relative to that of birds offered unmitigated feed. The iBWG of birds offered diet TD4 was comparable to that of birds offered the uncontaminated ration. This was an indication that treatment diet TD4 which had better reduction in aflatoxins absorption, reduced levels of oxidative stress indices, reduced aflatoxins retention, reduced mortality, considerably lower residual aflatoxins in the liver and breast meatand a better BWG similar to those of birdsoffered the uncontaminated feed,effectively counteracted the adverse effects of aflatoxins in broiler chicken.

The absence of aflatoxins counteracting agents as was the case with birds offered unmitigated diet, led to the production of birds that had higher average production cost (₦686.31) per bird, than the prevailingmarket liveweight value (₦650.97) per kilogramme weightas at the time of the current study. This resulted innegative marginal return per bird, in birdsthat consumed unmitigated treatment diet. Average feed cost/bird was the least in birds offered unmitigated treatment diet, perhaps due to reduction in feed intake brought about by the appetitesupressing attribute of aflatoxins. Consequent upon reduced feed intake and uponperformance depressing effectsthat resulted via aflatoxins metabolism, the average final bodyweight of birds offeredunmitigateddiet (1,001.49g) was significantly reduced up to50% of the bodyweight of birds (2,011.82g) fed the uncontaminated feed. Irrespective of the reduction in feed cost in birds fed unmitigated treatment diet, average total raising cost was very high relative to the final bodyweight, as all other costs incurred were the same for all birds offeredother treatmentrations. Hence, the average cost of raising each bird fedunmitigated diet to market weight was higher than the prevailing market value of the bodyweight. This gave rise to an average loss of ₦35.34 per every bird raised with unmitigated contaminateddiet.Addition of beta-glucans and supplemental dietary antioxidants to the mitigated diets was observed to have resulted in a gradual increase in marginal return/bird over average raising cost from ₦47.08 in birds fed TD1 to ₦109.50, ₦156.80 and ₦303.80 in birds offeredtreatment diets TD2, TD3 and TD4, respectively. Mitigation at the level of treatment diet TD4, the observed marginal return per bird wascomparableto that obtained from birds offered the uncontaminated ration.

Photomicrographs, showing microscopic sections of liver, kidney and ileum of birds offereduncontaminated, unmitigated aflatoxin-contaminateddietand aflatoxin-contaminated diets with mitigation revealed thatbirds fed aflatoxin-free diet basically had no observable lesion in samples of liver, kidney and ileum sections. Liver sections of birds fed unmitigateddiet showed gross multifocal hepatocellular coagulation necrosis. Kidney sections of birds fed unmitigated feed had multifocal tubular epithelial coagulation and defoliation and disruption of basement membrane, while the microscopic sections of ileum also showed necrosis and loss of enterocytes, cryptal hyperplasia with infiltration of inflammatory cells in the mucosa. These were suggestive of aflatoxins damages to these tissues and similar to the report documented

by Peles *et al*. (2019).Liver sections of birds fedtreatment diets TD1 and TD2 had moderate hepatocellular atrophy, which showed that addition of 250ppm beta-glucans reduced the quantity ofaflatoxins absorbed, as this resulted in reduced hepatic damage. However, renal microscopy showed that the quantity of the toxin that was inevitably absorbed was significantlysufficient to induce glomerular necrosis and tubular atrophy. Histology of ileum sections showed severe to moderate villi atrophy. The level of improvement recorded in the bodyweight gain of birds offeredtreatment diets TD1 and TD2, reflected the reduction in the damages on these three tissues as shown by the histological report.Microscopic sections of liver, kidney and ileum of birds offered treatment diets TD3 and TD4 showed that 375ppm beta-glucans addition to the contaminated diets was effective on the overall, in preventing hepatocellular coagulation necrosis, as observed in birds offered unmitigateddiet, and also prevented the necrosis on renal and ileum tissues.

Liver and kidney samples were more affected during aflatoxins ingestion than the ileum. It was also observed that treatment diets TD1 and TD2, with 250ppm beta-glucans were less effective inameliorating the adverse consequences of aflatoxins in liver, kidney and ileum samples. Birds offeredtreatment diets TD3 and TD4 with 375ppm beta-glucanshad no observable lesion in their liver, kidney and ileum tissues on the aggregate. Birds fedtreatment diet TD4which had the full complement of the mitigants performed better with overall assessment of noobservable lesion in theliver, kidney and ileum tissue samples, similar to that seen in birds feduncontaminated feed.

