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Potential of Tanzanian local clay and ash based materials for binding aflatoxins in animal feeds

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NM-AIST

POTENTIAL OF TANZANIAN LOCAL CLAY AND ASH BASED MATERIALS FOR BINDING AFLATOXINS IN ANIMAL FEEDS

Emmanuel Mathayo Ayo

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Potential of Tanzanian local materials was explored for alleviating aflatoxin-contamination of feeds. Preliminarily, farmers' awareness of aflatoxins was assessed using data collected from a random sample of 258 households in Meru District in Arusha, Tanzania. An *in-vitro* experiment, was used to evaluate aflatoxin-binding capacity of test materials (TMs); clays from Arusha (AC), Kilimanjaro (KC), Coast (CC) and Morogoro (MC) and ash-materials identified as volcanic ash (VA) and rice-husk ash (RA) in buffered solution. The TMs were compared for binding capacity with a reference-binder (Mycobind®, R). An *in-vivo* complete randomized experiment was used to evaluate aflatoxin-binding capacity of the TMs in reducing bioavailability of dietary aflatoxins using 109 rats in unbalance eight groups. On basal diet, dietary treatments DAC, DKC, DCC, DMC, DVA, DRA, each containing 2% of one of the TMs, DR containing 2% of R and DC (control) were formulated. One rat-group was fed one of the diets. Effects of the diets on feed intake (FI), growth rate (GR), feed conversion efficiency (FCE), packed-cell-volume (PCV), serum-total protein, albumin, globulin and albumin/globulin ratio (AGR) and parameters of liver, kidney and spleen of the rats were assessed. About 52%, 8% and 32% of respondents were aware that fungal toxins may occur in feeds, be transferred into foods of animal origin and are detoxifiable, respectively. About 28% of the respondents had ever heard about aflatoxins. Significantly ($p<0.05$), ≥secondary education, biological/life science exposure and short-time in livestock industry, positively influenced farmers' awareness of aflatoxins. Statistically, *in-vitro* aflatoxin-binding capacity of RA (84.7%) or AC (72.6%) was comparable to that of R (98.1%). Each of TMs could bind >94% of aflatoxin-B1.Statistically, FCE (16.6%) of DKC and AGR of DVA (1.2) were comparable to that of DR (17.5%, 1.2), respectively. Relative weight of liver of DRA (3.8%) was statistically normal comparable to that of DR (3.7%). Only DVA showed normal tissues of liver, kidneys and spleen. Farmer' awareness of aflatoxins was low, calling for more sensitization. Based on the *in-vitro* and *in-vivo* experiments, RA seems to be the best aflatoxin-binding material. Further testing of the TMs using farm animals and their combined performance effect are recommended.

Key words: Aflatoxins, awareness, feeds, test-binding materials, *in-vitro test, in-vivo* test

DECLARATION

I, Emmanuel Mathayo Ayo do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

_____ _____

Emmanuel M. Ayo Date

Name and signature of candidate

The above declaration is confirmed

_____ _____

Prof. Martin E. Kimanya Date

Name and signature of supervisor

_____ _____

Prof. Germana H. Laswai Date

Name and signature of supervisor

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Dr. Athanasia O. Matemu Date

Name and signature of supervisor

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled "*Potential of Tanzanian local clay and ash based materials for binding aflatoxins in animal feeds*" in fulfilment of the requirements for the Degree of Doctor of Philosophy in the School of Life Sciences of the Nelson Mandela African Institution of Science and Technology (NM-AIST).

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Prof. Martin E. Kimanya Date

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Prof. Germana H. Laswai Date

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Dr. Athanasia O. Matemu Date

Name and signature of supervisor

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I thank all individuals and institutions that have not been mention here, but in one way or another have hands on this success. The glory of the God be with them.

DEDICATION

To my Wife

Eliaichi Makundi

To our sons, Agape and Baraka and daughters, Caroline and Debora

.…To everything there is a season, and a time for every purpose under the heaven…...

…..Eccles. 3:1…..

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AC	Arusha Clay
AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AGR	Albumin-Globulin Ratio
AOAC	Association of Official Analytical Chemists
A_s	Absorbance of Sample Solution
A_{std}	Absorbance of Standard Solution
BMs	Binding materials
BW	Body Weight
CC	Coast clay
CI	Confidence Interval
C_s	Concentration of Sample Solution
C_{std}	Concentration of Standard Solution
CVMBS	College of Veterinary Medicine and Biomedical Sciences
DAC	Dietary Treatment of Arusha Clay
DC	Dietary of Control Treatment
DCC	Dietary Treatment of Coastal Clay
DKC	Dietary Treatment of Kilimanjaro Clay
DMC	Dietary Treatment of Morogoro Clay
DR	Dietary Reference of Binder
DRA	Dietary Treatment of Rice-Husk Ash
DVA	Dietary Treatment of Volcanic Ash
EAC	East African Community
ELISA	Enzyme-Linked –Immunosorbent
EU	European Union
FAO	Food and Agriculture Organization
FBW	Final Body Weight

FCE	Feed Conversion Efficiency
FDA	Food and Drug Administration
FI	Daily Feed Intake
FS	Florescence Spectrophotometry
GC	Gas Chromatography
GR	Daily Growth Rate
HPLC	High Performance Liquid Chromatography
IBS	India Bureau of Standards
IRDP	Institute of Rural development Planning
KC	Kilimanjaro Clay
KW	Kidney Weight
LFD	Lateral Flow Devices
LITA	Livestock Training Agency
LSBE	Life Sciences and Bioengineering
LW	Liver Weight
MC	Morogoro Clay
MS-Excel	Microsoft-Excel
NAIC	National Artificial Insemination Centre.
ng/g	Nano-gram per gram
NM-AIST	Nelson Mandela African Institution of Science and Technology
NRC	National Research Council
NS	Non-significant
PCV	Packed Cell Volume
Ppb	Parts per Billion
Ra	Rice-Husk Ash
RAI	Radioimmunoassay
RESC	Research Ethics Sub-Committee
RWK	Relative Weight of Kidney
RWL	Relative Weight of Liver
RWO	Relative weight of organ
RWS	Relative Weight of Spleen
SPMC	SUA Pest Management Centre

SUA	Sokoine University of Agriculture
SW	Spleen Weight
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TICD	Tengeru Institute of Community Development
TLC	Thin Layer Chromatography
TMs	Test Materials
TUMA	Tumaini University Makumira
UA	University of Arusha
URT	United Republic of Tanzania
USA	United States of America
VA	Volcanic Ash
WHO	World Health Organization.

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Worldwide, larger proportions of population in developing countries are estimated to dwell in rural areas. In Tanzania, about 80% of the entire population is living in rural areas, majority working in agricultural activities, particularly in crop-livestock mixed system (Magali, 2013). Livestock keeping as an industry that involves raising different farm animals is an important element in the rural livelihood in Tanzania, employing about 36% of the rural population on itself (Matthew *et al*., 2016). The livestock sub-sector has been contributing about five percent of the national Gross Domestic Product (GDP) and about 22% to the cash income of households in the rural set-up. The sub-sector also contributes enormously to high quality nutrition of households and entire food security of the nation and provides manure to improve soil fertility (Engida *et al*, 2015). Nevertheless, the contribution of the sub-sector has been stagnant due to slow growth rates, high mortality rates, low production and reproductive rates, low off-take rates and poor quality of its final products (Engida *et al*., 2015). Some of the factors contributing to these negative outcomes include effects of livestock diseases and health related disorders (Matthew *et al.*, 2016). Among the health disorders are nutritional disorders due to presence of anti-nutritional and toxic factors in feeds and impair proper utilization of feed nutrients by animals or cause toxicities.

Among the known agents which occur naturally in feeds and cause adverse effects on animal health and productivity, are natural toxins produced by toxigenic fungal organisms. The most problematic fungal toxins also known as mycotoxins are aflatoxins (Atherstone *et al.*, 2011). Aflatoxins are among mycotoxins produced by toxigenic fungal organisms, mainly of *Aspergillus spp*, particularly *A. flavus* and *A. parasiticus* (Kaoud, 2012; Qureshi *et al.*, 2015). Four types of aflatoxins denoted as B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) occurring abundantly in environment are considered more important in health and agricultural economy at large (Dhanasekaran *et al.*, 2011; Jen & Chen, 2017; Lopez *et al.*, 2002). Among the four forms of the common aflatoxins, AFB1 is the most toxic and abundant (Feddern *et al*., 2013) and is categorized by the World Health Organization (WHO) as a confirmed carcinogen (Feddern *et al.*, 2013). Aflatoxins M1, a metabolite of AFB1, secreted in milk and eggs also cause chronic aflatoxicosis in human (Arapcheska *et al.*, 2015;

Grace, 2013; Khan *et al.*, 2013). Aflatoxins have been known for about six decades now since their discovery and have been associated with adverse impacts on human and animal health and production, causing great economic losses (Applegate *et al.*, 2009). Due to these health and economic problems caused by these toxins, a lot of efforts have been directed to study them, their occurrence, associated effects and means to manage them. Over years, a lot of information about aflatoxins has been gathered, yet their hazards are alarming showing the need to search for more information about these toxins. Information about aflatoxins are documented pertaining to issues related to their nature, source, properties, conditions for occurrence, their impacts on health and economy and means to manage them and their impacts. Of the major concerns about aflatoxins are on how to control their occurrence and mitigate hazardous effects of contamination of food and feeds. In this respect the importance of aflatoxins as their adverse impacts on animal health and production are explained.

