EFFECT OF SUPPLEMENTAL DIETARY VITAMIN A ON AFLATOXIN B_1 TOXICOSIS IN GROWING BROILERS

By

Joseph Felix Chibanga

A thesis submitted to the University of Zambia in partial fulfillment of the requirements for the degree of Master of Science in Animal Nutrition.

The University of Zambia

Lusaka

2014

DECLARATION

I, **Joseph Felix Chibanga**, to the best of my knowledge, declare that this research has not been done or presented for a degree in this or any other university. The results shown herein are a true reflection of what was obtained from the study.

……..… ……...

Signature Date

CERTIFICATE OF APPROVAL

This thesis of **Joseph Felix Chibanga** is approved as fulfilling the requirements or partial fulfillment of the requirements for the award of the Degree of Master of Science in Animal Nutrition by the University of Zambia.

Examiner	Signature	Date
…….	……	…….
…….	……	…….
…….	……	…….

COPYRIGHT

ABSTRACT

Aflatoxin B_1 negatively affects broiler performance and is also a public health hazard as it has been associated with cancer in humans. Several methods have been suggested for minimizing effects of aflatoxin B_1 contamination including use of anti-oxidizing agents such as vitamin A. This study was conducted to evaluate the effects of supplementing different levels of vitamin A on the performance of broiler chickens fed on diets contaminated with aflatoxin B_1 at 35µg/kg of the feed diets for 42 days. The study was designed as a Completely Randomized Design in which 150 Ross Breeder's Broiler Chicks were randomly allocated to five (5) dietary treatments with each treatment being replicated 3 times. Standard broiler chicken starter, grower and finisher rations were used as controls. In the starter phase, contamination of diets with aflatoxin B_1 significantly ($P \leq 0.05$) reduced feed intake, bodyweight gains and feed conversion ratios. However, this was not the case in the grower and finisher phases, where only bodyweight gains and feed conversion ratios were reduced by aflatoxin B_1 contamination. This also affected final body weights and dressing out percentages of broiler chickens, where those fed on contaminated diets performed poorly compared to those on control diets. It was also noted that performance of chickens improved significantly with increasing levels of vitamin A supplementation in aflatoxin B_1-contaminated diets. The toxic effects of aflatoxin B_1 on feed intake of broilers were ameliorated by dietary supplementation of vitamin A at 6000 IU/kg and above. However, amelioration of the deleterious effects of aflatoxin B_1 on bodyweight gains and feed conversion ratios of broilers was achieved when vitamin A was supplemented in the diets at 3000 IU/kg. Levels of aflatoxin B_1 contamination used in the current study did not cause any death. Furthermore, feeding aflatoxin B_1 contaminated diets significantly decreased serum concentrations of total protein, albumin, triglyceride and cholesterol. Feeding aflatoxin B_1-contaminated diets significantly increased concentrations of liver functional enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransaminase) in the blood. This was an indication that increasing the levels of vitamin A supplementation reduced the negative effects of aflatoxin B_1 contamination on protein and lipid metabolism in broiler chickens. It was thus, concluded that supplementing increasing levels of vitamin A to aflatoxin B_1-contaminated diets results in reduced expression of toxic effects in broiler chickens.

Key words: Broiler chickens performance, Mycotoxins, Aflatoxin B_1 contamination, Vitamin A supplementation, antioxidising agents.

ACKNOWLEDGEMENTS

I praise, honour and glorify the LORD God for His mighty working and divine enablement that assailed me to the end of this study. I am also grateful to God's unique vessel, the presiding Bishop of Praise Christian Centre churches and ministries, Apostle, Dr. E. Ng'ambi for his spiritual guidance and counsel.

This research received support from numerous persons to whom I gratefully acknowledge my deep indebtedness. These include my research supervisors Dr. D. Banda Nyirenda and Dr. J. Simbaya for their invaluable guidance, assistance and encouragement. The help of Professor Pandey, Dr. B. Mwenya, Dr. K. Munyinda and others not mentioned here are also acknowledged for their individual help, advice and comments on this research.

My gratitude also goes to all academic, laboratory and Field Station members of staff in the School of Agricultural Sciences at the University of Zambia.

Lastly, but far from the least, my sincere thanks go to my employer and sponsor the Ministry of Agriculture and Livestock. I also acknowledge my family, workmates and friends for their encouragement, advice and assistance during the course of my study.

DEDICATION

In gratitude and appreciation to my dear wife, children, late father, mother and sisters.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immuno-Deficiency Syndrome
AFB_1	Aflatoxin B_1
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransaminase
AST	Aspartate aminotransferase
BW	Body weights
BWG	Body weight gains
C	Cholesterol
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FI	Feed Intake
HIV	Human Immunodeficiency Virus
IARC	International Agency for Research on Cancer
IU	International Unit
NRC	National Research Council
TG	Triglyceride
TP	Total protein
WHO	World Health Organization of the United Nations

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Food safety is an essential prerequisite in ensuring human health among all nations and there is a worldwide concern about food poisoning (FAO, 2004). Foods may be contaminated with physical, chemical or microbiological toxic agents that affect human and animal health and productivity. Human health is also compromised when people consume contaminated foods and animal products. Of the major microbiological food and feed contaminants, mycotoxins are of greatest concern. Mycotoxins are secondary metabolites produced by different types of fungi with the most common ones being aflatoxins, ochratoxin and fumonsin (Daghir, 1995). Among these, aflatoxins are of greatest concern for tropical countries like Zambia. Aflatoxins are mostly derived from two species *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are field and storage mycotoxins that proliferate under hot and humid conditions. It is for this reason that aflatoxins are exclusively limited to tropical climates and are mostly found in grain legumes and cereals (Hell *et al*., 2000). Some fungi like *Fusarium verticillioides* may invade plant species such as maize during growth and may remain on cereal grains even after crop harvest (Hell *et al*., 2000; Hawkins *et al*., 2005; Turner and Sylla, 2005).

Aflatoxin contamination is usually a consequence of interactions among the causative fungi, the host animal and the environment. The response to mycotoxin contamination also depends on a number of factors including levels of exposure, environmental conditions, the type and susceptibility of the invaded species (Kellerman *et al*., 1988). High levels of aflatoxins

contamination in feed results in acute necrosis of the liver and hyperplasia of the bile duct resulting in reduced digestibility of fatty acids and protein synthesis (Yunus *et al.,* 2013). Aflatoxin B_1 contamination also exerts inhibitory effects on biological processes including protein and DNA synthesis (Denli *et al*., 2004). Lower levels of aflatoxin poisoning may have no clinical symptoms but are usually associated with reduced productivity and suppressed immunity (Tedesco *et al*., 2004; Bailey *et al*., 2006; Shi *et al*., 2006). This may be manifested in form of reduced feed intake, poor growth rates and feed efficiency. Normally there are residues in animal/livestock products that may have adverse effects on human health including liver cancer.

Control of aflatoxin poisoning is normally aimed at minimizing feed and food contamination through pre and postharvest practices and processing to decontaminate contaminated feed ingredients and products. Use of biological anti-oxidants such as vitamins A, C and E to reduce the toxic effects of AFB_1 has also been proposed in many studies. Thus, the objective of this study was to evaluate the effects of supplementing vitamin A; a potent biological anti-oxidant on the performance of broiler chickens fed on diets contaminated with AFB_1.

1.2 Statement of the Problem

According to the FAO (2004), up to 25% of the world grain is contaminated with different types of mycotoxins and because of dependence on grain cereals and legumes, poultry and pigs are the most susceptible livestock species to mycotoxin poisoning. Mycotoxin poisoning in livestock usually result in chronic or acute health disorders depending on the levels of contamination and susceptibility of the infected species. Chronic poisoning is often associated with reduced feed intake and poor performance of infected species resulting in reduced productivity and eventual

economic losses (FAO, 2004). On the other hand, acute poisoning often leads to malfunctioning of many body organs and sometimes even death (Shashidhara and Devegowda, 2003). There is also residual accumulation of mycotoxins in livestock products including meat, milk and eggs that become a health hazard to humans consuming these products (Micco *et al*., 1988; Oliveira *et al*., 2000; Binthivok *et al*., 2002; Rizzi *et al*., 2003). According to Kellerman *et al*., (1988), mycotoxins are known to cause cancer, liver failure and Reyes syndrome (an acute neurologic disease that may result in accumulation of fat in the liver and swelling of the brain) in humans. In addition, exposure to aflatoxins has been associated with child stunting and underweight, neurological impairment, immunosuppression and increased mortality; and could interact with HIV/AIDS (WHO, 2006). The cost of complying with food safety and agricultural health standards has been a major source of concern in the international development community and among developing countries (World Bank, 2005). Thus, in order to prevent economic losses and safeguard human health, there is need for developing cost effective methods for minimizing mycotoxins poisoning in poultry and other livestock species. This research was designed to explore the possibility of minimizing toxic effects of AFB_1 poisoning in broiler chickens through use of vitamin A supplementation.

1.3 Justification

Complete eradication of contamination of poultry feeds with different types of mycotoxins is not a feasible proposition in modern livestock management systems due to pre-existing environmental conditions that favour fungal growth throughout the year. Use of chemical decontamination methods as a way of eradicating mycotoxins in food and feed products have

often proved to be too expensive for most developing countries including Zambia. One of the feasible solutions is to look at possibility of minimizing mycotoxin poisoning through application of biological anti-oxidizing agents. Vitamin A functions as a non-enzymatic anti-oxidizing agent that has capacity to protect cells from oxidative stress-induced toxicity and transformation (Chandra and Sarchielli, 1993; Kubena and McMurray, 1996; Cramer *et al*., 2001; Nicolle *et al*., 2003; Devaraj *et al*., 2008). By virtue of these properties, vitamin A can be supplemented in feeds to help reduce toxic effects of AFB_1 contamination in poultry rations. It is hoped that this may result in better performance of broiler chickens and improved quality of poultry products that are safe for human consumption. Use of vitamin A as a supplement in poultry diets is also justifiable in that the product is readily available in many forms including inactive pro-vitamins like carotenes (Jain, 2004; Muzaffer *et al*., 2003).

1.4 Main Objective

The main objective for this study was to determine the effects of supplementing different levels vitamin A to AFB_1-contaminated diets on the productive performance of broiler chickens.

1.5 Specific Objectives

i. To determine the effects of AFB_1 contamination on feed intake, bodyweight gain, feed conversion ratio, mortality, dressed weight and dressing percentage of broiler chickens when fed contaminated diets.

ii. To determine the effect of supplementing different levels of vitamin A in diets contaminated with AFB_1 on the serum concentration of alkaline phosphatase, aspartate aminotransferase, alanine aminotransaminase, total proteins, albumin, triglycerides and cholesterol in broiler chickens.

iii. To determine appropriate levels of vitamin A supplementation that may be effective in reducing toxic effects of AFB_1 contamination in broiler chickens.

1.6 Hypotheses

Null Hypothesis (Ho): Supplementation of vitamin A to AFB_1-contaminated diets cannot reduce the toxic effects and result in improved performance of broiler chickens.