Results from the combined effects of yeast beta-glucans and supplemental dietary antioxidants and vitamin Kshowed that the null hypothesis statement which stated that: “combining yeast beta-glucans with different combinations of supplemental dietary antioxidantsand vitamin K will not be effective in mitigating the deleterious effects of aflatoxins in broiler chicken” is not true and was rejected. On the other hand, the alternate hypothesis that said:“the adverse effects of aflatoxins in broiler chicken can be prevented by the inclusion of yeast beta-glucans withdifferent combinations supplemental dietary antioxidantsand vitamin K in their diets, and that the effect of one treatment will be different from the other” was upheld. This was particularly true for birds offered TD4 as results obtained in this treatment group compared favourably with birds fed the uncontaminated ration.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Birds that consumed diet contaminated with 270ppb aflatoxinsand mitigated with 375ppm beta-glucansaddition had up to 79.30±10.38% of adsorbed aflatoxins. Correlation graph revealed a positive correlation between aflatoxins adsorption and the level of beta-glucans inclusion. As the level of beta-glucansinclusion increases up to 375ppm, the level of aflatoxins adsorbed also rises. Beyond 375ppm of beta-glucansaddition to aflatoxin-contaminated diets, reduced aflatoxins adsorption and decreased performance were observed. Mitigation with beta-glucans, resulted in reduced residual aflatoxins concentration in liver up to 0.50 and 0.73µg/kg in birds fed 250 and 375ppm beta-glucansdiets respectively, while birds fed 0ppm beta-glucans addition had 2.57µg/kg residual aflatoxins in their liver samples.

The level of serum malondialdehydedeclined significantly in birds fed treatment diet containing the combination of 200mg vitamin E+250mg vitamin C+3mg vitamin K and 0.3mg Se, among all birds offered aflatoxin-contaminated diets. Birds offeredsupplemental dietary antioxidants' combination and vitamin K had the least residual aflatoxins concentration in their liver, compared with birds offered unmitigated contaminated diet. The addition of supplemental dietary antioxidants alone in different combinations was less effective in keeping mortality at bay. The least mortality rate obtained with supplemental dietary antioxidantscombinations alone to counteractthe upsurge in mortality characteristic of aflatoxins poisoning was 50%.

Addition of beta-glucans, supplemental dietary antioxidantsand vitamin Kdid not produce significant effect between the haematology of birds fed unmitigated contaminated diet and the mitigateddiets. Birds offered contaminated diets with 375ppm beta-glucansinclusion and the combination of 200mg vitamin E+250mg vita-

min C+3mg vitamin K and 0.3mg Se had the leastvalues of serum ALP and MDA.Feed intake, final bodyweight and oxidative stress ratio (GSH:GSSG) were significantlyimproved in birds that consumed contaminateddiet with thecombinations of beta-glucans and supplemental dietary antioxidants indicated above. With mitigation, aflatoxins retention in the body of the birds was reduced from 55.91% in the unmitigated contaminateddietto 12.09% in birds offeredmitigated treatment diet TD4. Levels of aflatoxins in blood, liver and breast meat in birds fedunmitigated contaminated diet were higher and were reduced significantly with beta-glucans and different combinations of supplemental dietary antioxidants and vitamin K. Mitigating with beta-glucanscombined withsupplemental dietary antioxidantsand vitamin K also lengthened the feed aflatoxin to tissue aflatoxin carry-over ratio, as compared to result from unmitigated contaminateddiet.

6.2 Conclusion

Elevated serum ALP level arising from the damage to the lining of the biliary tract during aflatoxins poisoning was effectively mitigated in the current study, similar to the ALP level observed in birds fed the uncontaminated diet. Elevated concentration of serum MDA, one of the terminal products of membrane PUFA oxidation or lipid peroxidation, that can cause functional abnormalities and pathological changes in bio-membranes, and oxidative stress, measured in the current study as GSH:GSSG, which is an index of imbalance between oxidants and antioxidants, with a potential for cellular damage when the former overwhelms the latter, were both significantly improved in birds that consumed the contaminated diet having addition of 375ppm beta-glucans combined with 200mg vitamin E+250mg vitamin C+3mg vitamin K and 0.3mg Se. These consequently resulted in increased BWG, high uniformity of bodyweight (a very important parameter of performance in commercial broiler production), reduced mortality, reduction in bodyweight loss, reduction in aflatoxins retention within the birds and reduced residual aflatoxins in liver and breast meat meant for human consumption. Marginal return per bird was also increased, similar to that obtained in birds feduncontaminated diet. Aflatoxins residue in edible products, such as breast meat and liver of broiler chickens, arising from the ingestion of aflatoxin-contaminated feed is usually not a route of risk, and may not be of concern to human’s as a possible source of exposure to aflatoxins, as the level of residual aflatoxins in edible products is usually below the permissible limit. However, with

very high level of aflatoxins contamination in feed, residual aflatoxins may shoot up in edible tissues but mitigation as seen in treatment diets TD2, TD3 and TD4 were effective in preventing this possibility.

