EFFECT OF CALCIUM AND SODIUM BENTONITE CLAYS FROM THE ALBERTINE GRABEN REGION OF UGANDA ON PERFORMANCE OF BROILER AND LAYER CHICKENS FED AFLATOXIN-CONTAMINATED DIETS

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DECLARATION

I declare that this thesis is my work and all external sources of materials used in its development have been duly acknowledged. I hereby declare that the work presented in this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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DEDICATION

To my Mother Nandawula Ruth who encouraged me to upgrade in this carrier.

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Glory be to the almighty Allah who has given me life, strength, and persistence to move this far and see the results of this study.

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May the Almighty bless you all, Amen

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LIST OF ABBREVIATIONS

AF	Aflatoxins
AOAC	Association of Official Analytical Chemists
AFB_1	Aflatoxin B_1
AFM_1	Aflatoxin M_1
BWG	Body Weight Gain
CaB	Calcium Bentonite
CAST	Council for Agricultural Science and Technology
CWG	Cumulative Weight Gain
CDMI	Cumulative Dry Matter Intake
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
CGMT	Cumulative Geometric Mean Titre
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
FI	Feed Intake
GDP	Gross Domestic Product
GIT	Gastrointestinal Tract
HASCAS	Hydrated Sodium Calcium Aluminosilicates
HDEP	Hen Day Egg Production
HI	Haemagglutination Inhibition
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IBD	Infectious Bursal Disease (Gumboro)
ILRI	International Livestock Research Institute
MUARIK	Makerere University Agricultural Research Institute Kabanyolo
NaB	Sodium Bentonite
NCD	Newcastle Disease
NGMT	Newcastle Disease Geometric Mean titer
NDP	National Development Plan
NPA	National Planning Authority
NRC	National Research Council
PBS	Phosphate Buffer Solution
PDA	Potato-dextrose Agar
PER	Protein Efficiency Ratio
TB	Toxiban

ABSTRACT

Aflatoxins are secondary metabolites of the species belonging to the genus *aspergillus* which are highly hepatocellular carcinogenic and are of great concern in the grain and feed industry. The most promising approach to detoxifying aflatoxin-contaminated feed is the use of high-affinity non-nutritive adsorbents like bentonite clays. This study was therefore conducted to assess the performance of broilers and layers fed aflatoxin-contaminated diets as influenced by the addition of bentonite clays from the Albertine Graben region of Uganda. Two experiments were conducted. In experiment 1, balanced diets for broiler growers and finishers were each laced with aflatoxins to levels of 250 ppb during weeks 3-4 and 5-7, respectively. Three aflatoxin binders including a commercial toxin binder (TB) as a control, and two Albertine bentonite clays (Calcium bentonite (CaB) and sodium bentonite (NaB)) were each separately added at graded levels of 0.0, 0.25, 0.5, 1.0, and 2.0% w/w to the contaminated diets. The treatments were then randomly assigned to a group of birds as experimental units in a completely randomized experimental design replicated 3 times (n = 20 birds per group replicate). The broilers were evaluated during weeks 3-7 after which they were slaughtered for relative organ weights, residual aflatoxins, and blood antibody titer determination. In experiment 2, assessment of layer performance as influenced by the level of binder inclusion to aflatoxin-contaminated diets was conducted during weeks 20-31. An experimental design similar to that of the broilers was used but with (n=15 birds per group replicate). Largely, voluntary dry matter intake increased quadratically ($p<0.05$) with increasing binder inclusion levels except for TB and NaB in the broiler grower phase which showed a linear trend ($p<0.05$). Broiler daily weight gain (BWG) increased quadratically ($p<0.05$) with inclusion levels in grower and finisher. However, increase due to TB and CaB inclusion followed a linear trend ($p<0.05$) in the growers. Cumulative feed conversion ratio (FCR) decreased with increasing binder levels. Consequently, optimum cumulative FCR of 1.862, 1.864, and 1.877 g of feed/g of BWG was obtained at 1.35, 1.70, and 1.5% inclusion levels of TB, CaB, and NaB, respectively. The infectious bursal disease antibody titer increased to optimum values of 2761, 2559, and 2532 corresponding to 1.33, 1.53, and 1.46% inclusion levels of TB, CaB, and NaB, respectively. The antibody titers of Newcastle disease increased to optima of 7.62, 6.89, and 6.91 corresponding to 1.37, 1.48, and 1.48% inclusion levels of TB, CaB, and NaB, respectively. While the decrease in broiler mortality tended towards linearity ($p=0.07$) due to increasing CaB inclusion levels, a quadratic decrease ($p<0.05$) was observed with both TB and NaB. The relative weight of liver decreased quadratically ($p<0.05$) while that of the kidney decreased linearly ($p<0.01$). However, the relative weight of the bursa of Fabricius increased linearly ($p<0.001$) with binder inclusions. Aflatoxin carry-over in the liver tissues generally decreased quadratically ($p<0.05$) with the binder inclusions while that of the kidney tissues decreased linearly ($p<0.05$). Whereas a quadratic increase ($p<0.05$) in hen day egg production was obtained with increasing levels of TB, CaB and NaB inclusion resulted into a linear increase ($p<0.01$). Consequently, FCR decreased for clay binders decreased linearly ($p<0.05$) except for the commercial binder which resulted in a quadratic response ($p<0.05$). As a result, optimum FCR of 3.99, 4.03, and 4.12 were obtained at inclusion levels of 1.30, 1.83, and 1.76% of TB, CaB, and NaB, respectively. Although commercial binder outperformed the Albertine bentonite clays, performance of layers and broilers improves with the addition of bentonite clays due to aflatoxin decontamination. This implies that, optimum performance can be obtained at 1.5 and 1.7% inclusion levels of NaB and CaB, respectively in the aflatoxin-contaminated broiler diets. For the case of layer chickens, however, optimum performance can be obtained at 1.76 and 1.83% inclusion levels of NaB and CaB, respectively.

CHAPTER ONE

1.0 Introduction

1.1 Background

Africa accounts for more than 33% of the world's nutrition insecurity and toxin-related deaths (Wiseman, 2006; Schmidhuber & Tublello, 2007). Like all African developing countries, Uganda still faces challenges of nutrition insecurity in humans and poultry feed toxicity due to utilization of toxin-contaminated agricultural products (Schmidhuber & Tublello, 2007). Since the utilization of such products is associated with chronic human and poultry health defects as well as reduced performance, feed decontamination is considered a key prerequisite for improving commercial poultry productivity (Wu et al., 2011).

In Uganda, commercial poultry production has a substantial contribution to the livelihoods of many people especially the low-income smallholder farmers (UBOS, 2014). This is attributed to the fact that poultry production has relatively lower startup capital as compared to other livestock enterprises (Zimbe, 2012). However, the poultry industry is heavily dependent on commercial feeds which are highly contaminated with mycotoxins beyond the recommended maximum of 20 ppb (Kaaya & Warren, 2005). This implies that, as Uganda seeks to improve the contribution of livestock to the gross domestic product (NDP III, 2020), mycotoxin decontamination will play a principal role.

Defined as secondary toxic fungal metabolites, mycotoxins are the greatest feed safety concerns in tropical regions (Wu et al., 2011). They are also the most potent natural carcinogens linked to severe illnesses and increase in the risk of liver cancer in humans as well as performance decline in commercial poultry (Verma et al., 2003; Wang & Tang, 2004). Nonetheless, several agricultural products used for poultry feed formulation are highly susceptible to mycotoxin contamination under humid conditions (Kamika & Takoy, 2011). As a result, these toxins are more prevalent in tropical areas where humid environmental conditions favor fungal growth and mycotoxin production (D'mello, 2003; Klich, 2007). For that reason, many countries and multilateral agencies have established regulations to protect humans and poultry from consuming such contaminated products (Kamika & Takoy, 2011). On the contrary, Uganda has no regulations on poultry feed safety, leaving farmers with the burden of battling the mycotoxin-associated production constraints.

Among the numerous mycotoxins, aflatoxins (AF) are the most predominant toxins hence largely contributing to the pool of toxins in the poultry feeds (CAST, 2003; Osuret et al., 2016). These toxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* which are the common contaminants in cereal grains, oilseeds, nuts, and animal-based feed formulation ingredients (Banerjee, 2010; Osuret et al., 2016). Since these are the key feed formulation ingredients, the risks of aflatoxin toxicity are high in intensive poultry production systems. However, defined as clinical defects associated with aflatoxin ingestion, aflatoxicosis is associated with increased susceptibility to microbial stress, reduction in immune function, feed utilization, egg-laying percentage, and organ health in poultry (Dersjant-Li et al., 2007; Banerjee, 2010). Since most of the poultry feeds in Uganda are heavily contaminated with aflatoxins (Kaaya & Warren, 2005), a grave threat to commercial poultry production resulting from reduced vaccine efficacy, organ health and feed conversion efficiency is inevitable (Banerjee, 2010; Mugerwa et al., 2013).

On the other hand, there is no practically effective means of decontaminating feeds through destroying aflatoxins without compromising their nutritional quality (Teleb et al., 2004). This implies that, utilization of chemically inert aflatoxin sequestering agents like bentonite clays and activated carbon needs to be prioritized. However, the choice of any binder is greatly dependent on its availability and the economic feasibility of its utilization.

Among the substances investigated as potential aflatoxin-binding agents, bentonites clays and activated charcoal have proved to be the most promising (Diaz et al., 2002). However, the cost-effectiveness of activated charcoal has been so variable (Huwig et al. 2001) while different bentonite clays have yielded consistently promising results (Dersjant-Li et al., 2007). Recognized for forming stable complexes with aflatoxins, bentonites clays have strong affinities for aflatoxin sequestration in the gastrointestinal tract (GIT) (Upadhaya et al., 2010). Such sequestration is achieved through bentonite clay hydration in which the polar water molecules weaken the interaction between the closely packed bentonite layers for aflatoxin molecule intercalation (Deng et al., 2010; McClure, 2014; Kang et al., 2016). Consequently, the resultant complex can be eliminated in the fecal materials hence preventing aflatoxin absorption into the bloodstream (Bintvihok, 2006; Prulovic et al., 2008; Russell et al., 2014). Therefore among the

several aflatoxin decontamination approaches, bentonite clay utilization is envisaged to offer the most reliable responses.

1.2 Statement of the problem

Most of the poultry feeds in Uganda are highly contaminated with aflatoxins (65-1000 ppb) far beyond the acceptable limits of 20 ppb. Due to the dependence on such feeds, commercial poultry in Uganda is highly susceptible to aflatoxicosis, which is reflected in the reduced growth and health performance (Mugerwa et al., 2013; Iqbal et al., 2014). As a result, severe losses due to poor poultry productive performance and health defects are common due to the consumption of aflatoxin-contaminated feeds (Huwig et al., 2001; Phillips et al., 2002).

On the other hand, commercial aflatoxin decontaminants are not readily accessible which culminates into both technological and logistical challenges. Nonetheless, Phillips et al. (2008) and Hesham et al. (2004) studied the utilization of dissimilar bentonites in broilers and recommended contradictory inclusion levels of 0.5% and 1.0%, respectively. In addition, bentonites are very specific in toxin adsorption, yet they also exhibit great diversities in nature (Mangnoli et al., 2011; Ravindra et al., 2017). This implies that, any attempts to improve poultry performance through bentonite clay-based aflatoxin-decontamination yield inconsistent results for different bentonite clay deposits. Such inconsistencies are attributed to the highly variable location-specific physiochemical properties among the bentonites clays hence the need for location-specific optimum inclusion level determination (Ravindra et al., 2017).

Furthermore, comparable studies on other bentonites clays have been conducted, while none has been done particularly on the Albertine bentonite clays. Besides, these studies also do not highlight responses of broiler and layer chickens with respect to aflatoxin carry-over, relative organ health, blood antibody titers alongside egg production indices. In addition, no studies have compared the performance of commercially available aflatoxin binders with the Albertine bentonite clays. Therefore, due to the inconsistent and insufficient information regarding the optimum bentonite clay inclusion levels in poultry feeds coupled with a dearth of information on the Albertine bentonites clays as aflatoxin binders (Mugerwa et al., 2013), a study to assess the effect of adding these clays and commercial binders as aflatoxin decontaminants was necessary.

1.3 Justification of the study

The consumption of aflatoxin-contaminated feeds poses vast aflatoxicosis-related production deficits as well as contamination of poultry products (Verma et al., 2004). The resultant aflatoxicosis, which is associated with compromised feed conversion efficiency, reduced vaccine efficacy, and histological organ deterioration, becomes a key production challenge (Dersjant-Li et al., 2007; Banerjee, 2010). Such challenges not only increase the cost of production but also the risk of losing birds due to highly infectious diseases like Newcastle and infectious bursal disease, which are known to cause up to 100% chicken mortality (Verma et al., 2004; Mugerwa et al., 2013). For that reason, poultry productivity cannot be improved without embracing aflatoxin-decontamination technologies from the feeds.

Whereas research has shown that aflatoxin decontamination improves commercial poultry performance (Verma et al., 2004), the spectrum of decontamination techniques is limited to chemically inert bentonite clay binders and activated carbon. However, the costs involved in the production of activated carbon are prohibitive which results in its scarcity while bentonite clays are readily available as waste in oil extraction (Mugerwa et al., 2013). Fortunately, Uganda is endowed with huge deposits of bentonite clays in the Albertine graben region. Elsewhere, these bentonite clays have been reported to have aflatoxin sequestration abilities but with noticeable variations due to physiochemical diversity (Hesham et al., 2004; Phillips et al., 2008; Ravindra et al., 2018). In addition, there is also a scarcity of literature regarding the optimum inclusion levels of the different Albertine bentonite clays as well as their comparative performance with respect to the commercial binders in poultry feeds. Consequently, despite the availability of such bentonite clays, farmers still make huge aflatoxicosis-induced losses due to poor feed utilization, reduced vaccine potency, and hence high mortalities. Therefore, the use of the Albertine bentonite clays as aflatoxin-binders would be a more sustainable decontamination method of averting aflatoxicosis to offset the heavy poultry production losses and the carcinogenic consequences of consuming contaminated poultry products.

1.4 Significance of the study

Results from this study will contribute to increased commercial poultry productivity due to improved feed conversion efficiency, vaccine efficacy, and reduced mortality. Such improvements shall arise from increased utilization of the local bentonite clays as aflatoxin

decontaminating agents instead of the dependence on the expensive and inaccessible commercial aflatoxin binders. The increased use of Uganda bentonite clays will in addition have a substantial contribution towards feed utilization hence improved productive performance among commercial poultry farmers. Furthermore, the use of bentonites will reduce the risk of consumption of aflatoxin-contaminated poultry products which have been reported to be the major causes of liver cancer and malnutrition-related illnesses in humans. Information on the optimum inclusion level of the respective bentonites in commercial poultry diets was not well established. Therefore, establishing the optimum inclusion level of bentonites in commercial poultry diets will help farmers to make informed decisions on the feed additives to use as aflatoxin binders.

1.5 Overall Objective

The overall objective of this study was to assess the performance of broilers and layers fed aflatoxin-contaminated diets as influenced by the addition of graded levels of calcium and sodium bentonite clays from the Albertine graben region of Uganda.

1.6 Specific objectives

1. To determine the optimum inclusion levels of the Albertine graben bentonite clays (calcium bentonite and sodium bentonite) as aflatoxin binders in commercial poultry diets.
2. To compare the aflatoxin carry-over as a measure of the binding potential of the Albertine bentonites clays with that of the existing commercial binder.
3. To evaluate relative organ weights and aflatoxin carry-over in the liver, kidney, gizzard, and muscles as influenced by addition of graded levels of Albertine graben bentonite clays.

1.7 Hypotheses

1. Feed conversion efficiency in poultry is not affected by addition of calcium and sodium bentonite clays to aflatoxin-contaminated diets.
2. There is no difference in the aflatoxin carry-over reduction due to calcium and sodium bentonites clays inclusion and the commercial aflatoxin binder inclusion.
3. Trends in relative organ weight and tissue aflatoxin carry-over in the liver, kidney, gizzard, and muscles are not influenced by addition of graded levels of calcium and sodium bentonite clays to aflatoxin-contaminated broiler diets.

CHAPTER TWO

Literature review

2.1 Occurrence of mycotoxins in foods

Defined as secondary toxic metabolites of *Aspergillus* mycotoxins can be produced on a variety of products from plant and animal origin containing more than 95% water activity (Milićević et al., 2010; Yunus et al., 2011). This term mycotoxin comes from the Greek word 'mykes' meaning mold, and 'toxicum' meaning poison that causes a clinical condition referred to as mycotoxicosis (Viljoen, 2003; Kamika, 2013). Besides, mycotoxins are also reported to be harmful to both humans and animals when absorbed either through ingestion, inhalation, or dermal absorption (CAST, 2003). However, it was later discovered that ingestion through contaminated foods is the main source of exposure to both humans and livestock (Milićević et al., 2010). This implies that, besides the direct consumption of contaminated products, humans can also get intox icated during livestock feed preparation (CAST, 2003).

Recognized for their toxicity, mycotoxins have been reported to be more serious in tropical humid regions where climatic conditions favor the growth of *Aspergillus species* (Hell, 2000; Kaaya and Warren, 2005). Such humid conditions, poor postharvest handling, and poor storage facilities make poultry feeds highly susceptible to fungal contamination (Hell, 2000). Consequently, most poultry feeds in Uganda are found to be highly contaminated with aflatoxins ranging between 65 and 1000 ppb (Kaaya & Warren, 2005). However, such contamination levels are far beyond the limit of 20 ppb beyond which feeds become unsafe for consumption (Shi et al., 2009).

Among the many mycotoxins discovered, aflatoxins are the most toxic and highly carcinogenic metabolites of *Aspergillus species* (CAST, 2003). Moreover, these toxins which also naturally occur in combination are identified as class one human hepatocellular carcinogens (IARC, 1993). In addition, aflatoxins B_1 can also be metabolized in animal tissues to aflatoxin M_1 from bio-activation pathways and excreted in milk (Alekseyev et al., 2004) hence propagating the effects to infants and higher in the food chain.

2.2 Occurrence of aflatoxins in livestock feeds

Aflatoxins were first isolated and characterized from *Aspergillus species* in poorly stored grains (Klich, 2007). They were later identified to be associated with the killing of young turkeys in England and ducklings in Kenya as well as Uganda (Klich, 2007; Meleki et al., 2013). Therefore, these outbreaks gave way to a multitude of discoveries in determining the different types of aflatoxins alongside the associated clinical signs (Meleki et al., 2013). In addition, these toxins were discovered to be hepatotoxic with their susceptibility being dependent on species, age, nutritional status, and duration of exposure (Richard, 2007).

Currently, aflatoxins are known to be mainly produced by *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* which use livestock feeds as substrates (Do and Choi, 2007). Whereas 20 aflatoxins have been identified, only four (B_1, B_2, G1, and G_2) have been widely studied due to their magnitudes of toxicity (Hussein & Brasel, 2001). Among the four, aflatoxin B_1 is the most common and dangerous to both humans and livestock (Kamika and Takoy, 2011). However, aflatoxins naturally exist in combinations implying that focus has to be put on their synergistic effects rather than the individual effects (Lewis et al., 2005).

Aflatoxins B_1, B_2, G_1, and G_2 are widely studied due to their high prevalence in food products and toxic effects (Wangikar et al., 2005; Yunus et al., 2011). Identified to be metabolites of aflatoxin B_1 and B_2, respectively, aflatoxins M_1 and M_2 occur in both milk and eggs of species fed aflatoxin-contaminated diets (Lewis et al., 2005). Besides the active aflatoxins, significant quantities of conjugated aflatoxins have also been reported to exist in feeds as well (Berthiller et al., 2005). Such conjugated aflatoxins can cause unexpected toxicity when they are hydrolyzed to the precursor toxins in the gastro intestinal tract (GIT) (Berthiller et al., 2005). Furthermore, as part of plant metabolism, some aflatoxins can be transformed into conjugated forms presenting additional forms of toxicity (Freire et al., 2018). For instance, zearalenone-4-beta-d-glucopyranoside, zearalenone and deoxynivalenol glucosides which are precursors can be metabolized to the respective active toxins in the GIT (Gareis et al., 1990; Schneweis et al., 2002). Unfortunately, these precursors cannot be detected during routine analysis yet they can hydrolyze during digestion contributing to the toxin pool in the GIT (Gareiset et al., 1990).

2.3 Perseverance of aflatoxicosis

More than ten thousand million metric tons of grains are lost annually due to aflatoxin contamination (Bhat et al., 2010). In addition, more than four billion individuals in developing countries get chronically exposed to aflatoxicosis every year due to contaminated food consumption (Williams et al., 2004). Such chronic exposure is largely attributed to heavy dependence on subsistence farming, unregulated local markets, and the lack of policy regulations on food and feed safety (Shepherd, 2008). Consequently, the large aflatoxicosis outbreaks like those in Kenya and Togo claimed many lives (Lewis et al. 2005) while survivors suffered from jaundice, leg edema, and hepatomegaly as well as malnutrition-related symptoms (Gong et al., 2002; Muture, 2005). In fact, these outbreaks are traced to be originating from homegrown maize by subsistence farmers who have poor storage facilities (Lewis et al., 2005). Typically, in this crisis 55% of maize products had contamination levels greater than 20 ppb, 35% had levels exceeding 100 ppb, and 7% beyond 1000 ppb (Lewis et al., 2005). Similarly, related cases were reported in India and China (Brera et al. 2008) which cases were all established after detecting the clinical signs of acute aflatoxicosis (Williams et al., 2004).

2.4 Effects of aflatoxins on human health

Recognized as a risk factor for cancer (CAST, 2003), aflatoxicosis is characterized by adverse immunological and nutritional effects (Williams et al., 2004). Among the aflatoxins, AFB_1 and mixtures of AFG_1, and AFM_1 have been reported to be the major causes of chronic aflatoxicosis and human carcinogens (IARC, 1993). Besides the carcinogenic effects, ingestion of foods contaminated with relatively lower aflatoxin levels over a long period has been reported to cause jaundice, liver cancer, chronic hepatitis (Azziz et al. 2005), infertility, and impaired nutrient utilization in infants (Walker et al., 2013).

On the other hand, the ingestion of highly contaminated feeds results in acute hepatic failure, hemorrhages, digestion alterations, mental changes, and coma (Williams et al., 2004). Besides direct chronic aflatoxicosis, aflatoxins have also been reported to complement hepatitis B in the causation of liver cancer (Turner & Sylla, 2005). Ultimately, the lack of policy regulations in developing countries of Southeast Asia and sub-Saharan Africa accounts for the high incidences of aflatoxicosis (Lewis et al., 2005). Such incidences, therefore, account for the fact that hepatocellular carcinoma is the fifth leading cause of cancer mortality in the world (Faromby,

2006). Therefore, this fact justifies the correlation between the high levels of aflatoxins contamination in maize with a high incidence of human hepatocellular carcinomas.

2.5 Biochemical properties and aflatoxin metabolism

Given suitable conditions of 80-85% relative humidity and temperature of 28-35°C up to 20 types of aflatoxins can be produced on a variety of feed substrates. However, among these naturally occurring toxins, aflatoxin B_1, B_2, G_1, and G_2 have been reported to be the most potent with aflatoxin B_1 (AFB_1) posing the highest degree of carcinogenicity (Romoser et al., 2013). Aflatoxins are characterized by high melting points of 260°C and a thermal degradation temperature of 269°C (Maye, 1999). This implies that aflatoxins exhibit high thermostability although they can be decomposed by strong oxidizing agents.

Aflatoxin metabolism plays the biggest role in determining the degree of toxicity in animal cells especially in the liver (Romoser et al., 2013). The liver is considered the principal target organ for aflatoxins basing on the first pass effect (Denli et al., 2004). Since AFB_1 is the most biochemically reactive, most research is focused on its metabolic pathways which justify its carcinogenic properties (Romoser et al., 2013). When aflatoxins are absorbed by the liver, they undergo biotransformation where AFB_1 is metabolized through activation by cytochrome P_{450} enzymes to form the intermediate adduct AFB_1-8, 9-epoxide. Characterized by its low stability, this adduct is responsible for the covalent modification of DNA, preferentially at the N_7 position of guanine bases there by affecting the complimentary base pairing mechanisms (Yunus et al., 2013). Besides DNA modification, AFB_1-8, 9-epoxide undergoes base-catalyzed reactions to form a more stable AFB1-8, 9-dihydrodiol which is reported to be the most mutagenic metabolite (Alekseyev et al., 2004). In addition, Kellerman et al. (1988) reported that AFB_1 can also be bio-activated to a highly active and labile intermediate AFB1 2, 3-epoxide, which reacts with various nucleophiles in the cell besides binding with DNA, RNA, and proteins. Furthermore, according to Yunus et al. (2013), AFB_1 can also be converted to hydroxylated metabolites (via mono-oxygenases) which are then metabolized to glucuronide and sulfate conjugate in the endoplasmic reticulum. Consequently, aflatoxicosis results in mutagenesis, carcinogenesis, and teratogenesis due to the alkylation of nuclear DNA by highly electrophilic adducts. In addition, chronic aflatoxicosis culminates into reduced protein synthesis, production of essential metabolic enzymes and structural proteins for growth (Yang et al., 2000), and immunosuppression hence poor poultry performance (Devaraj et al., 2008).