Globally, the region lying between 40 $^{\circ}$N and 40 $^{\circ}$S latitudes is generally at a risk of aflatoxin contamination of foods and feeds (Unnevehr & Grace, 2013; Williams *et al.*, 2004). The regions within 35 $^{\circ}$N and 35 $^{\circ}$S where most of developing low-income countries are found are even at higher risk of exposure of these toxins (Abyaneh *et al.*, 2014). Food crops are contaminated with aflatoxins originally from the field following infection by toxigenic fungi, though under normal environmental conditions these fungal organisms are sporadic and less toxigenic. Susceptibility of crops to the fungi increases with field stressful conditions such as drought and pest invasion of the harvested crops produced under high temperature and humidity (Atanda *et al.*, 2011).

Conditions favouring aflatoxin production are typically found in the humid tropical and sub-tropical regions (WHO, 2018). Aflatoxins occur and extend all the way along the food chain from field, during transportation, storage, processing to consumption point, provided that the conducive conditions for growth of the toxigenic fungi are attained (Atanda *et al*., 2011).

Contaminated crops are the primary source of aflatoxins to humans and animals when ingested as foods or feeds. Humans also consume metabolites of the aflatoxins in foods of animal origins such as milk, dairy products, eggs and meat from animals fed on aflatoxin contaminated feeds (Grace, 2013). Ingestion of aflatoxins by both humans and animals may lead to aflatoxicosis which is a condition of aflatoxin toxicity (Khan *et al*., 2013). Aflatoxicosis may be either acute or chronic if the level of aflatoxin intake is high in a short term or low and prolonged respectively. Acute aflatoxicosis may lead to fatal cases, which

are mostly reported in humans than in animals (Atherstone *et al*., 2016). Chronic aflatoxicosis is more problematic since it is associated with long term adverse impacts on health of humans and animals. In humans, it is associated with stunting in infants and children, low immunity, liver cancer, renal failure and mental disturbance (Bbosa *et al*., 2012).

According to Grenier and Applegate (2013) chronic aflatoxicosis impairs metabolic functions leading to long-term ailments, nutritional disorders, poor production performance and ultimately economic losses. Specifically, chronic aflatoxicosis in animals cause poor feed conversion efficiency and lead to decreased average daily gain and overall growth (Andretta *et al.*, 2012; Atherstone *et al*., 2016). Furthermore, it predisposes animals to more health risks by suppressing immunity (Atherstone *et al*., 2016; Dhanasekaran *et al*., 2011; Paulín *et al*., 2011).

Larger proportion of aflatoxins ingested by animals come from supplementary feeds which are mainly by-products of crops such as cereal brans and oil seed cakes (McDonald *et al.*, 2011; Joseph & Aikoh, 2017). These by-products accumulate the larger part of the total aflatoxins formed in crops prior crop processing (Nziramasanga *et al*., 2005). Any faulty handling of these feeds, mostly during storage, which is common at farm level, can lead to more formation of aflatoxins in the feeds. Studies show that chances to have aflatoxin-free feeds are a bit narrow in tropical and subtropical regions where environmental temperatures and humidity support high growth of the toxigenic fungi (Grace, 2013; WHO, 2018). Any level of aflatoxins in feeds adds up to the risk of the aflatoxicosis (Sassahara *et al*., 2005). This implies that aflatoxin menace may be regarded as a sensitive public health concern demanding for proper means of mitigation.

In the current study, aflatoxin alleviation as a concept refers to reduction of the adverse effects of aflatoxins on well-being of animals consuming the toxins in contaminated diets. In economic perspective as is also applied in this study, mitigation of aflatoxins means to minimize degree of any loss or harm due to aflatoxin impacts on animal production.

Though aflatoxin hazards are alarming, the general public in developing nations seems to be less informed that about the toxins and their associated impacts (Grace, 2013; Unnevehr & Grace, 2013). In addition, information about the level of awareness of aflatoxins in relation to socio-economic factors in various localities in Tanzania is scanty (Kamala *et al*., 2016). Farmers seem to be less aware about aflatoxin contamination of feed (Kajuna *et al.*, 2013).

Less or no information is available on awareness of aflatoxin contamination of feeds even in the risky areas of high aflatoxin contamination. Farmers' awareness in solving a farming problem may be considered as the first step towards designing mitigation measures. Therefore, documenting the level of awareness of aflatoxins and the impacts of their contamination of feeds such farmers is important in setting plans to roll back risks of the toxins.

Assessment of awareness of aflatoxin contamination of feeds is necessary as a starting point in managing the toxins in feeds. In addition, developing practical strategies to counteract aflatoxins in animal feeds, which are acceptable at local level is also important. A number of practical strategies have been devised and are explained with mix of successes and limitations. The strategies include first to reduce the growth of toxigenic fungi and aflatoxin production by applying pre-and post-harvest strategies in farm crops. Pre-harvest strategies include control of plant pests and weeds, breeding of fungal-resistant crop varieties, biocontrol using atoxigenic fungi and timed harvesting (Kumar *et al*., 2017). Pre-harvest strategies involve maintenance of field conditions aiming to suppress plant infection by the toxigenic fungi. Post-harvest strategies, targeting reduction of the toxigenic fungi and production of aflatoxins in storage, transportation and processing chain including sorting and drying (Waliyar *et al.*, 2015). All of these strategies do not ensure total control of aflatoxin formation in feeds since some factors such as those related to climate and whether are difficult to control. The toxigenic fungi may still grow and enter the food/feed chain and proliferates.

The second category of strategies for control of aflatoxins in foods and feeds are those involving direct detoxification of contaminated products using physical and chemical techniques. The physical strategies include thermal inactivation and irradiation while chemical strategies include treatment of the foods and feeds with acidic or alkaline solutions, ozone treatment and ammoniation and biological strategies are such as detoxification by microbial agents (Bandyopadhyay *et al.*, 2016). These technical strategies are also hampered by some limitations including cost implications, requirement of complicated facilities, reduction of dietary palatability and nutritional values and may create dangers of unsafe residues of the applied chemicals and agents (Devreese, 2013).

Binding aflatoxins in feeds to lower the systemic availability of the toxins once ingested by an animal, has been found to be the most feasible measure to control dietary impact of the toxins (Kolosova & Stroka, 2012). Use of binders (also called adsorbents or sequesters) of aflatoxins is regarded salient means since the potential binders bind aflatoxins in the gastrointestinal tract of the animal to form aflatoxin-binder complexes. The complexes pass out of the animal through faeces, limiting absorption and bioavailability of the toxins into the animal system (Phillips *et al*., 2002). However, integration of the available strategies is instrumental to mitigate aflatoxins in feeds. The aflatoxin-binding technology may be considered superior over several strategies especially on the convenience of use, but also the fact that the toxins are blocked prior to absorption (Phillips *et al*., 2002) and thus limit the toxins getting into the animal body system.

1.2 Statement of the problem

Contamination of animal feeds by aflatoxins have adverse impacts on health, production of animals and ultimately cause economic losses in livestock industry. Despite of the efforts that have been applied to combat aflatoxin contamination of feeds using various strategies, the challenge is still prevailing. Some factors need to be considered so as to contribute to the solution regarding the challenges of aflatoxin contamination of feeds. Information on the status of awareness status of aflatoxin of contamination in feeds among farmers is one of the key strategies in safe handling of feeds. The information may be the entry point for mitigation of aflatoxin contamination of feeds. In Tanzania, available information about aflatoxin awareness is mostly related to human food resources while contamination of feed is scanty reported. The current study endeavoured to feel the gap. Furthermore, there is a need of physical intervention to address aflatoxin contamination of feed. Aflatoxin contamination of feeds in the country is high that may create great health threats and economic losses (Kajuna *et al*., 2013; Mushi *et al*., 2018), yet no local strategies are in place for mitigation. The study endeavoured to explore means to address the challenge using clay and ash-based materials available in Tanzania.

Use of the binders that can hold the toxins in feed and block their entry into the vital systems of the animal body practically efficacious. Some materials in certain countries have been tested and refined as binders of aflatoxins in feeds. Few of these commercial binders such as Mycobind® are imported to Tanzania, but they are relatively too expensive for farmers to afford that may limit their applicability.

1.3 Rationale of the study

Efforts to alleviate impacts of aflatoxins on animal health and ultimately on the performance of animals in Tanzania are necessary through development of means to utilize available materials on local context. This goes with generation of information on these materials to build as data base, but also contributing to strategies done in other nations trying to reduce exposure of aflatoxins to animals and humans. The strategies may be considered in two main approaches. One of approach is social based, associated with involving farmers as main actors in day to day processes where aflatoxins are encountered. So efforts particularly assessment of awareness for the purpose raising it through public sensitisation about aflatoxins is imperative. This was part of the current study aiming to build common understanding between farmers and developers of technologies for aflatoxin mitigation. The second approach is physical based, associated with practical means to render aflatoxins less hazardous once they occur in feeds. In this study efforts to assess possibilities of utilizing local materials deemed to be potential in immobilizing aflatoxins in feeds were applied and give promising results. Tanzania is endowed with clay and ash-based materials deemed to bind aflatoxins in feeds. However, these materials being in crude form, need to be tested to explore for their potential capacity to bind aflatoxins as well as the inherent properties that render them capable of binding the toxins. Studies on possibilities of using clay and ash-based materials of Tanzanian origin for the purpose of binding aflatoxins have never been carried in the country. The results obtained may be utilized for further studies, academic purposes, industrial purposes and in other applications.

1.4 Objectives

1.4.1 General objective

To assess the potential of Tanzanian local clay and ash based materials for binding aflatoxins in animal feeds.

1.4.2 Specific objectives

(i) To assess the socio-economic factors influencing awareness of aflatoxins among livestock farmers in relation to animal feeding.

(ii) To evaluate the capacity of selected local clay and ash-based materials to bind aflatoxins in an *in-vitro* solution.

(iii) To explore the chemical properties influencing aflatoxin-binding capacity of the clay and ash-based materials.