6.3 Recommendations

To establish the effect of beta-glucans on the haematology of broilers fed aflatoxin-contaminated diet, further studies need to be conducted to have some treatments groups without aflatoxins contamination but havingbeta-glucans inclusion alone. This will enable the effect of beta-glucans alone on the haematology of broiler chickens to be separated from the combined effects of beta-glucans and aflatoxins on blood parameters, as no glaring differences were observed in haematology among the treated groups in the current study.

Broiler chicken producers and poultry farmers in general should note that the use of an antidote,such as toxin binders, should never be taken as a remedy for feeding toxins (contaminated feeds) to their birds, in view of the fact presented in this report that none of the birds offered the mitigated aflatoxin-contaminated diets performed better than birds offereduncontaminateddiet. However, if a situation of feed material scarcity arises and aflatoxin-contaminated feed materials are unavoidably presented to farmers for use or the volume ofaflatoxin-contaminated feed material in the farmers custody is so large, in the face of scarcity, to be destroyed, then adding beta-glucansat 375ppm combined with 200mg of vitamin E + 250mg of vitamin C + 3mg of vitamin K and 0.3mg of selenium will preventsignificantly, the induction of aflatoxicosis, improveperformance and also ensure better marginal return/bird to the farmer.

Finally, as much as possible, avoidance of the use ofaflatoxin-contaminated poultry feed/or feed materials in practical broiler chicken production is the only guaranteed option to preventing the adverse consequences that may emanate from aflatoxins ingestion. This can be achieved by an act of law, regulating aflatoxins level in feed and feed materials, backed up with effective monitoring and enforcement to ensure compliance. Regular advocacies aimed at enlightening stakeholders in the industry about the dangers posed by aflatoxins towards animals' productivity and human health should be heightened through public shows and seminars, organized jointly by the ministries of Agriculture and Health, professionals in nutritional toxicology and all other

relevant stakeholders in the industry such as ASAN and NIAS.

6.4 Contributions to knowledge

a) Varied inclusion levels of beta-glucans revealed that there is a limit to the use of yeast cell wall beta-glucans in animal nutrition as aflatoxins adsorbent. Beyond 375ppm the quantity of aflatoxins adsorbed declined while absorbed aflatoxins increased significantly.

b) Current study results also revealed that beta-glucans could reduce aflatoxins absorption not only *in vitro* as earlier documented but also *in vivo*.

c) Mitigation of unavoidably ingested and aflatoxins with beta-glucans addition synergised effectively with supplemental dietary antioxidants and vitamin K inclusion, in reducing cellular oxidative activities which characterised the deleterious or production depressing attributes of aflatoxins' poisoning in broiler chicken, hence, leading to improvements in performance.

d) The current study also showed that mitigation with beta-glucans,supplemental dietary antioxidants and vitamin K could result in the production of safe edible broiler chicken's meat for human consumption.

e) Data from current study revealed that without mitigation, the production of broiler chicken feeding on aflatoxin-contaminated feed may not be profitable and sustainable.