Alternative Hypothesis (Ha): Supplementation of vitamin A to AFB_1-contaminated diets can reduce the toxic effects and result in improved performance of broiler chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxins

The term mycotoxins refers to a group of toxic secondary metabolites derived from different types of fungi that grow on agricultural products (Jewers, 1990 and Daghir, 1995). They are termed secondary metabolites because they are produced by fungi as a result of interactions between the host and the environment normally as a protective measure (Mannon and Johnson, 1985). There are hundreds of different types of mycotoxins that have been identified and most of them are known to be pathogenic in nature (Shlig, 2009). The most common ones include aflatoxins, ochratoxins, fumonisins, ergotoxin, citrinin, oosporein, cyclopiazonic acid and sterigmatocystin (Yunus *et al.*, 2013). Rarely do these mycotoxins occur in isolation, but rather in various combinations and may produce effects that may be additive or even synergistic to each other. In feeds, mycotoxins may cause nutrient losses or organoleptic changes which ultimately result in deterioration of feed quality. They are also capable of accumulating in soft tissues of livestock or induce nutritional deficiencies in animals fed on contaminated diets (FAO, 2004). According to the IARC (1993), the toxic effects of mycotoxins in humans have been found to be carcinogenic, mutagenic, teratogenic and even estrogenic.

Many of the mycotoxins are chemically stable and may maintain their toxic effects over extended periods of time (Shlig, 2009). Almost all agricultural commodities tend to support fungal growth and formation of mycotoxins may occur during plant growth in the field, crop storage after harvest or indeed during transportation of commodities (Shlig, 2009). Thus,

conditions exposed to agricultural commodities during plant growth, harvesting, storage, processing and handling of feedstuffs have a bearing on mycotoxin contaminations. Different types of mycotoxins tend to favour different types of conditions for growth and that is the main reason that different types are found in their selected geographical areas. Warm and humid conditions are conducive for fungal growth and therefore for the existence of the mycotoxins in the tropics (Daghir, 1995).

2.2 Aflatoxins

Aflatoxins were discovered in 1960 in the United Kingdom following the death of about 100,000 turkey poults that consumed groundnut-based feeds from Brazil that were later found to have contained a group of compounds that have now come to be termed as aflatoxins (Jewers, 1990). Almost all agricultural commodities support growth of aflatoxin producing fungi including *Aspergillus flavus* and *Aspergillus parasiticus* (Shlig, 2009).

The aflatoxin producing fungi require temperatures of 24 to 35˚C and relative humidity above 70% to grow and produce aflatoxins (Williams *et al*., 2004). A heavy rain during harvest increases the moisture content of the crop and the risk of infection (Bankole and Mabekoje, 2004). Damage of pods by insects, both prior to harvest and during storage helps in the colonization of fungi and toxin production (Brown *et al*., 2001). Above all, storing of feeds without proper drying or in moist places readily attracts *Aspergillus* infection and toxin production (Hell *et al*., 2000; Hawkins *et al*., 2005; Turner *et al*., 2005). According to Wogan *et al.*, (1992), the structure of aflatoxins consists of a coumarin nucleus attached to a bifuran and either pentanone (AFB_1 and AFB_2) or a six-membered lactone (AFG_1 and AFG_2). Aflatoxins include B_1, B_2, G_1, G_2, M_1, and M_2 as shown in Figure 1.

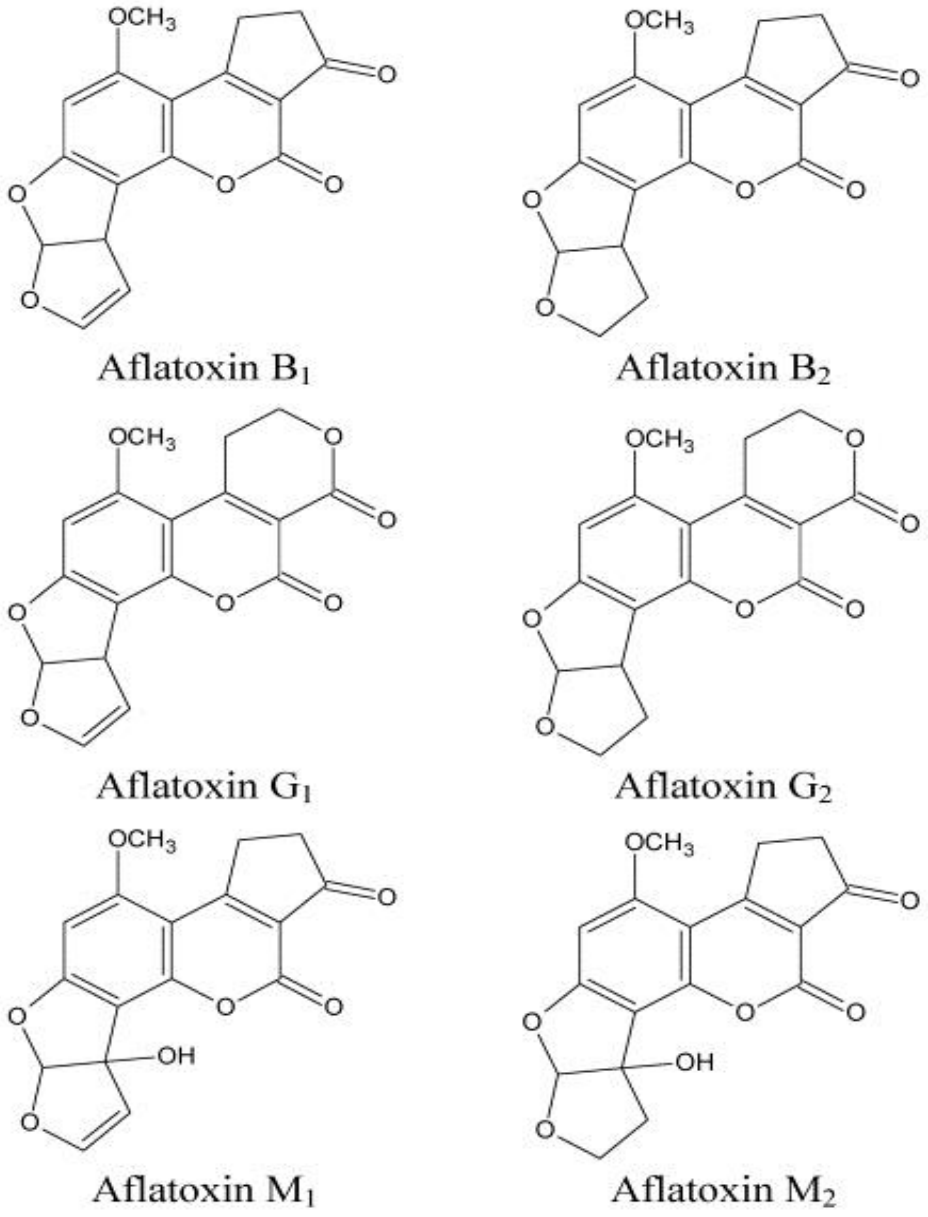

Figure 1: The different chemical structures of aflatoxins (Yunus *et al.*, 2013).

Aflatoxin B_1, B_2, G_1 and G_2 are the four main naturally occurring aflatoxins whose molecular formulae and weights are $C_{17}H_{12}O_6$, $C_{17}H_{14}O_6$, $C_{17}H_{12}O_7$ and $C_{17}H_{14}O_7$; and 312.3, 314.3, 328.3 and 330.3, respectively (Yunus *et al.*, 2013). The letters B and G, refer to the aflatoxins' colour

under UV light with B standing for Blue and G for Green, while the subscripts 1 and 2 refer to their relative positions on a developed thin-layer chromatography plate (Wogan *et al.,* 1992). Aflatoxins M_1 and M_2 however, mainly occur in milk in small quantities and have also been reported in eggs as metabolites of the B_1 and B_2. Aflatoxin B_1 is the most abundant and toxic form of all naturally occurring aflatoxins (Hussein and Brasel, 2001) and represents 75% of all aflatoxins found in contaminated foods and feeds (Shlig, 2009).

2.3 Metabolism of Aflatoxin B_1

Metabolism plays a major role in determining the degree of toxicity (Eaton *et al.,* 1994). The liver is considered the principal target organ for aflatoxins (Denli *et al*, 2004).

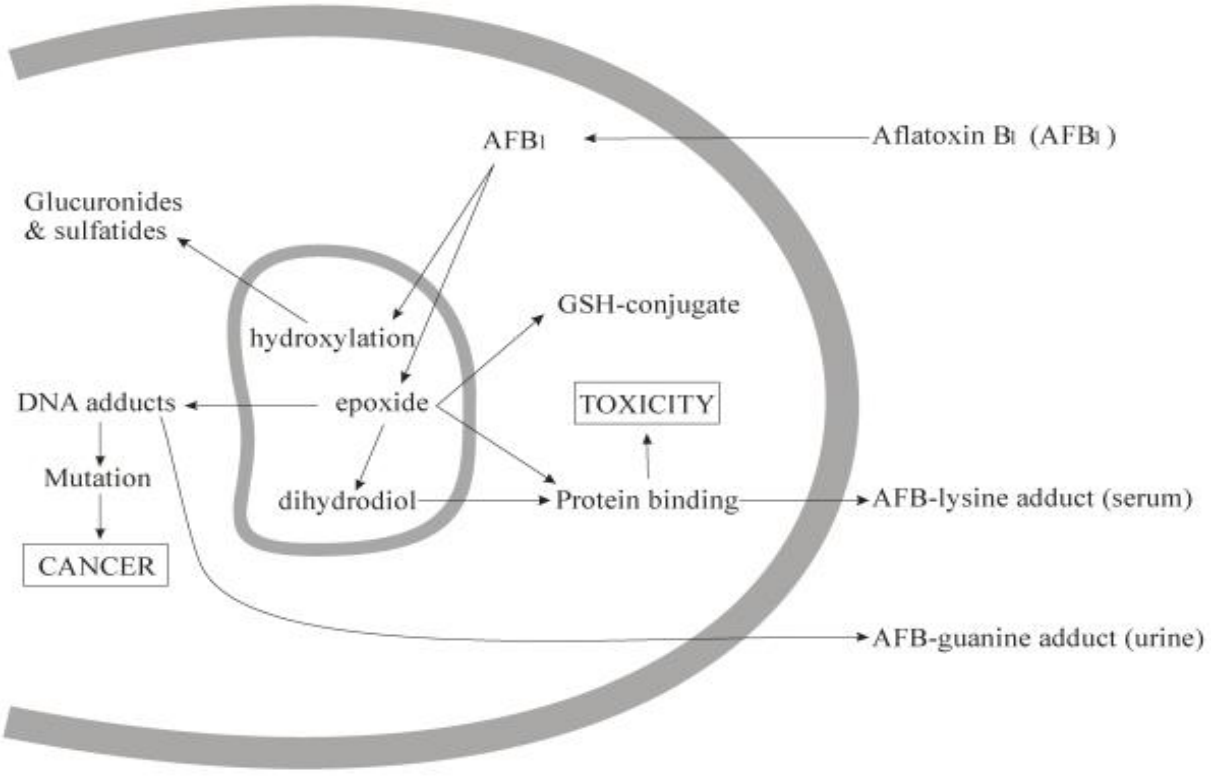

Figure 2: Mechanisms of Aflatoxin B_1 toxicity (Yunus *et al.*, 2013).