(iv) To assess the *in-vivo* potential of the selected local clay and ash-based materials in reducing bio availability of dietary aflatoxins to animals.

1.5 Research questions

(i) What are the socio-economic factors influencing awareness of aflatoxins among livestock farmers in relation to animal feeding?

(ii) Are the selected local clays and ash-materials capable of binding aflatoxins in an *in-vitro* solution?

(iii) What are the chemical properties influencing aflatoxin-binding capacity of the clay and ash-based materials?

(iv) What is the *in-vivo* potential of the selected local clay and ash-based materials in reducing bioavailability of dietary aflatoxins to animals?

1.6 Significance of the study

Generated information about awareness of aflatoxins is instrumental in designing intervention aiming to involve farmers in mitigating contamination of feeds, particularly to be used in extension services. Information about potential capacity of the local resources of clay and ash based materials is also instrumental in developing aflatoxin binders in feeds. The information is useful to policy makers, researchers and dealers of animal feeds. Combination of the two sets of information generated can contribute in efforts tomitigate aflatoxin exposure and impacts to safeguard public health and economy.

1.7 Delineation of the study

Results of the study are based on the explored measures to alleviate adverse effects of aflatoxin-contaminated feeds for improving performance of animals. Specifically, information was presented in relation to: (a) the factors influencing awareness of farmers

about aflatoxin contamination of feeds; (b) the capacity of selected local clay and ash-based materials to bind aflatoxins in buffered solution and (c) the chemical properties influencing aflatoxin-binding capacity of the studied clay and ash-based materials and their potential in reducing bioavailability impact of dietary aflatoxins on the animal health and production performance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background of aflatoxins

Aflatoxins are problematic natural toxins formed in many crops. Crops with high calorific value such as corn, peanuts, cottonseed, rice, sweet potatoes, potatoes, wheat, oats, barley, millet, sesame, sorghum, cacao beans and almonds and other nuts are more prone to aflatoxin formation (Coppock, 2018). The crops which are important source of food of humans and animals, are increasingly affected by fungal infections, due to a number of factors including climate change effects (Medina *et al.,* 2014). Some of the fungi are toxigenic such that they produce potent toxins on crops and silently, can harm humans and animals. Some of these deadly fungal toxins are aflatoxins which are secondary metabolites of toxigenic fungi, mainly the *Aspergillus* spp, particularly *Aspergillus flavus* and *Aspergillus parasiticus* (Chase *et al*., 2013; Feddern *et al.*, 2013). Aflatoxins were discovered in early 1960s in England when scientists were investigating the agent responsible for death of more than 100 000 turkey birds that had died of unknown disease termed "X" disease of Turkeys, and later it was diagnosed to have been caused by aflatoxins formed in peanut meals (Applegate *et al.*, 2009). Figure 1 summarises the chronological background of the discovery of aflatoxins and the great research work that has been done since early 1960s to 2000s.

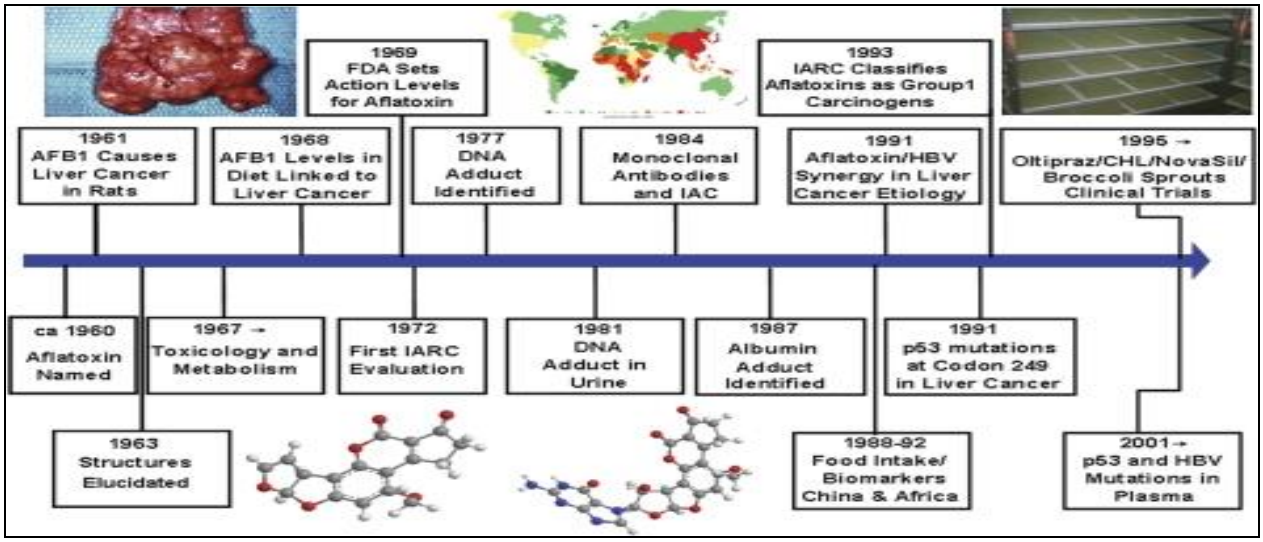


Figure 1: Timeline for aflatoxin discovery and the consequential events (Kensler *et al.*, 2011)

Chemically, aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) are related difuranocoumarin compounds (Fig. 2). The groups B and G of aflatoxins are produced by *Aspergillus flavus and A. Parasiticus* (Chase *et al*., 2013; Feddern *et al.*, 2013) under conditions favouring the fungi. The aflatoxin groups have different molecular structures where the B-group aflatoxins have cyclopentane ring whereas G-group has lactone ring (Wacoo *et al.*, 2014). The two groups are easily distinguished using ultraviolet (UV) light. Under the UV light, the aflatoxins in B-group and G-group display bluish and green is in fluorescence respectively (Wacoo *et al*., 2014). Varying combinations of water activity, temperature on expression of aflatoxin-producing gene of *Aspergillus spp* are major factor determining production of either B or G groups of aflatoxins. Temperature is the key favourable factor for aflatoxin B synthesis while water activity favours more G-group of the toxins (Heydt *et al*., 2010).

Aflatoxins are the most potent toxins among fungal toxins (mycotoxins) and abundantly occurring in many edible products that may predispose human and animals to chronic and acute or fatal episodes. Acute aflatoxicosis, is the condition that results when humans or animals ingest food or feed containing moderate to high levels of aflatoxins and can lead to death. Chronic aflatoxicosis occurs on prolonged ingestion of low levels of aflatoxins and is associated with, digestive disorders, stunting, immunosupressions, central nervous system interference, liver cancer, fertility impairment, faetal malformations and low birth weight (Bbosa *et al*., 2013; Dhanasekaran *et al*., 2011; Paulín *et al*., 2011; Williams *et al.*, 2004).

Aflatoxins are widely spread in nature and occur in many consumable organic materials used as food for human and animals, favoured by moisture exceeding 7% at temperature range between 24 °C and 35 °C (Williams *et al*., 2004). When ingested by animals in contaminated feeds, their metabolites become intermediate toxic residues in the foods of animal origin through which humans may encounter chronic exposure to aflatoxins (Atherstone *et al*., 2016). It is estimated that globally the region lying between 40 °N and 40 °S latitudes is generally at high risk of aflatoxin exposure through foods and feeds (Grace, 2013; Williams *et al*., 2004). Almost all feed resources may contain aflatoxins especially when invaded by the mouldy organisms (Sassahara *et al*., 2005).

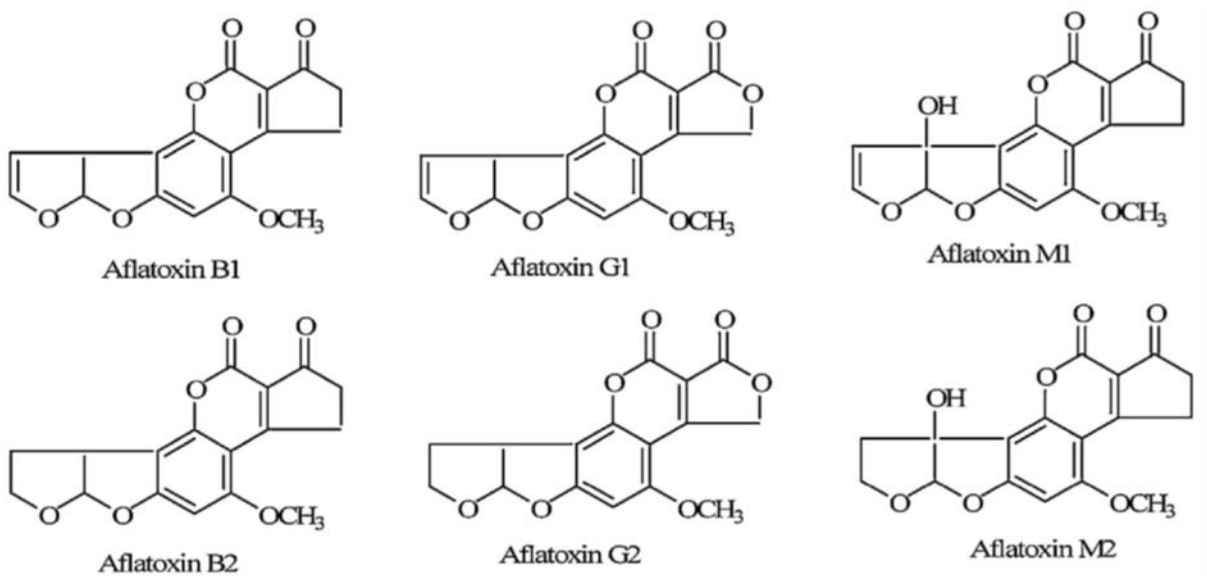


Figure 2: Structures of aflatoxins B, G and M (Zhang *et al*., 2014)