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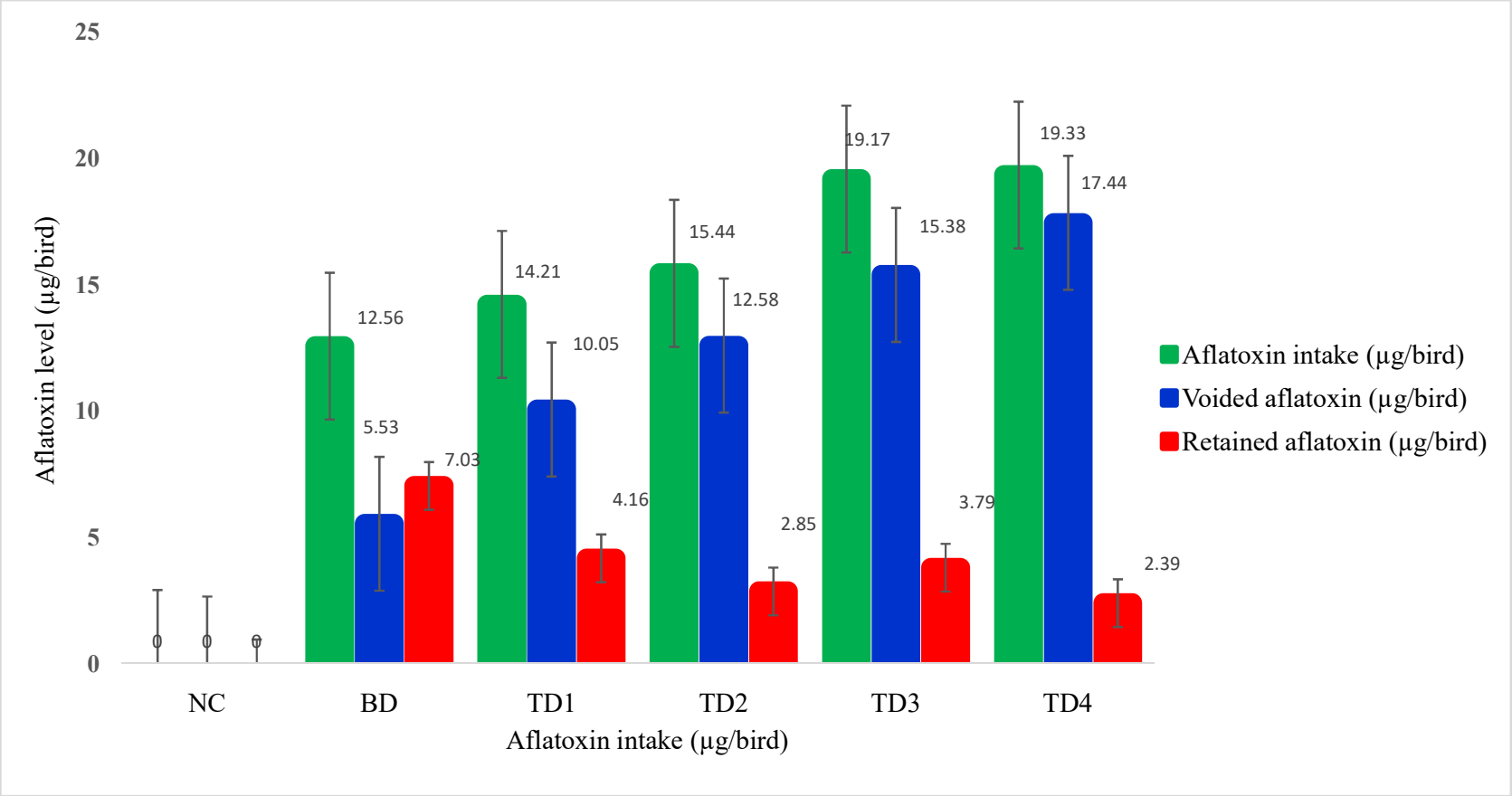