2.6 Aflatoxin prevalence in poultry feeds

Aflatoxins are produced on a wide range of food commodities especially cereals and oil-seeds which are the key ingredients in livestock feed formulation (He, 2013). Identified as the third most consumed cereal worldwide (Weismann, 2006), maize is most likely to be contaminated with aflatoxins at higher levels than any other feed ingredients (Atehnkeng et al., 2016). Consequently, most of the maize-based poultry feeds in Uganda are highly contaminated with aflatoxins beyond recommended levels of 20 ppb (Kaaya & Warren, 2005).

The key predisposing factor to poultry feed contamination is poor post-harvest handling maize which leads to high contamination levels by *Aspergillus Flavus* (Bhat et al., 2010). Such practices lead to high levels and prevalence of aflatoxin contamination in maize (Lillehoj, 2009). In addition, poultry feeds are stored at a relative humidity between 80% - 90% which is within the optimum range for fungal development (Lillehoj, 2009). Such conditions render the stored feeds more susceptible to fungal attack within the normal feed storage time (Hell, 2000). Whereas fungal contamination depends on postharvest handling, fungal development and aflatoxin production are dependent on storage time (Lillehoj, 2009). This implies that, as traders tend to hold maize back waiting for better market prices after harvest, high aflatoxin contamination is inevitable. Consequently, in an attempt to mitigate the aflatoxicosis-induced production declines, the Food and Drug Administration legislations set up an action level of 20 ppb as the maximum residue limit allowed in poultry feeds (Otsuki et al., 2001).

2.7 Effects of aflatoxicosis on poultry performance

2.7.1 Effets of aflatoxins on chicken Physiology

Due to the heavy aflatoxin contamination of feed ingredients, commercial poultry is highly susceptible to chronic and acute aflatoxicosis (Yildiz et al., 2004; Richard, 2007; Osuret et al., 2016). Whereas acute is associated with the consumption of highly contaminated feeds (Muture, 2005), prolonged consumption of feeds with relatively low aflatoxin contamination levels is associated with chronic aflatoxicosis (Tedesco et al., 2004). As a consequence, chronic aflatoxicosis is associated with poor feed utilization and increased susceptibility to microbial stress which is reflected in performance declines (Shi et al., 2009; Bhat et al., 2010). Furthermore, chronic aflatoxicosis has been reported to be the primary cause of teratogenicity, carcinogenicity, and mutagenicity as well as growth inhibitory effects in poultry (Oguz et al.,

2000; Celik & Sur, 2012). These defects, therefore, result in biochemical, hematological, and immunological alterations in the chronically affected poultry (Ortatatli & Oguz, 2001; Basmacioglu et al., 2005).

2.7.2 Effects of aflatoxicosis on feed intake, energy and amino acid utilization

While feed intake and utilization are key determinants of poultry performance, aflatoxicosis has been reported to reduce feed acceptability, utilization, and enzyme efficiency (Verma et al., 2003; Van Rensburg et al., 2006). In addition, aflatoxins interfere with calcium uptake and organic nutrient utilization in poultry (Hamdi et al., 2015) while excess calcium impedes phytase enzyme activity due to insoluble complex formation with phytate phosphorous (Angel et al., 2002). Furthermore, chronic aflatoxicosis decreases amino acid and energy utilization due to declines in cell metabolic efficiency (Li et al., 2002). Since the interaction between sulfur-containing amino acids and synthesis of aflatoxin B_1-epoxide (Bhattacharya et al., 1989) deter protein utilization and hence weight gain, it's worth investing in available local options for sustainable aflatoxin decontamination for improved feed utilization.

2.7.3 Effects of aflatoxicosis on humoral immunity and organ health

Besides hindering nutrient utilization, aflatoxicosis results in a reduction in antibody titers of Newcastle and Infectious Bursal disease as well as regression of the bursa of Fabricius (Oguz et al., 2003). This implies that, through regression of the bursa of Fabricius, aflatoxins interact with the T-cells to affect cell-mediated immunity in poultry to suppress vaccine efficacy (Oguz et al., 2003). In addition, a reversal in chemo-static inhibition of phagocytic abilities of leucocytes as well as heterophils has been reported to be associated with aflatoxicosis (Radhika, 2006). Therefore, the synergistic effect of both antibody synthesis and phagocytotic suppression contributes to the increased response to environmental stress in poultry.

Relative organ weight is a key indicator of organ health, whereas chronic aflatoxicosis has been reported to increase relative liver and kidney weights it results in the regression of the Bursa of Fabricius (Girish et al., 2006; Lehman et al., 2008; Kumar et al., 2009). In fact, the liver and kidney are considered to be the target organs for aflatoxicosis in all the affected species (Ortatatli et al., 2001). As a consequence, the accumulation of nitrogenous wastes and aflatoxin metabolites results in histopathological changes as well as inflammatory thickening of the uterine mucosa in the tissues (Girish et al., 2006). This implies that the reduced efficiency in nitrogenous

waster excretion which results from liver and kidney damage greatly accounts for aflatoxicosis-related stress in poultry.

2.7.4 Effect of aflatoxicosis on layer production and eggshell characteristics

Poultry layers are bred to convert feed nutrients into egg production rather than body muscles. Aflatoxicosis has been reported to reduce egg production without noticeable variations in layer weight gains (Osweiler et al., 2010; Cheng et al., 2014). Aflatoxicosis in layers has been reported to cause cytoplasmic vacuolation of the hepatocytes through impaired lipid transport and synthesis (Tung et al., 1972; Kumal, 2009). Such impairment is accompanied by lipid deposition in the liver, and suppressed lipid levels in the blood circulation (Tung et al., 1972). This implies that aflatoxicosis induces phospholipid and cholesterol inhibition in the liver which results in far-reaching effects on layer performance (Kumal, 2009).

Defined as the fastest bio-ceramic calcifying process among biological systems (Hincke et al., 2012), eggshell strength is a key aspect in egg storage and transportation. However, aflatoxicosis has been reported to affect eggshell characteristics by interfering with the absorption and transportation of calcium and phosphorous (Huff et al., 1975). In addition, aflatoxins have been reported to interfere with vitamin D_3 metabolism (Verma et al., 2003; Chowdhury & Smith 2004) hence affecting calcium immobilization for eggshell development. Furthermore, chronic aflatoxicosis results in hepatic damage and a reduction in hepatic zinc levels in poultry (Goel et al., 2005). Yet, the enzyme-dependent conversion of bone calcium into egg calcium is grossly affected by the generation and composition of zinc bicarbonate in the blood circulation (Joose, 1983). Therefore, in addition to aflatoxin decontamination, calcium supplementation through calcium bentonite clay inclusion may not only sequester aflatoxins but also possibly improve dietary calcium availability in layers.

2.8 Aflatoxin decontamination approaches

Whereas preventing feed contamination offers the best option it is practically impossible to get reasonable quantities of aflatoxin-free feeds. This implies that prevention of fungal contamination during harvesting and storage (Bintvihok & Kositcharoekul, 2006) as well as fungal growth inhibition offer more practical solutions (Huwing et al., 2001). On the contrary, most of the poultry farmers in Uganda don't produce their feed ingredients hence the lack of

such phytosanitary adherence. This, therefore, leaves feed fortification and decontamination as the most practically viable option to control aflatoxicosis.

Fortification of poultry rations with synthetic methionine alleviates aflatoxicosis-induced growth depression (NRC, 1994). This is attributed to the ability of sulfur-containing amino acids to inhibit AFB_1 mutagenicity and AFB_1-epoxide synthesis during biotransformation (Bhattacharya et al., 1989). Nevertheless, sulfur amino acids are some of the essential and limiting amino acids in poultry diets rendering this approach less cost-effective. Therefore, aflatoxin adsorption renders one of the most effective approaches since it reduces their bioavailability in the liver (Huwig et al., 2001; Phillips et al., 2008). Consequently, since there is a relatively broader spectrum of nonnutritive aflatoxin sequestering feed additives decontamination remains an economically viable option.

Besides amino acid fortification, biological approaches on the other hand can be used to absorb mycotoxins through enzymatic degradation and modification (Morteza et al., 2013). As a result, these characteristics led to the creation of bio-filters for fluid decontamination and probiotics which also bind and remove aflatoxins (Morteza et al., 2013). However, such biological decontamination works mainly through sorption and enzymatic degradation making it a limited approach for biological systems (Yan-ni, 2008). Besides, biological control approaches developed for decontamination of aflatoxins in feeds have equally had limited attention and a multitude of criticisms as well (Shetty, 2006).

Similarly, chemical decontamination using acids, salts, oxidizing, and reducing agents which require drying and cleaning have equally had setbacks (Huwing et al., 2001). These setbacks are associated with the health decline of the animals and many chemical residues in animal systems (Kolossova et al., 2009). However, Huwing et al. (2001) suggested that chemicals like calcium propionate successfully inhibited mold proliferation without affecting livestock health but with significant cost implications. For that reason, toxin decontamination using inorganic and chemically inert binders like some bentonites renders the most practical and economic approach.

2.9 Aflatoxin decontamination using binders

The most cost-effective approach to aflatoxin decontamination known is deactivation using aflatoxin binders like activated carbon and bentonite clays (Kolossova et al., 2009; Murugesan et al., 2015). However, the chemical structure of both the aflatoxin and the binder determines the effectiveness of adsorption and binder-aflatoxin compatibility (Russell et al., 2014). The important characteristics of the adsorbent to be taken into consideration, therefore, including its physical structure, charge, pore size, and surface area (Kolossova et al. 2009) in addition to the inert effect on other livestock physiological processes. Consequently, given such requirements activated carbon and hydrated sodium calcium aluminosilicates (bentonites) stand out to be the most suitable candidates for aflatoxin decontamination.

Identified as a general toxin adsorptive material with a high surface to mass ratio, activated charcoal has been reported to show great potential for aflatoxin decontamination in biological systems (Whitlow, 2006). However, the effects of activated charcoal on livestock performance have been variable (Huwig et al., 2001) while responses in poultry resulted in lower economic viability than clay-based binders (Wu & Khlangwiset, 2010). Such challenges consequently rule out the possibility of using activated carbon as long as bentonite clays can technically substitute them.

Aflatoxin decontamination by clay binders like bentonites on the other hand is associated with the hygroscopicity, polarity, and solubility which favor aflatoxin intercalation with the bentonites in water (Deng et al., 2010). This implies that, as the bentonite clay gets hydrated in the gastrointestinal tract, the polar water molecules weaken the interaction between the closely packed bentonite layers by lowering the charge density of the sequestered exchangeable cations such as Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} (Kang et al., 2016). Therefore, the mechanism of aflatoxin absorption is primarily through the exchangeable cations which neutralize the interlayer charges in aluminosilicates and are involved in the binding mechanism of aflatoxin B_1 (Phillips et al., 2008; Deng et al., 2010) and Hydrogen bonding (Dasheng et al., 2005; Tenorio et al., 2008). Since hydrated sodium calcium aluminosilicates have a high affinity for aflatoxin specifically, this character results in the formation of strong aflatoxin-clay complexes hence adsorption (Neef et al., 2013). However, the properties of bentonites vary from one geological deposit to another

hence the need to test the efficiency of each batch of bentonites clays as aflatoxin detoxifiers (Magnoli et al., 2008).

2.10. Practical methods of aflatoxin decontamination

The practicability of any aflatoxin decontamination approach depends on its effectiveness and the costs associated with its application. However, the application of physical, biological, and chemical methods of decontamination separately or in combination provides varying degrees of effectiveness (Pluyer et al., 1987). While physical means are less practical, with biological methods the degrading microorganisms have limited degradation mechanisms and stability in the GIT at different pH levels (Upadhaya et al., 2010). This consequently leaves chemically inert bentonite clays as the most suitable adsorbents for aflatoxin sequestration.

Defined as a smectite clay mineral generated from the crystallization of volcanic ash, bentonite clays adopt a sheet-like structure where double layers of tetrahedral sandwich an octahedral layer to make up individual sheets. They adopt negative charges which are derived from the replacement of Al^{3+} with divalent Ca^{2+} or Mg^{2+} ions. Then the sheets are interconnected by the exchangeable Na^{+} and Ca^{2+} whose predominance differentiates calcium bentonite from sodium bentonite (Ravindra et al., 2017). Due to its absorptive and colloidal effects, bentonite is used as a filler in pharmaceuticals and oil purification as well as additives in livestock feeds. However, their utilization in livestock feeds is limited by the fact that their physiochemical properties which are its aflatoxin sequestration signature are highly variable hence the need for localized performance evaluations.

Characterized as hydrated sodium calcium aluminosilicates, bentonites effectively bind aflatoxins in the GIT hence preventing their absorption into the bloodstream (Prvulovic et al., 2008). Moreover, Hesham et al. (2004) and Shi et al. (2009) reported an improvement in body weight gain and feed conversion ratio of broilers fed on diets fortified with bentonites. Consequently, this effectiveness of bentonites is attributed to the expanding clay lattice, interstitial water, exchangeable cations hence the aflatoxin binding ability (Kang et al., 2016). In addition, bentonites have been also reported to be efficient against a broad spectrum of mycotoxins making them more suitable for the most frequent cases of multi-contaminated feeds (Oguz et al., 2000). Using bentonite clays hence targets binding the aflatoxins and prevent them

from being absorbed from the animal's digestive tract, and then excrete them through the fecal matter (Bintvihok, 2002; Deng et al., 2010)

Characterized by the ability to form stable complexes with aflatoxins at temperatures between 25 °C and 37 °C, bentonites clays are capable of adsorbing aflatoxins under the condition in the GIT (Magnoli et al., 2008). As a result, certain bentonite at 0.5% inclusion levels was able to reduce aflatoxin bioavailability in broilers (Phillips et al., 2008; Hesham et al., 2004). However, bentonite clays from several sources have been reported to improve pellet stability (Huwing et al., 2001) and poultry performance through aflatoxin decontamination but with varying degrees of effectiveness (Magnoli et al., 2008). This implies that the different bentonite deposits in the Albertine Graben region of Uganda can provide a potential solution to feed AF decontamination (Mugerwa et al., 2013). However, the variation in properties among bentonites warrants assessing the performance.

2.11 The knowledge gap

The high affinity for aflatoxins by the bentonites justifies their utilization given the high levels of aflatoxin contamination in the poultry feeds (Kaaya and Warren 2005). However, the aflatoxin decontamination potential among bentonite clays is highly dependent on the geographical location and nature of the bentonite clay parent materials (Magnoli et al., 2011). Consequently, there is inconsistent and insufficient information about the inclusion levels of the different bentonite clay in poultry diets (Mugerwa et al., 2013). In addition, none of the previous studies has compared commercial aflatoxin binders with other bentonite clays as well as establishing the optimum inclusion levels of Albertine bentonite clays. Yet, beyond certain optima, bentonite clays are hypothesized to hinder nutrient utilization alongside the negative effects of excess calcium for the case of calcium bentonite. Furthermore, due to the transfer of aflatoxins and their metabolites to poultry edible products and their effect on human health, efforts have been made to set contamination limits of 20 ppb. Therefore, studies towards the exploitation of this resource are necessary since Uganda is endowed with huge bentonite deposits in the Albertine region (Mugerwa et al., 2013).

CHAPTER THREE

STUDY 1

Effect of calcium and sodium bentonite clays from the Albertine graben region of Uganda as aflatoxin binders on broiler chickens fed contaminated diets

ABSTRACT

This study was conducted to determine the optimum inclusion levels of Albertine bentonite clays, their effects on relative organ weights as well as aflatoxin carry-over in the liver, kidney, gizzard, and muscles of broilers fed aflatoxin-contaminated diets. Balanced diets for broiler growers and finishers were each laced with aflatoxins to levels of 250 ppb during weeks 3-4 and 5-7, respectively. Three aflatoxin binders including a commercial toxin binder (TB), and two Albertine bentonite clays (Calcium bentonite (CaB) and sodium bentonite (NaB) were each separately added at graded levels of 0.0, 0.25, 0.5, 1.0, and 2.0% w/w to the contaminated diets. The birds were then subjected to the treatments in a completely randomized experimental design replicated 3 times (n = 20 birds per replicate group) for 5 weeks, after which the broilers were slaughtered for tissue evaluation at the end of week 7. Largely, voluntary dry matter intake increased quadratically ($p<0.05$) with increasing binder inclusion levels except for TB and NaB in the broiler grower phase which increased linearly ($p<0.05$). Daily weight gain (BWG) increased quadratically ($p<0.05$) with inclusion levels in both phases except for TB and CaB which followed a linear trend ($p<0.05$) in the growers. Cumulative feed conversion ratio (FCR) decreased with increasing binder inclusion levels. Consequently, optimum FCR was recorded at 1.862, 1.864, and 1.877 g of feed/g of BWG at 1.35, 1.70, and 1.5% inclusion levels of TB, CaB, and NaB, respectively. The infectious bursal disease antibody titer increased to optimum values of 2761, 2559, and 2532 corresponding to 1.33, 1.53, and 1.46% inclusion levels of TB, CaB, and NaB respectively. The antibody titers of Newcastle disease increased to optimum titer values of 7.62, 6.89, and 6.91 corresponding to 1.37, 1.48, and 1.48% inclusion levels of TB, CaB, and NaB, respectively. While the decrease in broiler mortality tended towards linearity ($p=0.07$) due to increasing CaB inclusion levels, a quadratic decrease ($p<0.05$) was observed with both TB and NaB. The relative liver and kidney weights decreased quadratically ($p<0.05$) and linearly ($p<0.01$), respectively. However, that of the bursa of Fabricius increased linearly ($p<0.001$) with binder inclusion. Aflatoxin carry-over in the liver tissues generally decreased quadratically ($p<0.05$) with binder inclusion while that of the kidney tissues decreased linearly ($p<0.05$). Although the commercial binder outperformed the Albertine bentonites, relative organ health and tissue aflatoxin carry-over in broilers improves with the addition of the bentonite clays due to aflatoxin decontamination. This implies that these bentonite clays can be included at 1.5 and 1.7% inclusion levels of NaB and CaB, respectively in the broiler diets for optimum performance.

3.1 Introduction

Most of the poultry feeds in Uganda are heavily contaminated with aflatoxins beyond acceptable levels of 20 ppb (Kaaya & Warren, 2005). Due to heavy dependence on such contaminated feeds, broiler production is greatly challenged by aflatoxicosis. Moreover, aflatoxicosis is associated with low feed utilization efficiency, reduced vaccine efficacy, increased microbial stress, and mortality in poultry (Girish & Devegowda 2006; Magnoli et al., 2010). Furthermore, aflatoxins have been reported to have effects on metabolism by decreasing activities of several enzymes that catalyze the hydrolysis of starch, proteins, lipids, and nucleic acids (Mahmood et al., 2017). Therefore, it is postulated that the associated reduced feed utilization and vaccine efficacy can be mitigated through aflatoxin decontamination of broiler feeds (Sadeghi et al., 2012).

Recognized for their high aflatoxin binding affinity, bentonites have the potential to decontaminate broiler feeds through forming bentonite-aflatoxin complexes which can be eliminated in the fecal material (Deng et al., 2010). It was therefore hypothesized that aflatoxin binder type and inclusion levels could affect feed intake, body weight gain, feed utilization efficiency, and protein utilization by broiler birds. While Dale & Wyatt (1995) have suggested bentonite inclusion rates of 0.3% - 0·5% for poultry feeds, others have reported inclusion rates of 0.4% - 0.6 % in broiler diets to be protective against aflatoxicosis through toxin binding (Prvulovic et al., 2008; Mugerwa et al., 2013).

However, Watts et al. (1986) found that calcium-based bentonite clays have different hygroscopicity characteristics from those of sodium-based bentonite clays. In addition, different bentonites have been reported to have different naturally adsorbed ions, which determine their adsorption characteristics (Watts et al., 1986; Bailey et al. 2006). This implies that the different bentonite deposits have different properties and aflatoxin decontamination potentials (Mugerwa et al., 2013). This variation in properties thus warrants specifying the aflatoxin binding ability of the different types of bentonites in the Albertine region of Uganda. Consequently, the objective of this study was to assess the effects of fortifying poultry feeds with different types and levels of bentonite on broiler productivity and efficacy of Newcastle and Infectious Bursal Disease vaccines.

3.2 Materials and methods

3.2.1 Study site

This study was conducted between March and May 2016 at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), which is located 20 km North of Kampala but within the fertile lake Victoria crescent (0° 28′ N, 32° 27′ E, altitude 1204 m a.s.l). The area is characterized by a tropical climate with average maximum temperatures of 28.5°C and average minimum temperatures of 14.5°C.

3.2.2 Feed ingredients and aflatoxin binders

Before experimentation, feed ingredients used in the formulation of experimental diets were sourced from local input stockiest. The ingredients used included broken maize meal, maize bran, soybean meal, fishmeal, lake shells, di-calcium phosphate, methionine, lysine, broiler premix, and table salt (NaCl). Bentonite clay, a waste produced during drilling operations by oil prospective companies in the Albertine Graben region of Uganda was used. The experimental clay was sourced from Knights Mining Company and was characterized as calcium and sodium bentonite, respectively, by a laboratory in the Department of Geological Survey and Mines in the Ministry of Energy and Mineral Development. A commercial aflatoxin binder branded Toxiban from Novus International outlet in Uganda was used in the experimentation. A protocol to produce aflatoxins by fermentation of rice sterilized by autoclaving and inoculation with *Aspergilus flavus* before incubation at 28°C in the school of food technology, nutrition, and bioengineering at Makerere University was used.

3.2.3 Aflatoxin production protocol

Aflatoxins were produced by incubation of rice inoculated with *Aspergillus* spores. The spores were produced by removing the testa and hulling the groundnut grains followed by soaking in water for 30 minutes. The resultant moist ground nuts were then incubated at 28°C for seven days to produce mixed strains of green spores. After seven days, a Potato-Dextrose-Agar (PDA) was prepared by mixing potato extract, agar, and dextrose.

The potato extract was prepared by slicing 250 g of potato into 3.0 mm slices and cooked for 30 minutes in an open vessel. The potato extract was then collected by filtering the resultant medium through a muslin cloth before 20 g of dextrose was added. Simultaneously, the molten

agar was prepared by boiling 20 g of agar in 500 ml of distilled water for 30 minutes in a separate glass beaker. Finally, the PDA was prepared by mixing the resultant molten agar with the potato dextrose mixture and the volume made up to 1.0 liter using distilled water. The pH of the resultant PDA medium was then adjusted to 7.0 and autoclaved at 121°C before plating in the lamina flow hood. After the PDA had cooled to room temperature, *Aspergillus* spores were identified and carefully picked from the incubated ground nuts under a light microscope at low and high magnification (4×, 10×, 20× and 40×) using a sterile spatula and inoculated onto the PDA slant plates. The resultant inoculated PDA slant plates were then incubated at 28°C for 10 days to produce the spore mass which was later used to inoculate a rice medium.

After 10 days, a rice medium was prepared by soaking rice at a rate of 1 g of rice for 0.5 g of distilled water for 120 minutes, drained off excess water autoclaved at 121°C for 30 minutes. After cooling to room temperature, the rice medium was inoculated with the *Aspergillus* spores produced from the PDA medium. The spores on the PDA medium were scraped loose with a sterile loop, sterile water added before the slants were shaken to give uniform spore suspensions. Finally, 0.5 ml of the spore suspension was used to inoculate 50 g of the autoclaved rice medium before incubation for another 10 days to produce pure cultures of green conidia of *Aspergillus*.

After ten days, the cultures which had a heavy crop of green conidia were scraped loose with a sterile loop and agitated with sterile distilled water to give a uniform suspension of green spores. The resultant spore suspension (0.5 ml) was then used to inoculate the autoclaved rice medium in each Erlenmeyer flasks before incubation.

After inoculation, the subsequent incubations were carried out in 2,000 ml Erlenmeyer flasks containing 400 g of inoculated rice at 28°C for 21 days. At the end of the incubation (21 days), the flasks were briefly steamed to destroy the fungus and the resultant pale yellow medium was dried at 105°C. The oven-dry medium was then standardized using Vicam Aflatest procedure (950 ppb) before it was used to contaminate the respective experimental diets.

3.2.4 Aflatoxin determination using fluorometry

Total aflatoxins in the feed samples were determined using the Association of Official Analytical Chemists method. The maximum weight of 1.0 kg samples of each feed sample was collected from each feedlot immediately after mixing the ingredients. The moisture content of each sample on a dry basis was determined immediately using a moisture analyzer.

A ground feed sample aliquot of 25 g was weighed out together with 5 g of sodium chloride and placed in a blender jar. 125 ml of methanol: water in the ratio of 70:30 was added to the jar and the sample was blended at a high speed for 2 minutes. The blended sample was filtered through a filter paper into a clean beaker. After filtering, 15.0 ml of the resultant filtrate was diluted with 30 ml distilled water. The diluted extract was then filtered through a micro-fiber filter into to an affinity Afla-Test column to collect 15 ml of the filtered extract in a syringe. The extract was then passed through an affinity column at the rate of 2 drops per second with the aid of a pressure pump. Then, 10 ml of distilled water was passed through the Afla-Test affinity column at the rate of about 2 drops per second until air came through the column. The affinity column was eluted by passing 2 ml of HPLC grade methanol through the column at the rate of 2 drops per second while collecting the entire sample elute in a glass cuvette. Afla-Test developer was added to the eluant, thereafter it was mixed well and the aflatoxin concentration (ppb) was determined in the sample after 60 seconds by using a calibrated fluorometer.