2.2 Global burden of aflatoxins

Worldwide, aflatoxins cause a number of problems related to health and economic outcomes. It is now well-known that aflatoxins are carcinogens. The AFB1produced naturally by fungi of the *Aspergillus* spp, mostly by *Aspergillus flavus* (Feddern *et al*., 2013). International Agency for Research on Cancer (IARC) has classified AFB1 as a class 1 carcinogen (Udomkun *et al*., 2017). The Food and Agriculture Organization (FAO) has estimated that 25% of the food produced worldwide is contaminated with aflatoxins (Williams *et al*., 2004). About 4.5 billion people worldwide, majority in low-income countries face risk of unknown levels of aflatoxin exposure (Ghahfarokhi *et al.*, 2013). As previously stated, the risk is high in the global area within 40 °N and 40 °S which include many tropical countries (Fig. 3) (Abyaneh, 2014; Williams *et al*., 2004). Humans encounter aflatoxin exposure, mainly through direct consumption of contaminated food crops and contaminated foods of animal origin from animals ingested aflatoxin-contaminated feeds. The former may lead to acute level and cause fatal cases. Examples of acute exposure of aflatoxins to humans are those that occurred in 2004 in Kenya with 317 cases of which 125 were deaths (Lewis *et al.*, 2005). In Tanzania, 68 cases were confirmed and 20 died in 2016 following consumption of aflatoxin contaminated maize (Kamala *et al*., 2018). Intake of aflatoxins from foods of animal origin is also important as it predisposes humans to chronic consummation of aflatoxin metabolites which are also hazardous (Gong *et al.*, 2016). Animals encounter aflatoxins directly from

ingestion of contaminated feeds. In this review health and economic impacts of aflatoxins in relation to aflatoxin contamination of feeds are of major concern.

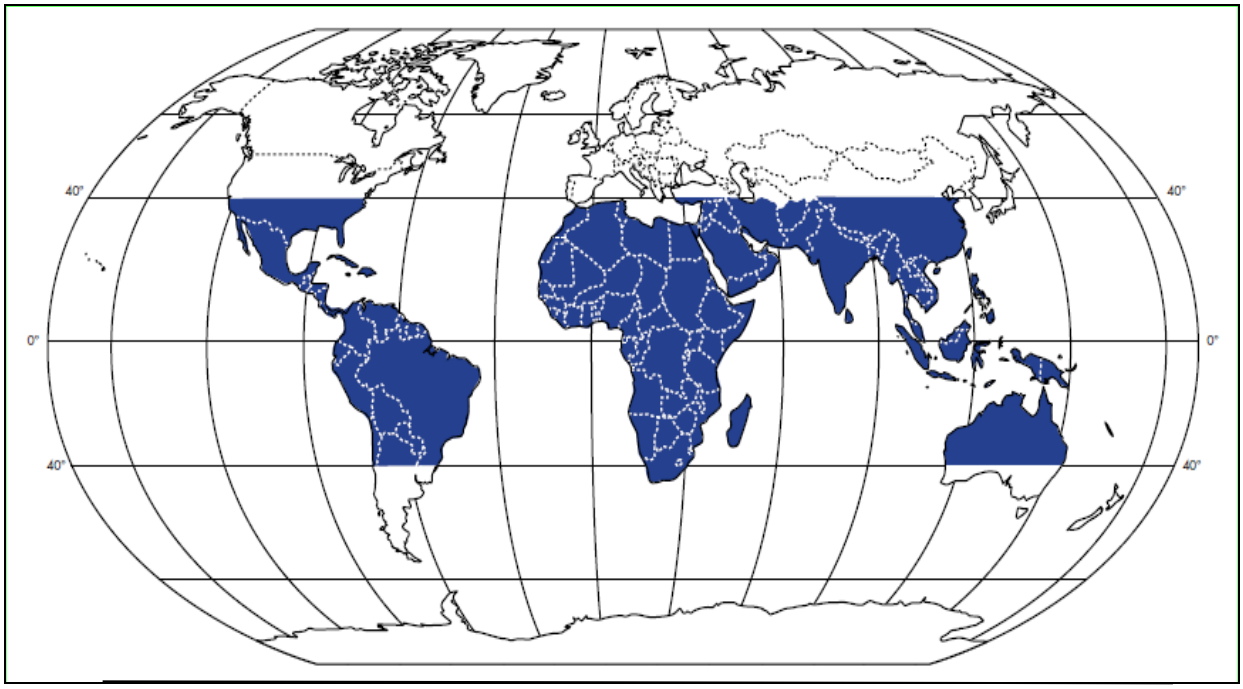


Figure 3: Global regions at risk of chronic aflatoxin exposure of uncontrolled aflatoxin contamination (William *et al.*, 2004 cited by Atherstone *et al.*, 2014)

2.2.1 Animal susceptibility to aflatoxin toxicity

According to Atherstone *et al.* (2014) all animals are variably affected by aflatoxins in diet. Sex, age, breed, species, nutritional status and stressful situation determine susceptibility of animals to aflatoxins. The orders of susceptibility are such that male > female, young > mature. Refined/exotic breeds > indigenous while specie wise, ducklings > turkeys > chicks > quail > rabbits > swine > cattle > sheep. Ruminants than non-ruminants, if old enough to have a functioning rumen, are relatively resistant. Other farm animals such as dogs and cats, laboratory animals such as rats and mice are also affected. Mice are relatively resistant than rats (Atherstone *et al.*, 2014; Feddern *et al*., 2013). The susceptibility to different categories is determined by the way aflatoxins are metabolised in the liver, which in turn governed by enzymatic activities of each category. Aflatoxin susceptibility of different categories of animals has been explained by Melissa *et al.* (2015). Capacity to produce hepatic cytochromes P450 enzymes that metabolize aflatoxins in the liver into highly reactive and electrophilic *exo*-AFB1-8, 9-epoxide (AFBO) varies with types/species of animals (Kuilman *et al.*, 2000 cited by Battacone *et al.*, 2009). So, animals highly efficient in producing

cytochromes P450 enzymes and hence high AFBO, a common nature in birds such as turkeys, are more sensitive to aflatoxins. On the other side, the murine alpha-class GST (GSTA) enzyme mGSTA3 has high affinity for AFBO which is a detoxifying effect (Dohnal *et al*., 2014). This is inherent in some rodents such as mice, making them extremely resistant to aflatoxicity (Melissa *et al*., 2015).

2.2.2 Economic losses

In both humans and animals, health and economic impacts of aflatoxins occur together. When animals ingest aflatoxin-contaminated diet, their health is ruined leading to impaired production that also diminishes the marginal benefit of animal keeping business. It is reported that ingested aflatoxins lower immunity of the animals leading to a number of different infections whose major implications are treatment costs and loss of animals through increased deaths. Aflatoxins bind vitamins and limit protein synthesis (Atherstone *et al*., 2016). For instance, layers' diet containing 10 ppm AFB1 can reduce egg laying by about 70% and dramatically lowering egg quality and size (Feddern *et al.*, 2013).

The most important economic effect of poultry ingesting aflatoxin-contaminated feed is the increase of the mortality index as reported in many studies (Nazar *et al.,* 2012). About every additional 1000 ppb in the diet of pigs found associated with about 3.9% reduction in body weight gain (Andretta *et al*., 2012). Importantly, aflatoxin-contaminated diets may lead to contaminated foods of animal origin, making them rejected from the market on safety grounds. The AFB1 in feeds can be metabolized to aflatoxin M1 (AFM1) in the liver and then carried over in the foods of animal origin (Hussain *et al.*, 2016; Iqbal *et al*., 2014; Khan *et al.*, 2013; Sassahara *et al*., 2005). The AFM1 is also toxic to the consumers of these foods, though not as potent as AFB1 and rarely can cause acute aflatoxicosis except for developing embryos (Çelik *et al*., 2000). This is one of the cases where aflatoxins are associated with infertility. In Tanzania, breast milk has been found to contain AFM1 as one of the metabolites of AFB1 ingested in diets by lactating mother that may cause chronic aflatoxicosis to suckling infants (Magoha *et al*., 2014).

2.2.3 Aflatoxin toxicity and impacts on animal health and production

The toxic effects of aflatoxins appear sometime prior to ingestion in diet. These effects can be observed as impairment of biomarkers in animals including reduced daily feed intake (FI), daily growth rate (GR), feed conversion efficiency (FCE) and packed-cell-volume (PCV)

(Medina *et al.*, 2014). Others impairments are maladjusted levels of serum proteins mainly total proteins, albumin, globulin and albumin/globulin ratio (AGR) as well as defective relative weights of liver, kidney and spleen associated with histopathological changes of internal organs such as liver, kidney and spleen (Rotimi *et al*., 2018). Various reports show that dietary aflatoxins in various animals reduce FI as observed in broilers (Yang *et al*., 2012), white shrimps (Salazar *et al*., 2012) and quail (Mahmood *et al*., 2017).

Dietary aflatoxins have detrimental effects on FCE and GR. Yang *et al*. (2012) and Nasrabadi *et al*. (2013) reported that impairment of FCE and GR is caused by reduced ratio of villus height to the intestinal crypt depth in the intestine which decreases with increasing aflatoxin contamination of diets and diminish nutrient absorption from the gut (Applegate *et al*., 2009). It has been reported that dietary aflatoxins reduce value of PCV and favour increase of serum globulin level at the expense of albumin and diminish AGR (Kaneko *et al.,* 2008 cited by Dónmez *et al*., 2012). However, susceptibilities of different groups of animals to the toxic effects of aflatoxins are caused by different forms of the enzymes such as cytochrome P450s, glutathione and S-transferases that metabolize aflatoxins (Dohnal *et al*., 2014). Chronic aflatoxicosis which is a more noxious form in animals, appears in various types of toxicity as negative health impacts; explained here in relation to animal health and production.