According to Yunus *et al.*, (2013), in the endoplasmic reticulum, AFB_1 is converted to hydroxylated metabolites (via monooxygenases) which are then metabolized to glucuronide and sulfate conjugates. An alternate pathway is through oxidation of AFB_1 to form AFB_1-8,9-epoxide which can further undergo hydrolysis to form AFB_1-8,9-dihydrodiol. The epoxide can also be conjugated (to form GSH-conjugate) and thus detoxified by glutathione *S*-transferases. Furthermore, Kellerman *et al*., (1988) reported that AFB_1 is bioactivated in the liver to a highly active and labile intermediate AFB_1 2,3-epoxide, which reacts with various nucleophiles in the cell and binds covalently with DNA, RNA and protein. Toxic effects of AFB_1 include mutagenesis due to alkylation of nuclear DNA, carcinogenesis, teratogenesis, reduced protein synthesis and immunosuppression (Chandra and Sarchielli, 1993; Kubena and McMurray, 1996; Cramer *et al*., 2001; Nicolle *et al*., 2003; Devaraj *et al*., 2008). Reduced protein synthesis results in decreased production of essential metabolic enzymes and structural proteins for growth (Yang *et al*., 2000).

2.4 Susceptibility of Broilers to Aflatoxin B_1

The toxic effects and susceptibility of broilers to AFB_1 mainly depends on its liver detoxification systems, genetic makeup, age, health and nutritional factors (Ramdell and Eaton, 1990). In addition, environmental factors, contamination level and duration of exposure can influence the toxicity (Jewers, 1990). In general, young animals (e.g. broiler chickens) are more susceptible to AFB_1 poisoning than older ones, and males are more susceptible than females (Kellerman *et al*., 1988). Aflatoxicosis in livestock can follow either an acute or chronic course, depending on the dosage level and exposure time to the toxin (Kellerman *et al*., 1988).

2.5 Public Health Hazard of Aflatoxin B_1 and Safety Levels

Aflatoxin B_1 and its metabolites can be transmitted from feed to animal edible tissues and products, such as the liver, milk and eggs (Micco *et al*., 1988; Oliveira *et al*., 2000; Binthivok *et al*., 2002; Rizzi *et al*., 2003). Apart from the danger aflatoxicosis poses to the poultry and livestock industries, aflatoxins are also a serious public health hazard, responsible for acute and chronic liver failure and Reyes syndrome in humans (Kellerman *et al*., 1988). In April 2004, a severe aflatoxicosis outbreak occurred in Kenya resulting in 317 cases and 125 deaths of humans and was due to contamination of improperly stored maize (Azziz-Baumgartner *et al*., 2005 and Probst *et al*., 2007). Aflatoxin B_1 is a Group 1 human liver carcinogen which acts synergistically with hepatitis B virus to increase the risk of liver cancer 12-fold (WHO, 2006). Furthermore, exposure to aflatoxins is causative factor in child stunting and underweight, neurological impairment, immunosuppression and child mortality; and could interact with HIV/AIDS (WHO, 2006).

Due to the transfer of aflatoxins into edible products and its effects on livestock species and humans, many countries have attempted to limit exposure to AFB_1 by imposing regulatory restrictions of commodities intended for use as food and feed. According to the regulations of the South African Department of Health (WHO, 2006), foodstuffs and livestock feed are deemed contaminated if they contain more than 10 µg/kg of total aflatoxin of which should not exceed 5 µg/kg may be AFB_1. Also, the Food and Drug Administration (FDA, 2004) of the United States of America allows aflatoxin level of not be more than 20 µg/kg in animal feed as well as maize, peanuts and other grains meant for human consumption. Similarly, it has been suggested that as a

general rule, feed for growing poultry should not receive greater than 20 µg/kg of aflatoxin in the diet (FAO, 2004; Muzaffer *et al*., 2003 and WHO, 2006).

2.6 Clinical Signs, Lesions and Diagnosis of Aflatoxin B_1 Toxicity

Aflatoxicosis, the poisoning that occurs from ingesting aflatoxins, is characterized in broiler chickens by decreased feed intake and inferior growth rates, poor feed utilization and increased mortality (Tedesco *et al*., 2004; Bailey *et al*., 2006; Shi *et al*., 2006). Similarly, Yunus *et al*., (2013) reported that low levels of AFB_1 in the rations have been implicated in reducing weight gains of broilers in a 3 weeks feeding study. According to Bintvihok and Kositcharoenkul, (2006); Denli *et al*., (2004); and Basmacioglu *et al*., (2005), the productive deterioration in aflatoxicosis is also associated with changes in biochemical and hematological parameters. Exposure of poultry to AFB_1 results in liver and kidney abnormalities, and impaired immunity, which is able to enhance susceptibility to infectious diseases (Shashidhara and Devegowda, 2003).

Prolonged feeding of low concentrations of AFB_1 may result in Steatorrhoea, diffuse liver fibrosis (cirrhosis), and carcinoma of the bile ducts or the liver (Fernandez *et al*., 1995). Steatorrhoea (an increase in faecal lipid content caused by fat malabsorption) is one of the crucial symptoms of aflatoxicosis, caused by decrease in concentration of gall, which leads to an increase in unabsorbed lipid content in the caecum. Thus in the case of chronic aflatoxicosis of poultry, feed conversion is greatly increased (Yunus *et al.,* 2013). Metabolic alterations caused by aflatoxins in chickens lead to elevated lipid levels, disruptions in hepatic protein synthesis, which result in several blood coagulation disorders, immunosuppression and decreased plasma amino acid concentrations (Sumit *et al*., 2010). In chronic exposures, lipid metabolism is

affected due to the reduction in enzyme synthesis and activity (Hussein and Brasel, 2001). However, acute toxicity of AFB_1 results in elevation of total lipid concentration in the liver as well as decrease in total lipids and triacylglycerol concentrations in the plasma (Sachan & Ayub, 1991; Sachan & Ayub, 1992). The toxic effects of aflatoxins in broilers are characterized by decreased total blood protein, albumin, cholesterol, glucose, uric acid, inorganic phosphorus and calcium concentrations; and increase in the liver function indicator enzymes (Amer *et al*., 1998). Aflatoxins reduce levels of proteins in the serum (Sumit *et al*., 2010). Furthermore, the synthesis of albumin and most of the globulins takes place in the liver and thus in chronic hepatic diseases, hypoalbuminaenia occurs (Fernandez *et al*., 1995). Aflatoxin B_1 increases serum levels of AST and ALT as well as decrease serum concentration of total protein and albumin (Oguz *et al*., 2000). Therefore, exposure of broilers to aflatoxins may significantly alter productivity, which could mean the difference between profit and loss to the poultry industry (Hamilton, 1984 and Kubena *et al*., 1998).

2.7 Interactions of Aflatoxin B_1 with Antioxidising Agents and Amino Acid Nutrition

Aflatoxins interact with the anti-oxidising agents including vitamins A, C and E. They have been shown to depress hepatic storage of the vitamin A and increase the dietary requirement for Vitamin D3 (Bird, 1978). Requirements for fat-soluble vitamins (vitamin A, D and E) for chickens are increased during aflatoxicosis, as well as specific amino acid such as methionine and proteins in general (Daghir, 1995). A fortification of poultry rations with synthentic methionine, has been shown to alleviate the growth depression usually seen during aflatoxicosis (NRC, 1984). In a study on rats, Bhattacharya *et al*., (1989) observed that sulphur-containing amino acids i.e. cystein, cystine and methionine were able to inhibit AFB_1 mutagenicity in

microbial systems. They further suggested that the inhibition was due to amino acids affecting the synthesis of AFB_1-epoxide (Bhattacharya *et al*., 1989).

2.8 Potential of Antioxidants in Reducing Aflatoxin B_1 Toxicity

In recent years, there has been increased interest among poultry scientists on the use of antioxidants against the toxic effects of aflatoxins. This is because aflatoxins have been demonstrated to induce the production of reactive oxygen species and oxidative stress has been suggested as one of the underlying mechanisms for AFB_1 induced cell injury and DNA damage (Yang *et al*., 2000). The antioxidant defense system has both enzymatic and nonezymatic components that prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules and could be effective means for preventing changes in liver and kidney functions. In addition, antioxidants function as protective agents against inducing mutagenic effects in genetic materials (Chandra and Sarchielli, 1993; Kubena and McMurray, 1996; Cramer *et al*., 2001; Nicolle *et al*., 2003; Devaraj *et al*., 2008). Vitamin A helps guard against cancer by protecting cells from undesirable oxidation, and scavenging the products of oxidation-free radicals, which are linked to the development of cancer (Jain, 2004). Webster *et al*., (1996) also reported that vitamin A may control carcinogenesis by manipulating molecular events at the initiation stage. In essence, vitamin A is anti-mutagenic, both *in vivo* and *in vitro* to prevent aflatoxin induced liver damage (Denli *et al.*, 2005).

Vitamin A supplementation in rats inhibits AFB_1-DNA binding (Bhattacharya *et al*., 1989). The protective effects of retinoids such as retinol, retinal, retinoic acid and retinal esters on AFB_1 carcinogenicity are due to inhibition of AFB_1-DNA adduct formation by affecting the CYP_{450} systems resulting in less epoxide being formed (Bhattacharya *et al*., 1984; Firozi *et al.*, 1997).

Retinal has the same inhibitory effect on the formation of AFB_1-protein adducts (Bhattacharya *et al*., 1989). Vitamin A has been shown to induce the activity of glutathione S-transferase, thereby enhancing the detoxification of AFB_1-epoxide. On the other hand, vitamin A deficiency decreased glutathione S-transferase activity. In a study employing Woodchuck (a giant North American ground squirrel) hepatocytes to find the role of vitamins A, C and E, and β-carotene on the initiation of AFB_1-induced carcinogenesis, Bhattacharya *et al*., (1989), found that vitamin A was more effective than vitamin C in inhibiting DNA adduct formation. In contrast, vitamin E and β-carotene enhanced the binding (Yu *et al*., 1994). Furthermore, carotenoids are less effective in reducing DNA damage than vitamin A (Denli *et al*., 2005). Lastly, vitamin A significantly prevents aflatoxin induced alterations in the tissue such as liver, kidney and gizzard of chicks (Muzaffer *et al*., 2003).

Available literature has shown that the liver is the principle target of AFB_1 and major storage organ of vitamin A (Denli *et al*, 2004); and that vitamin A is more effective in ameliorating the toxic effects of this mycotoxin than other antioxidants (Bhattacharya *et al*., 1989; Yu *et al*., 1994; Denli *et al*., 2005 and Muzaffer *et al*., 2003), necessitated its use in this study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Study Site and Source of Materials

This study was conducted at the University of Zambia in the School of Agricultural Sciences, Department of Animal Science. The materials used for the study included 150 unsexed day-old broiler chicks weighing on average 46.3 g from a local hatchery, Ross Breeders Zambia Ltd. Feed ingredients for the formulation of experimental diets were purchased from Livestock Services Cooperative Society, Lusaka, Zambia. These included maize meal No.3, Soybean meal, Limestone flour, Di-calcium phosphate, methionine, lysine, broiler premix and salt. Retinol (Vitamin A) acetate and Aflatoxin (aflatoxin B_1) were sourced from HiMedia Laboratories, Mumbai, India.