3.2.5 Experimental diets and design

Diets were formulated to meet the nutritional requirements of broilers in their different physiological status as starters for feeding broilers between 1-14 days (3190.4 kcal/kg ME, 23% CP), growers for feeding broilers between 15-28 days (3188.2 kcal/kg ME, 20% CP), and finisher for feeding broilers between 29-49 days (3187.3 kcal/kg ME, 18% CP) according to the National Research Council (NRC, 1994). All the chicks (1-14 days) were subjected to a uniform broiler starter diet for the first two weeks. Thereafter, the broiler growers' and finisher diets for feeding the experimental birds in weeks 3-4 and 5-7, respectively were formulated and analyzed for aflatoxin contamination. The standardized diets were then laced with aflatoxins to levels of 250 ppb using the aflatoxins from the incubated rice medium. For each of the contaminated diets, each of the 3 aflatoxin binders including a commercial toxin binder (TB), Calcium bentonite (CaB), and sodium bentonite (NaB) were separately added at graded levels of 0.0, 0.25, 0.5, 1.0, and 2.0% w/w. A total of 900 birds were then allocated to experimental units (n = 20 per replicate group), at 5 levels for each of the 3 binders replicated 3 times. Finally, each of the diets for the respective stages of broiler growth was randomly assigned to experimental groups in a completely randomized experimental design.

2.6 Management of the experimental birds

A total of 950-day old chicks (Cobb-500) weighing 46.5±1.71 g which had been sourced from Uga-chick Poultry Breeders were delivered and introduced to the brooding facility at MUARIK. After brooding for two weeks, 900 out of the 950 chicks were sexed, weighed, and randomly allocated to each of the dietary treatments. Birds in each replicate were then housed in a deep litter system where plywood was used to separate the floor space. The experimental unit comprised of 20 broilers in a pen whose length, breadth, and height were 2m × 2m × 2.5m, respectively. The experimental birds were subjected to routine management practices including a 24-hour lighting Programme using fluorescent tubes (Phillips 240V/36W). The experimental birds were also provided with drinking water *ad-libitum* at a rate of 20 birds per drinker, while the feeds were provided in (100 cm × 15 cm ×15 cm) linear feeders that provided a feeding space of 5 cm per bird. The experimental birds were also vaccinated by the ocular route against Newcastle disease on day 21 using the freeze-dried live lentogenic Newcastle disease virus, Lasota strain. On day 14, the experimental birds were also vaccinated against Infectious Bursal Disease (IBD) through the ocular route using freeze-dried live IBD virus, Virgo 7 intermediate-hot strain as recommended by Uga-chic Poultry Breeders. The litter was constantly raked and its conditions consistently monitored to ensure friability, while the broilers were observed for any unusual behavior.

3.2.7 Determination of antibody titers

The Haemagglutination Inhibition (HI) test was used to determine the antibody titers against Newcastle disease which was carried following the procedure described by Kapczynski & King (2005). The procedure involved the preparation of an antigen by inoculating embryonated eggs with a sample of Newcastle Disease virus and harvesting the allantoic fluid four days later. The standard amount of Newcastle Disease virus used in the HI test was 4HA units. A test suspension of Newcastle disease virus-containing 4HA units in order was prepared by titrating the stored suspension of virus that was used as the antigen in the HI test, this was followed by calculating the HA titer. The dilution factor required to produce 4 HA units was calculated by dividing the HA titer by 4. The original suspension of antigen was diluted in phosphate-buffered solution (PBS) to produce an adequate volume of 4HA antigen to carry out the HI test using the dilution factor. 25µl of PBS was dispensed into each well of the microwell plate. Serum samples were

aken and 25 µl dispensed into the first well and the last (control) well of a row of a micro-well ate.

multi-channel pipette was used to carry out two-fold serial dilutions along the row until the cond last well from the end, the last well was the serum control and was not diluted. This was llowed by adding 25µl of 4HI Ag in each of the wells except column 12. Samples were then haken in an orbital shaker for 30 minutes after which 50µl of 0.5% chicken red blood cells was dded to each of the wells and mixed using an orbital shaker. The plate was then left to stand for -5 minutes at room temperature while covered. Settling patterns for each serum sample were ead followed by the control serum well and lastly, the rest of the wells and the pattern observed n each well was recorded on a micro-well plate recording sheet and the endpoint was letermined.

To determine the infectious bursal disease antibody titers, serum was collected at the end of weeks three, four, five, six, and seven. The antibody titers against the infectious bursal disease were determined using the ELISA technique commercial test kits (Maryland 20879, USA). The plates were read at 405 on the ELISA reader (IDEXX Lab., CA, USA).

3.2.8 Blood sample collection

Two birds (male: female 1:1) were randomly selected from each replicate every week throughout the experimental period for a blood sample collection from day 21 to 49 for estimation of antibody tires against NCD and IBD. Blood samples (3ml) were collected from the wing vein of selected birds using sterile syringes and needles. The serum was separated from respective clotted blood samples by centrifugation at 3000 rpm for 10 minutes. After centrifugation, the blood was decanted into serum vials and frozen at -20°C until Haemagglutination Inhibition (HI) test was performed at the College of Veterinary Medicine and Biosecurity. The HI titers were determined in all sampled broilers, and the geometric mean titers of each group were calculated. The birds selected on a given day were used for the collection of blood samples for that date and were not returned to the experiment in any case. Sample collection was done before vaccination on a given day.

3.2.9 Tissue residual aflatoxin

At the end of week seven, two birds per replicate in each treatment were randomly selected and slaughtered. Weights of liver, kidney gizzard, and bursa of Fabricius were recorded and adjusted to 1.0 kg live weight to obtain the relative organ weights using the following formula: $(Organ\ weight \div Body\ weight) \times 1000$. To estimate residual aflatoxins, samples of organ tissues and muscles from the 2 birds per replicate were stored at -20°C till the analysis was done. During the analysis, aflatoxins in the tissues were extracted using immune affinity columns and estimated by HPLC fluorescent detector method. During aflatoxin extraction, 25 g of defrosted tissue samples were homogenized and blended with 5.0 g of NaCl in 100 ml of water: methanol (80:20) mixture for 3 minutes. The resultant suspension was then filtered and an aliquot of 10 ml of the filtrate was diluted with phosphate-buffered saline solution (PBS) and passed through an immune-affinity column. Aflatoxins were eluted with 1.0 ml methanol in a glass vial and dried under a nitrogen stream. Then pre-column derivation was performed with tri-fluroacetic acid and 20µl of the extract was injected into the HPLC system with a recovery rate of 89 % and 0.025µg / ml limit of detection.

3.2.10 Data collection on broiler performance

To monitor weekly body weight gains, birds were weighed just before allocating them to each of the experimental pens using a digital weighing scale (RS232, WT-X series, 0.1g accuracy. Max. Range 30 kg). Afterward, birds were weighed weekly, usually on the same day of the week in the morning before feeding. Daily feed intake for the group replicates was estimated by deducting the dry weight of feed refusals from the total dry weight of feed offered to each of the treatment groups each day. Daily feed intake per bird per day was then calculated by dividing the total dry matter weight by the number of birds per group replicate. Feed conversion ratio (FCR) among treatments was calculated by dividing weekly feed intake by body weight gains. Protein efficiency ratio (PER) was calculated as weight gain divided by protein intake. Mortality of birds was recorded as it occurred.

3.2.11 Data analysis

Polynomial contrasts were used to test for linear and quadratic effects of the inclusion of graded levels of each aflatoxin binder. Study parameters were considered significantly different at $P <$

0.05. Second-order polynomial regressions were also fitted to cumulative feed intake, body weight gains, feed conversion ratios, and antibody titers data to establish treatment responses. Furthermore, regressions equations were differentiated to determine the point of inflection on each response curve.

3.3 Results

3.3.1 Feed intake

Effect of inclusion levels of the commercial binder (TB) and Albertine bentonite clay ((calcium bentonite (CaB) and sodium bentonite (NaB)) on voluntary dry matter intake (DMI), weight gain, feed conversion ratio, protein efficiency ratio, and antibody titers of Newcastle and infectious bursal disease in broilers are presented in Table 3.1. During the grower phase, DMI linearly increased ($p<0.05$) with increasing commercial binder (TB) and sodium bentonite (NaB) inclusion levels. For the case of calcium bentonite (CaB) however, DMI only tended ($p=0.061$) to follow a quadratic trend. In the finisher phase, the increase in DMI followed a quadratic trend ($p<0.05$) for both TB and CaB while an increase at a decreasing rate ($p<0.05$) was obtained as a result of NaB inclusion. As a consequence, the highest DMI of feeds fortified with the Albertine bentonites clays were observed at 2.0% and 1.0% inclusion levels of NaB and CaB, respectively, while that of the commercial binder was observed at 1.0%. However, using the regression equations of the response curves in Fig.3.1, cumulative DMI increased with aflatoxin-binder inclusion at decreasing rate to optimum values of 4.64, 4.89, and 4.52 kg/bird for TB, CaB and NaB, respectively. The optimum cumulative DMI levels were obtained at percentage binder inclusions of 1.32, 1.43, and 1.75% levels of TB, CaB and NaB, respectively.

3.3.2 Daily weight gain

In the grower phase, the commercial binder (TB) and CaB inclusion resulted in a linear increase ($p<0.05$) in daily weight (g/day) whereas that of NaB increased at a decreasing rate ($p<0.05$). As a result, highest body weight gain (56.4 g/day) of the TB was observed at 2.0% inclusion level while for CaB and NaB highest gain of 51.9 g/day and 47.9 g/day, respectively were both observed at a 1.0% inclusion level. In addition, using the regression equations of the response curves (Fig. 3.1), body weight gain increased at a decreasing rate ($p<0.05$) with increasing binder level to optimum values of 53.3, 49.0, and 49.4g/bird/day for TB, CaB and NaB, respectively.

The optimum weight gains were obtained at percentage binder inclusion levels of 1.39, 1.61, and 1.32% levels of TB, CaB, and NaB, respectively.

In the finisher phase, for both the commercial binder (TB) and calcium bentonite (CaB), body weight gain of broilers increased quadratically ($p<0.05$) while that of NaB increased at a decreasing rate ($p<0.05$). Consequently, the highest body weight gain due to TB (72.7 g/bird/day) and CaB (71.0 g/bird/day) inclusion was obtained at 2.0% while that of NaB (66.9 g/bird/day) was observed at 1.0%. Furthermore, using regression equations of the response curves (Fig. 3.1), BWG was observed to increase at decreasing rates with optimum values of 73.2, 71.5, and 67.7 g/bird/day for TB, CaB and NaB, respectively. The optimum weight gains were obtained at percentage binder inclusion levels of 1.56, 1.55, and 1.43 % levels of TB, CaB, and NaB, respectively.

3.3.3 Feed conversion ratio

During the grower phase (Table 3.1), the feed conversion ratio (FCR) decreased at a decreasing rate ($p<0.05$) with increasing CaB and NaB inclusion whereas there was no significant ($p>0.05$) response due to TB inclusion. As a result, the FCR decreased to optimum values of 1.86 and 1.73 g of feed/ g BWG due to inclusion of NaB and CaB at respective inclusion levels of 1.0 and 2.0%. For the case of the commercial binder, however, least feed conversion ratio response (1.6 g of feed/g of BWG) was observed at 0.25% inclusion level. Precisely, using the regression equations of the response curves (Fig. 3.1), FCR generally decreased with increasing binder inclusion to optimum values of 1.62, 1.75, and 1.77g of feed/ g BWG for TB, CaB and NaB, respectively. Specifically, the optimum FCR were obtained at percentage binder inclusion levels of 1.5, 1.37, and 1.45% levels of TB, CaB, and NaB, respectively. Whereas the FCR response observed due to NaB inclusion was more than those of TB and CaB, the FCR responses due to TB and CaB inclusion were similar ($p>0.05$) at 1.0 and 2.0% inclusion levels (Fig. 3.2).

During the finisher phase (Table 3.1), the feed conversion ratio (FCR) decreased at a decreasing rate ($p<0.05$) with commercial binder (TB) inclusion while that of calcium bentonite (CaB) followed a linear trend ($p<0.05$). However, there was no observable trend ($p>0.05$) response in FCR as a consequence of NaB inclusion. Consequently, for TB and NaB the lowest FCR obtained at 2.0% inclusion levels were similar but lower than that of CaB (Fig. 3.2). Precisely, using the regression equations of the response curves (Fig. 3.1), FCR decreased with increasing

binder levels to optima of 1.62 and 1.27g of feed/g BWG for CaB and TB, respectively. The optimum feed conversion ratios were obtained at percentage binder inclusion levels of 1.93 and 2.03% levels of CaB and TB, respectively.

3.3.4 Protein efficiency ratio

During the grower phase (Table. 3.1), the addition of the sodium bentonite (NaB) resulted in a linear increase ($p<0.05$) in protein efficiency ratio (PER) with increasing binder inclusion while that of calcium bentonite (CaB) increased at a decreasing rate ($p<0.05$). As a result, maximum PER responses were observed at 2.0% inclusion levels of both CaB and NaB. During the finisher phase, PER increased linearly ($p<0.05$) with inclusion levels of CaB. Highest PER values were observed at 1.0% inclusion levels of CaB.

3.3.5. Newcastle and infectious bursal disease antibody titers

During the grower phase (Table. 3.1), Newcastle disease (NCD) antibody titers increased linearly ($p<0.05$) with the increase in commercial binder (TB) and calcium bentonite (CaB) inclusion levels while that of the sodium bentonite (NaB) increased at a decreasing rate ($p<0.05$). However, the highest NCD antibody titers were observed at 1.0% inclusion of TB and CaB while that of NaB was observed at 2.0%. Whereas there were no significant observable trends ($p>0.05$) in infectious bursal disease (IBD) antibody titer due TB and CaB inclusion, NaB inclusion resulted in a linear increase ($p<0.05$) in IBD antibody titers. However, the highest IBD antibody titers were observed at 2.0% inclusion levels of all the binders.

In the finisher phase, NCD antibody titers increased at decreasing rates ($p<0.05$) with increasing inclusion levels of TB and CaB while that of NaB resulted in a linear increase ($p<0.05$). However, both the commercial binder and Albertine bentonites inclusion resulted in highest NCD anti body titers at 1.0% inclusion levels. Using the regression equations of the response curves (Fig. 3.3), cumulative NCD antibody titers increased with increasing binder level of inclusion to optimum values of 5.62, 4.89, and 4.91 for TB, CaB and NaB, respectively. The optimum antibody titer responses correspond to 1.37, 1.48, and 1.48% inclusion levels of TB, CaB, and NaB respectively.

Whereas there was no observable trend in IBD titer response ($p>0.05$) due to NaB inclusion, the antibody titters increased at a decreasing rate ($p<0.05$) due to TB and CaB inclusion. The highest

IBD antibody titers were obtained at 2.0% inclusion levels of both CaB and TB. The cumulative IBD antibody titers increased to optimum values of 2161, 2159, and 2132 for TB, CaB and NaB, respectively. The optimum values correspond to 1.33, 1.53, and 1.46% inclusion levels of TB, CaB, and NaB, respectively (Fig. 3.3).

Table 3.1: Effect of aflatoxin binder inclusion level on growth, feed utilization, and antibody titers of broilers in the grower and finisher phases

	Grower phase								Finisher phase							
	Binder inclusion levels						Polynomial contrasts		Binder inclusion levels						Polynomial contrasts	
	0.00	0.25	0.50	1.00	2.00	SEM	Linear	Quadratic	0.00	0.25	0.50	1.00	2.00	SEM	Linear	Quadratic
Commercial binder (TB)																
DMI (g/day)	84.8	89.3	92.9	95.4	98.8	3.509	0.018	0.42	149.6	161.6	164.0	164.5	161.5	1.891	0.097	<0.001
BWG (g/day)	44.6	50.3	54.8	54.7	56.4	1.810	0.017	0.137	60.8	66.3	70.9	71.4	72.7	0.880	0.053	<0.001
FCR (g of feed /g of BWG)	2.15	1.64	1.71	1.75	1.72	0.170	0.229	0.106	2.47	2.43	2.30	2.29	2.21	0.040	<0.001	0.029
PER	2.58	2.23	2.76	2.68	2.57	0.189	0.557	0.349	2.37	2.29	2.34	2.37	2.39	0.050	0.163	0.632
IBD	407.4	409.4	419.0	415.3	424.1	5.360	0.238	0.867	2055	2080	2099	2124	2147	26.83	0.002	<0.001
NCD	3.48	2.91	3.65	3.89	3.87	0.016	0.036	0.425	4.50	5.36	5.34	5.73	5.70	0.1140	0.002	0.001
Sodium bentonite (NaB)																
DMI (g/day)	79.3	83.5	85.3	87.1	89.2	1.851	<0.001	0.180	140.2	144.5	152.9	152.8	156.7	3.551	0.001	0.027
BWG (g/day)	38.7	44.0	44.9	47.9	47.4	1.450	0.049	<0.001	60.2	63.3	64.5	66.9	66.8	0.710	0.019	<0.001
FCR (g of feed /g of BWG)	2.21	2.08	1.98	1.86	1.92	0.121	0.023	0.016	2.32	2.28	2.36	2.27	2.33	0.062	0.867	0.557
PER	2.52	2.44	2.54	2.68	2.73	0.169	0.037	0.126	2.36	2.36	2.35	2.40	2.37	0.070	0.709	0.56
IBD	406.7	410	418	415	423	4.570	0.020	0.339	2060	2017	2055	2038	2076	25.22	0.173	0.223
NCD	3.15	3.29	3.33	3.55	3.56	0.071	<0.001	0.002	4.49	4.43	4.44	4.74	4.71	0.103	0.005	0.441
Calcium bentonite (CaB)																
DMI (g/day)	84.2	84.6	83.6	90.6	86.7	1.501	0.10	0.061	149.8	152.0	153.5	161.2	158.0	2.102	0.053	0.002
BWG (g/day)	43.2	39.2	45.9	51.9	51.3	4.011	0.007	0.226	61.2	65.4	67.0	70.0	71.0	1.406	0.101	0.001
FCR (g of feed /g of BWG)	2.08	1.98	1.86	1.76	1.73	0.101	0.019	0.001	2.45	2.31	2.29	2.29	2.22	0.103	0.002	0.123
PER	2.54	2.55	2.51	2.55	2.73	0.103	0.003	0.037	2.32	2.31	2.38	2.43	2.40	0.202	0.025	0.325
IBD	406	410	417	414	422	4.601	0.24	0.862	2058	2202	2246	2227	2271	28.032	0.005	0.014
NCD	3.22	3.46	3.58	3.81	3.73	0.102	0.011	0.086	4.51	4.76	4.782	5.11	5.07	0.103	<0.001	0.002

SEM: standard error of the mean. DMI; feed intake; BWG: average daily body weight gain; FCR: feed conversion ratio; IBD: infectious bursal disease antibody geometric mean titers; NCD: Newcastle disease antibody geometric mean titers; PER: protein efficiency ratio.

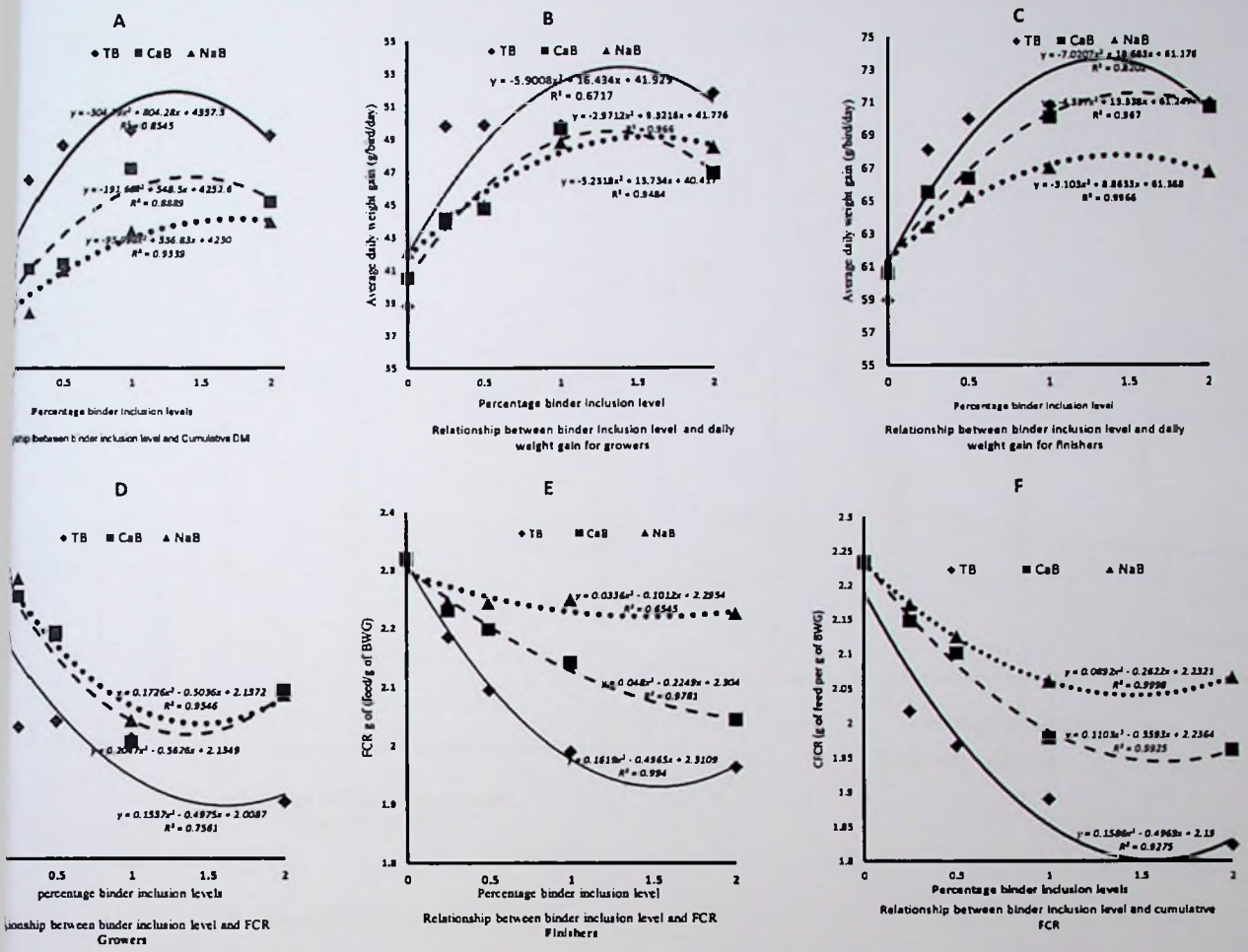


Figure 3.1: Relationship between binder inclusion levels and feed intake and utilization in broilers

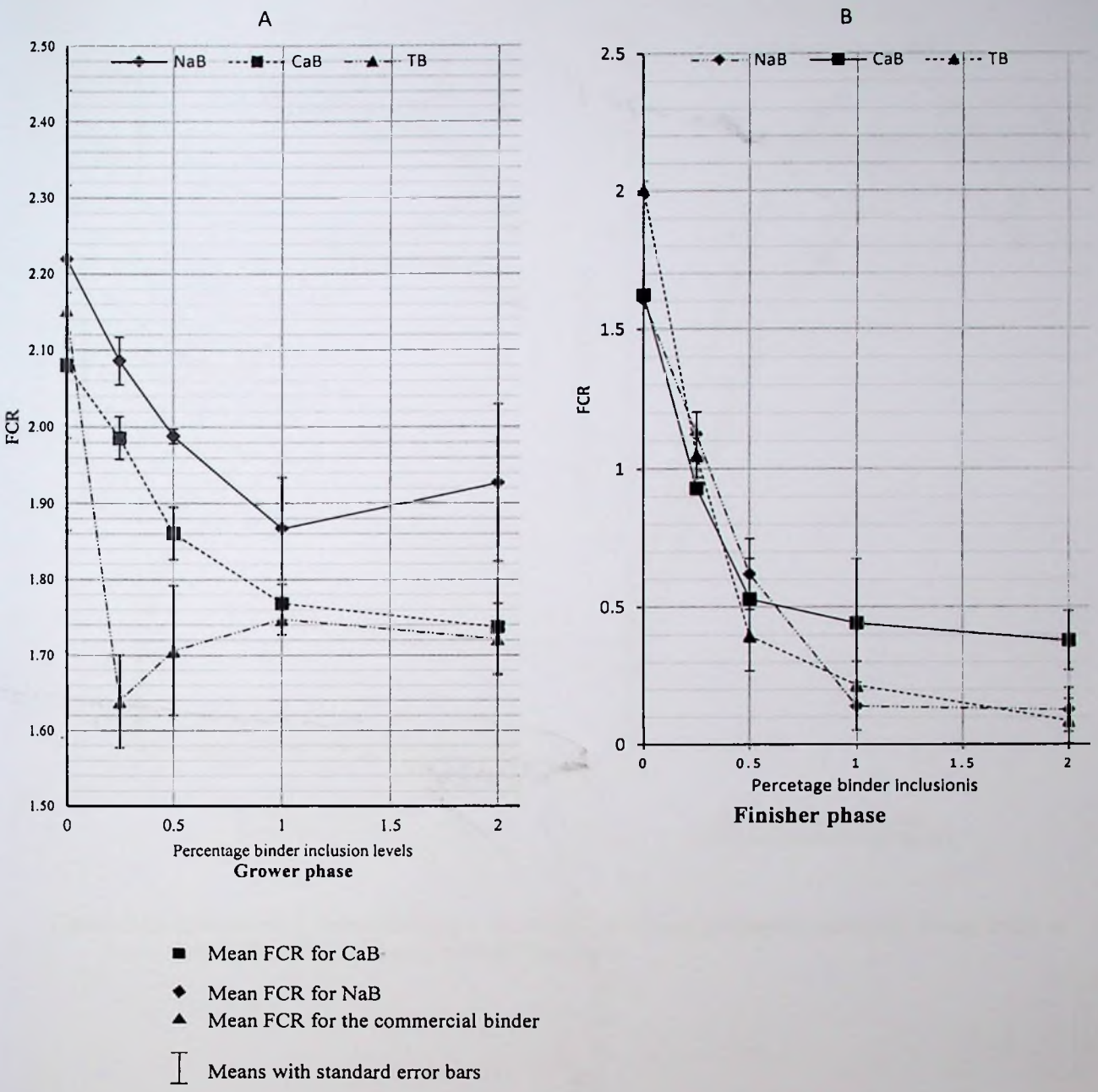


Figure 3.2: Relationship between binder inclusion level and feed conversion ratio in grower and finisher broilers