(i) The AFB1 adducts

This is a reaction of AFB1 and DNA or RNA forming AFB1-DNA and AFB1-RNA adducts (Muhammad *et al.,* 2019). These can inhibit transcription and translation, to cause DNA mutation, carcinogenesis and other conditions detrimental to animal health. Through a series of reactions, AFB1 can produce adducts with lysine residues in proteins which then can cause toxicity through impairment of protein synthesis and function the vital organs (Wogan *et al*., 2012).

(ii) Mutagenicity

This is a detrimental effect of AFB1 caused by binding of AFB1 to hepatic DNA and form mutation in liver DNA (Feddern *et al*., 2013). Aflatoxins are mutagenic in the sense that the effect leads to mutation of genetic code and cause DNA alteration and breakage of chromosome, gene rearrangements and malformation of genetic information (Woo *et al*., 2011). This condition has great impacts leading to many health challenges which can occur in all animals and in humans.

(iii) Hepatotoxicity and nephrotoxicity

Hepatotoxicity is a condition of toxicity in liver characterised by increased relative weight and pale or yellow pigmentation of liver which also becomes soft and friable (Hinton *et al*., 2003). Chronic exposure of AFB1 that may combine with hepatitis-B infections is likely to result into liver cancer. Activation of liver by AFB1 may result into the hepatotoxicity commonly known as hepatocellular carcinoma. Similarly, aflatoxins may cause nephrotoxicity which is a toxicity condition in kidneys brought about by accumulation of any potent toxic agent in the renal tubules (Devendran *et al.*, 2011).

(iv) Immunotoxicity

This is impairment of immune system of an animal and humans by a toxic agent leading to reduced body immunity. Poultry which is highly vulnerable to aflatoxicosis encounter immune-toxicity very easily. Birds depend on the bursa of Fabricius, thymus and spleen to produce leukocytes for active immunity (Hinton *et al*., 2003). It is reported that even at low level of dietary aflatoxins these organs are likely to be challenged and injured and lower immunity of the birds. The mechanism of aflatoxin immunotoxicity is not clearly known. However, according to Mehrzad *et al. (*2014), the AFB1 can quickly impair the phagocytic capacity of dendritic cells, up-regulating the membrane expression levels of dendritic cell activation markers and lead to poor T-cell stimulatory capacity.

(v) Intestinal toxicity

The intestinal toxicity occurs as a result of AFB1 lowering the size (length/weight) of the duodenum and jejunum (Yunus *et al.,* 2011) and affect tissue morphology. Particularly in chickens, AFB1 has been shown to raise crypt depth in the jejunum, decrease villus height in the duodenum, then reduce the ratio between villus height/crypt depth in all three parts of the small intestine and reduce feed efficiency and growth (Yang *et al.,* 2012).

(vi) Embryo toxicity

The effect of embryonic exposure to toxins has been proved to be risky to poultry embryo. AFB1 and its metabolites can be transferred from contaminated diet ingested by laying hen into the albumen and yolk of the egg as AFM1 (Çelik *et al*., 2000; Devendran *et al*., 2011). The AFB1 is hydroxilated into AFM1 in the liver by hepatic microsomal cytochrome P450

enzyme family (Battacone *et al.*, 2009; Britzi *et al.*, 2013). The AFM1 is a common metabolite detected in milk, eggs and meat. Though not as carcinogenic as AFB1, the AFM1 can cause acute toxicity in developing embryos (Çelik *et al*., 2000).

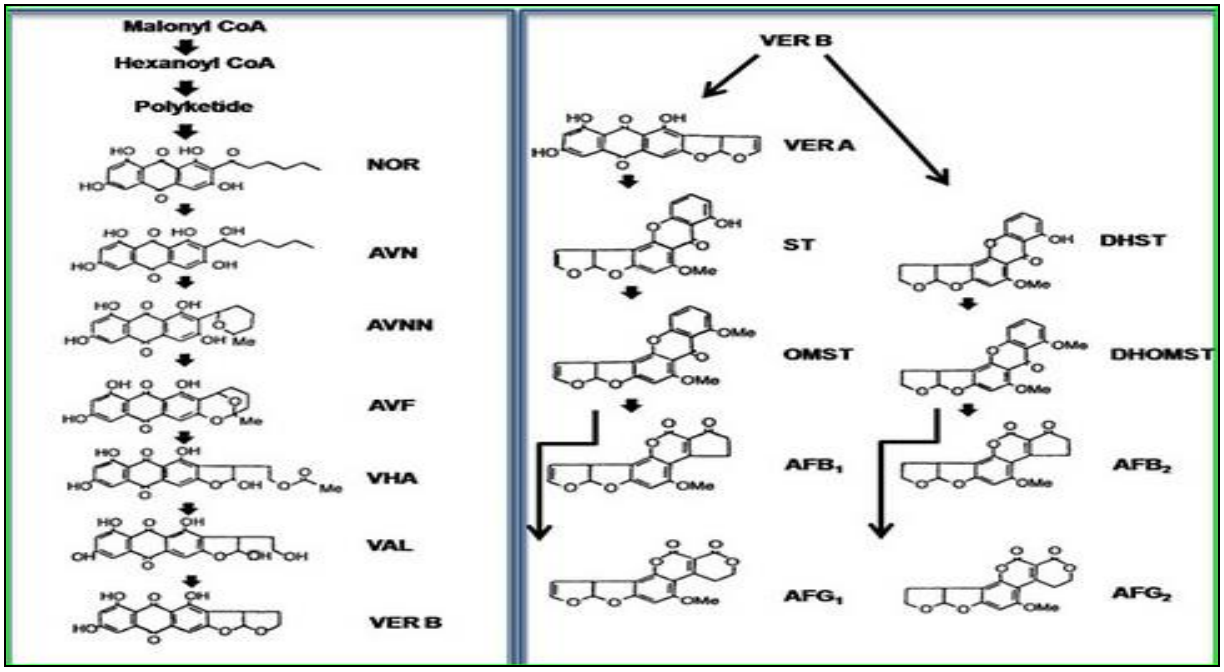
(vii) Production losses

Aflatoxins, particularly AFB1, negatively affect production values and result into economic losses in livestock industry, particularly in poultry. The adverse production effects are observed as low weight gain, reduced feed intake and reduced feed conversion efficiency (Thieu *et al*., 2008). However, these parameters abnormally may vary with type of animals and probably modality of feeding and dietary balance. For instance studies showed that AFB1 contaminated diet resulted into reduced feed intake and weight gain in chicken and turkeys without effect on feed conversion efficiency (Applegate *et al*., 2009; Devendran *et al.*, 2011).

2.3 Aflatoxin biosynthesis and contamination of crops and feeds

2.3.1 Aflatoxin production process and crop contamination

Aflatoxins are naturally produced as secondary metabolites by a complex biosynthetic process of fungi as their adaptive mechanism (Varga *et al.*, 2009; Kunzler *et al.*, 2018). The biosynthetic path way for aflatoxin is shown in Fig. 4. The enzyme Malonyl CoA of the toxigenic fungi is the initiator of the process, where through a complex chain of reaction the chemical process ends up with AFB1, AFB2, AFG1 and AFG2.



NOR=Norsolorinic acid, AVN=Averantin, HAVN=5'-hydroxyaverantin, OAVN=Oxoaverantin, AVNN=Averufanin, AVF=Averufin, VHA=Versiconal hemiacetal acetat, VAL=Versiconal, VERB=Versicolorin B, VERA=Versicolorin A, DMST=Demethylsterigmatocystin DHDMST= Dihydrodemethylsterigmatocystin, ST=Sterigmatocystin, DHST=Dihydrosterigmatocystin, OMST=O-Methylsterigmatocystin, DHOMST= Dihydro-O-methylsterigmatocystin

Figure 4: Schematic diagram of aflatoxin synthesis pathway (Kumar, 2015)

2.3.2 Factors influencing aflatoxin production in crops pre and post-harvest

Several factors influence growth of toxigenic fungi and formation of aflatoxins in crops, pre and post-harvest. These may be categorised into physical, chemical and biological factors (Dhanasekaran *et al*., 2011; Milani *et al.,* 2013). The physical factors include the moisture and temperature factors (Hassane *et al*., 2017). Chemical factors include composition of the substrate and circulating air while among biological factors host susceptibility plays the major role (Klich *et al*., 2007).

(i) Moisture and temperature

Warm and moist weather which is the typical tropical and subtropical condition, favour toxigenic fungal growth and aflatoxin production in the field. In Uganda Kaaya *et al.* (2006) found that proportion of samples of maize harvested in moist mid-altitude, dry mid-altitude and highland zones, with aflatoxin content (ppb) were found contaminated in the order of 83% (9.7), 70% (7.7) and 55% (3.9) respectively. The reported data show the importance of levels of moisture and temperature in aflatoxin formation as found in these sub-zones. Post-

harvest optimum levels of relative humidity and moisture in crops have been reported to be 62% and 14% respectively (Russo & Yanong, 2006).

(ii) Composition of substrate

Aflatoxigenic (aflatoxin-producing) fungi require specific nutrients in form of minerals, vitamins, lipids, proteins and energy sources, prioritized as, zinc, B-complex, fatty acids, amino acids and starch respectively (Agag *et al.,* 2005). Furthermore, the author reported that stuff with high concentration of carbohydrates such as cereals; and lipids such as oilseeds favour lager production of aflatoxins. Most of the fungal organisms including the aflatoxigenic Aspergillus group require sugars and lipids as a source of carbon and energy (Hamad *et al.,* 2015) for proliferation. They prefer carbon sources in from of glucose, sucrose or fructose which are highly found in cereals and fatty acids richly found in oil seeds (Hamad *et al*., 2015; Kollia *et al*., 2017).