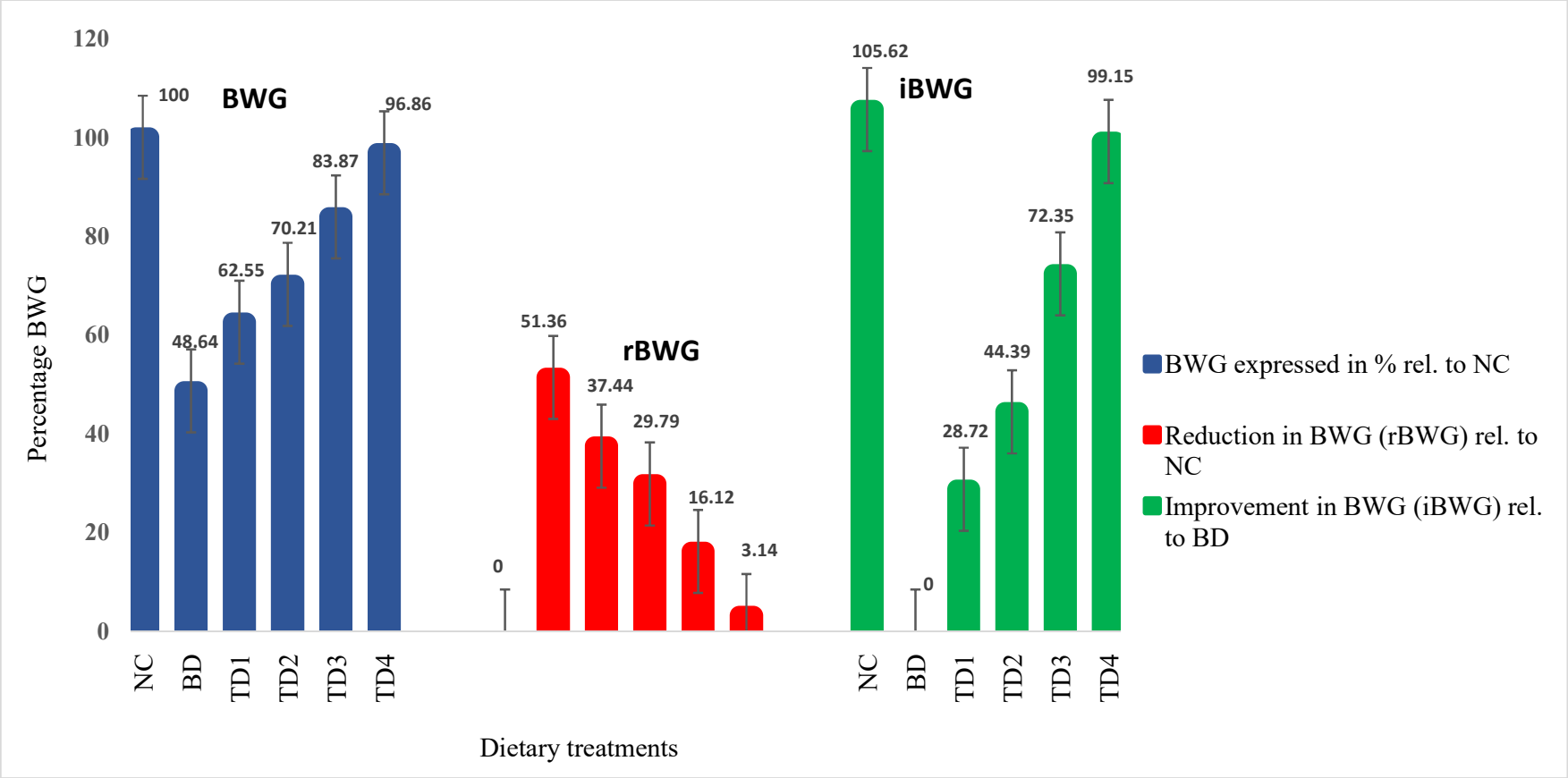
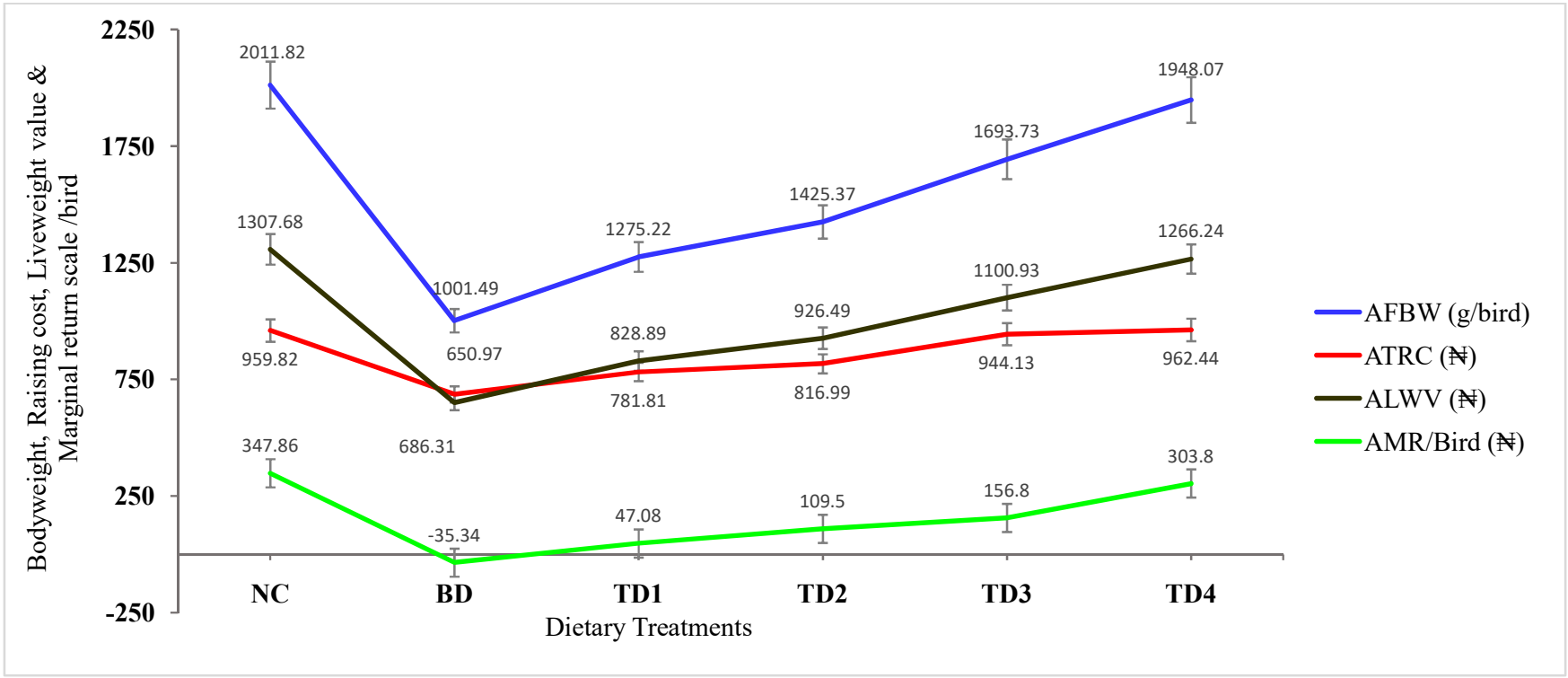
APPENDICES