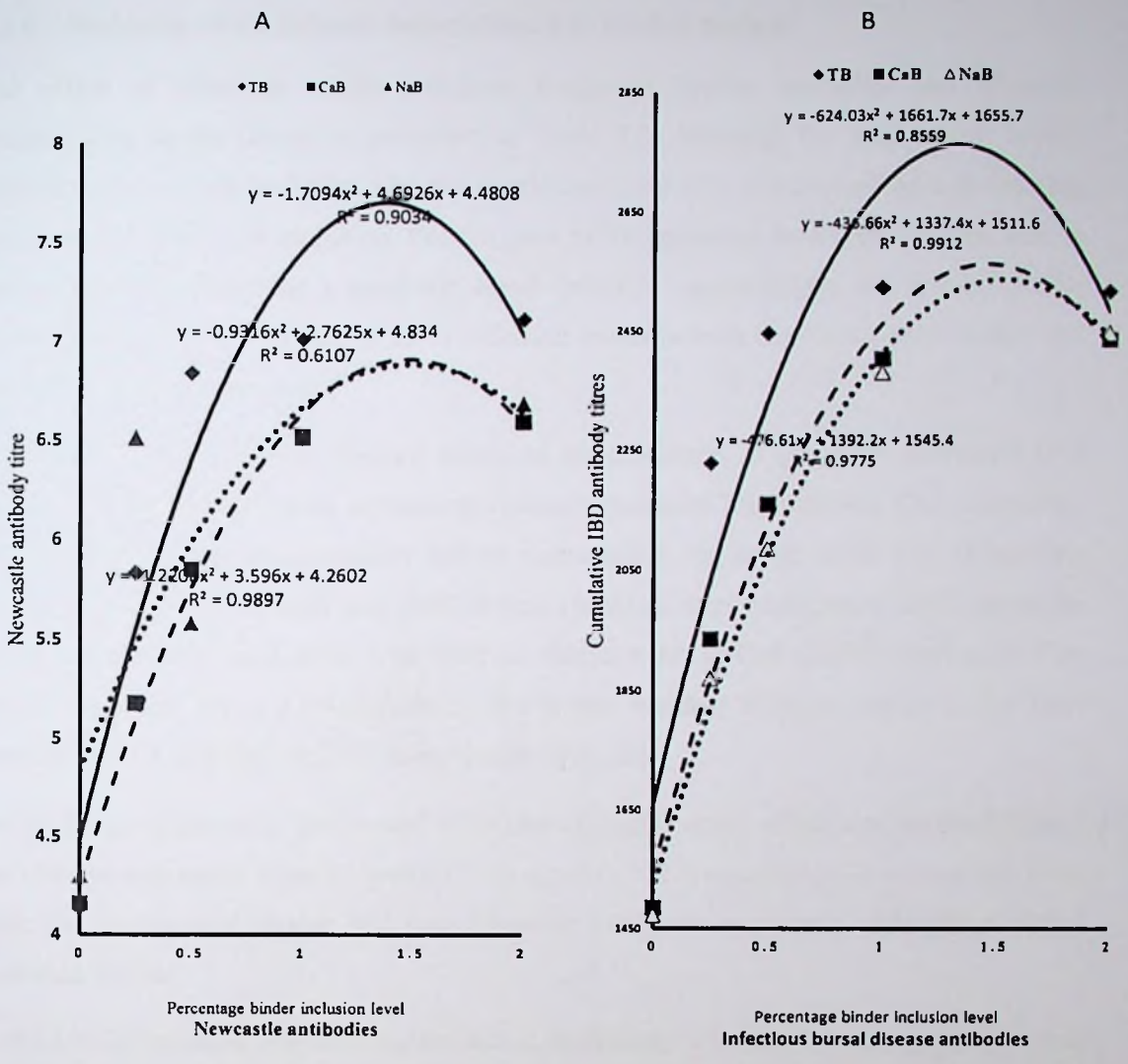


Figure 3.3: Relationship between binder inclusion level and geometric antibody mean titers of Newcastle and infectious bursal diseases

3.3.6. Mortality and aflatoxin accumulation in broiler tissues

The effect of aflatoxin binder inclusion levels on broiler mortality and aflatoxin accumulation in the tissues is presented in Table 3.2. Although the response in broiler mortality due to CaB inclusion was not significant ($p>0.05$), it decreased at a decreasing rate ($p<0.05$) with NaB inclusion. For the case of TB inclusion however, the decrease in broiler mortality followed a quadratic trend ($p<0.05$). Accordingly, the lowest broiler mortalities were first observed at 1.0% inclusion levels in both the commercial binder and the Albertine bentonite clays.

Among the three aflatoxin binders, aflatoxin accumulation in the liver decreased at a decreasing rate ($p<0.05$) with increasing inclusion levels of TB, NaB and CaB. However, the lowest aflatoxin accumulation due to commercial binder at 2.0% was below the detection limit while in CaB and NaB, lowest residuals were obtained at 2.0% inclusion levels. On the other hand, the lowest residual aflatoxin due to CaB (2.0%) was higher than that of NaB and TB at 2.0%. However, the lowest residual aflatoxin levels in the liver were due to TB and NaB at 2.0% were similar (Fig. 3.4).

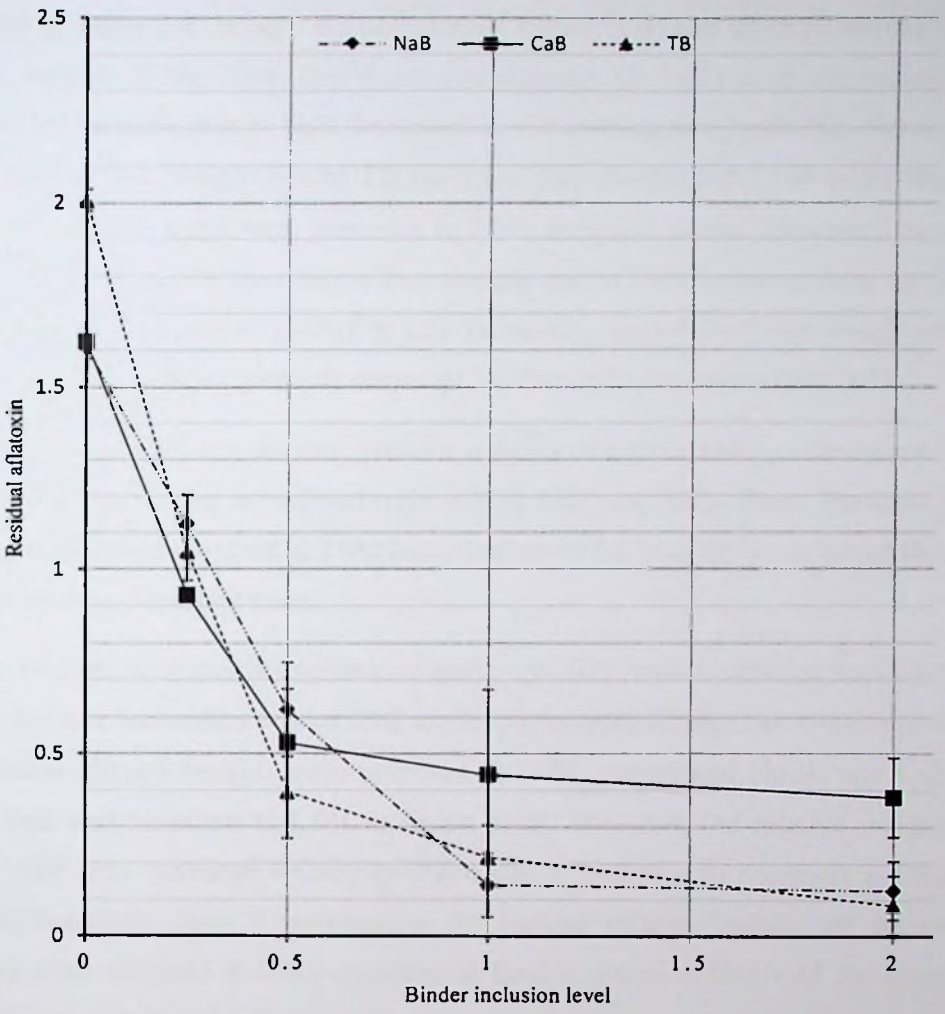
For both the commercial binder and Albertine bentonite clays, aflatoxin accumulation in the kidneys decreased linearly ($p<0.05$). As a result, the lowest aflatoxin accumulation in both the commercial binder and test Albertine bentonite clays was obtained at 2.0% inclusion levels.

In the broiler muscles, aflatoxin accumulation decreased following a linearly ($p<0.05$) due to TB inclusion. However, beyond 0.5% inclusion level of TB, the residual aflatoxins in the liver were below the detection limit. For the case of Albertine bentonites, aflatoxin accumulation in the muscles decreased at a decreasing rate of NaB ($p<0.05$) inclusion while that of CaB followed a quadratic trend ($p<0.05$). Subsequently, the lowest aflatoxin detectable values were observed at 0.5 for TB and CaB, while that of NaB was obtained at 0.25% inclusion levels.

Table 3.2: Effect of aflatoxin binder inclusion levels on mortality and aflatoxin carry-over in organ tissues

	Percentage binder inclusion levels.						Polynomial contrasts	
	0.00	0.25	0.50	1.00	2.00	SEM	Linear	Quadratic
Commercial binder (TB)								
Mortality (%)	8.30	1.67	1.67	0.00	1.67	2.578	0.113	0.012
Liver (µ/kg)	2.01	1.11	0.39	0.21	ND	0.410	<0.001	0.020
Kidneys (µ/kg)	3.81	2.30	1.8	1.7	1.5	0.569	0.010	0.140
Muscles (µ/kg)	0.89	0.36	0.26	ND	ND	0.258	0.007	0.068
Sodium bentonite (NaB)								
Mortality (%)	8.33	6.67	1.67	0.00	0.00	2.227	0.008	0.039
Liver (µ/kg)	1.61	1.12	0.62	0.14	0.13	0.041	0.001	0.050
Kidneys (µ/kg)	3.13	2.16	1.94	1.14	1.05	0.510	0.001	0.140
Muscles (µ/kg)	0.56	0.26	ND	ND	ND	0.001	0.040	<0.001
Calcium bentonite (CaB)								
Mortality (%)	6.67	3.33	3.33	0.00	0.00	2.402	0.070	0.092
Liver (µ/kg)	1.62	0.93	0.53	0.44	0.38	0.301	0.050	0.003
Kidneys (µ/kg)	3.03	2.15	1.97	1.71	1.10	0.410	<0.001	0.203
Muscles (µ/kg)	0.69	0.35	0.27	ND	ND	0.103	0.052	0.010

ND: aflatoxins not detected (below the detection limit); SEM: standard error of mean.



■ Mean residual aflatoxin for CaB

◆ Mean residual aflatoxin for NaB

▲ Mean residual aflatoxin for the commercial binder

I Means with standard error bars

Figure 3.4: Relationship between binder inclusion levels and residual aflatoxins in the liver tissues

3.3.7. Relative organ weights

The effect of aflatoxin binder inclusion levels on relative organ weights in broilers is presented in Table 3.4. In both the commercial binder (TB) and calcium bentonite (CaB) relative weight of the liver decreased quadratically ($p<0.05$) with increasing binder inclusion levels while that of NaB decreased at a decreasing rate ($p<0.05$). Subsequently, lowest relative liver weight due to TB inclusion was observed at 1.0% while that of the Albertine bentonite clays were observed at 2.0% inclusion levels. However, beyond the inclusion level of 0.25% the relative liver weight due to NaB inclusion was significantly ($p<0.05$) higher than that of CaB (Fig 3.5). Generally, the relative liver weights between TB and CaB were similar ($p>0.05$) across all the five inclusion levels (Fig. 3.5).

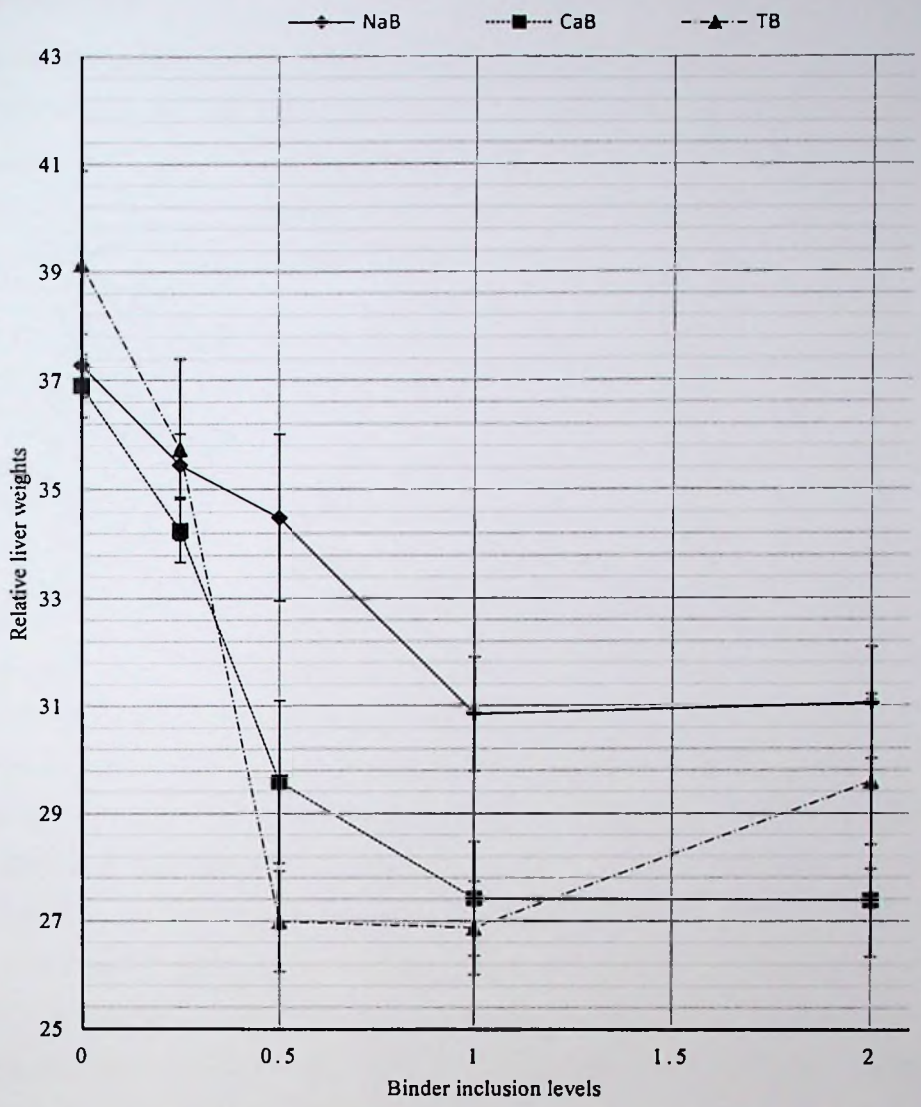
Among the three aflatoxin binders, relative weights of broiler kidneys decreased linearly ($p<0.05$) with increasing inclusion levels of TB, NaB and CaB. Thus, the least relative kidney weights were obtained at 2.0% inclusion levels for both the commercial binder and the test Albertine bentonite clays.

Relative weights of gizzards decreased linearly ($p<0.05$) with increasing inclusion levels of the Albertine bentonite clays as well as the commercial binder. As a consequence, the least relative gizzard weights were observed at 2.0% commercial binder and CaB while that of NaB was observed at 1.0% inclusion level. However, the relative weight of the bursa of Fabricius increased linearly ($p<0.05$) due to inclusion of commercial binder and Albertine bentonite clays. Consequently, the highest relative weights of the bursa of Fabricius were obtained at 2.0% commercial binder inclusion levels of the commercial binder and the Albertine bentonite clays.

Table 3.3. Effect of aflatoxin binder inclusion levels on relative organ weights in broilers

	Percentage binder inclusion						Polynomial Contrasts	
	0.00	0.25	0.50	1.00	2.00	SEM	Linear	Quadratic
Commercial binder (TB)								
Liver (g/kg)	39.1	35.7	26.9	26.8	29.6	2.90	0.420	0.001
Kidney (g/kg)	12.9	10.6	8.7	8.8	7.9	1.02	0.002	0.120
Gizzard (g/kg)	29.1	26.3	25.1	24.5	24.0	1.30	0.004	0.057
Bursa of Fabricius (g/kg)	2.21	2.22	2.22	2.35	3.1	0.24	<0.001	0.064
Sodium bentonite (NaB)								
Liver (g/kg)	37.2	35.4	34.5	31.1	30.8	2.09	0.002	0.046
Kidney (g/kg)	12.4	11.3	9.8	9.4	9.3	0.62	0.002	0.06
Gizzard (g/kg)	28.3	26.1	25.9	24.4	25.4	1.10	0.004	0.054
Bursa of Fabricius (g/kg)	2.23	2.22	2.41	2.49	2.70	0.02	<0.001	0.541
Calcium bentonite (CaB)								
Liver (g/kg)	36.9	34.2	29.6	27.5	27.4	1.70	0.061	0.001
Kidney (g/kg)	12.4	11.5	9.9	10.3	9.7	0.71	0.006	0.053
Gizzard (g/kg)	28.1	27.7	26.0	25.4	24.9	0.90	0.001	0.058
Bursa of Fabricius (g/kg)	2.3	2.2	2.23	2.53	2.8	0.11	<0.001	0.187

SEM: standard error of the mean.



■ Mean relative liver weight for CaB

◆ Mean relative liver weight for NaB

▲ Mean relative liver weigh for the commercial binder

I Means with standard error bars

Figure 3.5. Effect of aflatoxin binder inclusion levels on relative liver weights

3.4 Discussion

3.4.1 Feed intake

Defined as secondary toxic metabolites of *Aspergillus parasiticus* and *Aspergillus flavus,* aflatoxins are the key determinants of feed intake in poultry and hence performance. The use of high-affinity non-nutritive adsorbents like bentonite clays is one of the most promising decontamination techniques that can be used to combat the toxic effects of aflatoxins on feed utilization, organ health, and toxin carry-over in poultry products (Verma et al., 2004). Moreover, according to several authors (Verma et al., 2004; Verma et al., 2004; Ferket & Gernant, 2006) feed intake (DMI), weight gain, and organ health are key in assessing broiler response to aflatoxin decontamination. In this study, the general increase in DMI with aflatoxin binder inclusion to intermediate inclusion levels (Table. 3.1) demonstrates that DMI is dependent on the level of aflatoxin decontamination. Such trends are consistent with earlier observations of Verma et al. (2003) who reported an increase in feed intake of broiler poultry as a consequence of aflatoxin decontamination. Whereas Ferket & Gernant (2006) argued that broilers consume feeds to maximum gut fill and meet their energy requirements, they can also to a greater extent be limited by dietary toxicities. It can therefore be argued that the observed increase in DMI was attributed to an increase in feed acceptability and utilization as a result of aflatoxin decontamination (Van Rensburg et al., 2006). In fact, Abousadi et al. (2007) reported an improvement in feed utilization possibly attributed to reduced interference of specific enzymes involved in feed digestion. Therefore, the improved efficiency in digestion and assimilation of hydrolyzed nutrients in presence of aflatoxin binders possibly stimulates feed intake in broilers to maintain the gut fill and meet their energy requirements.

Whereas a general increase in DMI was observed with higher inclusion of aflatoxin binders up to intermediate levels, a comparatively higher response was obtained due to commercial binder (TB) inclusion than the bentonite clays (CaB and NaB). To that extent of binder inclusion, such disparities could be attributed to the higher effectiveness of TB in aflatoxin decontamination than the bentonite clays. The observed trends in DMI following the increase in binder inclusion are in agreement with earlier findings by several authors (Tedesco et al., 2004; Bailey et al., 2006; Shi et al., 2006;

Mohamood et al., 2011) who reported a decrease in feed intake and growth performance due to aflatoxicosis in broilers.

However, beyond the intermediate levels, the increase in DMI followed a quadratic trend with increasing levels of the commercial binder and bentonite clay inclusion. As a result, the optimum response in DMI (4.89 kg/bird) due to CaB inclusion was lower than that of TB but was higher than for Sodium bentonites (NaB) (4.52 kg/ bird). This could possibly be attributed to both the greater lattice separation of CaB and the larger surface area of the resultant hydrated complex of the CaB bentonite clay than that of NaB (Russell et al., 2014; Soufiani et al., 2016). In fact, Deng et al., (2010) argued that aflatoxin decontamination by bentonite clay is greatly associated with hygroscopicity, surface polarity, and solubility which favor aflatoxin intercalation with the bentonites in water. Kang et al, (2016) argued that, as the bentonite clay gets hydrated in the GIT, the polar water molecules weaken the interaction between the closely packed bentonite layers by lowering the charge density of the sequestered exchangeable cations ($Ca^{2+}$and Na^{+}). The exposed cations ($Ca^{2+}$and Na^{+}) between the clay layers then form complexes with aflatoxin molecules through hydrogen bonding on the cyclone rings (Kang et al., 2016). Furthermore, McClure (2014) and Soufiani et al. (2016) argued that the higher colloidal effect, accessibility of the exchangeable cations, and the higher water absorption capacity exhibited by CaB than NaB provides a larger surface area in CaB for better sequestration of aflatoxins. Besides, Kang et al. (2016) reported that the aflatoxin sequestration capacities of bentonite clays largely depend on the charge density of the exchangeable cations to induce dipoles on the aflatoxin molecules hence their attraction to the clay layers. Therefore, the higher charge density on Ca^{2+} than Na^{+}, the greater colloidal effect, and surface area possibly account for the higher aflatoxin adsorption potential of the calcium bentonite clay.

However, a reduction in DMI was observed beyond 1.32% inclusion levels of CaB, (Fig. 3.1). Such a quadratic trend could either be attributed to the negative effects of excess calcium or interference of CaB to nutrient absorption (Angel et al., 2002). But, excess calcium has been reported to form insoluble complexes with phytate phosphorous which results in the hindrance of phytase enzyme activity (Angel et al., 2002). Furthermore,

Gowda et al. (2009) and Neeff et al. (2013) reported no significant effects on feed intake and nutrient utilization of broilers fed more than 1.5% of bentonite clays. Since no reports have supported the claim that bentonites interfered with nutrient absorption, the drop in DMI could therefore be attributed to earlier reported effects of excess calcium from CaB.