(iii) Composition of the air around the substrate

Proportion of oxygen to CO_2 in the air around or circulating in the stored crop is important for toxigenic fungi to grow and produce aflatoxins. Since fungal organisms are aerobic, lower oxygen content relative to CO_2 inhibits growth of *A. flavus* and production of aflatoxins (Villers *et al*., 2014). Ellis *et al.* (1993) reported that extensive growth of *A. flavus* on a synthetic media in a storage packaged with 10–20% O_2 and 54–48% CO_2 at temperatures between 25–35 °C. According to Melissa *et al.* (2015) extensive growth of *A. flavus* aflatoxin production are favoured by temperatures near 30 °C and water activity of 0.99, although substrate, time, CO_2 levels and other environmental factors are also important.

(iv) Susceptibility of host plants

The nature of crop particularly genetic make-up, has influence on the susceptibility of the host substrate to be invaded by the toxigenic fungi. For instance maize is susceptible to invasion by aflatoxigenic fungi because it has genes that encode formation of enzymes that favour fungal growth, sporulation, and toxin production (Warburton *et al*., 2013). It is most likely that the genes render maize less resistant to many environmental stresses including challenges of toxigenic fungal invasion (Warburton *et al*., 2013).

2.3.3 Aflatoxin contamination of feeds

Worldwide animals are exposed to risk of aflatoxins owing to movement of feeds and other edible stuff mainly through international trade. However, due to conditions that favour development of toxigenic fungi, the tropical humid and sub-humid regions face major challenges of aflatoxins (WHO, 2018). Because of the favourable climatic and weather conditions for the toxigenic fungi, aflatoxin contamination of feeds are common in tropical areas of South America, Africa, Asia and Australia (Atanda *et al*., 2011). Almost the whole of the African region is found in this risky part of the world (Williams *et al*., 2004). Feed ingredients (cereal and oilseed), used to compound ration of various animal classes particularly poultry and pigs carry high levels of aflatoxins (Mushi *et al*., 2018). Aflatoxins have been detected in many types of feeds especially cereal and oilseed by-products. Crops that are frequently affected by the aflatoxigenic fungi, hence highly susceptible to aflatoxin formation include cereals (corn, sorghum, wheat and rice), oilseeds (soybean, peanut, sunflower and cotton seeds) (Kang'ethe & Lang'a, 2009; WHO, 2018). Also fish meal and other marine products such as silver fish common in compounding poultry feed, have been to found to contain aflatoxins (Dirican *et al*., 2013). Preserved fodder such as silage and hay may be contaminated by aflatoxin producing fungi, particularly when improperly stored to encourage aflatoxin production (Filazi & Tansel, 2013). When aflatoxins in contaminated feeds metabolized in the liver the metabolites mainly AFM1 are secreted in foods of animal origin such as milk, eggs and meat and ruin health people consuming the foods (Mohammed *et al.,* 2016).

2.4 Exposure of aflatoxins to animals and associated impacts

2.4.1 Hazards of aflatoxins to animals

Animals form a very potential economic sector particularly for the rural communities and at nation level at large (Bettencourt *et al*., 2015). However, animals are prone to many health challenges that hinder their development. Of these health challenges are toxicities caused by toxic agents inherent in feeds such as aflatoxins that cause aflatoxicoses (Grace *et al*., 2015b). Chronic aflatoxicosis caused by aflatoxin contamination of feeds is one the important health challenges hindering animal performance (Atherstone *et al*., 2016; Mok *et al*., 2013). Generally, animals encounter a lot of aflatoxins through consumption of crop by-products which after processing they act as a sink for crop contamination (Nziramasanga *et al*., 2005).

Any level of dietary contamination of aflatoxin in feeds consumed by animals is likely to pose a certain health risk to animals (Sassahara *et al*., 2005). Studies show that apart from the direct health challenges, aflatoxins in feeds depress development and production performance of animals (Andretta *et al*., 2012; Mok *et al*., 2013). As a spill-over effect, when animals are fed naturally aflatoxin-contaminated feeds, the toxins particularly AFB1 are secreted in milk or retained in eggs as AFM1 (Arapcheska *et al.*, 2015; Atherstone *et al*., 2016; Grace, 2013; Khan *et al.*, 2013). Concomitantly, milk is considered to be the most important food component for children in many localities in Tanzania and elsewhere, while this is the most susceptible group to dietary aflatoxin. Therefore, unlike adults, children succumb to higher risks of aflatoxin exposure, subjecting them to stunted growth, delayed development and other health disorders (Chan *et al*., 2003; Williams *et al*., 2004). Chronic exposure to aflatoxins is associated with liver cancer development, since aflatoxins metabolites can intercalate into genome and cause mutations in the *p53* gene plus other metabolic and reproductive problems (Chan *et al.*, 2003; Macé *et al*., 1997).

It has been reported feeds of maize and groundnut origin are mostly susceptible to the formation of AFB1, a problem well experience in Southeast Asian and Sub-Saharan African countries (Mahato *et al*., 2019). In East Africa, Kenya is the most affected country by aflatoxin exposure to human and animals, where outbreaks of aflatoxicosis have been frequently reported since 1978 in various rural areas (Lewis *et al.*, 2005). In Tanzania, the level of exposure through contaminated foods and feeds is also very high. Survey conducted in the country showed that about 45% of the collected maize samples were contamination to up to 269 μg/kg (Kamala *et al.*, 2016). Therefore, it is imperative to prevent and reduce hazards of aflatoxin contamination of feeds for the protection and promotion of human and animal health.

2.4.2 Prevalence of aflatoxins and aflatoxicosis outbreaks

Normally, prevalence of aflatoxin occurrences is proportionally associated with the level of exposure to human and animals. In this section, the status of abundance and distribution of aflatoxins with their impacts reported as aflatoxicosis cases particularly in East Africa are reviewed.

(i) Aflatoxin prevalence

In this review, acute aflatoxicosis and outbreak in humans has been used as evidence of high occurrence of aflatoxins in food and feed chains. In Africa humans and animals consume unsafe levels of aflatoxins whereas West and East Africa are known to be hotspots of aflatoxin poisoning (Stepman e*t al.,* 2018). For instance, in Nigeria as high as 138 000 µg/kg contamination has ever been reported in maize samples (Prasanna *et al.,* 2014) while the international Codex Alimentarius standards for safe food and agricultural products allows maximum limits of ≤ 10 µg/kg (Grace *et al.*, 2015a). In East Africa, many people and animals are suspected to have been consuming unsafe levels of aflatoxins in different foods and feeds also (Gong *et al.,* 2016). Survey conducted in Singida region of Tanzania showed that about 65% of feed samples were contaminated with AFB1 at a rate of about 20.5 µg/kg concomitantly, about 84% of fresh cow milk samples contained greater than 2 ng of AFM1 per millilitre of milk (Mohammed *et al*., 2016). Some examples of specific feeds and their respective levels of contamination with AFB1 according to location in Tanzania are shown in Table 1.

Table 1: Concentration of AFB1 in feeds from various locations in Tanzania

Location	Feeds	% of samples contaminated	AFB1 concentration (µg/kg)	% contaminated above 5ng/g	Source
Morogoro	Maize bran	50	9.4	73.0	Kajuna *et al.* (2013)
	Broiler mash	91	35.8		
	Sunflower seed cake	70	31.6		
	Layers mash	70	15.1		
Arusha	Starter feed	65	40.6 Range: 1.1-80.1	70.8	Mushi *et al.* (2018)
	Finisher feed	72.2			
	Layers mash	79			
	Maize bran	62.5			
	Sunflower seed cake	75			
Singida	Sunflower seed cake	80.0	27.4 Range: 2.0 – 52.8		Mmongoyo *et al*. (2017)
Dodoma	Sunflower seed cake		300.0 1.4 – 598.4		
Mbeya	Sunflower seed cake		50.3 Range: 2.8 – 97.7		
Morogoro	Sunflower seed cake				

(ii) Outbreaks of aflatoxicoses following aflatoxin contamination of crops

Generally, high occurrence of aflatoxins in crops and ultimately in foods and feeds subject humans and animals to either acute or chronic aflatoxicoses. Information on acute aflatoxicoses in livestock is rare or not available, probably they occur but not reported (Atherstone *et al.*, 2016). A number of cases of acute aflatoxicosis in humans in various localities have been reported in East Africa during different times. Among the countries of the region, Kenya is reported to be mostly affected by aflatoxins with acute cases that have caused a number of deaths. In 2004, the largest aflatoxicosis outbreaks occurred in rural Kenya in Makueni, Kitui, Machakos and Thika Districts, resulting to 317 cases of which 125 were deaths (Lewis *et al.*, 2005). The report indicated that the source of the outbreak was due to consumption of aflatoxin-contaminated home grown maize. In relation to the outbreaks of human aflatoxicosis, between 81-87% of feed samples collected in some urban areas of Kenya contained 13-21.4 ng/g AFB1 and 72-84% of fresh cow milk samples from the same area contained 5-780 pp AFM1 (Kang'ethe & Lang'a, 2009). These contents are relatively high compared to the maximum/safety limits recommended in East African Community (EAC) of 5 µg/kg for AFB1 and 10 µg/kg for total aflatoxins in selected foods, cereals and pulses and 5 µg/kg for AFM1 in milk (Gong *et al.*, 2016). Similarly, an acute aflatoxicosis outbreak in Tanzania occurred in 2016 in Dodoma and Manyara regions where 68 cases were confirmed and 20 died following consumption of maize diets with contaminated of aflatoxins (Kamala *et al.*, 2018).