3.2 METHODS

3.2.1 Experimental Diets

The study started with formulation of standard broiler chickens diets according to nutritional standards set by the National Research Council (NRC, 1994). These included the broiler starter (0-14 days), grower (15-28 days) and finisher diets (29-42 days). Each bird was allocated 622g, 1378g and 1500g of starter, grower and finisher diets, respectively.

The basal diets were contaminated with pure crystalline AFB_1 by first dissolving 1 mg of the mycotoxin in 10 ml of chloroform, which was followed by mixing the resulting solution with 1 kg of each basal diet to make a toxin-premix. The toxin-premix was then left overnight at room temperature to allow the solvent to evaporate. Thereafter, the rest of the basal diets (except for the Control) were mixed with the toxin premix to give a final 35µg/kg concentration of AFB_1 in the diet. This was slightly higher than 20 µg/kg total AFB_1 feed the regulatory limit and maximum level permitted for human food and most animal feeds (FAO, 2004; Muzaffer *et al*., 2003; FDA, 2004; and WHO, 2006).

Contaminated diets were then supplemented with different levels of vitamin A as shown in Table 1. Every assigned level of vitamin A (powder) supplementation per treatment diet shown in Table 1, was separately and sequentially mixed with 20 g (initially with 5 g and finally 15 g), 80 g and 900 g of broiler premix, dicalcium phosphate meal and maize meal, respectively. Lastly, the final premix was added to the dietary treatment. The quantities of broiler premixes as well as the dicalcium phosphate and maize meals that were used to play the role of carriers for supplemented vitamin A were part of the ingredients in the formulated feed diets.

Finally, the contaminated diets were then supplemented with different levels of vitamin A to evaluate its effects on broiler chickens performance when fed diets contaminated with AFB_1. The study was done during the entire broiler chicken growing period from day 1 to 42 days of age.

Table 1: Level of feeding and vitamin A supplementation in the dietary treatments

Treatment	Total Quantity of Feed per Treatment (kg)			Total Vitamin A Supplementation per Treatment (mg)		
	Starter	Grower	Finisher	Starter	Grower	Finisher
A (Control)	18.66	41.34	45	0	0	0
B	18.66	41.34	45	0	0	0
C	18.66	41.34	45	16.794	37.206	40.5
D	18.66	41.34	45	33.588	74.412	81
E	18.66	41.34	45	61.578	136.422	148.5

Before adding different levels of vitamin A, all basal diets contained 9,000 IU (2.7 mg) of vitamin A per kg. Finally, vitamin A was added to the treatment diets as shown in Table 2.

Table 2: Levels of AFB_1 contamination and vitamin A supplementation in the dietary treatments

Treatment	AFB_1 (µg/kg)	Level of Vitamin A Supplementation	
		(mg/kg)	(IU/kg)*
A (Control)	0	0	0
B	35	0	0
C	35	0.9	3,000
D	35	1.8	6,000
E	35	3.3	11,000

***1 IU = 0.0003 mg*

3.2.2 Experimental Design and Bird Management

The study was designed as Completely Randomized Design where birds were allocated to AFB_1 contaminated diets based on the different levels of vitamin A supplementation. The study had five treatments with each treatment being replicated three times. The feed diets (i.e. different levels of vitamin A supplementation and AFB_1 contamination) were the treatments and replications were the blocks. Each replicate consisted of ten (10) chickens with each one serving as an experimental unit on which data was collected.

The birds were housed in a deep-litter system where wire gauge frames were used to separate the floor space into treatment pens whose length, breadth and height were 2m x 1m x 1m, respectively. Each pen consisted of 10 chicks. The birds were allocated to treatment diets at random and water *ad libitum.* The birds were subjected to routine management practices including 23 hours of lighting every day. During brooding, heating in the poultry house was provided through use of 250 watts infra-red bulbs. The birds were also vaccinated by ocular route against Newcastle disease (ND) at 14 and 24 days of age using the freeze-dried live lentogenic ND virus, Lasota strain. While at days 10 and 28 of age, the birds were vaccinated by ocular route against Infectious Bursal Disease (IBD) using the freeze-dried live IBD virus, Virgo 7 intermediate-hot strain. The ND and IBD vaccines were sourced from Biovac Ltd, Or-Akiva 30600, Israel. During the growing period, the chickens were also observed for any unusual behavior and litter conditions in the pens were checked consistently.

3.2.3 Data Collection and Study Parameters

3.2.3.1 Growth Performance

To monitor weekly body weight changes, the birds were weighed just before housing to get initial body weights in each experimental pen. Afterwards, the birds were weighed on a weekly basis, usually the same day of the week in the morning before feeding. Feed intake was monitored daily during the study by weighing the feed left over from the previous day and the amount given on that particular day. Intake of feed was calculated as the difference between the amount of feed given the previous day and the remainder on that particular day. The Feed Conversion Ratio (FCR) among treatments was calculated by dividing weekly feed intake by body weight gains. Mortality was recorded when it occurs so as to associate causes of death with treatment diets. Where possible, the cause of death was to be ascertained by postmortem examinations in order to ensure that deaths were linked with treatment diets. On day 43, the chickens were slaughtered in order to ascertain their dressed weights and dressing out percentages.

3.2.3.2 Liver Functional Enzymes, Lipids and Proteins in the Serum

At the end of the feeding trail, on day 43, 2.0 ml of blood samples were randomly collected by vein puncture (ulnar vein) from 15 birds in each treatment group (i.e. 5 birds from each replicate) for analysis of serum liver functional enzyme concentrations including; Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransaminase (ALT). Protein synthesis was estimated by analyzing for total proteins (TP) and albumin (ALB) and the

metabolism of fatty acids by assessing the profile of lipid metabolites including total triglycerides (TG) and Cholesterol (C) in the blood serum. The blood metabolites were analyzed from serum, which was obtained by centrifuging blood samples in glass tubes (2,500 × *g* for 15 minutes) within one hour after collection. The collected serum was then placed in sterile microtubes and stored in a freezer at -80.0°C until all analyses were completed. Serum biochemical parameters were measured by using the UV-Spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Germany) and commercial kits (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany), following the manufacturer's instructions.

3.2.4 Statistical Analysis

All collected data were compiled and summarized using Microsoft Excel computer software and analyzed by using the GenStat statistical software package (GenStat 14th Edition, 2012). Tukey's test was used for multiple comparisons when a significant interaction was detected. All statements of significance were based on probability ($P \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Productive Performance Parameters

4.1.1.1 Starter Phase (0 – 14 days)

The results on the performance of broiler chickens fed on AFB_1 contaminated diets that were supplemented with different levels of vitamin A during the Starter Phase were as presented in Table 3. The results showed that contamination of broiler starter diets with AFB_1 tends to reduce feed intake in broiler chickens. This was demonstrated by the significant difference ($P \leq 0.05$) in feed intake between the chickens fed on uncontaminated (Treatment A) and contaminated (Treatment B) diets. The decreased feed intake in the other dietary treatments was attributed to susceptibility of young broiler chickens to AFB_1 toxicity. There was also a clear indication that supplementing increasing levels of vitamin A to AFB_1-contaminated diets had a reducing effect on its toxicity as demonstrated by the increasing feed intake levels in chickens exposed to Treatments D and E that were not significantly different to that of Treatment A, the Control diet. The results on bodyweight gains also reflected similar trends to that of feed intake in that the chickens fed the Control diet had significantly ($P \leq 0.05$) higher bodyweight gains than those that were fed on AFB_1 contaminated diet without any vitamin A supplementation. The chickens that were fed on diets supplemented with different levels of vitamin A (except for those in Treatment C) gained more weights than those fed on contaminated diets without vitamin A supplementation. There were no significant differences in bodyweight gains of broilers chickens

whose diets were supplemented with vitamin A at 0 IU (Control/Treatment A) and 11000 IU per kilogram. The bodyweight gains of broilers exposed to AFB_1-contaminated feed diets that were supplemented with 3000 IU, 6000 IU and 11000 IU per kilogram showed no significant differences. The results on feed conversion ratios reflected feed intake and bodyweight gains with the Control having significantly superior ratios than those on unsupplemented diets. However, it was statistically observed that there were no significant differences in feed conversion ratios of broilers in the Control and those whose AFB_1-contaminated feed diets were supplemented with vitamin A at 11000 IU/kg. In addition, broilers whose AFB_1-contaminated diets were supplemented with vitamin A at 0 IU (Treatment B), 3000 IU and 6000 IU per kilogram showed no significant differences in their feed conversion ratios. There were no cases of mortality in this study which may be an indication that the contamination levels used were not acutely toxic which is often associated with necrosis and increased chicken mortality.

Table 3: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Starter Phase (0 – 14 days)

Treatment	**Vitamin A (IU/kg)**	**AFB_1 (µg/kg)**	**Parameter**		
			FI (g)	**BWG (g)**	**FCR**
A (Control)	0	0	622.00^{a}	294.5^{a}	2.11^{c}
B	0	35	604.33^{b}	252.5^{c}	2.39^{a}
C	3,000	35	612.67^{b}	264.4^{bc}	2.32^{ab}
D	6,000	35	622.00^{a}	268.2^{bc}	2.32^{ab}
E	11,000	35	622.00^{a}	282.0^{ab}	2.21^{bc}
Lsd			4.20	10.3	0.08

^{abc}Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

4.1.1.2 Grower Phase (15 – 28 days)

The performance of broiler chickens during the grower phase in terms of feed intake, body weight gains and feed conversion ratios were as tabulated in Table 4. Unlike in the starter phase, there were no significant differences in feed intake among birds fed different treatment diets. This may be an indication of increased tolerance in broiler chickens to feeding on AFB_1-contaminated diet as they advance in age. The results on bodyweight gains of broilers showed significant differences ($P \leq 0.05$) between the birds fed Control diets and those fed diets that were contaminated with AFB_1 and diets supplemented with vitamin A at 11000 IU/kg. No significant differences in the bodyweight gains of broilers were also observed between chickens whose AFB_1-contaminated diets were supplemented with vitamin A at 3000 IU and 6000 IU per kilogram. However, no significant differences in the bodyweights of broilers were observed between those whose AFB_1-contaminated diets were supplemented with vitamin A at 0 IU and 3000 IU per kilogram. The highest bodyweight gains and superior feed conversion ratios were recorded in chickens fed on the Control diet that was not contaminated with AFB_1. The lowest bodyweight gains and worst feed conversion ratios were observed in birds fed on AFB_1-contaminated diet that had no vitamin A supplementation.

Table 4: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Grower Phase (15 – 28 days)

Treatment	Vitamin A (IU/kg)	AFB_1 (µg/kg)	Parameter		
			FI (g)	BWG (g)	FCR
A (Control)	0	0	1378	864.20[a]	1.60[d]
B	0	35	1378	650.87[d]	2.12[a]
C	3,000	35	1378	653.43[cd]	2.11[ab]
D	6,000	35	1378	663.07[c]	2.08[b]
E	11,000	35	1378	837.70[b]	1.65[c]
Lsd			0.0	5.88	0.02

[a-d]Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

4.1.1.3 Finisher Phase (29 – 42 days)

Like in the Starter and Grower Phases, there were no cases of mortality in the Finisher Phase. Furthermore, there were no significant differences among treatment means on feed intake among chickens fed on different treatment diets (Table 5). This again confirms the tolerance of older chickens to feeding on AFB_1-contaminated diets. The results on bodyweight gains and feed conversion ratios of broilers showed no significant differences between birds whose diets were not contaminated with AFB_1 and those fed on AFB_1-contaminated diet that was supplemented with vitamin A at 11000 IU per kg. The bodyweight gains and feed conversion ratios of broilers exposed to AFB_1-contaminated feed diets that were supplemented with 3000 IU and 6000 IU per kilogram and those fed Control (Treatment A) diets showed no significant differences. However, bodyweight gains and feed conversion ratios of broilers whose AFB_1-contaminated feed diets were supplemented with 0 IU (Treatment B), 3000 IU and 6000 IU per kilogram showed no significant differences.