3.4.2 Effect of aflatoxin binder inclusion on daily weight gain

Recognized as a key feed utilization indicator, weight gain (BWG) is one of the measures for investigating broiler response to aflatoxin decontamination. Since DMI alone cannot depict broiler performance, BWG was investigated to compare broiler performance as influenced by aflatoxin decontamination. The general increase in BWG with aflatoxin binder inclusion to intermediate binder inclusion levels (Table. 3.1) demonstrates that BWG is dependent on the level of aflatoxin decontamination. Besides, Li et al. (2002) and Arab-Abousadi et al. (2007) argued that such an increase in BWG due to aflatoxin decontamination could be mainly attributed to an increase in metabolic efficiency, protein synthesis, and its eventual accretion into muscles. In fact, aflatoxicosis is associated with a decrease in protein synthesis, energy metabolism as well as utilization of protein and energy (Li et al., 2002). McIntosh et al. (1975) further attributed such reductions to the inhibition of RNA synthesis by the aflatoxin metabolites through nucleolar DNA template impairment and inhibition of RNA polymerase II activity in the metabolizing cells. The observed response in BWG is consistent with findings by Yunus et al. (2013) and Chen et al. (2014) who reported a decrease in feed intake and weight gain due to chronic aflatoxicosis in broilers. Similarly, Lopez et al. (2006) reported a 9.5 % improvement in weight gain due to the addition of 0.1% bentonite clay to a diet containing 3.0 µg/kg aflatoxins. However, the observed optimum BWG responses (71.5-67.7g/bird/day) resulting from bentonite clay inclusion in this study were lower than the 78.08 g/bird/day obtained by Zhu et al. (2014) who evaluated a combination of mycotoxins. Such a discrepancy possibly suggests that a combination of different mycotoxins exerts a greater effect on BWG than aflatoxins alone. So, cases of multiple toxin contamination might therefore necessitate different decontamination approaches or binder combinations.

Nonetheless, beyond the intermediate levels, the increase in BWG followed a quadratic trend with increasing levels of the commercial binder and bentonite clay inclusion. As a result, the optimum response in BWG (71.5 g/bird/day) due to CaB inclusion was lower than that of TB but was higher than for Sodium bentonites (NaB) (67.7 g/bird/day) (Fig. 3.1). This demonstrates that besides aflatoxin decontamination, BWG response was also dependent on dietary calcium availability. Moreover, several authors (Kaplan et al., 1976; Hamdi et al., 2015) argued that reduced dietary calcium reduces feed intake and utilization through lowering RNA concentration and inhibiting protein synthesis. In addition, aflatoxicosis has been reported to induce calcium deficits yet CaB is reported to release calcium in the GIT (Soufian et al., 2016). Although Yanovski et al. (2009) reported no direct effect of Ca^{2+} on BWG, it anticipated that the released Ca^{2+} from CaB improved the aflatoxicosis-induced calcium-deficit which possibly promoted DMI and feed utilization hence BWG.

Bodyweight gain in the broiler finisher phase remarkably descended beyond 1.5 and 1.43% inclusion levels of CaB and NaB, respectively (Fig. 3.1). This quadratic response demonstrates that besides the reduction in feed intake, such a decline could also be attributed to the mechanism of aflatoxin adsorption by the bentonite clays through their exchangeable cations (Russel et al., 2014). Arguably, bentonites sequester aflatoxins through hydration of the exchangeable cations of Ca^{2+} and Na^{+} which adsorb the aflatoxins through the cyclone bridges (Russel et al., 2014). These exchangeable cations create hydrophilic environments on the bentonite surfaces, which shifts the equilibrium in a way that promotes aflatoxin absorption and release of calcium in the GIT (Stoj, 2001). The released Ca^{2+} promotes lipid flux by lowering plasma concentrations of vitamin D and parathyroid hormone, which are both known to promote intra-adipocyte Ca^{2+} concentrations hence affecting the rate of lipogenesis and lipolysis (Shamik et al., 2003). The resultant reduced lipogenesis and stimulated lipolysis possibly results in energy wastage and hence reduced body weight gain (Shamik et al., 2003). In addition, Soares et al. (2010) argued that increased fecal energy loss due to the formation of none absorbable calcium-fat complexes in the GIT further contributes to reducing BWG in broilers. Consequently, excess calcium released by CaB possibly created an energy utilization inefficiency yet energy is a key requirement in protein synthesis and hence BWG.

Therefore, for optimum BWG responses in broiler chickens fed aflatoxin-contaminated diets, bentonite clay inclusion should be at intermediate inclusion levels corresponding to optimum responses.

3.4.3 Effect of aflatoxin binder inclusion on the feed conversion ratio

Aflatoxicosis-induced performance decline in poultry can be reflected in reduced DMI, BWG, and damage of key organs. However, obtaining optimum performance due to aflatoxin-decontamination using DMI and BWG has offered inconsistent optimum responses as earlier indicated. As a measure of feed utilization efficiency, feed conversion ratio (FCR) is the best parameter for determining the optimum inclusion levels of aflatoxin binders since it accounts for both DMI and BWG responses. Therefore, determining the respective optimum FCR for bentonite clay binders gives the respective optimum binder inclusion levels. In this study, broiler FCR generally decreased at a decreasing rate with aflatoxin binder inclusion levels (Fig. 3.1). Broiler FCR was measured as a ratio of DMI to BWG, both DMI and BWG generally followed similar trends with binder inclusion levels. This demonstrates that aflatoxin decontamination increases both DMI and BWG but only up to intermediate levels of binder inclusion. The decrease in FCR with increasing binder inclusion suggests an increase in feed utilization efficiency resulting in higher BWG for less feed intake in the broilers. Tung et al. (1972) and Li et al. (2002) argued that an increase in feed utilization efficiency due to aflatoxin decontamination could possibly be through improved efficiency in energy and protein utilization. In fact, aflatoxin-decontamination has been reported to improve the utilization of sulfur-containing amino acids as well as energy utilization efficiency (Bhattacharya et al., 1989; Kermanshahi et al., 2009). Moreover, Kermanshahi et al. (2009) argued that aflatoxin detoxification in the liver results in metabolic intermediates that are produced largely at the expense of sulfur-containing amino acids (methionine and cysteine). Since protein synthesis is an all-or-nothing process, limiting the availability of any amino acid results in protein wasting and a reduction in BWG hence lowering feed utilization efficiency. The observed responses are in agreement with findings elsewhere which indicated improved feed utilization in broiler birds due to aflatoxin decontamination that resulted in improved protein utilization (Verma et al., 2003; Pandey &Chauhan, 2007; Oguz, 2011).

However, calcium bentonite clay resulted in a better optimum FCR response (1.86 g of feed/g of BWG) than sodium bentonite clay (1.88 g of feed/g of BWG) (Fig 3.1). This demonstrates that CaB exhibits higher degrees of aflatoxin decontamination than NaB. Arguably, besides aflatoxin decontamination, CaB exhibits a greater calcium recovery through releasing calcium in the GIT as reported by Stoj (2001) and Soufian et al. (2016). However, aflatoxicosis has been reported to create a calcium deficit that impedes feed utilization in poultry as earlier reported by Hamdi et al. (2015). As a consequence, the combined effects on aflatoxin decontamination and improved calcium availability by CaB synergistically could have contributed to the better FCR optimum response due to CaB inclusion than NaB.

Furthermore, beyond the point of inflection more DMI per unit of BWG was observed with CaB inclusion (Fig. 3.1). This demonstrates that feed utilization efficiency decreased when CaB was included beyond the optimum inclusion level of 1.7% w/w. Such a response could be attributed to the effects of excess calcium on weight gain and feed intake as earlier reported by Angel et al. (2002). Besides, calcium has also been reported to interact with inorganic phosphorous in the gut lumen forming insoluble calcium orthophosphate complexes (Angel et al., 2002; Plumstead et al., 2008). For that reason, this interaction probably makes inorganic calcium and phosphorous less available for assimilation yet they are key nutrients in the efficiency of energy and protein utilization for broiler growth. Therefore, whereas aflatoxin decontamination improved DMI and BWG, the increase in feed utilization efficiency was evident up to intermediate binder inclusion levels. So, for optimum broiler performance CaB and NaB can be included in aflatoxin-contaminated feeds at 1.7 and 1.5% w/w inclusion levels, respectively.

3.4.4 Effect of aflatoxin binder inclusion on the protein efficiency ratio

The effect of aflatoxin decontamination on BWG and FCR reveals the adverse effect of aflatoxicosis on the ratio of daily weight gain in grams to voluntary DMI in broilers (protein efficiency ratio(PER)). Although there was no specific trend in PER response due to TB inclusion, a general increase was observed with Albertine bentonite clays (CaB and NaB) inclusion (Table. 3.1). Such a response could be attributed to the fact that aflatoxicosis is associated with a decrease in protein utilization as reported by Li et al.

(2002). Arguably, the metabolism of aflatoxins to less toxic metabolites is carried out at the expense of sulfur-containing amino acids which upsets the amino acid balance in broilers (Bhattacharya et al., 1989; Kermanshahi et al., 2009). Since protein synthesis is an all-or-nothing process, limiting the availability of any amino acid results in amino acid deamination hence protein and energy wasting. Therefore, the increased aflatoxin decontamination levels possibly reduced aflatoxin metabolism hence conserving the essential amino acids. These observations did not differ from those earlier made by Verma et al. (2003), who reported a reduction in protein utilization as well as weight gain in broilers due to aflatoxin contamination. In addition, aflatoxins have also been reported to impair liver functions especially its protein utilization mechanisms (Hossain, 2011). It can therefore be argued that aflatoxin decontamination improves protein utilization efficiency through conserving the commonly limiting sulfur-containing amino acids in broilers.

3.4.5 Newcastle and Infectious Bursal Disease antibody titers

The antibody titers of Newcastle (NCD) and infectious bursal disease (IBD) measured the degree of broiler responses to vaccination (Vaccine efficacy) as affected by aflatoxin-decontamination. The antibody titers were used to measure NCD and IBD vaccine efficacies. In response to vaccination, antibodies are produced through antigen-lymphocyte interaction mechanisms to provide immunity in the broiler chickens (Mohiuddin, 1993). In this study, NCD and IBD antibody titers increased with aflatoxin binder inclusion levels (Fig. 3.3). The variations in the antibody titers with aflatoxin binder inclusion demonstrate that NCD and IBD vaccine efficacies increase with increasing aflatoxin decontamination. Furthermore, results from this study also indicated a reduction in aflatoxicosis-induced regression in the bursa of Fabricius with aflatoxin decontamination (Table. 3.3). Although limited information on antibody-aflatoxin interaction is available, the results are consistent with findings by Verma et al. (2004), who reported a reduction in NCD and IBD antibody titers as well as a regression of the bursa of Fabricius due to aflatoxicosis in broilers. Meissonier et al. (2008) argued that such a variation in antibody titer could be attributed to a reduction in aflatoxicosis-induced inflammatory responses and the enhancement of cell-mediated immune

responses due to aflatoxin decontamination. In fact, reductions in antibody quantities are primarily attributed to inhibitory effects of inflammatory cytokine IL-6 on antigen detection as well as antigen-specific immune responses (Meissonier et al., 2008). This implies that aflatoxicosis induces cytokine IL-6 which interferes with antigen detection by lymphocytes which reduces their proliferative response to antigens (Meissonier et al., 2008). Such alterations possibly reduced the sensitivity of lymphocytes to vaccine-based antigens leading to reduced response hence reduced antibody production as earlier reported by Mohiuddin (1993).

In addition, just like aflatoxicosis, IBD has been reported to cause a regression in the bursa of Fabricius hence suppressing its IBD antibody production ability in poultry (Verma et al., 2004). So, possibly, the observed reduction in aflatoxicosis-induced regression in the bursa of Fabricius improved its ability to produce IBD antibodies, hence the increase in IBD antibody titers. Therefore, whereas the increase in IBD antibody titer could be attributed to decreased regression in the bursa of Fabricius that of NCD could be attributed to improved sensitivity of lymphocytes to vaccine-based antigens.

Furthermore, aflatoxins have been reported to have negative effects on cell-mediated immunity through interacting with the T-cells hence blocking the antigen receptor sites (Oguz et al., 2003). Such competitive inhibitions result in immunosuppression through greatly inhibiting antibody synthesis as reported by Qureshi et al. (1998) and Radhika (2006). It can therefore be envisaged that aflatoxin decontamination reduces the regression of bursa of Fabricius, increases antigen-antibody sensitivity as well as chemo-static stimulation of T-cells hence increasing broiler response to vaccination (Radhika, 2006). Since cell-mediated and antibody-based immunity is key sensitive complementary aspects of the immune system, poultry farmers need to be advised to prioritize aflatoxin-decontamination for reduced NCD and IBD challenges.

3.4.7 Relative organ weights

Aflatoxicosis in poultry results in tissue damage which is reflected in the relative weights of the respective organs. In the process of metabolizing aflatoxins to less toxic derivatives, the key target organs are the liver, kidney, gizzard, and bursa of Fabricius. Results indicated that, whereas the relative weight of the bursa of Fabricius increased, that

of liver, kidney, and gizzard decreased with aflatoxin binder inclusion (Table. 3.3). Such responses in relative organ weights suggest that aflatoxin-decontamination improved organ health and functionality. These observations are in agreement with previous findings (Girish et al., 2006; Lehman et al., 2008; Kumar et al., 2009) which also indicated an increase in organ weights due to glomerular basement membrane thickening and histopathological changes due to toxicities in broiler liver, kidneys, and gizzards. Arguably, the increase in relative kidney and gizzard weight could be attributed to a reduction in the aflatoxicosis induced thickening of the glomerular basement membrane as earlier reported by Kumar et al. (2009). Results also indicate that among the organs evaluated, the liver was the most affected followed by the kidneys. On that note, Yunus et al. (2013) argued that first pass metabolic effect of aflatoxins through bio-activation of aflatoxins to less toxic intermediate metabolites in the liver, followed by elimination in the kidneys could explain the greater influence of aflatoxin decontamination in the liver. In fact, Girish et al. (2006) associated the histopathological changes with an accumulation of nitrogenous wastes and aflatoxin metabolites due to inflammatory thickening of the uterine mucosa. For that reason, the liver and kidney are considered to be the target organs for aflatoxicosis in all the affected species as earlier reported by Oruamabi et al. (2001). It can therefore be argued that aflatoxin-decontamination prevented aflatoxicosis induced histopathological changes (cellular dissociation and necrosis) in the respective organs hence the decrease in the relative organ weights (Ekhlas, 2012).

However, an increase in the relative weight of the bursa of Fabricius with increasing inclusion levels of the aflatoxin binders was detected which demonstrates that aflatoxicosis induced regression in the bursa of Fabricius. So aflatoxin decontamination reversed the regression of the bursa of Fabricius thereby improving its health and prominence. This observation is in agreement with findings by Verma et al. (2004) who reported a regression in the bursa of Fabricius due to aflatoxicosis in poultry. In fact, Choi et al. (2010) reported an aflatoxicosis induced an increase in lipid peroxidation and production of highly reactive oxygen species in the cell of the bursa of Fabricius. So when such biochemical species attack the membrane systems in the mitochondria, they interact with proteins and nucleic acids to affect its prominence (Sajan et al., 1996). Possibly, the resultant, impairment in cell physiology inhibits cell division and differentiation in the

tissues of the bursa of Fabricius hence accelerated regression (Peng et al., 2015). Therefore, reversing such detrimental lipid peroxidation through aflatoxin decontamination possibly restored the functionality of the bursa of Fabricius hence its progression.

3.4.8 Aflatoxin carry-over in tissues

Whereas active metabolic breakdown of aflatoxins to less toxic metabolites successively reduces the accumulation in the consumable poultry products, some residual toxins still find their way into the consumable tissues. Aflatoxin, carryover is dependent on both the amounts absorbed from the GIT and the enzymatically detoxified quantities by the tissues in the bird. The general decrease in residual aflatoxins with binder inclusion levels (Fig. 3.2) suggests that residual aflatoxins in the tissues decrease with aflatoxin decontamination. The variations also indicate that the commercial binder was the most effective in aflatoxin decontamination but comparable to the bentonite clays. Furthermore, the relatively lower residual aflatoxin levels were detected in the liver tissues than in the kidney tissues indicate that the rate of aflatoxin detoxification in the liver tissues is higher than that in the tissues of other organs. The relatively lower residual aflatoxins in the liver than in the kidneys is in agreement with findings by (Verma et al. 2004) who also reported lower levels of aflatoxin accumulation in the liver than in the kidneys for broilers fed aflatoxin-contaminated diets. On that note, Yunus et al. (2013) and Romoser et al. (2013) argued that the rapid cytochrome P_{450}-catalyzed bio-activation and detoxification in the liver due to the first-pass metabolic effect largely account for that variance. The high residual aflatoxin levels in the kidney on the other hand could be attributed to the fact that the kidneys accumulate and eliminate these toxins with their metabolites in the glomerular filtrate yet the kidney has limited aflatoxin detoxification activities (Kumar et al., 2009). Therefore, whereas the kidneys are challenged by both aflatoxin-induced inflammation and aflatoxin accumulation, the liver is enriched with aflatoxin detoxifying enzymes for quick metabolic detoxification (Yunus et al., 2013). So the addition of bentonite clays reduced the amounts of aflatoxins absorbed from that GIT to the levels that could be safely detoxified by the bird to the extent that between 1.0 - 2.0 levels of binder inclusion, no residual aflatoxins could be detected in the muscles.

3.5. Conclusion

Results from this study are interpreted to mean that feeding aflatoxin-contaminated diets adversely affects feed utilization efficiency, Newcastle, and infectious bursal disease vaccine efficacy as well as organ health in broiler chickens. The inclusion of Albertine bentonite clays mitigates the aflatoxicosis-induced decline in broiler productive performance, response to NCD and IBD vaccination as well as organ health. This implies that the Albertine bentonites can effectively be used in the decontamination of aflatoxin-contaminated broiler feeds. The overall results also indicate that calcium bentonite is more efficacious than sodium bentonite although slightly inferior to the commercial aflatoxin binder. Therefore, whereas most response parameters showed overlapping optimum values in ranges between 1.3 and 1.8% inclusions, the inclusion of calcium and sodium bentonites at 1.70 and 1.5%, respectively, results in optimum broiler growth performance. However, further investigations need to be conducted to close the performance gap between the commercial binder and the respective bentonites.

CHAPTER FOUR

STUDY 2

Effect of calcium and sodium bentonite clays from the Albertine graben region as aflatoxin binders on layer chickens fed contaminated diets

ABSTRACT

The study was conducted to determine the optimum inclusion levels of calcium and sodium bentonite clays from the Albertine graben region of Uganda in layer diets contaminated with aflatoxins. Balanced diets were formulated to meet the nutritional requirements of layers in their different physiological status as developer, grower, pre-layer, and layers. The grower, pre-layer, and layer diets were each laced with aflatoxins to levels of 250 ppb. Then, three aflatoxin binders including a commercial toxin binder (TB) as a control, and two Albertine bentonite clays (Calcium bentonite (CaB) and sodium bentonite (NaB) were each separately added at graded levels of 0.0, 0.25, 0.5, 1.0, and 2.0% w/w to the aflatoxin-contaminated diets. The diets included; grower, pre-layer, and layer diets for feeding the layers between 11-18 weeks, 19-start of laying, and during lying, respectively. The treatments were then allocated to the experimental layer in a completely randomized experimental design replicated 3 times (n = 15 layer birds per replicate group). The results indicated that hen day egg production increased with increasing commercial binder inclusion levels following a quadratic trend ($p<0.05$) while those of CaB and TB followed a linear trend ($p<0.01$). As a result, optimum responses of 52.6 and 48.9% were obtained at 1.21 and 1.76 % inclusion levels of TB and CaB, respectively. On the other hand, egg weight increased quadratically ($p<0.05$) with increasing commercial binder inclusion level while the increase due to CaB as well as NaB inclusion followed linear trends ($p<0.05$). As a consequence, optimum responses of 52.93, 52.3, and 52.0 g were obtained at 1.49, 1.47, and 1.79% inclusion levels of CaB, TB, and NaB, respectively. Feed conversion ratio decreased with an increase in TB inclusion levels following a quadratic trend ($p<0.01$) whereas the addition of CaB as well as NaB resulted in linear responses ($p<0.001$). As a result, optimum responses of 3.99, 4.03, and 4.12 were obtained at 1.30, 1.83, and 1.76 inclusion levels of TB, CaB, and NaB, respectively. This implies that CaB is highly comparable to the commercial binder in aflatoxin sequestration. Based on the feed conversion ratio, the inclusion of calcium and sodium bentonites from the Albertine region at inclusion levels of 1.83 and 1.76%, respectively to aflatoxin-contaminated feeds results in optimum layer performance.

4.1 Introduction

Due to the rapidly growing egg market in urban centers and cities (UBOS, 2009), more income can be earned through poultry layer production. However, the cost of commercial egg production restricts the advancement of the poultry layer industry, in which over 70% of the production costs go into feeding. This high cost of feeds has been amplified by poor feed utilization efficiency and layer performance (Brayden, 2012). Furthermore, reduced layer performance is also largely attributed to chronic aflatoxicosis (Zanghini et al., 2005).

As a result of long-term exposure to aflatoxins, chronic aflatoxicosis places commercial layers at a greater risk of a consequence of bioaccumulation (Verma et al., 2003). During such chronic aflatoxicosis, reduced feed utilization, growth rates, and egg production are inevitable (Oznurlu et al., 2012). In addition, feeds contaminated with aflatoxins beyond 200 ppb have been reported to reduce body weight gain, egg weight, and percentage hen day egg production in commercial layers (Verma et al., 2003). Since most layer feeds in Uganda are contaminated to levels between 65-1000 ppb layer productivity is highly compromised by aflatoxicosis (Kaaya & Warren, 2005).

However, the use of clay-based adsorbents in poultry diets like bentonites has proved to be effective in aflatoxin decontamination (Fowler et al., 2014). Consequently, the addition of bentonites to aflatoxin-contaminated feeds at a rate of 0.3 - 0.5% also reduces bioavailability and residues in the liver and kidney (Prvulovic et al., 2008; Neeff et al., 2013,). However, the aflatoxin sequestering ability of the bentonites varies from one deposit to another given the nature of the parent material and composition (Deng et al., 2010).

It was therefore hypothesized that aflatoxin binder type and inclusions level affects feed intake and utilization in the layer birds. The objective of this study hence was to assess the effects of fortifying layer feeds with Albertine bentonite clays on performance.

4.2 Materials and methods

4.2.1 Study site

This study was conducted between March and December 2017 at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), which is located 20 km North of Kampala but within the fertile lake Victoria crescent (0° 28′ N, 32° 27′ E, altitude 1204 m a.s.l). The area is characterized by a tropical climate with average maximum temperatures of 28.5°C and average minimum temperatures of 14.5°C.

4.2.2 Aflatoxin production protocol

Aflatoxins were produced by incubation of rice inoculated with *Aspergillus* spores. The spores were produced by removing the testa and hulling the groundnut grains followed by soaking in water for 30 minutes. The resultant moist ground nuts were then incubated at 28°C for seven days to produce mixed strains of green spores. After seven days, a Potato-Dextrose-Agar (PDA) was prepared by mixing potato extract, agar, and dextrose.

The potato extract was prepared by slicing 250 g of potato into 3.0 mm slices and cooking for 30 minutes in an open vessel. The potato extract was then collected by filtering the resultant medium through a muslin cloth before 20 g of dextrose was added. Simultaneously, the molten agar was prepared by boiling 20 g of agar in 500 ml of distilled water for 30 minutes in a separate glass beaker. Finally, the PDA was prepared by mixing the resultant molten agar with the potato dextrose mixture and the volume made up to 1 liter using distilled water. The pH of the resultant PDA medium was then adjusted to 7.0 and autoclaved at 121°C before plating in the lamina flow hood. After the PDA had cooled to room temperature, *Aspergillus* spores were identified and carefully picked from the incubated ground nuts under a light microscope at low and high magnification (4×, 10×, 20× and 40×) using a sterile spatula and inoculated onto the PDA slant plates. The resultant inoculated PDA slant plates were then incubated at 28°C for 10 days to produce the spore mass which was later used to inoculate a rice medium.

After 10 days, a rice medium was prepared by soaking rice at a rate of 1 g of rice for 0.5 g of distilled water for 120 minutes, drained off excess water autoclaved at 121°C for 30 minutes. After cooling to room temperature, the rice medium was inoculated with the

Aspergillus spores produced from the PDA medium. The spores on the PDA medium were scraped loose with a sterile loop, sterile water added before the slants were shaken to give uniform spore suspensions. Finally, 0.5 ml of the spore suspension was used to inoculate 50 g of the autoclaved rice medium before incubation for another 10 days to produce pure cultures of green conidia of *Aspergillus*.

After ten days, the cultures which had a heavy crop of green conidia were scraped loose with a sterile loop and agitated with sterile distilled water to give a uniform suspension of green spores. The resultant spore suspension (0.5 ml) was then used to inoculate the autoclaved rice medium in each Erlenmeyer flask before incubation.

After inoculation, the subsequent incubations were carried out in 2,000 ml Erlenmeyer flasks containing 400 g of inoculated rice at 28°C for 21 days. At the end of the incubation (21 days), the flasks were briefly steamed to destroy the fungus and the resultant pale yellow medium was dried at 105°C. The oven-dry medium was then standardized using the Vicam Aflatest procedure (950 ppb) before it was used to contaminate the respective experimental diets.

4.2.3 Aflatoxin determination using fluorometry

Total aflatoxins in the feed samples were determined using the Association of Official Analytical Chemists method. The maximum weight of 1.0 kg samples of each feed sample was collected from each feedlot immediately after mixing the ingredients. The moisture content of each sample on a dry basis was determined immediately using a moisture analyzer.