In Uganda, 90% of serum samples from 713 people were positive for AFB-Lys (Kang *et al.*, 2015). A review by Agbetiameh *et al*. (2018) showed that consumption of maize and groundnuts contaminated by AFB1 increases the risk of chronic aflatoxin exposure especially among vulnerable groups in African. Furthermore, Xu *et al*. (2018) reported aflatoxin-albumin adduct levels during acute aflatoxicosis ranging from 9.7 pg/mg albumin in Ugandan children to 578 pg/mg albumin in Kenyan adolescents. In Rwanda about 85% and 80% of peanut and maize samples collected in various locations were found with unsafe high levels of aflatoxins respectively (Nyinawabali, 2013). In another study by Nishimwe *et al.* (2017) maize and groundnut feeds samples were reported to contain AFB1 at rates >45 µg/kg and >100 µg/kg respectively. The information represents current cases of high aflatoxin load in human and animals in African region. Prevalence of aflatoxins in Burundi and South-Sudan is scantly reported. However, this does not that these two member countries of EAC like in

many other countries in tropical areas are not exceptionally free from aflatoxin menace. These cases call for coming together to design control measures and their impacts on human and animal health as well as the consequential economic losses.

2.5 Control and management of aflatoxins

Control of aflatoxins in crops is instrumental in managing feed contamination by aflatoxins since most of the feeds are either whole crop or by-products of the crops. Aflatoxins can be controlled firstly by raising public awareness. Secondly, applying preventive measures right away from the field and along the food chain to the final point of consumption, involving control measures for pre-harvest, at harvest, and post-harvest as suggested by Kumar *et al*. (2017). Thirdly, applying regulatory mechanisms that involve imposition of strict measures against use, distribution and sale of contaminated products.

2.5.1 Raising public awareness about aflatoxins

Majority of the people in developing nations seems to know little about aflatoxins and the associated health and economic impacts (Grace, 2013; Unnevehr & Grace, 2013; WHO, 2015). For instance, in a study conducted in Kenya, farmers perceived that eating mouldy food may be harmful, but considered meat from animals fed on mouldy feeds to be safe (Kiama e*t al.*, 2016). This shows that the scenario of aflatoxin contamination of feeds is even less known that with food cases. Studies done in other localities indicate that levels of awareness of aflatoxins are low. Some of the documented levels of aflatoxins are such as: 25% in Vietnam (Lee *et al*., 2017), 6% in Zimbabwe (Nleya *et al.*, 2017), 12% in The Greater Addis Ababa milk shed of Ethiopia (Gizachew *et al.*, 2015) and 20% in Tanzania (Kiama *et al*., 2016; Ngoma *et al.*, 2017). In Rwanda, awareness about aflatoxins was 7.3% among soybean farmers (Niyibituronsa *et al*., 2016) and nil among vendours of maize based flour and feeds (Nishimwe *et al*., 2017).

Levels of awareness about aflatoxins and other fungal toxins have been found to vary with various socio-economic set-ups. For instance, in Kenya, women were found more informed of the dangers of fungal toxins and cautious to mouldy feeds than men (Kiama *et al*., 2016). In Vietnam, young farmers (at age of 21–29) were found to be more informed about aflatoxins in crops than older groups (Lee *et al*., 2017). In Tanzania, studies have shown that education level has positive effect on aflatoxin level of awareness (Ngoma *et al.*, 2017; Magembe *et al.*, 2016). In Ghana, it was found that field of study particularly life sciences

has positive impact on aflatoxin awareness (Awuah *et al.,* 2008). In Ethiopia, farmers were found less informed of aflatoxins than individuals in other occupations (Ephrem *et al.,* 2015).

Scanty information on the level of awareness about aflatoxins is available in Tanzania, particularly in relation to socioeconomic characteristics/factors. Also the available reports are more deflected to awareness of aflatoxins in food crops such as ground nuts and maize than feeds. Where reports touching awareness on aflatoxins in feeds are available, they still lack some vital details required for mitigation of challenges related to aflatoxins occurrence. Moreover, the reports are less informing about terms of location specificity. Little is known about awareness of aflatoxin contamination of feeds among farmers based on socio-economic characteristics, even in the aflatoxin risky areas. Farmers' awareness in solving a farming problem may be considered as the first step towards identification and designing mitigation measures (Walker & Davies, 2013). Therefore, knowing the level of awareness of aflatoxins in feeds among livestock farmers is important in setting plans to reduce risks of aflatoxin exposure through feeds.

Studies show that, in intensive systems use of crop by-products such as maize bran and oil seed cakes as supplementary feeds is very high (Chadd *et al*., 2002). These by-products are the potential sources of aflatoxin exposure to animals; yet it is not well known whether farmers are aware of this concern. In countries and places where acute aflatoxicoses have ever occurred and cause deaths, the level of awareness of aflatoxins is relatively high. In Kenya for example, report on awareness of milk consumers in urban areas of Nairobi revealed that about 80% of the surveyed respondents had ever heard about aflatoxins (Mtimet *et al.*, 2015). In contrast, in Tanzania where cases of aflatoxicosis were yet to be reported several years past and without public sensitisation, awareness of aflatoxins has been found as low as 20 – 30% based on having ever heard about aflatoxins (Kiama *et al*., 2016; Magembe *et al*., 2016; Ngoma *et al.*, 2017). It has also been reported that aflatoxins are not widely known even to some of health and agricultural professionals (Jolly *et al*., 2009). This may imply a lack of mention of the importance of the aflatoxins in curricula of various professions particularly those related to health, agriculture, trade and allied training programs.

2.5.2 Pre-harvest measures to control aflatoxins

Pre-harvest measures that apply Good Agricultural Practices (GAP) involve selection of stress resistant varieties of crops, well timely planting, pest and weed control as well as soil moisture and fertility maintenance (Mukanga *et al.*, 2019). Adopting crop rotation is also important to break the cycle of the toxigenic fungi and accumulation in soil (Hell *et al.*, 2010; Torres *et al*., 2014). These can minimize overall stress to plants which is the major predisposing factor for growth of aflatoxigenic fungi and aflatoxin production. Recently, application of biocontrol techniques that make use of atoxigenic fungi to inhibit growth of toxigenic fungi, such as use of Aflasafe has given great achievements (Udomkun *et al.*, 2017). The technology is customised per country or region, such that in Tanzania an Aflasafe-TZ that can reduce aflatoxin contamination in food and feed by 85% has been tested and show that great success (Mahuku, 2017).

2.5.3 Measures to control aflatoxins in crops during harvest

Simple best practices can be applied to prevent aflatoxin contamination during harvest time (Hell *et al*., 2010). The salient practices are such as timely harvesting at maturity, avoiding premature and over-matured harvesting since these predispose crops to contamination (Zuza *et al.*, 2019). Also, timely harvesting by avoiding wet or rainy days that may cause moisture harbouring in the harvested crops are useful. Use of appropriate methods to avoid damage to the crop is important and avoiding contact with soil during harvesting time (Torres *et al*., 2014).

2.5.4 Post-harvest measures to control aflatoxins in crops

Post-harvest techniques target immediate collection of crops from the field, transporting the produce in clean dry containers and vehicles (WHO, 2015). Dirty handling of harvested crops may initiate toxigenic fungi in the produce while moist condition encourage proliferation of the organism to increase aflatoxin boom (Hell *et al.*, 2010). In addition sorting to remove damaged and defective crops can reduce contamination of uncontaminated (WHO, 2018). Avoiding contact of the harvested crops with soil minimizes contamination as soil is a rich source toxigenic fungi (Torres *et al*., 2014). Ensuring proper drying on raised mesh, storing the dried crops in a clean dry place and controlling of damage of post-harvest pests (Lavkor & Var, 2017; Torres *et al*., 2014). Preferable moisture content and ambient temperature for most crops are below 14% and 25 °C respectively (Dhanasekaran *et al*., 2011; WHO, 2018).

According to Waliyar *et al*. (2015) some of the improved post-harvest strategies to control aflatoxins in crops are for ultimate reduction of the toxins in feed are listed as follows:

(i) Lowering moisture content during storage to ≤8%.

(ii) Adding preservatives to prevent insect infestation and fungal contamination during storage.

(iii) Sorting of contaminated grains/parts.

(iv) Re-drying of harvested crops.

(v) Appropriate storage conditions to avoid favourable conditions for mould growth.

(vi) Avoidance of re-moistening of harvested crops.

(vii) Detoxification of contaminated products and use of aflatoxin binders. These strategies may be achieved through use of the following tools and methods, mostly in combination:

- Proper post-harvest grain handling involving cleaning, grading, transportation, storage, processing, packaging and retailing at the market.
- Post-harvest machinery use, involving threshers, dryers and shellers that help to increase yield and reduce post-harvest processing and drying time.
- Physical separation to remove discoloured or damaged/shrivelled parts/grains to minimise aflatoxin levels.
- Storage methods and conditions, such as uses of hermetic triple-layer bags that is Purdue Improved Crop Storage (PICS) for grain storage of several crops that is gaining popularity over traditional storage devices, and
- Other methods and means such as disinfestation and detoxification, inactivation, filtration, binding agents and use of antifungal compounds.

2.5.5 Detoxification of aflatoxins

(i) Detoxification techniques

Some techniques have been developed to reduce toxic effects of aflatoxins in contamination of feeds. These include physical techniques such as thermal inactivation and irradiation; chemical techniques such as the treatment of the feeds with acidic or alkaline solutions, ozone and ammoniation and biological techniques such as use of microbial agents (Kolosova & Stroka, 2012). These techniques are mostly applied in the animal feed industry, and are reported to have some limitations including cost implications, demand for some complicated facilities, the reduction of dietary palatability and nutritional values and danger of unsafe residues, chemical or microbial agent residues (Devreese, 2013). Use of aflatoxin binders (also called adsorbents or sequesters) is a relatively simple technique found economically feasible, easy to apply and nutritionally safe (Binder, 2007). A vast number of types of materials expected to have capacity to bind aflatoxins in feeds so as to render the toxins unavailable to the animal body as they pass out in the faeces.