Appendix I: Aflatoxin intake, voided and retained in broiler chickens fed aflatoxin-contaminated diets with varied inclusion levels of beta-glucans and selenium supplementation



Appendix II: Effect of varying levels of beta-glucans and seleniumsupplementation on Bodyweight Changes (BWC) of broiler chickens fed aflatoxin-contaminated diets



Appendix III: Final bodyweight (g/bird), total raising cost (₦), and marginal return/bird (₦) of broiler chickens fed aflatoxin-contaminated diets, mitigated with varying levels of beta-glucans and antioxidant vitamins

Inoculated maize grains arranged on shelves

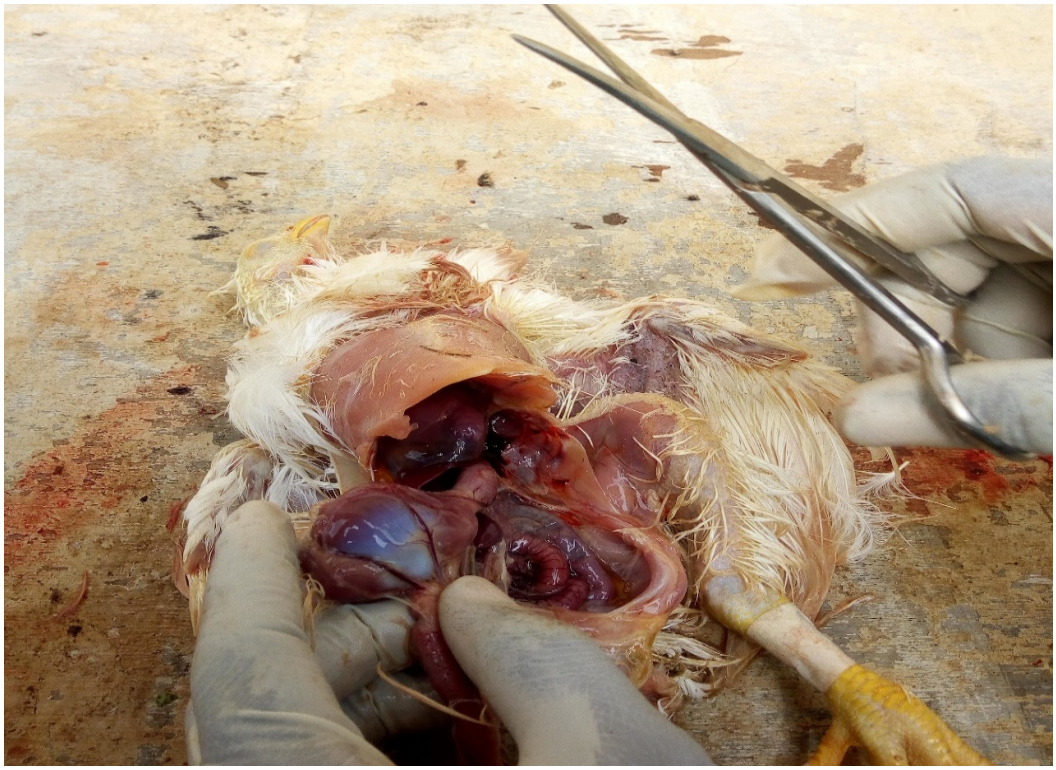
Appendix IV: Intensely colonised maize grains ready for spores destruction and drying