A ground feed sample aliquot of 25 g was weighed out together with 5 g of sodium chloride and placed in a blender jar. 125 ml of methanol: water in the ratio of 70:30 was added to the jar and the sample was blended at a high speed for 2 minutes. The blended sample was filtered through a filter paper into a clean beaker. After filtering, 15.0 ml of the resultant filtrate was diluted with 30 ml distilled water. The diluted extract was then filtered through a micro-fiber filter into an affinity Afla-Test column to collect 15 ml of the filtered extract in a syringe. The extract was then passed through an affinity column at the rate of 2 drops per second with the aid of a pressure pump. Then, 10 ml of distilled water was passed through the Afla-Test affinity column at the rate of

about 2 drops per second until air came through the column. The affinity column was eluted by passing 2 ml of HPLC grade methanol through the column at the rate of 2 drops per second while collecting the entire sample elute in a glass cuvette. Afla-Test developer was added to the eluent, thereafter it was mixed well and the aflatoxin concentration (ppb) was determined in the sample after 60 seconds by using a calibrated fluorometer.

4.2.4 Source and description of feed ingredients

Before experimentation, feed ingredients used in the formulation of experimental diets were sourced from local input stockiest. The ingredients used included broken maize meal, maize bran, soybean meal, fishmeal, lake shells, di-calcium phosphate, methionine, lysine, layer premix, and table salt (NaCl). The experimental clay was sourced from Knights Mining Company and was characterized as calcium and sodium bentonite, respectively, by a laboratory in the department of geological survey and mines in the ministry of energy and mineral development. A commercial aflatoxin binder branded Toxiban from Novus International outlet in Uganda was used in the experimentation while aflatoxins were produced as described in study I (section 4.2.3).

4.2.5 Experimental diet and design

Diets were formulated to meet the nutritional requirements of layers in their different physiological status as starters for layers between 1-6 weeks (2780 kcal/kg ME, 18% CP), developer for layers between 7-10 weeks (2740 kcal/kg ME, 16% CP) grower diet between 11-18weeks (2640 kcal/kg ME, 15% CP), pre-layer diets between 19 weeks to start off laying (17% CP, 2580 kcal/kg ME) and layers' diet, during laying (2610 kcal/kg ME, 17% CP) according to the National Research Council (NRC, 1994). Thereafter, the developer, pre-layer, and layers' diets were formulated and analyzed for aflatoxin contamination after which they were laced with aflatoxins to levels of 250 µg / kg DM using the aflatoxins produced on the rice medium. Then, each of the 3 aflatoxin binders was added at 5 graded levels (0.0, 0.25, 0.5, 1.0, and 2.0% w/w) to the respective contaminated layer diets. However, all the chicks were subjected to a uniform layer starter diet after which a total of 675 birds were selected and allocated to the 3 binders at

5 inclusion levels replicated 3 times (n=15). Finally, the experimental treatments were allocated to the experimental layers in a completely randomized experimental design till week 31.

4.2.6 Management of experimental birds

Both brooder and raring house equipment were cleaned, washed, and sun-dried before fumigation with (35 ml formalin + 17.5 g potassium permanganate) a week before the bird's arrival. In study 1, before the arrival of the day-old chicks, the brooder was prepared to provide conditions required for intensive broiler chick rearing. The floor of the brooder was covered with a dry litter of coffee husks that was 7.5 cm deep covered with paper boards to prevent the chicks from pecking the litter. The brooder was also fitted with paper egg trays, which were used as feeders for the chicks in the first 4 days. The heat was provided using perforated clay pots with burning charcoal alongside installed low-hanging light bulbs (Phillips, 240V/100W). Clean drinking water containing glucose and water-soluble vitamins was provided in round drinkers at a rate of 25 chicks per drinker. The brooder was also surrounded by a brooder guard that was 45 cm high and was heated until attainment of a steady peripheral temperature of about 32°C before the day-old chicks were introduced.

After attaining a steady temperature a total of 950-day old layer chicks weighing 44.6 ± 0.63 g were introduced to the brooding facility at MUARIK. The day-old chicks were given warm drinking water on arrival by dipping their beaks in the water. On day 5, linear feeders (100 cm × 8 cm × 5 cm) that provided a feeding space of 20 chicks per feeder replaced the paper trays and later fed layer starter diets up to week 8 at rates 35 g per bird which was gradually increased to 75g /bird/day by week eight. In addition, small drinkers were replaced with larger round drinkers (3-liter capacity) at a rate of 25 chicks per drinker as recommended by the supplier. The chicks were vaccinated against Newcastle diseases and IB on days 7, 21, and week 8 through the ocular route while Gumboro was vaccinated in weeks 4 and 6 through the oral route.

After raising the chicks for eight weeks, 675 out of the 950 chicks were weighed and randomly allocated to each of the dietary treatments. Birds in each replicate were housed in a deep litter system where plywood was used to separate the floor space. The

experimental unit was a pen whose length, breadth, and height were 2m × 2m × 2.5m, respectively. The experimental birds were subjected to routine management practices including a 12-hour lighting program using daylight. The experimental birds were also provided with drinking water *ad-libitum* at a rate of 15 pullets per drinker, while the feeds were provided in (100 cm × 15 cm ×15 cm) linear feeders. After the 19th week, the pullets were provided with laying nests at a rate of 30 cm^2 per pullet as recommended by Uga-chic Poultry Breeders.

The experimental birds were also vaccinated through the intramuscular route against fowl typhoid in week 10 using the fowl typhoid vaccine while in week 12 they were vaccinated against Fowlpox through wing web using the fowlpox vaccine. After 5 months they were vaccinated against Newcastle + IB and later vaccinated against Newcastle after every 2 months. Experimental birds were also observed for any unusual behavior while the litter was constantly raked and its conditions consistently monitored to ensure friability.

4.2.7 Data collection

Body weights of birds were recorded at the start of lay and the end of the experiment using a digital weighing scale (RS232, WT-X series, 0.1g accuracy. Max. Range 30 kg). Daily feed intake for the group replicates was estimated by deducting the dry weight of feed refusal from the total dry weight of feed offered to each of the groups. Daily feed intake per bird per day was then calculated by dividing the total daily feed intake by the number of birds in the respective replicates. In addition, weekly DMI was also determined for each replicate while the mortality of birds was recorded as it occurred. Hen-day egg production (HDEP) was determined daily using the formula:

$$HDEP = (number\ of\ eggs\ laid\ in\ the\ day \div total\ number\ of\ hens) \times 100.$$

The eggs were collected 5 times daily, weighed immediately and weights recorded to the nearest of 1.0 g accuracy. Eggshell thickness was measured at three points equidistant along the equator of the shell after drying the shells at 100 °C for 2 hours using a micrometer screw gauge. The eggshell percentages were determined after removing the inner shell membranes, drying for 24 hours before it was determined using the digital

weighing scale. The average egg weight for each experimental unit was also determined weekly while the total egg mass of all the eggs laid in the ten weeks was also calculated at the end of the study. The feed conversion ratio was also calculated as feed consumed per unit eggs weight and weight gain using the following formula:

$$FCR = \frac{Fc}{Em + Bw}$$

Where, Fc = Feed consumed

Em = Egg mass-produced

Bw = Body weight gain or loss

Egg quality measured was shell thickness and percentage shell proportion.

4.2.8 Data analysis

Polynomial contrasts were used to test for linear and quadratic effects of the inclusion of graded levels of each aflatoxin binder. Parameters were considered significantly different at $P < 0.05$. Second-order polynomial regressions were fitted to body weight gains, hen day egg production, feed conversion ratios, egg mass per bird, and egg weight data to establish treatment responses, and the regressions equations were differentiated to determine the point of inflection on each response curve.

4.3 Results

4.3.1 Layer productive performance and eggshell characteristics

The effect of aflatoxin binder inclusion levels on the productive performance of layers is presented in Table 4.1. Among the layers, daily feed intake (DMI) increased quadratically ($p<0.05$) with increasing commercial binder (TB) and calcium bentonite (CaB) inclusion levels. For the case of sodium bentonite (NaB) however, DMI increased linearly ($p>0.01$) with increasing binder inclusion levels. As a consequence, the highest DMI of feeds fortified with the Albertine bentonite clays were observed at 2.0% and 1.0% inclusion levels of NaB and CaB, respectively while that of the commercial binder was observed at 1.0%.

Whereas body weight gain only tended to follow quadratic trends due to the commercial binder ($p=0.052$) and Calcium bentonite ($p=0.064$) inclusion, layer body weight increased with increasing NaB inclusion levels following a linear trend ($p<0.05$). Hen day egg production increased quadratically ($p<0.05$) with increasing TB inclusion levels. For the case of Albertine bentonite clays, however, hen day egg production increased linearly ($p<0.01$). Subsequently, the highest hen day egg production responses were obtained at 0.5% inclusion levels of the commercial binder while that of the Albertine bentonite clays were observed at 2.0%. Although the observed maximum hen day egg production responses were similar ($p>0.05$) in both the commercial and NaB, that of CaB was significantly ($p<0.05$) higher than that of NaB and the commercial binder (Fig. 4.1). Furthermore, using the regression equations of the response curves (Fig. 4.2) hen day egg production generally increased with increasing binder level of inclusion levels to optimum responses of 52.6 and 48.9% at 1.21 and 1.76 % inclusion levels of TB and CaB, respectively.

Precisely, due to the commercial binder inclusion, egg weight increased quadratically ($p<0.05$) with increasing inclusion level while the increase due to Albertine bentonite clays inclusion followed a linear trend ($p<0.05$). As consequence, the highest egg weights were observed at 2.0% inclusion levels of the commercial binder as well as CaB, while that of NaB was obtained at 0.5% inclusion level. Generally, using the regression equations of the response curves (Fig. 4.2), egg weight increased with increasing binder

level of inclusion to optimum values of 52.93, 52.3, and 52.0 g at 1.49, 1.47, and 1.79% inclusion levels of CaB, TB, and NaB, respectively.

Due to commercial binder inclusion, FCR decreased quadratically ($p<0.01$) whereas Albertine bentonite clay inclusion resulted in linear responses ($p<0.001$). Subsequently, the lowest FCR responses were obtained at 0.5% inclusion levels of the commercial binder while those of the Albertine bentonites were obtained at 2.0%. In addition, using the regression equations of the response curves (Fig. 4.2), FCR decreased with an increase in binder inclusion levels to optimum responses of 3.99, 4.03, and 4.12 at 1.30, 1.83, and 1.76 inclusion levels of the commercial binder, CaB, and NaB, respectively. However, whereas the observed minimum values of FCR observed at 0.5% inclusion levels of the commercial binder were significantly lower ($p<0.05$) than those of the Albertine bentonites, it was not significantly ($p>0.05$) different from minimum values observed at 2.0% (Fig. 4.1).

The egg mass per bird increased with increasing binder inclusion levels due to both the commercial binder and Albertine bentonite clay addition following quadratic trends ($p<0.01$). As a result, the highest values of egg mass per bird were obtained at 0.5% inclusion levels of the commercial bentonite and 2.0% inclusion levels of the Albertine bentonite clay. On the other hand, using regression equations of the response curves (Fig 4.2), egg mass per bird increased with an increase in binder inclusion level to optima of 1.83, 1.73, and 1.62 kg. The optimum responses were obtained at 1.86, 1.28, and beyond 2.0% inclusion levels of TB, CaB, and NaB, respectively.

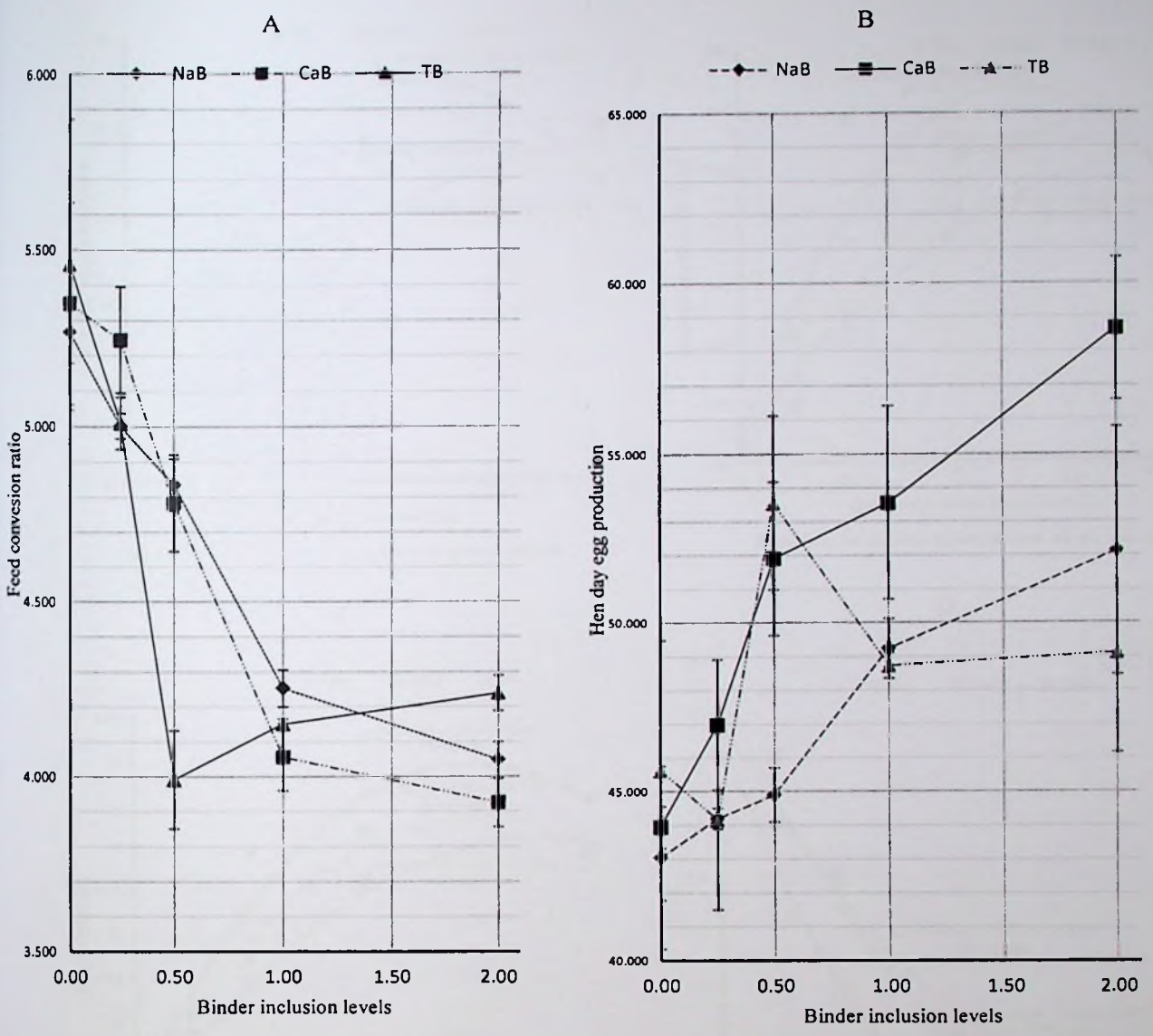
Eggshell percentage increased quadratically ($p<0.05$) with increasing aflatoxin binder inclusion levels due to commercial and CaB addition while that of NaB followed a linear trend ($p<0.05$). As a result, the highest shell percentages were obtained at 2.0 for both the commercial and NaB while that of CaB was first observed at 1.0%.

Precisely, due to the commercial binder and CaB inclusion, eggshell thickness increased with increasing inclusion levels following quadratic and linear trends respectively ($p<0.05$). As a consequence, the highest values were obtained at 1.0 inclusion of the commercial binder while that of the Albertine bentonite clays were obtained at 0.25 and 2.0 % inclusion levels of NaB and CaB, respectively.

Table 4.1. Effect of aflatoxin binder inclusion on the productive performance and eggshell characteristics in layers

	Percentage binder inclusion						Polynomial contrasts	
	0.00	0.25	0.50	1.00	2.00	SEM	Linear	Quadratic
Commercial binder (TB)								
DMI (g/day)	119.6	128.6	131.0	131.5	128.5	1.891	0.097	<0.001
BWG (g/day)	108.4	116.3	137.3	135.3	128.3	12.57	0.16	0.052
HDEP (%)	45.6	44.1	53.5	48.7	49.1	2.70	0.314	0.016
Egg weight (g)	50.5	51.8	52.5	53.6	53.7	0.76	0.073	0.004
FCR (g/bird)	5.40	5.00	3.90	4.10	4.20	0.190	0.17	0.001
Egg mass/bird (kg)	1.23	1.40	1.78	1.710	1.66	0.055	0.021	<0.001
Egg shell percentage (%)	8.90	9.00	9.01	9.10	9.20	0.007	0.101	<0.001
Shell thickness ($x10^{-2}$mm)	32.4	32.2	33.0	33.0	33.0	0.048	0.049	0.001
Sodium bentonite (NaB)								
DMI (g/day)	112.2	115.2	152.9	121.8	124.8	3.52	0.001	0.027
BWG (g/day)	118.1	127.4	129.0	123.6	107.0	6.79	0.048	0.062
HDEP (%)	43.0	44.2	44.9	49.2	52.1	2.44	<0.001	0.497
Egg weight (g)	51.3	51.5	54.5	53.3	53.9	0.54	0.007	0.092
FCR	5.26	5.00	4.83	4.25	4.04	0.058	<0.001	0.051
Egg mass/bird (kg)	1.29	1.40	1.40	1.69	1.72	0.078	0.05	0.002
Egg shell percentage (%)	8.94	8.95	8.95	8.95	8.97	0.01	0.040	0.387
Shell thickness ($x10^{-2}$mm)	32.0	33.3	32.9	33.0	32.9	0.36	0.314	0.131
Calcium bentonite (CaB)								
DMI (g/day	119.8	121.0	153.5	122.2	126.4	2.102	0.055	0.003
BWG (g/day)	117.4	121.5	125.9	129.6	125.4	6.80	0.191	0.064
HDEP (%)	43.9	46.9	51.9	53.5	58.7	3.71	0.001	0.190
Egg weight (g)	51.2	53.8	53.8	54.1	54.6	0.71	0.049	0.061
FCR	5.3	5.2	4.7	4.0	3.9	0.23	<0.001	0.090
Egg mass/bird (kg)	1.25	1.20	1.40	1.72	1.78	0.073	0.061	0.001
Egg shell percentage (%)	8.9	9.0	9.0	9.1	9.1	0.010	0.061	0.001
Shell thickness ($x10^{-2}$mm)	32.0	32.5	32.8	33.0	33.3	0.360	0.001	0.125

BWG: average daily weight gain; HDEP: Hen day egg production; FCR: feed conversion ratio; SEM: standard error of the mean.



■ Mean FCR for CaB

◆ Mean FCR for NaB

▲ Mean FCR for the commercial binder

I Means with standard error bars

■ Mean hen day egg production for CaB

◆ Mean hen day egg production for NaB

▲ Mean hen day egg production for the commercial binder

I Means with standard error bars

Figure 4.1: Relationship between aflatoxin binder inclusion level and performance of layers

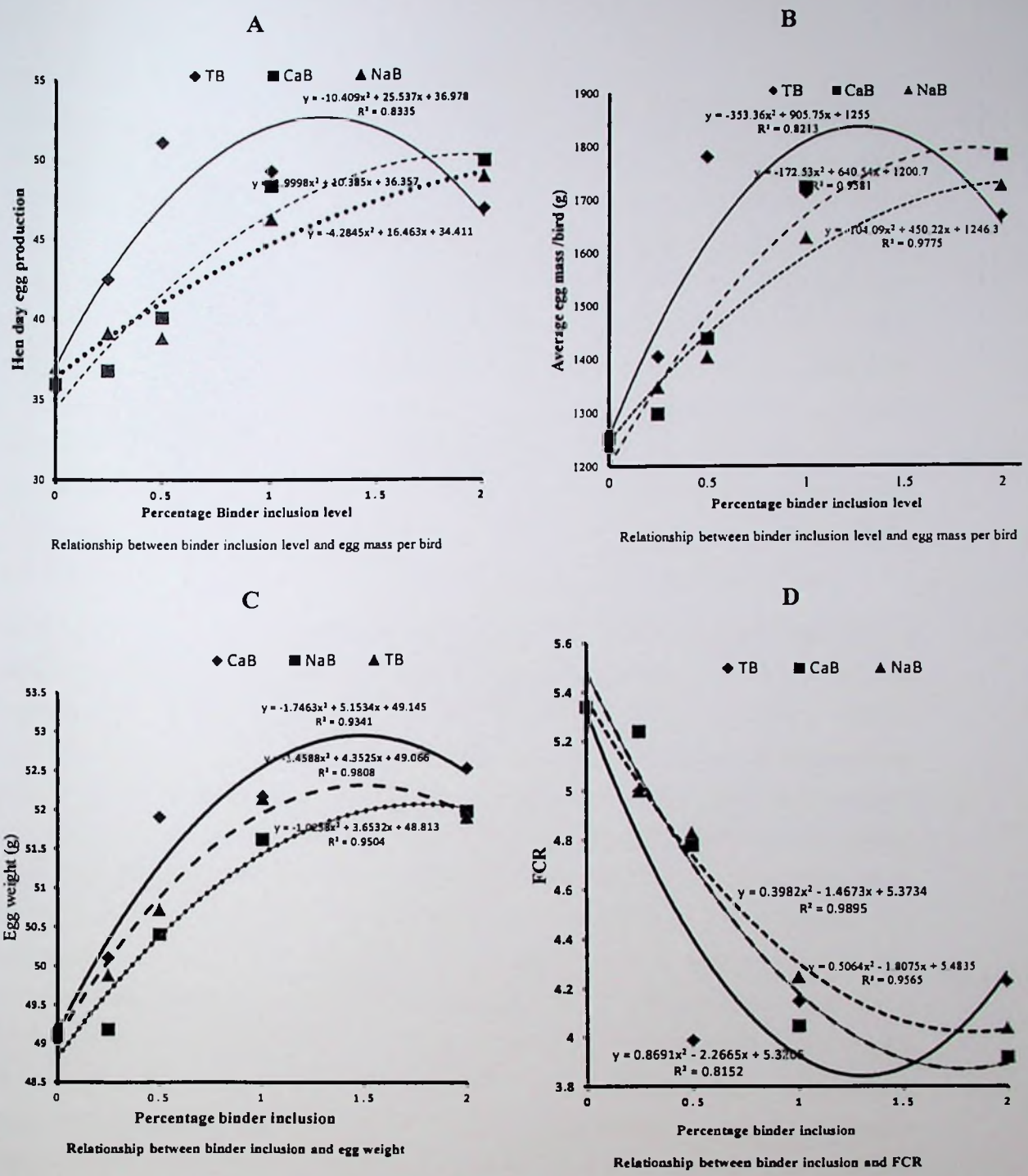


Figure 4.2. Relationship between binder inclusion levels and layer productive performence

4.4 Discussion

4.4.1 Hen day egg production and feed utilization

Although several bentonite clays have been evaluated for aflatoxin decontamination, nothing is known about the influence of the Albertine bentonite clays of Uganda in layers fed aflatoxin-contaminated diets. Even though there were no significant trends in live weight gain variations, results from this study display linear responses in the hen day egg production due to Albertine bentonite clay inclusion (Table 4.1). This is supported by the fact that layers are bred to convert feed nutrients into egg production rather than body muscles. These results are in agreement with previous reports (Verma et al., 2003; Chen et al., 2014), which also indicated that egg production increased due to mycotoxin decontamination without noticeable variations in layer weight gains. The increase in hen day egg production is perhaps due to the fact that bentonites mitigate aflatoxicosis-induced declines through improving the enzyme-dependent egg development processes and nutrient assimilation in the layers (Khan et al., 2010; Chaudhry et al., 2011).

On the other hand, the feed conversion ratio (FCR) decreased at constant rates with an increase in Albertine bentonite clay inclusion levels. This implies that feed utilization improves with increasing levels of aflatoxin decontamination. However, calcium bentonite resulted in a lower optimum FCR response (4.03) than sodium bentonite (4.12) (Fig.4.2). As a consequence, layers fed diets fortified with calcium bentonites required less feed per unit of egg produced than those fed on diets fortified with sodium bentonites. These trends are consistent with earlier observations of Kermanshahi et al. (2007) and Barati et al. (2018) who reported similarly improved feed utilization and blood protein composition in layers due to mycotoxin decontamination. Arguably, the increased feed utilization could be attributed to the fact that aflatoxicosis is manifested through impaired enzyme-dependent calcium utilization, protein assimilation, and hepatic damage (Sharline et al., 1980). In fact, Zaghini et al. (2005) and Khan et al. (2014) reported an impairment in protein synthesis and pancreatic digestive enzyme production due to chronic aflatoxicosis in poultry. Therefore, among the test bentonites, calcium bentonite possibly poses a greater improvement in the aflatoxicosis-induced decline in calcium utilization and protein synthesis than sodium bentonite. Hence, the disparities in responses in both hen day egg production and feed conversion ratio among the binders reflect

the ability of the Albertine bentonite clays to sequester aflatoxins though slightly inferior to the commercial binder. Furthermore, the linearity in FCR response implies that optimum inclusion levels of the bentonites are obtainable beyond close to 2.0% inclusion levels of the bentonite clays.