Table 5: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Finisher Phase (29 – 42 days)

Treatment	Vitamin A (IU/kg)	AFB_1 (µg/kg)	Parameter		
			FI (g)	BWG (g)	FCR
A (Control)	0	0	1500	938.0^{ab}	1.60^{bc}
B	0	35	1500	885.2^{c}	1.70^{a}
C	3,000	35	1500	906.8^{bc}	1.65^{ab}
D	6,000	35	1500	916.1^{bc}	1.64^{ab}
E	11,000	35	1500	967.8^{a}	1.55^{c}
Lsd			0.0	20.4	0.04

^{abc}Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

4.1.1.4 Body Weights, Dressed Weights and Dressing Percentages

Results on bodyweights, dressed weights and dressing percentages are shown in Table 6. In the Starter Phase, significant differences ($P \leq 0.05$) were observed in bodyweights of broilers fed Control diets and those exposed to AFB_1-contaminated diets but supplemented with vitamin A at 0 IU, 3000 IU and 6000 IU per kg. However, broilers fed AFB_1-contaminated diets but supplemented with vitamin A at 0 IU, 3000 IU and 6000 IU per kg showed no significant differences in their bodyweights. Bodyweights of broilers whose diets were supplemented with 6000 IU and 11000 IU per kg of vitamin A showed no significant differences. Bodyweights in the Grower Phase showed significant differences ($P \leq 0.05$) between broilers fed Control diets and those exposed to AFB_1-contaminated diets that were supplemented with vitamin A at 11000 IU per kg. However, no significant differences were observed between the broilers which were fed AFB_1-contaminated diets but supplemented with vitamin A at 0 IU and 3000 IU per kilogram. Broilers exposed to AFB_1-contaminated diets that were supplemented with 3000 IU

and 6000 IU per kg of vitamin A also showed significant differences. In the Finisher Phase, bodyweights of broilers were non-significant between the Control diets and those supplemented with vitamin A at 11000 IU per kg. However, their (Control and Treatment E) bodyweights showed significant differences ($P \leq 0.05$) with the broilers in other treatment diets (Treatments B, C and D). Bodyweights were non-significant between chickens whose diets were supplemented with 3000 IU and 6000 IU vitamin A per kilogram. Also no significant differences were observed between the broilers which were fed AFB_1-contaminated diets but supplemented with vitamin A at 0 IU and 3000 IU per kilogram. Results on dressed weights and dressing percentage showed significant differences ($P \leq 0.05$) among all treatments. However, there were no significant differences in dressed weights of birds whose diets were supplemented with 3000 IU and 6000 IU/kg of vitamin A.

The results in all treatment diets were an indication that supplementing increasing levels of vitamin A to AFB_1-contaminated diets at the tested level improved bodyweight gains in broiler chickens. A similar pattern was observed for dressed weights and dressing percentages which increased with increasing levels of vitamin A supplementation to AFB_1-contaminated diets. Thus, despite reduced effects of vitamin A supplementation in AFB_1-contaminated diets in the Finisher Phase, the overall performance of birds fed on AFB_1-contaminated diets demonstrated beneficial effects in supplementing broiler chickens with increasing levels of vitamin A to reduce toxic effects of AFB_1 (Table 6).

Table 6: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on body weights, dressed weights and dressing percentages in broiler chickens

Treatment	Vitamin A (IU/kg)	AFB_1 (µg/kg)	Study Parameters				
			Starter Phase BW (g)	Grower Phase BW (g)	Finisher Phase BW (g)	Dressed Weight (g)	Dressing Percentage (%)
A (Control)	0	0	340.1^{a}	1204.3^{a}	2142.3^{a}	1520.7^{a}	70.99^{a}
B	0	35	299.2^{c}	950.1^{d}	1835.3^{c}	1118.1^{d}	60.92^{e}
C	3,000	35	311.0^{c}	964.4^{cd}	1871.2^{bc}	1194.3^{c}	63.83^{d}
D	6,000	35	313.8^{bc}	976.9^{c}	1892.9^{b}	1229.6^{c}	64.96^{c}
E	11,000	35	329.3^{ab}	1167.0^{b}	2134.8^{a}	1473.0^{b}	69.00^{b}
Lsd			8.5	11.1	27.3	17.4	0.26

$^{a-e}$Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1 and BW = Bodyweight.

4.1.2 Serum Biochemical Parameters

4.1.2.1 Concentrations of Liver Function Enzymes

The results on the concentrations of liver functional enzymes in blood serum including ALP, AST and ALT were as presented in Table 7. Levels of ALP and AST were significantly different ($P \leq 0.05$) in all the chickens fed different treatment diets. They were found to be lower in chickens fed the control diets and highest in those that were exposed to AFB_1-contaminated diets that did not have any vitamin A supplementation. The concentrations of the enzymes, however, reduced with increasing levels of vitamin A supplementation. As for ALT, no significant differences were observed between broilers fed the Control diets and those that were given AFB_1-contaminated diets with highest level of vitamin A supplementation (11000 IU/kg). There were, however, significant differences ($P \leq 0.05$) among treatments for chickens that were fed contaminated diets that decreased with increasing levels of vitamin A supplementation. This

showed that feeding AFB_1 contaminated diets to growing broiler chickens increased concentration of liver functional enzymes that decreased with increasing levels of vitamin A supplementation. The increase in the concentrations of these enzymes was an indication of the negative effects of AFB_1 on liver function. The reduction in blood enzyme concentrations with increasing levels of vitamin A supplementation demonstrates the reducing effect of the vitamin A on AFB_1 toxicity.

Table 7: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on the concentration of liver function enzymes in broiler chickens

Treatment	**Vitamin A (IU/kg)**	**AFB_1 (µg/kg)**	**Parameter**		
			ALP (u/L)	**AST (u/L)**	**ALT (u/L)**
A (Control)	0	0	984.37^{e}	10.00^{e}	1.75^{d}
B	0	35	1668.63^{a}	26.83^{a}	7.00^{a}
C	3,000	35	1459.57^{b}	22.67^{b}	5.25^{b}
D	6,000	35	1337.83^{c}	15.65^{c}	3.50^{c}
E	11,000	35	1053.43^{d}	13.42^{d}	1.75^{d}
Lsd			4.13	1.98	0.00

$^{a-e}$Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, ALT = Alanine aminotransaminase.

4.1.2.2 Concentration of Proteins and Lipids

The results on the blood serum concentration of protein and lipid metabolites were as presented in Table 8 and clearly indicate that the concentrations of TG, C, TP and ALB in blood serum of broiler chickens fed various treatment diets were significantly different ($P \leq 0.05$). It is well-known that ALT and AST belong to a group of enzymes that catalyse interconversion of amino acids and oxoacids by transfer of amino groups while ALP is involved in energy transfer for

exchange of ions across the cell membrane. Thus, the impaired levels of these liver function enzymes caused by the toxic effects of AFB_1 on the liver, greatly affected the synthesis of proteins and lipids. However, it was also observed that increasing levels of vitamin A supplementation in AFB_1-contaminated diets improved productive performance of the broilers which was indicated by improving serum concentration of the proteins and lipids. This was as a result of the antioxidant defense systems of vitamin A which have both the enzymatic and nonezymatic components that prevent free radical formation, remove radicals before damage occurs, repair oxidative damage, eliminate damaged molecules and being effective in preventing changes in the liver by AFB_1.

Table 8: Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on the concentration of lipids and proteins in broiler chickens

Treatment	**Vitamin A (IU/kg)**	**AFB_1 (µg/kg)**	**Parameter**			
			TG (mg/dL)	**C (mg/dL)**	**TP (g/dL)**	**ALB (g/dL)**
A (Control)	0	0	216.00[a]	282.77[a]	6.210[a]	3.813[a]
B	0	35	97.93[e]	121.13[e]	2.833[e]	1.507[e]
C	3000	35	106.07[d]	157.00[d]	3.203[d]	1.723[d]
D	6000	35	122.87[c]	171.90[c]	3.680[c]	2.107[c]
E	11000	35	144.20[b]	182.97[b]	4.537[b]	2.660[b]
Lsd			1.87	2.30	0.150	0.055

[a-e]Means within a column with different superscript letters differ significantly ($P \leq 0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, ALB = Albumin, TP = Total Protein, TG = Triglycerides and C = Cholesterol.

4.2 DISCUSSIONS

Results from this study have shown that AFB_1 contamination at 35µg/kg of basal diets has toxic effects on broilers leading to impaired productive performance and serum biochemistry. However, it was also observed that increased vitamin A supplementation reduced AFB_1 toxicity in broilers fed on diets contaminated with this mycotoxin. This resulted in improved serum concentrations of liver function enzymes, proteins and lipids as well as better productive performance.

Results of from this study indicated that dietary AFB_1 affected the FI, BW, BWG, FCR, dressed weights and dressing percentages of broilers. However, increase in vitamin A supplementation greatly improved the productive performance of broilers. These observations agree well with those of Yunus *et al*., (2013) who reported that AFB_1 decrease weight gain of broilers in their 3 weeks feeding study. Similarly, Tedesco *et al*., (2004); Bailey *et al*., (2006); and Shi *et al*., (2006), found that aflatoxicosis that occurs from ingesting aflatoxins, is characterized in broiler chickens by decreased feed intake and poor growth rates. In this study, no mortalities were observed and this was in agreement with those of Denli *et al*., (2005) who found out that low level exposure of chicks to AFB_1 (< 50 µg/kg of diets) may not lead to their death but merely lower their performance and productivity. The effects of AFB_1 on growth performance have been associated with a decrease in protein and energy utilization (Dalvi and Ademoyero, 1984; Verma *et al*., 2002), probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds.

In this study, results showed that AFB_1 lowers productivity and impairs levels of blood biochemical parameters in broilers thereby altering protein and lipid synthesis. These results agree with Denli *et al*., (2004); Basmacioglu *et al*., (2005); Bintvihok and Kositcharoenkul, (2006) who observed in their studies that productive deterioration is also associated with changes in biochemical and hematological parameters (liver function enzymes, lipids and proteins).