Heavily cultured maize grains

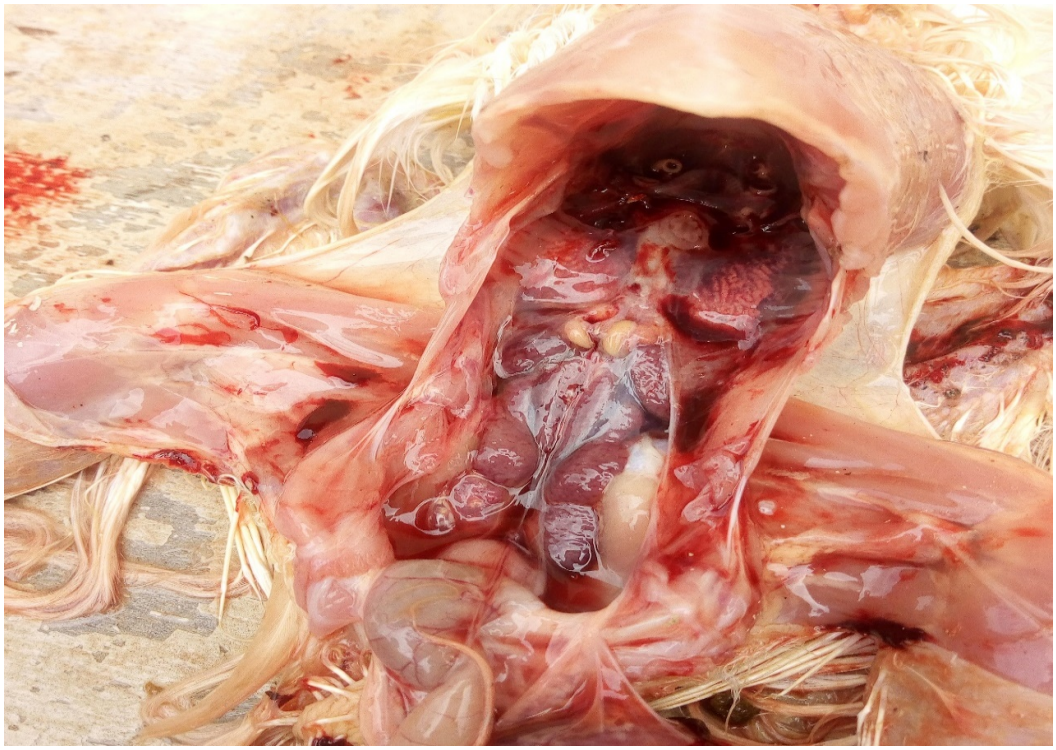
Contaminated maize grains

Appendix V: Sun-drying the cultured maize grains

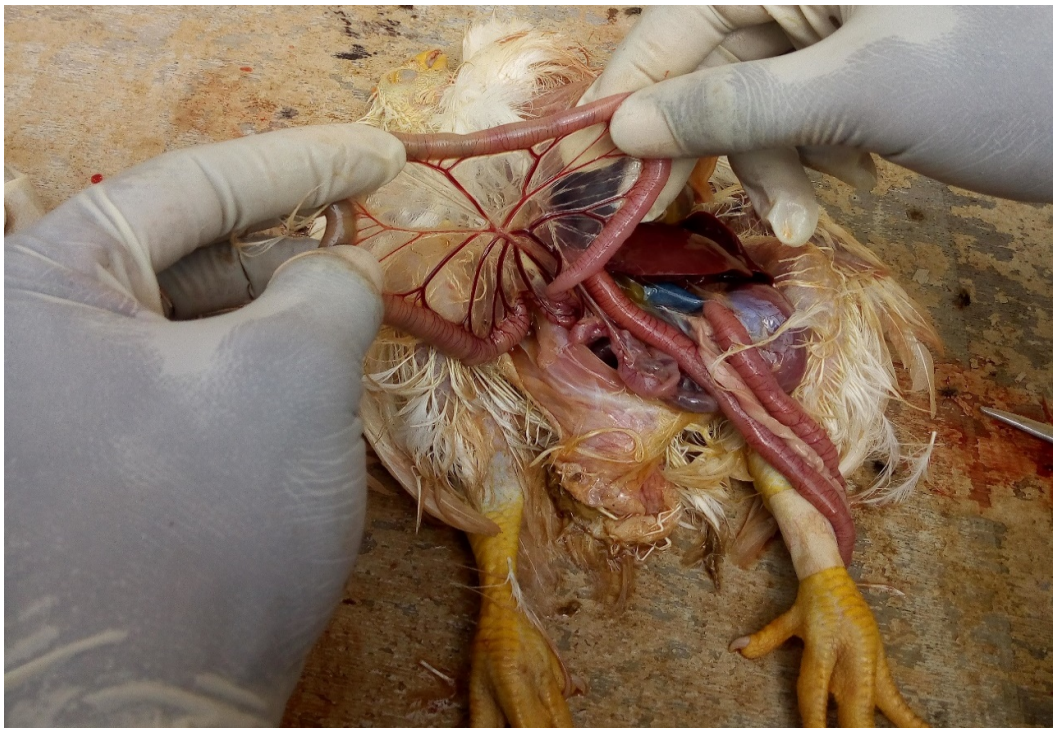
Opening up for tissues and organs harvesting



Appendix VI: Sectioning to harvest liver for histology