4.4.2 Egg weight and egg mass per bird

The trends in egg weight and egg mass per bird with binder inclusion (Table 4.1) reveal that aflatoxin binder inclusion increases the performance in the egg production indices. Although similar trends in egg weight were reported by Verma et al. (2003), calcium bentonite clay inclusion resulted in a relatively higher egg weight than sodium bentonite clay but comparable to the commercial binder. Such disparities could be attributed to the higher effectiveness of calcium bentonite clay in aflatoxin decontamination due to its better lattice separation and release of exchangeable calcium (Billey et al., 2006; McClure, 2014). Subsequently, the released calcium from calcium bentonite alleviates the aflatoxicosis-induced calcium insufficiency hence improving feed utilization as earlier reported by Hamdi et al. (2015).

Whereas calcium bentonite clay inclusion resulted in a higher optimum response in egg weight (52.9 g) than the commercial binder (52.3 g), the commercial binder exhibited a higher optimum egg mass per bird (1.83 kg) than calcium bentonite clay (1.73 kg). This implies that improved egg weight due to calcium bentonite clay inclusion could be at the expense of hen day egg production percentage. In this study, the observed trends in the egg weight are in agreement with findings elsewhere (Hamdi et al., 2015) who also reported higher egg weight and egg mass per bird due to the abundance of calcium in layer diets. Therefore, in addition to aflatoxin decontamination, calcium bentonite clay also provides additional calcium which corrects the aflatoxicosis-induced blood calcium deficit. Yet, aflatoxicosis is reported to cause a reduction in calcium and phosphorous in circulation in the blood (Eraslan et al., 2005). However, calcium and phosphorous are key compliments in egg synthesis as well as feed utilization in poultry (Hamdi et al., 2015). Therefore, in addition to aflatoxin sequestration, calcium bentonite clay also corrects the aflatoxicosis-induced calcium deficit in layers hence outperforming sodium bentonite clay in layers.

The comparable responses in egg mass per bird between calcium bentonite clay and the commercial binder (Fig 4.2) could imply that the increase in egg weight due to calcium bentonite clay inclusion is at expense of hen day egg production. This could imply that the effect of aflatoxin decontamination by CaB outweighs its effect on improved calcium availability and utilization. Although no similar study has reported on this before, such responses can be attributed to the fact that aflatoxicosis causes cytoplasmic vacuolation of the hepatocytes through impaired lipid transport and synthesis (Tung et al., 1972; Kumal, 2009). This impairment is accompanied by lipid deposition in the liver, which suppresses the lipid levels in the blood circulation (Tung et al., 1972). This implies that calcium bentonite inclusion improves enzyme-dependent phospholipid and cholesterol synthesis in the liver (Kumal, 2009). Since Phospholipids and cholesterol are key requirements in egg formation, it implies that egg mass per bird could be affected by the antagonism of these metabolic pathways (Abdel et al., 2012). Probably, the aflatoxicosis-induced calcium deficit inhibited phospholipid metabolism and cholesterol synthesis synergistically affected the egg quality indices in the experimental layers. It's therefore the correction of these metabolic defects through the addition of CaB at 1.86 % inclusion level which yields the best response in egg mass hence the feed utilization.

4.4.3 Effect on the eggshell percentage

The variations in eggshell percentage with aflatoxin binder inclusion levels suggest that eggshell formation and egg calcium depositions are influenced by the extent of aflatoxin decontamination. Largely, the observation is in agreement with the findings by Chowdhury & Smith (2004) and Zangihi et al. (2005), who reported a reduction in eggshell mass due to chronic aflatoxicosis. Furthermore, chronic aflatoxicosis results in hepatic damage and a reduction in hepatic zinc levels in poultry (Goel et al., 2005). Yet, the enzyme-dependent conversion of bone calcium into egg calcium is grossly affected by the generation and composition of zinc bicarbonate (Joose, 1983). As a consequence, the increase in shell percentage due to aflatoxin decontamination could have resulted in the enhanced conversion of bone calcium into egg calcium through reversing hepatic damage and improving zinc carbonate generation.

On the other hand, the greater variation in eggshell thickness due to calcium bentonite clay inclusion implies that calcium is the first limiting nutrient in the eggshell formation process. Arguably, shell formation is the fastest bio-ceramic calcifying process among biological systems (Hincke et al., 2012). So, the completed eggshell is deposited daily through ion transport across the uterine mucosa during its daily production cycle (Bar, 2009; Hincke et al., 2012). However, the aflatoxins have been reported to interfere with the absorption and transportation of calcium and phosphorous (Huff et al., 1975) as well as interfere with vitamin D_3 metabolism (Verma et al., 2003). Therefore, the resultant increase in shell percentage could be attributed to the improved utilization of Chole-calciferol hence the increase in 25hyroxy vitamin D and 1,25hyroxy vitamin D levels (Talapatra et al., 2016). As a result, the increase in the composition of 25hyroxy vitamin D and 1,25hyroxy vitamin D levels increases the composition of calcium and phosphorous in blood circulation (Eraslan et al., 2005). Therefore, in addition to aflatoxin decontamination, the calcium supplementation by the calcium bentonite possibly improved dietary calcium utilization in layers, which corrected the aflatoxicosis-induced calcium deficit.

The slightly lower response in eggshell thickness and percentage due to sodium bentonite inclusion indicates that it had lower aflatoxin decontamination and calcium supplementation capacities than calcium bentonite. This can further be supported by the fact that aflatoxins inhibit the synthesis of calcium-binding phosvitins, which are synthesized by the cells in the liver tissues (Chowdhury & Smith, 2005). Since the liver tissues are the primary targets by aflatoxins, damage to these cells also could account for the observed disparities. Likewise, aflatoxins have also been reported to deplete vitamin A, which vitamin is known to maintain the shell gland mucosal secretory activities (Pimpunkdee et al., 2004). Therefore, the observed disparities in eggshell composition could be attributed to vitamin A-related utilization and calcium-binding phosvitins deficiencies in the layers.

4.5 Conclusion

Results from this study indicate that feeding aflatoxin-contaminated diets greatly affects egg production and eggshell quality characteristics in layers. Since this was the first study

of its kind in layers, results indicate a high potential for utilization of the bentonite clays in the decontamination of aflatoxin-contaminated feeds. The results imply to mean that Albertine bentonite inclusion reduces aflatoxicosis-induced decline in laying performance eggshell quality indices. Therefore, the Albertine bentonite clays can be effectively used in improving the utilization of aflatoxin-contaminated layer feeds. However, overall results indicate that calcium bentonite is more efficacious than sodium bentonite and greatly comparable to the commercial aflatoxin binder. Hence, based on the feed conversion ratio, the inclusion of calcium and sodium bentonites from the Albertine region at inclusion levels of 1.83 and 1.76%, respectively to aflatoxin-contaminated feeds results in optimum layer performance.

CHAPTER FIVE

5 General discussion, conclusions, and recommendations

5.1 General discussion

Aflatoxicosis in poultry is associated with reduced productive performance and vaccine efficacy. The performance decline is attributed to the fact that aflatoxicosis affects the utilization of key nutrients like proteins, carbohydrates, calcium, and phosphorous. As a result, the compromised nutrient utilization hence the decrease in productivity affects the profitability of the poultry enterprise. However, the aflatoxin contamination thresholds beyond which binder inclusion is necessary are not established. Similarly, the optimum bentonite inclusion levels in aflatoxin-contaminated diets are not well defined as well (Williams et al., 2004). On the contrary, the performance broilers are less affected by low aflatoxin contamination levels while layers are highly affected by even low aflatoxin contamination levels. But, even such low-level aflatoxin exposures have been observed to significantly affect feed utilization and vaccine efficacy in both layers and broilers just like it was reported by Oguz et al., (2003). Moreover, poultry productivity is largely dependent on feed utilization efficiency whereas survival is highly dependent on vaccine efficacy and tolerance to microbial stress. Therefore, since feed utilization, vaccine efficacy, and microbial tolerance are affected by aflatoxicosis, aflatoxin decontamination remains a key requirement for improved poultry productivity.

Whereas several studies have suggested the use of biological aflatoxin degradation approaches, such decontamination methods have equally had a multitude of criticisms (Shetty, 2006). The limitations of such approaches have been attributed to their mechanisms of decontamination which result in alterations of the nutritive value of the feeds (Yan-Ni, 2008). Yet, aflatoxin decontamination approaches should be effective under low binder inclusion levels with no significant alterations in the nutritive values and acceptability of feeds. In addition, any effective detoxification approach must be economical, accessible, and capable of eliminating a broad spectrum of toxins. For these reasons, bentonites have been identified to best mitigate aflatoxicosis since they have been reported to be closest to meeting such requirements. Therefore, since the extraction and

utilization of Albertine bentonites as aflatoxin-binders is still in its infancy, it implies that adoption of the technology largely depends on bridging the information given the trend in the growth of the Ugandan oil industry.

Identified as cheap wastes from oil extraction, results from these studies have indicated that the Ugandan bentonites are effective aflatoxin decontamination agents. This implies that bentonite utilization has the potential to offset the aflatoxicosis-induced increase the poultry production costs. This is justified by the fact that most poultry farmers stabilize production costs through bulk feed purchase (Katongole et al., 2012). However, their post-harvest and feed storage conditions predispose the feeds to aflatoxin contamination. Moreover, according to Byarugaba (2007), aflatoxin contamination in poultry feeds has been reported to be the biggest threat to poultry production in Uganda. But, as earlier reported the reduced feed utilization efficiency and decreased vaccine efficacy which is due to aflatoxicosis greatly contribute to the increased costs of production. Therefore, in response to this threat, the study focused on evaluating the effect of adding the cheap Ugandan bentonites on poultry performance and identifying the optimum inclusion levels of these bentonites into the aflatoxin-contaminated diets.

Besides feed utilization, results from these studies also indicated an increase in feed intake, feed conversion ratio, and protein utilization due to increased aflatoxin decontamination levels. Whereas broilers have been observed to be less responsive to dietary toxin-related influences on feed utilization, laying hens were highly sensitive to dietary toxicity. This implies that the broilers had a higher degree of tolerance to chronic aflatoxicosis than the layer birds. Arguably, broilers tend to consume to maximum gut fill unless limited by acute toxicities and disease factors while layers could be limited by mild toxicities as reported by Ferket &Gernat, (2006).

On the other hand, similar trends in the Newcastle and IBD antibody titers as well as relative organ weights were observed. This implied that the addition of Ugandan bentonites improved vaccine potency hence the survival of the experimental birds. Such an observation can be explained by the increased tolerance to microbial and environmental stress as earlier reported to be affected by aflatoxicosis. Consequently, the

risks of losing birds to infectious challenges can be mitigated through the inclusion of bentonites in contaminated feeds.

The observed improvement in feed utilization and survival of the experimental birds treated with the test bentonites consequently confirms the ability of the bentonites to sequester aflatoxins through binding. Furthermore, calcium bentonite had a better performance than sodium bentonite on both layers and broilers. Arguably, such a performance was not only attributed to its better lattice separation but also the ability of calcium bentonite to offset the calcium deficit created by aflatoxicosis. Similarly, the increased eggshell percentage and feed conversion efficiency in layers implied that calcium bentonite could also become a potential source of calcium for layers besides aflatoxin decontamination. Results from both studies hence are interpreted to mean that layer productivity was more responsive to aflatoxicosis than broilers productivity. Consequently, this was due to the fact that broilers were exposed to contaminated feeds for a shorter time (7 weeks) than the layers which spent more than 30 weeks in experimentation. Moreover, the effects of aflatoxicosis are largely dependent on the duration of exposure as well as levels of contamination.

Consequently, as poultry farmers embrace the bulk feed purchase strategies to stabilize poultry production costs, they also need to adopt bentonite inclusion to cub the aflatoxicosis-induced production decline. Unfortunately, most commercial poultry farmers have not embraced this technology. This is due to the fact that most farmers lack information about the dangers of aflatoxicosis as well as the ability of Ugandan bentonites to mitigate these dangers. On the contrary, the few informed farmers have adopted the utilization of commercial feed additives, which include the expensively imported commercial binders in addition to the antibiotics. Possibly, it is because such farmers have been impacted by extension messages which made them appreciate the role of aflatoxin binders given the high levels of aflatoxin contamination reported in the feeds.

Therefore, as farmers tend to improve profitability in a commercial poultry enterprise they should be able to cheaper inputs without compromising performance (Thirumalaisamy et al., 2016). Moreover, the Ugandan bentonites were observed to be comparable to the commercial bentonites in performance yet they are wastes obtained

during the petroleum drilling process. Ultimately, the use of these bentonites in aflatoxin decontamination has the potential to improve the self-sufficiency of Ugandan poultry farms by reducing their dependence on imported toxin-binders.

5.2 Conclusion

Commercial poultry production is greatly constrained by the high levels of aflatoxin contamination in commercial feeds. As a coping strategy to this challenge, some of the elite farmers resort to using less accessible and expensive commercial toxin binders. However, the performance of calcium bentonite clay is exceedingly comparable to that of the commercial aflatoxin binder. This implies that there is a great potential in the utilization of the readily available inexpensive Albertine bentonite clay of Uganda in poultry diets. This is justified by the fact that Albertine bentonite clay inclusion was effective in mitigating the aflatoxicosis-induced decline in commercial poultry performance thus underscoring the utmost need to use commercial aflatoxin binders for decontamination. Therefore due to disparities in their performance, Albertine bentonite clays can be included at 1.5 and 1.7% inclusion levels of NaB and CaB, respectively in the broiler diets while in layer diets optimum performance is achievable at 1.76 and 1.83% inclusion levels of NaB and CaB, respectively.

5.3 Recommendation and further studies

5.3.1. Recommendation

1. Given the levels of aflatoxin contamination in the Ugandan poultry feeds, the addition of aflatoxin binders is unavoidable.
2. Farmers need to adhere to proper feed storage practices to lower the risks of aflatoxin contamination.
3. Smallholder poultry farmers can use Albertine bentonites to mitigate the aflatoxicosis induced performance decline in commercial poultry
4. The Albertine bentonites should be characterized, further purified for enhanced aflatoxin adsorption to acquire optimum responses at relatively lower inclusion levels.
5. Farmers and poultry feed dealers need to be trained in techniques of feed processing and storage to ensure lower aflatoxin contamination levels.

5.3.2 Further studies

The areas of further study arising from this research include, but are not limited to the Characterization of all Ugandan bentonites based on their mycotoxin binding abilities.

References

Alekseyev, Y. O., Hamm, M. L., & Essigmann, J. M. (2004). Aflatoxin B_1 formamidopyrimidine adducts are preferentially repaired by the nucleotide excision repair pathway in vivo. *Carcinogenesis*, *25*(6), 1045-1051.

Angel, R., N. Tamim, T., Applegate, A., Dhandu., J. Ellestad. (2002). Phytic acid chemistry: Influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11: 471–480.

Arab Abousadi, M., Rowghani, E., & Ebrahimi Honarmand, M. (2007). The efficacy of various additives to reduce the toxicity of aflatoxin B1 in broiler chicks. *Iranian Journal of Veterinary Research*, *8*(2), 144-150.

Atehnkeng, J., Donner, M., Ojiambo, P. S., Ikotun, B., Augusto, J., Cotty, P. J., & Bandyopadhyay, R. (2016). Environmental distribution and genetic diversity of vegetative compatibility groups determine biocontrol strategies to mitigate aflatoxin contamination of maize by *Aspergillus flavus*. *Microbial biotechnology*, *9*(1), 75-88.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H. S., Kieszak, S., Njapau, H., Rubin, C. (2005). Case–control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environmental health perspectives*, *113*(12), 1779-1783.

Bailey, C. A., Latimer, G. W., Barr, A. C., Wigle, W. L., Haq, A. U., Balthrop, J. E., & Kubena, L. F. (2006). Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *Journal of applied poultry research*, *15*(2), 198-206.

Banerjee, D., Wang, F., Liu, Y., Chen, X., & Liu, X. (2010). Up-conversion nanoparticles in biological labeling, imaging, and therapy. *Analyst*, *135*(8), 1839-1854.

Bar, A. (2009). Differential regulation of calbindin in the calcium-transporting organs of birds with high calcium requirements. *The journal of poultry science*, *46*(4), 267-285.

Barati, M., Chamani, M., Mousavi, S. N., Hoseini, S. A., & Taj Abadi Ebrahimi, M. (2018). Effects of biological and mineral compounds in aflatoxin-contaminated diets on blood parameters and immune response of broiler chickens. *Journal of Applied Animal Research*, *46*(1), 707-713.

Basmacioglu, H., Oguz, H., Ergul, M., Col, R., & Birdane, Y. O. (2005). Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin. *Czech Journal of Animal Science*, *50*(1), 31-39.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G., & Krska, R. (2005). Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography– tandem mass spectrometry. *Journal of agricultural and food chemistry*, *53*(9), 3421-3425.

Bhat, R., Rai, R. V., & Karim, A. A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive reviews in food science and food safety*, *9*(1), 57-81.

Bhattacharya, T., DiBenedetto, E., & Manfredi, J. (1989). Limits as $p \to \infty$ ofΔ pup= f and related extremal problems. *Rend. Sem. Mat. Univ. Politec. Torino*, *47*, 15-68.

Bintvihok, A., Kositcharoenkul, S., (2006). Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon 47, 41-46.

Brera, C., Debegnach, F., Minardi, V., Prantera, E., Pannunzi, E., Faleo, S. & Miraglia, M. (2008). Ochratoxin A contamination in Italian wine samples and evaluation of the exposure in the Italian population. *Journal of agricultural and food chemistry*, *56*(22), 10611-10618.

Bryden, W. L. (2012). Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. *Animal Feed Science and Technology*, *173*(1-2), 134-158.

CAST Report (2003). Mycotoxins: risks in plant, animal, and human systems (Richard, J. L and Payne, G. A. Ed.) Council for Agricultural Science and Technology Task Force report No. 139, Ames, Iowa, USA.

Celik. I, Sur. E., T. Ozaydın , Y. Oznurlu , H. Oğuz & K. Altunbaş (2012) Determination of the effects of aflatoxin B1 given in ovo on the proximal tibial growth plate of broiler chickens: histological, histometric and immunohistochemical findings, Avian Pathology, 41:5, 469-477.

Chaudhry, A., Samstein, R. M., Treuting, P., Liang, Y., Pils, M. C., Heinrich, J. M., Rudensky, A. Y. (2011). Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity*, *34*(4), 566-578.

Chen, X., Horn, N., & Applegate, T. J. (2014). Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin B1 in broiler chicks. *Poultry Science*, *93*(8), 2037-2047.

Choi, K. C., Chung, W. T., Kwon, J. K., Yu, J. Y., Jang, Y. S., Park, S. M., ... & Lee, J. C. (2010). Inhibitory effects of quercetin on aflatoxin B1-induced hepatic damage in mice. *Food and Chemical Toxicology*, *48*(10), 2747-2753.

Chowdhury, S. R., & Smith, T. K. (2004). Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on performance and metabolism of laying hens. *Poultry science*, *83*(11), 1849-1856.

Coulombe, R.A., J.A. Guarisco, P.J. Klein and J.O. Hall, (2005). Chemoprevention of aflatoxicosis in poultry by dietary butylated hydroxytoluene. J. Anim. Feed Sci. Technol., 121: 217-225.

Council for Agricultural Science. (2003). *Mycotoxins: Risks in plant, animal, and human systems* (No. 139). Council for Agricultural.

D'mello, J.P.F. (2003). Mycotoxins in cereal grains, nuts and other plant products. In: D'Mello J.P.F. (ed.) *Foods safety: Contaminants & toxins*. Wallingford: Cab International Publishing. pp. 65-88.

Daniel, V. C., Marchionni, L., Hierman, J. S., Rhodes, J. T., Devereux, W. L., Rudin, C. M., Watkins, D. N. (2009). A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. *Cancer research*, *69*(8), 3364-3373.

Deng, Y., Velázquez, A. L. B., Billes, F., & Dixon, J. B. (2010). Bonding mechanisms between aflatoxin B1 and smectite. *Applied Clay Science*, *50*(1), 92-98.

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 B1." South African Journal of Animal Science, 34, 97–103.

Dersjant-Li Yueming, Martin W.A. Verstegen, Walter J.J. Gerrit. (2007). The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. https://doi.org/10.1079/NRR200368

Desheng. Q, L. Fan Y. Yanhu Z. Niya (2005) Adsorption of aflatoxin B1 on montmorillonite *Poultry Science*, Volume 84, Issue 6, 1 June 2005, 959–961.

Devaraj, S., S. Mathur, A. Basu, H. H. Aung, V. . 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

Diaz, D.E., Hagler, W.M. Jr., Hopkins, B.A., Whitlow, L.W (2002). Aflatoxin binders In-vitro binding assay for aflatoxin B1 by several potential sequestering agents. Mycopathologia, 2002; 156: 223-226.

Do, J. H., & Choi, D. K. (2007). Aflatoxins: detection, toxicity, and biosynthesis. *Biotechnology and Bioprocess Engineering*, *12*(6), 585-593.

Ekhlas, K. H. (2012). Histopathological changes of some internal organs in broilers fed aflatoxin. *AL-Qadisiya Journal of Veterinary Medicine Sciences*, *11*, 70-79.

Eraslan, G., Eşsiz, D., Akdoğan, M., Şahindokuyucu, F., & Altintaş, L. (2005). The effects of aflatoxin and sodium bentonite combined and alone on some blood electrolyte levels in broiler chickens. *Turkish Journal of Veterinary and Animal Sciences*, *29*(3), 601-605.

Farombi, O. E. (2006). Aflatoxin contamination of foods in developing countries: Implications for hepatocellular carcinoma and chemopreventive strategies. *African Journal of Biotechnology*, *5*(1), 1-14.FDA. (2011).

Ferket, R. P., & Gernat, G. A. (2006). Fctors that affect feed intake inatake of meat birds: Areview. *International Journal of Poultry Science*, *10*(5), 905–911.

Food and Drug Administration (FDA). (2000). "Action Levels for Aflatoxins in Animal Feeds." Compliance Policy Guide, 683.100, 384-385.

Freire, L., Guerreiro, T. M., Caramês, E. T., Lopes, L. S., Orlando, E. A., Pereira, G. E., ... & Sant'Ana, A. S. (2018). Influence of Maturation Stages in Different Varieties of Wine Grapes (Vitis vinifera) on the Production of Ochratoxin A and Its Modified Forms by Aspergillus carbonarius and Aspergillus niger. *Journal of agricultural and food chemistry*, *66*(33), 8824-8831.

Gareis, M., Bauer, J., Thiem, J., Plank, G., Grabley, S., & Gedek, B. (1990). Cleavage of zearalenone-glycoside, a "masked" mycotoxin, during digestion in swine. *Journal of Veterinary Medicine, Series B*, *37*(1-10), 236-240.

Girish, C. K., & Devegowda, G. (2006). Efficacy of Glucomannan-containing Yeast Product (Mycosorb) and Hydrated Sodium Calcium Aluminosilicate in Preventing the Individual and Combined Toxicity of Aflatoxin and T-2 Toxin in Commercial Broilers. *Asian-Australasian Journal of Animal Sciences*, *19*(6), 877-883.

Goel, A., Dani, V., & Dhawan, D. K. (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chemico-Biological Interactions*, *156*(2-3), 131-140.

Gong, Y. Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P. C., Hall, A. J., & Wild, C. P. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *Bmj*, *325*(7354), 20-21.

Gowda, N. K. S., Ledoux, D. R., Rottinghaus, G. E., Bermudez, A. J., and Chen, Y. C. (2009). Antioxidant efficacy of curcuminoids from turmeric (Curcuma longa L.) powder in broiler chickens fed diets containing aflatoxin B1. The British Journal of Nutrition, 102(11), 1629–34.

Hamdi, M. S. López-Vergé, E. G. Manzanilla, A. C. Barroeta, J. F. Pérez. 2015. Effect of different levels of calcium and phosphorus and their interaction on the performance of young broilers, *Poultry Science*, Volume 94, Issue 9, 1 September 2015, Pages 2144–2151.

Hamilton, P.B. (1971): "A natural and extremely severe occurrence of aflatoxicosis in laying hens." Poult. Sci., 50: 1880-1882.

He, M., Miyajima, F., Roberts, P., Ellison, L., Pickard, D. J., Martin, M. J & D'Arc, S. (2013). Emergence and global spread of epidemic healthcare-associated Clostridium difficile. *Nature genetics*, *45*(1), 109

Hell, K., Cardwell, K. F., Setamou, M., & Poehling, H. M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. *Journal of Stored Products Research*, *36*(4), 365-382.

Hesham M. Teleb, Ali A Hegazy and Yehia A Hussein (2004). Efficiency of Kaolin and Activated Charcoal to Reduce the Toxicity of Low Level of Aflatoxin in Broilers Scientific Journal of King Faisal University (Basic and Applied Sciences) Vol.5 No.1 pp 145-160.

Hincke, M. T., Nys, Y., Gautron, J., Mann, K., Rodriguez-Navarro, A. B., & McKee, M. D. (2012). The eggshell: structure, composition and mineralization. *Front Biosci*, *17*(1266), 120.

Hossain, S. A., Haque, N., Kumar, M., Sontakke, U. B., & Tyagi, A. K. (2011). Mycotoxins residues in poultry product: Their effect on human health and control. *Wayamba J. Anim. Sci*, *9296*.