Cholesterol is a major lipid that is a precursor of all the steroid hormones and bile acids as well as a component of the plasma membranes of cells. In this study, decreased serum level of cholesterol was associated with effects of AFB_1 on the liver which synthesize this main lipid. Triglycerides are the main storage form of lipids, and are a major energy source. They are synthesized in the intestinal mucosa and liver from the components of fat digestion and absorption. In this study, the toxic effect of AFB_1 decreased serum levels of triglycerides and this was due to the breakdown of fats and proteins, primarily from the muscle tissue, through gluconeogenesis in the liver. In this research, serum levels of triglycerides and cholesterol were lowest in broilers fed AFB_1-contaminated diets and concentrations improved with increase in vitamin A supplementation. These results agree with findings of Hussein and Brasel (2001) who reported that lipid metabolism is affected by AFB_1 due to the reduction on enzymes synthesis and activity, mainly in chronic exposures. Similarly, other researchers reported that acute toxicity of AFB_1 results in elevation of total lipid concentration in the liver and a decrease of total lipids and triacylglycerol concentrations in the plasma (Sachan & Ayub, 1991; Sachan & Ayub, 1992).

Results on the serum level of lipids in this study disagree with those in a study conducted by Sumit *et al*., (2010). These workers observed that metabolic alterations caused by aflatoxins in

chickens led to elevated serum levels of lipids and disruptions in hepatic protein synthesis. The findings on elevated serum concentration of lipids disagree with those of this study probably because Sumit *et al*., (2010) used naturally occurring aflatoxins at levels >35 µg/kg of dietary feeds. Besides, they made these observations at day 14, 21, 28 and 35. However, in this study, unsexed broiler chickens were exposed to low levels (35µg/kg) of AFB_1 and that the serum lipid concentrations were only measured at day 42 when the birds were more tolerant to its toxic effects. Therefore, it can be suggested that exposure of broiler chickens to low levels (35 µg/kg) of AFB_1 decreases the concentration of gall, which leads to an increase in unabsorbed lipid content and that this results in reduced serum levels of cholesterol and triglycerides.

Most plasma proteins, with the exception of immunoglobulins and protein hormones, are synthesized in the liver and they form the basis of organ and tissue structure, operate as enzymes in biochemical reactions, are regulators and are transport and carrier compounds for most of the constituents of plasma. In this trial, serum levels of TP and ALB were low and this was due to the toxic effect of AFB_1 on the liver (i.e. impaired protein synthesis) particularly in the Starter and Grower Phases. These findings agreed with those of Oguz *et al*., (2000) who indicated that AFB_1 decreases serum concentration of TP and ALB. In addition, Fernandez *et al*., (1995) also reported that the synthesis of albumin and most of the globulins takes place in the liver and in chronic aflatoxicosis, hypoalbuminaenia occurs.

In this study, the increased serum levels of the liver function enzymes (ALP, AST and ALT) were associated with toxic effects of AFB_1 on the liver. This was also observed by Oguz *et al*., (2000) who reported that AFB_1 contamination increases serum levels of ALT and AST. Results on concentrations of serum liver function enzymes, lipids and proteins of broilers fed AFB_1-

contaminated diets were in agreement with observations made by Amer *et al*., 1998. These workers observed that aflatoxin toxicity in broilers is characterized by decreased total blood protein, albumin, cholesterol, glucose, uric acid, inorganic phosphorus and calcium concentrations, as well as by increase in the liver function indicator enzymes. Furthermore, in a research conducted by Sumit *et al*., (2010) they observed that metabolic alterations caused by aflatoxins in chickens led to elevated lipid levels, disruptions in hepatic protein synthesis, which resulted in blood coagulation disorders, immunosuppression and decreased plasma amino acid concentrations. Similarly, Miazzo *et al*., (2005); Bailey *et al*., (2006); and Pasha *et al*., (2007) reported that the liver is considered as the target organ for AFB_1 metabolism because it is where most aflatoxins are bioactivated to the reactive 8,9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures.

Vitamin A is a potent biological antioxidant and is known to help repair damaged tissue and therefore may be beneficial in counteracting free radical damage. In this study, vitamin A supplementation significantly ameliorated the toxic effects of AFB_1 on the broilers which counteracted the serum biochemical changes and resulted in improved productive performance. These results are comparable to those of Muzaffer *et al*., (2003) whose trial on Japanese quails revealed reduction in feed consumption in birds exposed to AFB_1 and the efficacy of vitamin A in reducing its toxicity. Muzaffer *et al*., (2003) concluded that vitamin A is a potent biological antioxidant. Similarly, Denli *et al*., (2004) found out that vitamin A is anti-mutagenic, both *in vivo* and *in vitro* to prevent AFB_1 induced liver damage thus enhancing broiler productivity.

It can be concluded that AFB_1 in the diet at levels of 35µg/kg resulted in impaired productive performance and an alteration of concentrations of liver function enzymes, protein and lipids of

broilers. However, vitamin A supplementation greatly ameliorated the toxic effects of AFB_1 and demonstrated that this fat-soluble vitamin plays a role in the process of chemical aflatoxicosis and when supplemented in the diet can provide protection against the toxic effects of AFB_1 in broilers.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The combined adverse effects of AFB_1 on hepatic metabolism and protein synthesis typically decrease production of structural protein for growth resulting in reduced broiler chickens productive efficiency. Suppression of liver protein synthesis by AFB_1 is a main factor resulting in poor broiler performance. In addition, aflatoxin metabolites negatively affect carbohydrate and lipid metabolism leading to low broiler productivity. It was observed that susceptibility of broilers to the toxic effects of AFB_1 decrease with age and also vitamin A supplementation resulting in improved productive performance. In this study, it was observed that feeding AFB_1-contaminated diets can adversely affect liver function of broiler chickens as characterized by depressed FI (at early stage of growth), BW, BWG, FCR, dressed weights and dressing percentages; reduced levels of serum TG, C, ALB and TP; and increased concentrations of serum ALP, AST and ALT. However, vitamin A supplementation at 6000 IU per kg ameliorated the toxic effects of AFB_1 (35µg/kg) on feed intake. While amelioration of the deleterious effects of aflatoxin B_1 on bodyweight gains and feed conversion ratios of broilers was achieved when vitamin A was supplemented in the diets at beyond 3000 IU per kg.

5.2 RECOMMENDATIONS

It is recommended that further research be done to explore the interaction of vitamin A with AFB_1 at different levels of supplementation and contamination. There is need to investigate the effects of AFB_1 contamination at more than two (2) levels (e.g. 50, 75 and 100 µg/kg) and observe its impact on broiler performance, liver pathology as well as serum concentration of liver-function enzymes, proteins and lipids in the first, second, third and fourth weeks of production. In the same assessment, the efficacy of vitamin A supplementation in ameliorating the toxic effects of AFB_1 at more than two (2) levels can as well be investigated. It would also be interesting to evaluate the effect of other oxidizing agents such as vitamins C and E on suppressing the toxic effects of aflatoxins in broiler chickens.

REFERENCES

Amer, A. M. M., E. M. M. Fahin and R. K. Ibrahim. 1998. "Effect of Aflatoxicosis on Kinetic Behavior of Ceftiofur in Chickens." Research Veterinary Science, 65, 115-118.

Azziz-Baumgartner, E., K. Lindblade, K. Gieseker, H.S. Rogers, S. Kieszak and H. Njapau. 2005. Case–control Study of an Acute Aflatoxicosis Outbreak in Kenya. Environmental Health Perspective, 113, 1779–1783.

Bailey, C.A., G.W. Latimer, A.C. Barr, W.L. Wigle, A.U. Haq, J.E. Balthrop and L.F. Kubena. 2006. "Efficacy of Montmorillonite Clay (NovaSil PLUS) for Protecting Full-term Broilers from Aflatoxicosis." Journal of Applied Poultry Research, 15, 198–206.

Bankole, SA and O.O. Mabekoje. 2004. "Occurence of Aflatoxins and Fumonisins in Preharvested Maize from South-Western Nigeria." Food Additive Contaminants, 21, 251-5.

Basmacioglu, H., H. Oguz, M. Ergul, R. Col and Y.O. Birdane. 2005. "Effect of Dietary Esterified Glucomannan on Performance, Serum Biochemistry and Haematology in Broilers Exposed to Aflatoxin." Czech Journal of Animal Science, 50, 31–39.

Bhattacharya R. K., A. L. Prabhu and V. S. Aboobaker. 1989. "*In vivo* Effect of Dietary Factors on the Molecular Action of Aflatoxin B_1: Role of Vitamin A on the Catalytic Activity of Liver Fractions." Cancer Research, 44, 83-88.

Bintvihok, A. and S. Kositcharoenkul. 2006. "Effect of Dietary Calcium Propionate on Performance, Hepatic Enzyme Activities and Aflatoxin Residues in Broilers Fed a Diet Containing Low Levels of Aflatoxin B_1." Toxicon, 47, 41–46.

Bintvihok, A., S. Thiengnin, L. Doi and S. Kamagai. 2002. "Residues of Aflatoxin in Liver Muscles and Eggs of Domestic Fowls." Journal of Veterinary Science, 64, 1037 – 1039.

Bird, F. H. (1978). "The Effect of Aflatoxin B_1 on the Utilisation of Cholecalciferol by Chicks." Poultry Science, 57, 1293-1296.

Brown, R. L., T. E. Cleveland, Z. Y. Chen, A. Menkir, K. Cardwell and J. King. 2001. "Resistance to Aflatoxin Accumulation in Kennels of Maize Inbreds Selected for Ear-rot Resistance in West and Central Africa." Journal of Food Protection, 64, 396-400.

Chandra, R. K. and P. Sarchielli. 1993. "Nutritional Status and Immune Responses." Clinical Laboratory Medicine, 13, 455-641.

Cramer, P, D. A. D. A. Bushnell and R. D. Kornberg. 2001. "Structural Basis of Transcription: RNA Polymerase II at 2.8 angstrom Resolution." Science Journal, 292 (5523), 1863-76.

Daghir, N.J. 1995. Poultry Production in Hot Climates. CAB International. Wallingford Oxom OX10 8DE, UK.

Dalvi, R. R., and A. A. Ademoyero. 1984. "Toxic Effects of Aflatoxin B_1 in Chickens Given Feed Contaminated With *Aspergillus flavus* and Reduction of the Toxicity by Activated Charcoal and Some Chemical Agents." Avian Diseases, 28, 61–69.

Denli, M., F. Okan and F. Doran. 2004. "Effect of Conjugated Linoleic Acid on the Performance and Serum Variables of Broiler Chickens Intoxicated With Aflatoxin B_1." South African Journal of Animal Science, 34, 97–103.

Denli, M., F. Okan, F. Doran, and T. C. Inal. 2005. "Effect of Dietary Conjugated Linoleic Acid on Carcass Quality, Serum Lipid Variables and Histopathological Changes of Broiler Chickens Infected With Aflatoxin B_1." South African Journal of Animal Science, 35, 109–116.

Devaraj, S., S. Mathur, A. Basu, H. H. Aung, V. T. Vasu, S. Meyers and I. Jialal. 2008. "A Dose-response Study on the Effects of Purified Lycopene Supplementation on Biomarkers of Oxidative Stress." Journal of the American College of Nutrition, 27 (2), 267-273.

Eaton, D.L. and J.D. Groopman. 1994. The Toxicology of Aflatoxins. Academic Press, New York, 383-426. Eaton D.L., Gallagher E.P. 1994. "Mechanisms of Aflatoxin Carcinogenesis." Annual Review in Pharmacology and Toxicology. 34, 135–172.

Fernandez, A., M. T. Verde, J. Gomez, M. Gascon and J. J. Ramos. 1995. "Changes in the Prothrombin time, Haematology and Serum Proteins Experimental Aflatoxicosis in Hens and Broiler Chickens." Resaerch in Veterinary Science, 58, 119-122.