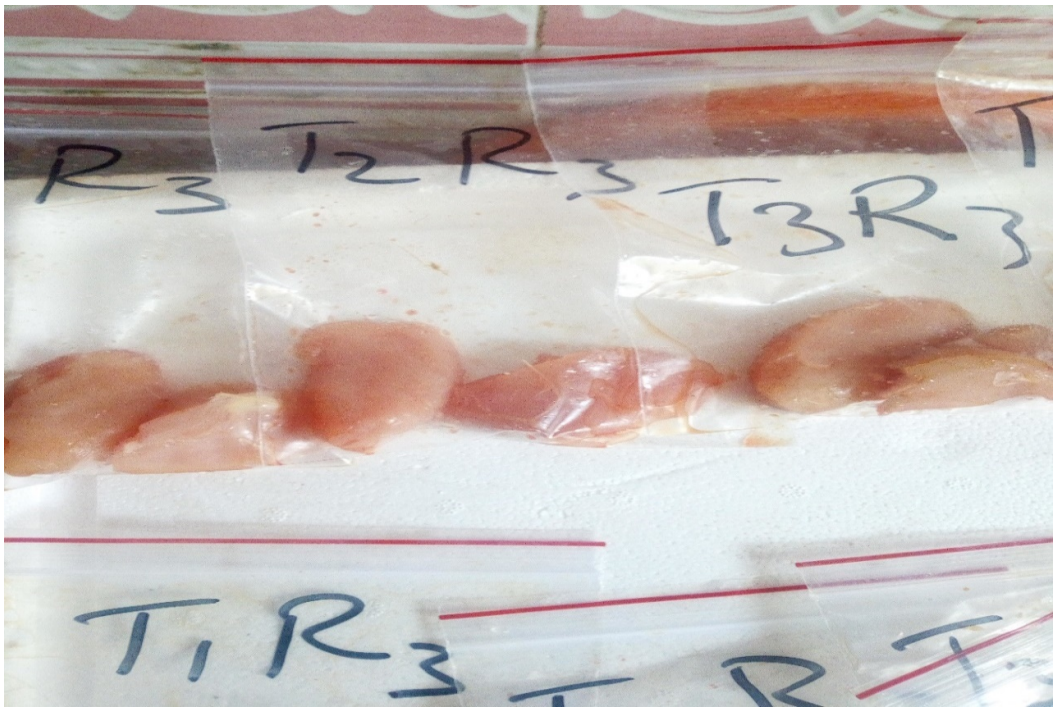


Harvesting kidney for histology



Appendix VII: Sectioning to harvest ileum section for histological examination

a) Liver samples



b) Breast meat samples

Appendix VIII: Harvested liver and breast meat for residual aflatoxins determination