Huff, W. E., Wyatt, R. D., & Hamilton, P. B. (1975). Nephrotoxicity of Dietary Ochratoxin A in Broiler Chikens. *Applied Microbiology*, *30*(1), 48-51.

Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, *167*(2), 101-134.

Huwig, A., Freimund, S., Kappeli, O. Dutler, H. (2001). "Mycotoxin detoxication of animal feed by different adsorbents." Toxicology Letters, 122, 179-188.

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.

Iqbal S. Z., Mustafa H. G., Asi M. R., Jinap S. (2014). Variation in vitamin E level and aflatoxins contamination in different rice varieties. *J. Cereal Sci.* 60 352–355. 10.1016/j.jcs.2014.05.012.

Joosse, J. (1983). Hormonal Regulation of Calcification, with Particular Reference to the Hormonal Control of Eggshell Formation in Birds and Shell Growth in Molluscs. In *Biomineralization and Biological Metal Accumulation* (pp. 133-144). Springer, Dordrecht.

Kaaya, NA., Warren, H.L. (2005). A review of past and present research on aflatoxin in Uganda. *African Journal of Food Agriculture Nutrition and Development, Vol. 5, No. 1.*

Kamika, I. (2013). Determination of aflatoxins in peanut (Arachis hypogaea L.) collected from Kinshasa, Democratic Republic of Congo and Pretoria, South Africa: a comparative study (Doctoral dissertation).

Kamika, I., & Takoy, L. L. (2011). Natural occurrence of Aflatoxin B_1 in peanut collected from Kinshasa, Democratic Republic of Congo. *Food Control*, *22*(11), 1760-1764.

Kang, F., Ge, Y., Hu, X., Goikavi, C., Waigi, M. G., Gao, Y., & Ling, W. (2016). Understanding the sorption mechanisms of aflatoxin B1 to kaolinite, illite, and smectite clays via a comparative computational study. *Journal of hazardous materials*, *320*, 80-87.

Kapczynski, D. R., & King, D. J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle. *Vaccine*, *23*, 3424–3433.

Kaplan, E., & Richman, H. G. (1976). Effect of calcium on RNA and protein synthesis in the hypertrophied myocardium. *Proceedings of the Society for Experimental Biology and Medicine*, *152*(1), 24-28.

Katongole, C. B., Nambi-Kasozi, J., Lumu, R., Bareeba, F., Presto, M., Ivarsson, E., & Lindberg, J. E. (2012). Strategies for coping with feed scarcity among urban and peri-urban livestock farmers in Kampala, Uganda. *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, *113*(2), 165-174.

Kawasugi. S, Kawasima. K, Prisnar Siriacha, Prawat Tonboon-EK. (1988). Experimental studies on the field-dried Thai maize inoculated with Aspergillus flavus; Volume 1988 (1988) Issue 28 Pages 17-22

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.

Kermanshahi, H., Hazegh, A. R., & Afzali, N. (2009). Effect of sodium bentonite in broiler chickens fed diets contaminated with aflatoxin B1. *Journal of animal and veterinary advances*, *8*.

Khan, R. A., Khan, M. R., Sahreen, S., & Bokhari, J. (2010). Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat. *Food and Chemical Toxicology*, *48*(8-9), 2469-2476.

Khan, W. A., Khan, M. Z., Khan, A., Hassan, Z. U., Rafique, S., Saleemi, M. K., & Ahad, A. (2014). Dietary vitamin E in White Leghorn layer breeder hens: a strategy to combat aflatoxin B1-induced damage. *Avian pathology*, *43*(5), 389-395.

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, *8*(6), 713-722.

Kolossova, A., Stroka, J., Breidbach, A., Kroeger, K., Ambrosio, M., Bouten, K. and Ulberth, F. (2009). Evaluation of the Effect of Mycotoxin Binders in Animal Feed on the Analytical Performance of Standardized Methods for the Determination of Mycotoxins in Feed. *Joint Research Centre*.

Kumar, R., & Balachandran, C. (2009). Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. *Vet. arhiv*, *79*, 31-40.

Lehman, E. M., Soliman, A. S., Ismail, K., Hablas, A., Seifeldin, I. A., Ramadan, M., Wilson, M. L. (2008). Patterns of hepatocellular carcinoma incidence in Egypt from a population-based cancer registry. *Hepatology Research*, *38*(5), 465-473.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S. & DeCock, K. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental health perspectives*, *113*(12), 1763.

Li, F. Q., Yoshizawa, T., Kawamura, O., Luo, X. Y., & Li, Y. W. (2001). Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. *Journal of agricultural and food chemistry*, *49*(8), 4122-4126.

Li, Q., & Verma, I. M. (2002). NF-κB regulation in the immune system. *Nature reviews immunology*, *2*(10), 725.

Lichtman, A. H., Segel, G. B., & Lichtman, M. A. (1983). The role of calcium in lymphocyte proliferation.(An interpretive review).

Lillehoj. EB, F. Elling (2009). Environmental Conditions That Facilitate Ochratoxin Contamination of Agricultural Commodities. Journal Acta Agriculturae Scandinavica, Volume 33, 1983 - Issue 2

Lopez, Juarez Morbini, Rutz, Fernando, Mallmann, Carlos Augusto, and Toledo, Geni Salete Pinto. (2006). Addition of sodium bentonite as adsorbent of aflatoxins in broiler rations. Rural Science, 36 (5), 1594-1599.

Magan,N.,M. Olsen. (2004). Woodhead Publishing Ltd.Cambridge, England.

Magnoli, A. P., Monge, M. P., Miazzo, R. D., Cavaglieri, L. R., Magnoli, C. E., Merkis, C. I & Chiacchiera, S. M. (2011). Effect of low levels of aflatoxin B1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite. *Poultry science*, *90* (1), 48-58

Magnoli, A. P., Tallone, L., Rosa, C. A., Dalcero, A. M., Chiacchiera, S. M., & Sanchez, R. M. T. (2008). Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. *Applied Clay Science*, *40*(1-4), 63-71.

Mahmood, S., Younus, M., Aslam, A., & Anjum, A. A. (2017). Toxicological effects of aflatoxin B1 on growth performance, humoral immune response and blood profile of japanese quail. *japs: journal of animal & plant sciences*, *27*(3).

Mahmood, T., Pasha, T. N., & Khattak, F. M. (2011). Comparative Evaluation of Different Techniques for Aflatoxin Detoxification in Poultry Feed and Its Effect on Broiler Performance. In *Aflatoxins-Detection, Measurement and Control.*

Maye D, K. (1999). *Incidence of aflatoxin contamination in selected spice samples in Andhra Pradesh* (Doctoral dissertation, Acharya NG Ranga Agricutlrual University).

McClure R., Smith C., Dixon J.B. Bentonite properties, formation, and distribution as adsorbents of aflatoxin in grain—The Texas case study. In: Dixon J.B., Barrientos-Velázquez A.L., Deng Y., editors. Aflatoxin Control: Safeguarding Animal Feed with Calcium Smectite. American Society of Agronomy and Soil Science Society of America; Madison, WI, USA: 2014. pp. 11–16

Meissonnier, G. M., Pinton, P., Laffitte, J., Cossalter, A. M., Gong, Y. Y., Wild, C. P., ... & Oswald, I. P. (2008). Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicology and applied pharmacology*, *231*(2), 142-149.

Meleki, L. P., Mbwette, T. S. A., & Judicate, N. N. food borne diseases in Mbeya city, Mbeya region, Tanzania Key words: Water activity, Aflatoxins, Food borne diseases. *Health career and food vendors.*

Milićević, D. R., Škrinjar, M., & Baltić, T. (2010). Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. *Toxins*, *2*(4), 572-592.

Mishra, N. K., and S K Daradhiyar (1991). Mold flora and aflatoxin contamination of stored and cooked samples of pearl millet in the Paharia tribal belt of Santhal paragana, Bihar, India, *J. applied and environmental micro biology.*

Morteza Azizollahi Aliabadi, Fatemeh (Elham) Alikhani, Mahya Mohammadi and Reza Kazemi Darsanaki (2013). Biological Control of Aflatoxins 161-165.

Mugerwa, S., Zziwa, E., & Kabirizi, J. (2013). Effect of fortifying Aflatoxin-contaminated feeds with Ugandan bentonite on performance of broiler birds. *Appl Sci Report*, *3*, 106-109.

Muhammad A, Faqir Muhammad Anjum, Masood Sadiq Butt, Imran Pasha, Muhammad Atif Randhawa, Farhan Saeed & Khalid Waqas (2012) Aflatoxins: Biosynthesis, Occurrence, Toxicity, and Remedies, Critical Reviews in Food Science and Nutrition, 53:8, 862-874, DOI: 10.1080/10408398.2011.563154

Murugesan, G. R., Ledoux, D. R., Naehrer, K., Berthiller, F., Applegate, T. J., Grenier, B., Phillips, T. D. and Schatzmayr, G. (2015). Prevalence and Effects of Mycotoxins on Poultry Health and Performance, and Recent Development in Mycotoxin Counteracting Strategies. *Poultry Science*, 00:1–18.

Muture, B. N., & Ogana, G. (2005). Aflatoxin levels in maize and maize products during the 2004 food poisoning outbreak in Eastern Province of Kenya. *East African Medical Journal*, *82*(6).

NDP III. (2020). National Development Plan, Ministry of Finance, Planning and Economic Development

Neeff, D. V., Ledoux, D. R., Rottinghaus, G. E., Bermudez, A. J., Dakovic, A., Murarolli, R. A., & Oliveira, C. A. F. (2013). In vitro and in vivo efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B1. *Poultry Science*, *92*(1), 131-137.

NRC, "Nutrient Requirements of Poultry". 8th Revised Ed. National Academy Press, Washington, D. C. (1994).

Oguz H, Kurtoglu V, (2000). Effect of CLI on fattening performance of broiler chickens during experimental aflatoxicosis. Brit Poult Sci, 41, 512–517

Oguz, H. (2011). A review from experimental trials on detoxification of aflatoxin in poultry feed. *Eurasian J Vet Sci*, *27*(1), 1-12.

Oguz, H., Hadimli, H. H., Kurtoglu, V., & Erganis, O. (2003). Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. *Revue de Medecine Veterinaire*, *154*(7), 483-486.

Ortatatli M, Oguz H, (2001). Ameliorative effects of dietary CLI on pathological changes in broiler chickens during aflatoxicosis. Res Vet Sci, 71, 59–66

Osuret, J., Musinguzi, G., Mukama, T., Halage, A. A., Natigo, A. K., Ssempebwa, J. C., & Wang, J. S. (2016). Aflatoxin contamination of selected staple foods sold for human consumption in Kampala markets, Uganda. *Journal of Biological Sciences*, *16*(1), 44.

Osweiler, G. D., Jagannatha, S., Trampel, D. W., Imerman, P. M., Ensley, S. M., Yoon, I., & Moore, D. T. (2010). Evaluation of XPC and prototypes on aflatoxin-challenged broilers. *Poultry science*, *89*(9), 1887-1893.

Otsuki, T, John S. W, and Mirvat. S, (200la). What Price Precaution? European Harmonisation of Aflatoxin Regulations and African Groundnuts Exports. European Review of Agricultural Economics

Oznurlu, Y., Celik, I., Sur, E., Ozaydın, T., Oğuz, H., & Altunbaş, K. (2012). Determination of the effects of aflatoxin B1 given in ovo on the proximal tibial growth plate of broiler chickens: histological, histometric and immunohistochemical findings. *Avian pathology*, *41*(5), 469-477.

Pandey, I., & Chauhan, S. S. (2007). Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of aflatoxin AFB1. *British poultry science*, *48*(6), 713-723.

Parikh, S. J., & Yanovski, J. A. (2003). Calcium intake and adiposity. *The American journal of clinical nutrition*, *77*(2), 281-287.

Peng, X., Bai, S., Ding, X., Zeng, Q., Zhang, K., & Fang, J. (2015). Pathological changes in the immune organs of broiler chickens fed on corn naturally contaminated with aflatoxins B1 and B2. *Avian Pathology*, *44*(3), 192-199.

Phillips T.D., Afriyie-Gyawu E., Williams J., Huebner H., Ankrah N.A., Ofori-Adjei D., Jolly P., Johnson N., Taylor J., Marroquin-Cardona A. (2008). Reducing human exposure to aflatoxin through the use of clay: A review. Food Addit. Contam. 2008; 25:134–145.

Phillips, T. D., Lemke, S. L. & Grant, P. G. (2002). "Characterization of clay-based enterosorbents for the prevention of aflatoxicosis." Mycotoxins and Food Safety, 504, 157-171.

Pimpukdee, K. L. F. K., Kubena, L. F., Bailey, C. A., Huebner, H. J., Afriyie-Gyawu, E., & Phillips, T. D. (2004). Aflatoxin-induced toxicity and depletion of hepatic vitamin A in young broiler chicks: Protection of chicks in the presence of low levels of NovaSil PLUS in the diet. *Poultry science*, *83*(5), 737-744

Plumstead, P. W., Leytem, A. B., Maguire, R. O., Spears, J. W., Kwanyuen, P., & Brake, J. (2008). Interaction of calcium and phytate in broiler diets; Effects on apparent pr-ececal digestibility and retention of phosphorus. *Poultry Science*, *87*(3), 449-458.

Pluyer H R, Ahmed E M and Wei C 1 (1987). Destruction of aflatoxin on peanuts by oven and microwave roasting. Jr. of Food protec. 50: 504-508.

Prvulovic, D., Kojic, D., Grubor-Lajsic, G., & Kosarcic, S. (2008). The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. *Turkish Journal of Veterinary and Animal Sciences*, *32*(3), 183-189.

Qureshi, M. A., J. Brake, P. B. Hamilton, W. M. Hagler, and S. Nesheim. (1998). Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. Poult. Sci. 77:812–819

Radhika, G., & Paul Raj, R. (2006). Aflatoxicosis and its amelioration in black tiger shrimp, Penaeus monodon Fabricius.

Ravindra, R., Kaneko, S., Endo, T., Lakshmi, R. (2017). Spectroscopic Characterization of Bentonite. Journal of lasers, Optics and photonics, *Vol 4, issue 3*.

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses; an overview. *International journal of food microbiology*, *119*(1-2), 3-10.

Romoser, A. A., Marroquin-Cardona, A., Phillips, T. D. and Eastridge, M. L. (2013). Managing Risks Associated With Feeding Aflatoxin Contaminated Feed. *Tri-State Dairy Nutrition Conference.* (pp. 35-50).

Russell, R., Smith, C., & Dixon, J. B. (2014). Bentonite properties, formation, and distribution as adsorbents of aflatoxin in grain. The Texas case study. *Aflatoxin Control: Safeguarding Animal Feed with Calcium Smectite*, (aflatoxin control), 11-16.

Sadeghi, A. A., Ahmadi-Mazhin, H., Shawrang, P., & Mohammadi-Sangcheshmeh, A. (2012). Effects of MOS and heat activated sodium bentonite as aflatoxin absorbents on antibody titers against Newcastle disease and infectious bursal disease viruses in broiler chickens. *World Appl Sci J*, *18*, 127-129.

Sajan, M. P., Satav, J. G., & Bhattacharya, R. K. (1996). Alteration of energy-linked functions in rat hepatic mitochondria following aflatoxin B1 administration. *Journal of biochemical toxicology*, *11*(5), 235-241.

Salari. S, Kermanshah. H, Nasisi, M. (2006). Effect of sodium bentonite and comparison of pellet vs. mash on performance of broiler chickens. International journal of poultry science

Schneweis, I., Meyer, K., Engelhardt, G., & Bauer, J. (2002). Occurrence of zearalenone-4-β-D-glucopyranoside in wheat. *Journal of agricultural and food chemistry*, *50*(6), 1736-1738.

Sharline, K.S.B., Howarth, B.J. & Wyatt, R.D. (1980). Effect of dietary aflatoxin on reproductive chicks. Poultry Science, 72, 651–657.

Shepherd, G. S. (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and contaminants*, *25*(2), 146-151.

Shetty, P.H., Jespersen, L. (2006). Trends Food Sci Technology, 17, 48-55.

Shi, Y., Xu, Z., Sun, Y., Wang, C, Feng, J. (2009). Effects of two different types of motmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. *Turkish Journal of Veterinary and Animal Science*, 33: 15–20.

Soares, M. J., & She-Ping-Delfos, W. L. C. (2010). Postprandial energy metabolism in the regulation of body weight: is there a mechanistic role for dietary calcium?. *Nutrients*, *2*(6), 586-598.

Soufiani, G. N., Razmara, M., Kermanshahi, H., Velázquez, A. B., & Daneshmand, A. (2016). Assessment of aflatoxin B1 adsorption efficacy of natural and processed bentonites: In vitro and in vivo assays. *Applied Clay Science*, *123*, 129-133.

Southern, E. M., Case-Green, S. C., Elder, J. K., Johnson, M., Mir, K. U., Wang, L., & Williams, J. C. (1994). Arrays of complementary oligonucleotides for analysing the hybridisation behaviour of nucleic acids. *Nucleic Acids Research*, *22*(8), 1368-1373.

Stoj, D. (2001). The effect of exchangeable cations in clinoptilolite and montmo-rillonite on the adsorption of aflatoxin B1. *J. Serb. Chem. Soc*, *66*(8), 555-561.

Talapatra, I., & O'Connell, I. P. M. (2016). An attempt to develop the management guidelines of vitamin D supplementation in patients with primary hyperparathyroidism and co-existent vitamin D in the United Kingdom. *Contains 84 pages from 85 to 164 (inclusive of all advertisements)*, *17*(2), 148

Tedesco, D., Steidler, S., Galletti, S., Tameni, M., Sonzogni, O., & Ravarotto, L. (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. *Poultry science*, *83*(11), 1839-1843.

Teleb, H.M., A.A. Hegazy and Y.A. Hussein, (2004). Efficiency of kaolin and activated charcoal to reduce the toxicity of low level of aflatoxin in broilers. Scient. J. King Faisal Univ. (Basic Applied Sci.), 5: 145-160.

Tenorio Arvide M.G., A.L.Barrientos Velazquez, J.B.DixonI. Kannewischer. (2008). Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan. Applied Clay Science Volume 40, Issues 1-4, pp,201-208

Thirumalaisamy, G., Muralidharan, J., Senthilkumar, S., Hema Sayee, R., & Priyadharsini, M. (2016). Cost-effective feeding of poultry. *International Journal of Science, Environment and Technology*, *5*(6), 3997-4005.

Tung, H. T., Wyatt, R. D., Thaxton, P., & Hamilton, P. B. (1975). Concentrations of serum proteins during aflatoxicosis. *Toxicology and applied pharmacology*, *34*(2), 320-326.

Tung, H.T., W.E. Donaldson and P.B. Hamilton. (1972). Altered lipid transport during aflatoxicosis. J. Toxicol. Applied Pharm., 22: 97-104.

Turner, P. C., Sylla, A., Gong, Y. Y., Diallo, M. S., Sutcliffe, A. E., Hall, A. J., & Wild, C. P. (2005). Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: a community-based intervention study. *The Lancet*, *365*(9475), 1950-1956.

UBOS (Uganda Bureau of Statistics) (2013). Statistical abstract for Uganda

Upadhaya, S. D., Park, M. A., & Ha, J. K. (2010). Mycotoxins and their biotransformation in the rumen: a review. *Asian-Australasian Journal of Animal Sciences*, *23*(9), 1250-1260.

Van Rensburg, C. J., Van Rensburg, C. E. J., Van Ryssen, J. B. J., Casey, N. H., & Rottinghaus, G. E. (2006). In vitro and in vivo assessment of humic acid as an aflatoxin binder in broiler chickens. *Poultry science*, *85*(9), 1576-1583.

Verma, J., Johri, T. S., & Swain, B. K. (2003). Effect of varying levels of aflatoxin, ochratoxin and their combinations on the performance and egg quality characteristics in laying hens. *Asian Australasian Journal of Animal Sciences*, *16*(7), 1015-1019.

Viljoen, J.H. (2003). Mycotoxins in grain and grain products in South Africa and proposals for their regulation. Ph.D. Thesis. Pretoria: University of Pretoria.

Walker, A. W., & Lawley, T. D. (2013). Therapeutic modulation of intestinal symbiosis. *Pharmacological Research, 69* (1), 75-86.

Wang, C., Waje, M., Wang, X., Tang, J. M., Haddon, R. C., & Yan, Y. (2004). Proton exchange membrane fuel cells with carbon nanotube based electrodes. *Nano letters, 4*(2), 345-348.

Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., & Telang, A. G. (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. *Toxicology, 215*(1-2), 37-47.

Watts, K. C., Bilanski, W. K., & Menzies, D. R. (1986). Comparison of drying corn using sodium and calcium bentonite. *Can. Agric. Eng, 28*, 35-41.

Whitlow, L.W., and W.M. Hagler, Jr. (2005). Mycotoxins in feeds. Feedstuffs 77 (No. 38):69-79.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American journal of clinical nutrition, 80*(5), 1106-1122.

Wiseman, D. (2006). *A global hunger index: Measurement concept, ranking of countries, and trends* (Vol. 212). Intl Food Policy Res Inst.

Wu, F. and Khlangwiset, P. (2010). Evaluating the Technical Feasibility of Aflatoxin Risk Reduction Strategies in Africa. *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment.* 27(5): 658–676

Wu, F., Narrod, C., Tiongo, M. and Liu, Y. (2011). The health economics of aflatoxin: global burden of disease. *aflaControl Internal Food Policy Research Institute*, working paper 4.

Wyatt, R. D., Doerr, J. A., Hamilton, P. B., & Burmeister, H. R. (1975). Egg production, shell thickness, and other physiological parameters of laying hens affected by T-2 toxin. *Applied microbiology, 29*(5), 641-645.

Yang, C. F., J. Liu, S. Wasser, H. M. Shen, C. E. C. N. Ong. (2000). "Inhibition of Ebseien on Aflatoxin B1-induced Hepatocarcinogenesis in Fischer 3 Rats." Carcinogenesis, 21, 2237-2243.

Yan-Ni Yin (2008). Biological control of aflatoxin contamination of crops, journal of Zhengjiang University.

Yanovski, J. A., Parikh, S. J., Yanoff, L. B., Denkinger, B. I., Calis, K. A., Reynolds, J. C., ... & McHugh, T. (2009). Effects of calcium supplementation on body weight and adiposity in overweight and obese adults: a randomized trial. *Annals of internal medicine, 150*(12), 821-829.

Yildiz, A. O., Parlat, S. S., & Yildirim, I. (2004). Effect of dietary addition of live yeast on some performance parameters of adult Japanese quail induced by aflatoxicosis. *Revue de médecine vétérinaire, 155*(1), 38-41.

Yunus, A. W., Razzazi-Fazeli, E., & Bohm, J. (2011). Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues. *Toxins, 3*(6), 566-590.

Yunus, A.W., E. Razzazi – Fazeli, and J. Bohm. (2013). "Aflatoxin B1 Affecting Broiler's Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues on Toxins." Basel, 3(6), 566–590.

Zaghini, A., Martelli, G., Roncada, P., Simioli, M., & Rizzi, L. (2005). Mannanoligosaccharides and aflatoxin B1 in feed for laying hens: effects on egg quality, aflatoxins B1 and M1 residues in eggs, and aflatoxin B1 levels in liver. *Poultry science*, *84*(6), 825-832.

Zhu, Y. J., Li, Y., Wang, C., Ma, Y., Li, X. L., Zhang, Y., Ji W. Y., Shao, C. M. (2014). Effects of HSCAS and YGM on mycotoxins challenged broilers raised under different environmental conditions. Journal of Food, Agriculture and Environment, 12(1), 474-479.

Zimbe, j. j. (2012). Socio-economic impact of dairy cattle zero grazing production system on the livelihoods of the rural poor in Uganda (a case study of sironko dairy heifer project) (doctoral dissertation, Makerere university).

Appendix 1: Dehulled ground nuts after soaking (a), after 7 day incubation. (b)

a

b

Appendix 2: Percentage Composition of key elements in calcium and sodium bentonites

Element (%)	Sodium bentonite (NaB)	Calcium bentonites (CaB)
O	51.21	48.76
Na	4.49	1.78
Mg	1.37	1.50
Al	8.342	9.07
Si	22.22	25.85
Cl	2.74	1.25
K	0.30	0.55
Ca	0.38	1.99
Ti	0.39	0.85
Fe	3.46	6.85
Cu	0.12	0.76
Zn	0.00	0.90
S	0.00	0.0
Na/Ca ratio	11.9	0.89

Appendix 3: Determined nutrient composition of ingredients used in experimental diet formulation

Nutrient	Fish meal	Soybean meal	Maize	Maize bran
Dry matter (g/kg)	925	890	915	918
Crude protein (g/kg DM)	703	503	96	139
Crude fat (g/kg DM)	105	12	33	49
Ash (g/kg DM)	216	88	21	57
Nitrogen free extract (g/kg DM)	801	397	98	109
Phosphorous (g/kg DM)	40	7.3	52	39
Gross energy (MJ/kg DM)	196	192	189	191
Calcium (g/kg DM)	40.2	41	54	43