Food and Agricultural Organisation (FAO). 2004. "Worldwide Regulations for Mycotoxins in Food and Feeds in 2003." FAO Food and Nutrition Paper 81, Rome, Italy.

Food and Drug Administration (FDA). 2004. "Action Levels for Aflatoxins in Animal Feeds." Compliance Policy Guide, 683.100, 384-385.

Firozi, P. F., V. S. Aboobaker and R. K. Bhattacharya. 1997. "Action of Curcumin on the Cytochrome P450-system Catalyzing the Activation of Aflatoxin B_1." Chemical Biology Interact, 100, 41-51.

GenStat 14thEdition. 2012. GenStat Procedure Library Release PL22.2. VSN International Ltd.

Hamilton, P. B. 1984: "Determining Safe Levels of Mycotoxins." Journal of Food Protection, 47, 67-73.

Hawkins, L. K., G. L. Windham and W. P. Williams. 2005. “Effect of Different Postharvest Drying Temperatures on Aspergillus Survival and Aflatoxins in Five Maize Hybrids.” Journal of Food Protection, 68, 1521-1524.

Hell, K. K. F. Cardwell, M. Setamou and H. M. Poehling. 2000. “The Influence of Storage Practices on Aflatoxin Contamination in Maize in Four Agroecological Zones of Benin, West Africa.” Journal of Stored Production Research, 36:356-382.

Hussein, H. S., and J. M. Brasel. 2001. “Rewiev: Toxicity, Metabolism and Impact of Mycotoxins on Humans and Animals.” Toxicology, 167, 101–134.

International Agency for Research on Cancer (IARC). 1993. “IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Some Naturally Occurring Substances: Food Items and Constituents, Heteroyclic Aromatic Amines and Mycotoxins.” 56, 362-395.

Jain, J. L. 2004. Fundamentals of Biochemistry. Fifth Revised and Enlarged Edition; S. Chand & Company Ltd, 7361, Ram Nagar, New Delhi-110 055.

Jewers, K. 1990. “Mycotoxins and their effect on Poultry Production.” Options Méditerranéennes, Sér. A/No. 7, 195–202.

Kellerman, T. S., Coetzer, J.A.W. and Naude`,T.W. 1988. Plant Poisonings and Mycotoxicoses of Livestock in Southern Africa. 2nd Edition. Oxiford University Press.

Kubena, K. S. and D. N. McMurray. 1996. "Nutrition and the Immune System – A Review of Nutrient-Nutrient Interractions." Journal of American Dietetic Association, 11, 1156-1164.

Kubena, L.F., R.B. Harvey, R.H. Bailey, S.A. Buckley and G.E. Rottinghaus. 1998. "Effects of Hydrated Sodium Calcium Aluminnosilicate (T-Bind™) on Mycotoxicosis in Young Broiler Chickens." Poultry Science, 77, 1502–1509.

Mannon, J and E. Johnson. 1985. "Fungi down on the Farm." New Scientist, 105, 12-16.

Miazzo, R., M. F. Peralta, C. Magnoli, M. Salvano, S. Ferrero, S. M. Chiacchiera, E. C. Q. Carvalho, C. A. R. Rosa, and A. Dalcero. 2005. "Efficacy of Sodium Bentonite as a Detoxifier of Broiler Feed Contaminated with Aflatoxin and Fumonisin." Poultry Science, 84, 1–8.

Micco, C., M. Miraglia, R. Onori, C. Brera, A. Mantovani, A. Loppolo and D. Stasolla. 1988. "Long-term Administration of Low Doses of Mycotoxins to Poultry. Residues of Aflatoxin B_1 and its Metabolites in Broilers and Laying Hens." Food Additive Contamination, 5, 303–308.

Muzaffer, D., C. Kemal and O. Ferda. 2003. "Effects of Vitamin A Supplementary in the Feed to Reduce Toxic Effects of Aflatoxin B_1 on Japanese Quails (*Coturnix contunix japonica*)." International Journal for Poultry Science, 2 (2), 174-177.

National Research Council (NRC). 1984. Nutrient Requirements of Poultry. 8th Edition. National Academy of Sciences, Washington D.C., USA.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th Edition. National Academy of Sciences, Washington D.C., USA.

Nicolle, C., N. Cardinault, O. Aprikian, J. Busserolles, P. Grolier, E. Rock, C. Demigne, A. Muzur, A. Scalbert, P. Amouroux and C. Remesy. 2003. "Effect of Carrot Intake on Cholesterol Metabolism and on Antioxidant Status in Cholesterol-free Rat." European Journal of Nutrition, 42(5), 254-61.

Oguz, H., T. Kececi, Y. O. Birdane, F. Onder and V. Kurtoglu. 2000. "Effect of Clinoptilolite on Serum Biochemical and Haematological Characters of Broiler Chickens during Aflatoxicosis." Research Veterinary Science, 69, 89-93.

Oliveira, C.A.F., E. Kobashigava, T.A. Reis, L. Mastieri, R. Albuquerque and B. Correa. 2000. "Aflatoxin B_1 Residues in Eggs of Laying Hens Fed a Diet Containing Different Levels of the Mycotoxin." Food Additive Contamination, 17, 459–462.

Pasha, T. N., M. U. Farooq, F. M. Khattak, M. A. Jabbar, and A. D. Khan. 2007. "Effectiveness of Sodium Bentonite and Two Commercial Products as Aflatoxin Absorbents in Diets for Broiler Chickens." Animal Feed Science Technology, 132, 103–110.

Probst, C., H. Njapau and P.J. Cotty. 2007. "Outbreak of an Acute Aflatoxicosis in Kenya in 2004: Identification of the Causal Agent." Application Environmental Microbiology, 73, 2762–2764.

Ramdell, H. S. and D. L. Eaton. 1990. "Species Susceptibility to Aflatoxin B_1 Carcinogenesis." Cancer Research, 50, 615-620.

Rizzi, L., M. Simioli, P. Roncada, and A. Zaghini. 2003. "Aflatoxin B_1 and Clinoptilolite in Feed for Laying Hens: Effects on Egg Quality, Mycotoxin Residues in Livers, and Hepatic Mixed-function Oxygenase Activities." Journal of Food Protection, 66, 860–865.

Sachan, D. S. and M. Y. Ayub. 1991. "Modulation of Aflatoxin B_1 Toxicity by L-carnitine." FASED J 5: A1987.

Sachan, D. S. and M. Y. Ayub. 1992. "Suppression of Aflatoxin B1-induced Lipid Abnormalities and Macromolecule-adduct Formation by L-carnitine." Journal of Environment, Pathology and Toxicology, 11, 205-210.

Shashidhara, R.G. and G. Devegowda. 2003. "Effect of Mannan Oligosaccharide on Broiler Breeder Production Traits and Immunity." Poultry Science, 82, 1319–1325.

Shi, Y.H., Z.R. Xu, J.L. Feng and C.Z. Wang. 2006. "Efficacy of Modified Montmorillonitenano Composite to Reduce the toxicity of Aflatoxin in Broiler Chicks." Animal Feed Science Technology, 129, 138–148.

Shlig, A.A. 2009. "Effects of Vitamin E and Selenium Supplement in Reducing Aflatoxicosis on Performance and Blood Parameters in Broiler Chicks." Iraq Journal of Veterinary Sciences, 23, 97-103.

Sumit, R., E. J. Kim and R. Coulombe. 2010. "Aflatoxin B_1 in Poultry: Toxicology, Metabolism and Prevention." Research in Veterinary Science, 89, 325-331.

Tedesco, D., S. Steidler, S. Galletti, M. Taneni, O. Sonzogni and L. Ravarotto. 2004. "Efficacy of Silymarin-phospholipid Complex in Reducing the Toxicity of Aflatoxin B_1 in Broiler Chicks." Poultry Science, 83, 1839–1843.

Turner, P. C. and A. Sylla. 2005. "Reduction in Exposure to Carcinogenic Aflatoxins by Postharvest Intervention Measures in West Africa: A Community-based Intervention Study." Lancet, 65, 950-956.

Verma, J., B. K. Swain, and T. S. Johri. 2002. "Effect of Various Levels of Aflatoxin and Ochratoxin A and Combinations Thereof on Protein and Energy Utilisation in Broilers." Journal of Science, Food and Agriculture, 82, 1412–1417.

Webster, R.P., Gawde M.D. and R.K. Bhattacharya. 1996. "Effects of Different Vitamin A on Carcinogenic-induced DNA damage and Repair Enzymes in Rats." *In vivo*, 10, 113-118.

Williams, D. M., T. D. Phillips, P. E. Jolly, J. K. Stiles, M. J. Curtis and D. Aggarwal. 2004. "Human Aflatoxicosis in Developing Countries: A Review of Toxicology, Exposure, Potential Health Consequences and Interventions." American Journal of Clinical Nutrition, 80, 1106-1122.

Wogan G.N., 1992. "Aflatoxin Carcinogenesis: Interspecies Potency Differences and Relevance for Human Risk Assesssment." Clinical Biology Research, 374, 23 - 37.

World Bank. 2005. "Food Safety and Agricultural Health Standards: Challenges and Opportunities for Developing Country Exports." World Bank, Washington D.C., USA.

World Health Organisation. 2006. "Impacts of Aflatoxins on Health and Nutrition." Report of an Expert Group Meeting.

Yang, C. F., J. Liu, S. Wasser, H. M. Shen, C. E. Tan and C. N. Ong. 2000. "Inhibition of Ebseien on Aflatoxin B_1-induced Hepatocarcinogenesis in Fischer 344 Rats." Carcinogenesis, 21, 2237-2243.

Yu, M. Y., Y. J. Zhang, W. S. Blaner and R. M. Santella. 1994. "Influence of Aflatoxin B_1 Binding to DNA in Woodchuck Hepatocytes." Cancer Research, 1, 596-604.

Yunus, A.W., E. Razzazi – Fazeli, and J. Bohm. 2013. "Aflatoxin B_1 Affecting Broiler's Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues on Toxins." Basel, 3(6), 566–590.

APPENDICES

APPENDIX 1: ANALYSIS OF MEAN FEED INTAKE (FI) IN THE STARTER PHASE

Analysis of variance

Variate: FI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	760.267	190.067	35.64	<.001
Residual	10	53.333	5.333		
Total	14	813.600			

Tables of means

Variate: FI

Grand mean 616.60

Treatment	A	B	C	D	E
	622.00	604.33	612.67	622.00	622.00

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	1.333

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	1.886

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	4.201

Stratum standard errors and coefficients of variation

Variate: FI

d.f.	s.e.	cv%
10	2.309	0.4

APPENDIX 2: ANALYSIS OF FEED INTAKE (FI) IN THE GROWER PHASE

Analysis of variance

Variate: FI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.	0.		
Residual	10	0.	0.		
Total	14	0.			

Tables of means

Variate: FI

Grand mean 1378.00

Treatment	A	B	C	D	E
	1378.00	1378.00	1378.00	1378.00	1378.00

Standard errors of means

Table	Treatment
rep.	3
d.f.	*
e.s.e.	0.000

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	*
s.e.d.	0.000

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	*
l.s.d.	*

Stratum standard errors and coefficients of variation

Variate: FI

d.f.	s.e.	cv%
10	0.000	0.0

APPENDIX 3: ANALYSIS OF FEED INTAKE (FI) IN THE FINISHER PHASE

Analysis of variance

Variate: FI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.	0.		
Residual	10	0.	0.		
Total	14	0.			

Tables of means

Variate: FI

Grand mean 1500.00

Treatment	A	B	C	D	E
	1500.00	1500.00	1500.00	1500.00	1500.00

Standard errors of means

Table	Treatment
rep.	3
d.f.	*
e.s.e.	0.000

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	*
s.e.d.	0.000

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	*
l.s.d.	*

Stratum standard errors and coefficients of variation

Variate: FI

d.f.	s.e.	cv%
10	0.000	0.0

APPENDIX 4: ANALYSIS OF BODY WEIGHT (BW) IN THE STARTER PHASE

Analysis of variance

Variate: BW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	3097.71	774.43	35.53	<.001
Residual	10	217.99	21.80		
Total	14	3315.70			

Tables of means

Variate: BW

Grand mean 318.7

Treatment	A	B	C	D	E
	340.1	299.2	311.0	313.8	329.3

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	2.70

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	3.81

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	8.49

Stratum standard errors and coefficients of variation

Variate: BW

d.f.	s.e.	cv%
10	4.67	1.5

APPENDIX 5: ANALYSIS OF BODYWEIGHT (BW) IN THE GROWER PHASE

Analysis of variance

Variate: BW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	180346.14	45086.54	1202.26	<.001
Residual	10	375.01	37.50		
Total	14	180721.16			

Tables of means

Variate: BW

Grand mean 1052.5

Treatment	A	B	C	D	E
	1204.3	950.1	964.4	976.9	1167.0

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	3.54

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	5.00

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	11.14

Stratum standard errors and coefficients of variation

Variate: BW

d.f.	s.e.	cv%
10	6.12	0.6

APPENDIX 6: ANALYSIS OF BODYWEIGHT (BW) IN THE FINISHER PHASE

Analysis of variance

Variate: BW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	271591.1	67897.8	300.72	<.001
Residual	10	2257.8	225.8		
Total	14	273848.9			

Tables of means

Variate: BW

Grand mean 1975.3

Treatment	A	B	C	D	E
	2142.3	1835.3	1871.2	1892.9	2134.8

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	8.68

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	12.27

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	27.34

Stratum standard errors and coefficients of variation

Variate: BW

d.f.	s.e.	cv%
10	15.03	0.8

APPENDIX 7: ANALYSIS OF BODYWEIGHT GAIN (BWG) IN THE STARTER PHASE

Analysis of variance

Variate: BWG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	3171.53	792.88	24.92	<.001
Residual	10	318.15	31.81		
Total	14	3489.67			

Tables of means

Variate: BWG

Grand mean 272.3

Treatment	A	B	C	D	E
	294.5	252.5	264.4	268.2	282.0

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	3.26

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	4.61

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	10.26

Stratum standard errors and coefficients of variation

Variate: BWG

d.f.	s.e.	cv%
10	5.64	2.1

APPENDIX 8: ANALYSIS OF BODYWEIGHT GAIN (BWG) IN THE GROWER PHASE

Analysis of variance

Variate: BWG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	138417.90	34604.47	3316.51	<.001
Residual	10	104.34	10.43		
Total	14	138522.24			

Tables of means

Variate: BWG

Grand mean 733.85

Treatment	A	B	C	D	E
	864.20	650.87	653.43	663.07	837.70

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	1.865

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	2.637

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	5.877

Stratum standard errors and coefficients of variation

Variate: BWG

d.f.	s.e.	cv%
10	3.230	0.4

APPENDIX 9: ANALYSIS OF BODYWEIGHT GAIN (BWG) IN THE FINISHER PHASE

Analysis of variance

Variate: BWG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	11900.1	2975.0	23.57	<.001
Residual	10	1262.4	126.2		
Total	14	13162.5			

Tables of means

Variate: BWG

Grand mean 922.8

Treatment	A	B	C	D	E
	938.0	885.2	906.8	916.1	967.8

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	6.49

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	9.17

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	20.44

Stratum standard errors and coefficients of variation

Variate: BWG

d.f.	s.e.	cv%
10	11.24	1.2

APPENDIX 10: ANALYSIS OF FEED CONVERSION RATIO (FCR) IN THE STARTER PHASE

Analysis of variance

Variate: FCR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.146590	0.036647	21.61	<.001
Residual	10	0.016960	0.001696		
Total	14	0.163550			

Tables of means

Variate: FCR

Grand mean 2.270

Treatment	A	B	C	D	E
	2.113	2.394	2.317	2.319	2.207

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.0238

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.0336

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.0749

Stratum standard errors and coefficients of variation

Variate: FCR

d.f.	s.e.	cv%
10	0.0412	1.8

APPENDIX 11: ANALYSIS OF FEED CONVERSION RATIO (FCR) IN THE GROWER PHASE

Analysis of variance

Variate: FCR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.84161179	0.21040295	2549.25	<.001
Residual	10	0.00082535	0.00008254		
Total	14	0.84243714			

Tables of means

Variate: FCR

Grand mean 1.9088

Treatment	A	B	C	D	E
	1.5946	2.1172	2.1089	2.0783	1.6450

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.00525

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.00742

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.01653

Stratum standard errors and coefficients of variation

Variate: FCR

d.f.	s.e.	cv%
10	0.00908	0.5

APPENDIX 12: ANALYSIS OF FEED CONVERSION RATIO (FCR) IN THE FINISHER PHASE

Analysis of variance

Variate: FCR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.0366831	0.0091708	19.49	<.001
Residual	10	0.0047063	0.0004706		
Total	14	0.0413894			

Tables of means

Variate: FCR

Grand mean 1.6272

Treatment	A	B	C	D	E
	1.5992	1.6953	1.6542	1.6374	1.5500

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.01253

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.01771

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.03947

Stratum standard errors and coefficients of variation

Variate: FCR

d.f.	s.e.	cv%
10	0.02169	1.3

APPENDIX 13: ANALYSIS OF MEAN DRESSED WEIGHT (DW)

Analysis of variance

Variate: DW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	382816.70	95704.18	1046.34	<.001
Residual	10	914.65	91.47		
Total	14	383731.36			

Tables of means

Variate: DW

Grand mean 1307.2

Treatment	A	B	C	D	E
	1520.7	1118.1	1194.3	1229.6	1473.0

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	5.52

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	7.81

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	17.40

Stratum standard errors and coefficients of variation

Variate: DW

d.f.	s.e.	cv%
10	9.56	0.7

APPENDIX 14: ANALYSIS OF MEAN DRESSING PERCENT (D%)

Analysis of variance

Variate: D%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	196.35801	49.08950	2403.02	<.001
Residual	10	0.20428	0.02043		
Total	14	196.56229			

Tables of means

Variate: D%

Grand mean 65.938

Treatment	A	B	C	D	E
	70.986	60.921	63.827	64.957	69.001

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.0825

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.1167

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.2600

Stratum standard errors and coefficients of variation

Variate: D%

d.f.	s.e.	cv%
10	0.1429	0.2

APPENDIX 15: ANALYSIS OF MEAN ALKALINE PHOSPHATASE (ALP)

Analysis of variance

Variate: ALP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	969600.000	242400.000	47147.30	<.001
Residual	10	51.413	5.141		
Total	14	969651.413			

Tables of means

Variate: ALP

Grand mean 1300.77

Treatment	A	B	C	D	E
	984.37	1668.63	1459.57	1337.83	1053.43

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	1.309

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	1.851

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	4.125

Stratum standard errors and coefficients of variation

Variate: ALP

d.f.	s.e.	cv%
10	2.267	0.2

APPENDIX 16: ANALYSIS OF MEAN ASPARTATE AMINOTRANSFERASE (AST)

Analysis of variance

Variate: AST

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	569.772	142.443	120.87	<.001
Residual	10	11.785	1.178		
Total	14	581.557			

Tables of means

Variate: AST

Grand mean 17.71

Treatment	A	B	C	D	E
	10.00	26.83	22.67	15.65	13.42

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.627

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.886

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	1.975

Stratum standard errors and coefficients of variation

Variate: AST

d.f.	s.e.	cv%
10	1.086	6.1

APPENDIX 17: ANALYSIS OF MEAN ALANINE AMINOTRANSAMINASE (ALT)

Analysis of variance

Variate: ALT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	62.4750	15.6188		
Residual	10	0.0000	0.0000		
Total	14	62.4750			

Tables of means

Variate: ALT

Grand mean 3.85

Treatment	A	B	C	D	E
	1.75	7.00	5.25	3.50	1.75

Standard errors of means

Table	Treatment
rep.	3
d.f.	*
e.s.e.	0.000

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	*
s.e.d.	0.000

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	*
l.s.d.	*

Stratum standard errors and coefficients of variation

Variate: ALT

d.f.	s.e.	cv%
10	0.000	0.0

APPENDIX 18: ANALYSIS OF MEAN TRIGLYCERIDES (TG)

Analysis of variance

Variate: TG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	26924.437	6731.109	6374.16	<.001
Residual	10	10.560	1.056		
Total	14	26934.997			

Tables of means

Variate: TG

Grand mean 137.41

Treatment	A	B	C	D	E
	216.00	97.93	106.07	122.87	144.20

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.593

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.839

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	1.870

Stratum standard errors and coefficients of variation

Variate: TG

d.f.	s.e.	cv%
10	1.028	0.7

APPENDIX 19: ANALYSIS OF MEAN CHOLESTEROL (C)

Analysis of variance

Variate: C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	43739.897	10934.974	6860.08	<.001
Residual	10	15.940	1.594		
Total	14	43755.837			

Tables of means

Variate: C

Grand mean 183.15

Treatment	A	B	C	D	E
	282.77	121.13	157.00	171.90	182.97

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.729

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	1.031

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	2.297

Stratum standard errors and coefficients of variation

Variate: C

d.f.	s.e.	cv%
10	1.263	0.7

APPENDIX 20: ANALYSIS OF MEAN TOTAL PROTEIN (TP)

Analysis of variance

Variate: TP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	21.682093	5.420523	799.49	<.001
Residual	10	0.067800	0.006780		
Total	14	21.749893			

Tables of means

Variate: TP

Grand mean 4.093

Treatment	A	B	C	D	E
	6.210	2.833	3.203	3.680	4.537

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.0475

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.0672

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.1498

Stratum standard errors and coefficients of variation

Variate: TP

d.f.	s.e.	cv%
10	0.0823	2.0

APPENDIX 21: ANALYSIS OF MEAN ALBUMIN (ALB)

Analysis of variance

Variate: ALB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	10.1009333	2.5252333	2725.07	<.001
Residual	10	0.0092667	0.0009267		
Total	14	10.1102000			

Tables of means

Variate: ALB

Grand mean 2.3500

Treatment	A	B	C	D	E
	3.8133	1.5067	1.7233	2.1067	2.6000

Standard errors of means

Table	Treatment
rep.	3
d.f.	10
e.s.e.	0.01758

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	10
s.e.d.	0.02486

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.05538

Stratum standard errors and coefficients of variation

Variate: ALB

d.f.	s.e.	cv%
10	0.03044	1.3