Aflatoxin-contaminated feeds can be rendered safe to animals when treated with materials capable of binding aflatoxins in the feeds. Many types of crude or refined materials, including clays, cellulose products, yeast cell wall, and activated charcoal are envisaged to have the ability to sequester or bind aflatoxins (Kong *et al*., 2014). The potential binding capacities of these materials are known to vary based on their nature and source (Vekiru *et al*., 2015). Clear information on the relationship between the binding capacity and the properties of aflatoxin-binding materials is scanty (Kannewischer *et al*., 2006; Vekiru *et al*., 2015). This demands for studying specific materials suspected to bind aflatoxins in feeds and reduce the toxin bioavailability and safeguard health of animals and that of humans consuming animal-origin foods.

Studies show that aluminosilicates, common aflatoxin-binders and other similar materials have a wide variation of chemical elements as shown (Table 2). Surveillance of occurrence of aflatoxins is considered very important in fighting dietary aflatoxins in animals, but parallel with it, emphasis is on initiatives to identify user-friendly technology to detoxify the toxins in contaminated feeds as suggested by Grace (2013).

Table 2: Elemental composition of clay and ash samples studied from various sources

Number of samples	Mean/Range	Percent structural components of clays and RHA samples					Source
		SiO_2	Al_2O_3	Fe_2O_3	CaO	K_2O	
Clay (11)	Mean	59.6	19.0	5.2	1.7	0.8	Karnland (2010)
	Range	1.1-69.0	0.5-21.7	0.2-14.8	0.1-6.8	0.1-3.3	
Clay(10)	Mean	55.3	13.7	4.4	1.4	1.3	Mukasa-Tebandeke *et al.* (2015)
	Range	44.3-71.0	8.4-20.1	1.4-8.0	0.1-2.4	0.1-2.6	
Bentonite (1)	One value	80.2	13.2	2.7	0.2	0.1	Anjos *et al.* (2016)
RA(1)	Mean	88.3	0.5	0.7	0.7	2.9	Habeeb and Mahmud (2010)
RA(1)	Mean	89.0	1.2	1.3	1.0	1.2	Mohamed *et al*. (2015)
RA(1)	Mean	93.4	0.1	0.1	0.3	1.4	Korotkova *et al.* (2016)

RA: Rice husk ash

Various clay and ash-based materials have been used in a number of practices where accidentally and unknowingly they might have been reducing aflatoxin load in foods and feeds (Rejeb *et al.*, 2019). For example, in South American countries, ashes such as soda ash and wood ash have been used in some food processes particularly in nixtamalization for corn tenderization, where dietary aflatoxin contamination load is also reduced, owing to the breakage of aflatoxin structures such as the aflatoxin lactone ring by the ash alkalinity (Pedraza *et al*., 2015).

(ii) Evaluation of potential capacity of materials to bind aflatoxins

In evaluation of aflatoxin-binding capacity of materials *in-vitro* and *in-vivo* techniques are applied. Normally, the *in-vitro* and *in-vivo* techniques serve as preliminary and confirmatory tests respectively (Gallo & Masoero, 2009; Devreese, 2013). Chances for results on *in-vitro* and *in-vivo* tests to come into one-to-one function for same binding materials are narrow, and it is likely that the *in-vivo* results are a bit superior and more informative than *in-vitro* tests (Devreese, 2013). Running both tests gives more enriched information by harnessing advantage of each technique and make comparison that can reveal salient potential of specific aflatoxin-binding materials.

The *in-vitro* binding tests make use of controlled conditions in digestion tubes to simulate binding processes that takes place in the gastro-intestinal tract of an animal (Kong *et al.*,

2014). The technique is useful for higher throughput in preliminary testing of capacity of a number of materials in short time. With *in-vivo* tests, actual system of live animals is employed. It is widely known that *in-vitro* test is more sensitive than the *in-vivo*, such that where no binding is detected with *in-vitro* testing of binders, then no effect is likely to be observed with *in-vivo* testing of the same binders (Devreese, 2013). With *in-vitro* testing, more practical and rapid results are obtained, serving as preliminary or screening test towards efficacy confirmation of the binding capacity of binders by using *in-vivo* testing.

2.5.6 Application of regulations for control and management of aflatoxins

Levels of aflatoxins in foods and feeds are monitored by regulations designed by Government authorities to control quality of these edible resources to safety standards (Grace *et al*., 2015a). These standards state the maximum limits of aflatoxins in foods and feeds. Standards in feeds are also designed to prevent carry-over of aflatoxins from animal feeds to humans (FAO, 2008). Levels in animal feeds are also regulated to protect humans from exposure of the toxic metabolites in foods of animal origin as a result of contaminated feeds. The regulations and standards differ across countries and regions and across food and feed types (Feddern *et al.*, 2013; WHO, 2018). Codex Alimentarius Commission (Codex) an international body formed jointly by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations has a coordination role (FAO, 2008). The Codex is vested with the responsibility of formulating food and feed safety standards, including Maximum Limits (MLs) for contaminants such as aflatoxins. However, the Codex recommends maximum levels of aflatoxin in foods and feeds as guidance for national authorities on setting appropriate standards for foods and feeds (Grace *et al*., 2015a).

2.6 Detection and quantification of aflatoxins

2.6.1 Methods for detection and quantification of feeds

Detection and determination of aflatoxins in feeds is an important aspect for mitigation strategies. Data of aflatoxin concentration in feeds are needed by stakeholders such as researchers, policymakers and risk managers. Detection of aflatoxins in feeds faces sampling challenge towards the required information due to distribution nature of the toxins in feeds (Wagner, 2015). Toxigenic fungi and aflatoxins are heterogeneously distributed in the lots of feeds (Wagner, 2015) and create difficulty in taking samples that ideally represent the real contamination levels in the feeds. To have representative samples effective sampling

protocols are developed parallel with the context of aflatoxin regulatory control for each type of feed (Grace *et al*., 2015a).

Analysis of feeds to detect and quantify aflatoxins has been employing several methods that are classified into three main categories namely, Chromatographic methods, Spectroscopic methods and Immunochemical methods (Goryacheva *et al.,* 2007; Lee *et al*., 2015). None of the aflatoxin testing methods is entirely accurate, but they differ in some respects such as in sensitivity, detection limit, specificity, cost, labour and operation technicalities (Mohamadi *et al*., 2012). On the other hand, each of the methods has some strengths and limitations (Table 3). As suggested by Wacoo *et al*. (2014) the method to be used should be dictated by the intention of detecting and quantifying aflatoxins in feeds and other edible stuff. For instance, where more accurate and sensitive detection of aflatoxin are required, chromatographic methods particularly High Performance (HPLC) are salient techniques to use, though they are cumbersome in use, laborious in sample preparation and expensive in terms of required materials and facilities in terms of facilities required (Gupta *et al.,* 2014).

Table 3: Methods for detection and determination of aflatoxins in feeds

Categories of the methods	Strengths	Limitations
Chromatographic methods		
Thin Layer Chromatography (TLC)	Can detect several types of mycotoxins in single test with detection limit 1 – 20ppb.	It lacks precision due error accumulation, expensive, requires pre-treatments of samples and skilled technicians.
High Performance Chromatography (HPLC)	It is fast and accurate results with sensitivity of detection 0.1ng/kg.	Requires high sample purification using immunoaffinity columns, pre and post injection derivatization of samples, skilled technicians and is expensive.
Gas Chromatography (GC)	Capable of detecting aflatoxins samples.	It is expensive, requires derivatization, few mycotoxins can be analysed and lacks linearity of calibration.
Spectroscopic methods		
Florescence Spectrophotometry (FS)	Has Sensitivity of detection 5-5000 ppp for < 5min.	Requires derivatization to increase fluoresce, less sensitive with detection limit > 4µg/kg.
Frontier Infrared Spectroscopy (FIS)	It works and give results fast.	Give categorical in form of either high or low, hence less informative.
Immunochemical methods		
Radioimmunoassay (RAI)	Has high specificity and sensitivity limit of 1µg/kg, determines qualitative and quantitative levels of aflatoxins B_1 in feeds and can perform multiple analyses simultaneously.	Require pure antigen, use radioactive materials with health hazards and pose problem in disposing radioactive wastes.
Enzyme-Linked - Immunosorbent (ELISA)	Can analyse large number of samples simultaneously, analysis kits are cheap and easy to use, do not require extensive sample clean up and safe health wise.	It requires multiple washing steps, hence laborious and time consuming.
Lateral Flow Devices (LFD) Immunodipsticks	It is cost effective and easy to use for day to a day needs.	Designed specific for detection of AFB1 in pig feeds and has low detection sensitivity of 5µg/kg aflatoxin.
Immunosensors		
Piezoelectric Quartz Crystal Microbalances	It is a very good label-free technology and can detect AFB1 in a range of 0.5–10 ppb in agro-products	Its direct use for detection of mycotoxins is limited by the small sizes of most mycotoxins.
Optical Immunosensors	Suitable for aflatoxin detection solution with detection range of 0.5 and 10 ng/mL.	Less adopted in determination of aflatoxins owing to false positive values.
Electrochemical Immunosensors	Has a dynamic range of 3.2–0.32 Pico moles and detection limit of one femtomole with excellent long-term stability.	Not well adopted in determination of aflatoxins owing to false positive values.

Wacoo *et al.* (2014)

Equipment and facilities necessary to complete aflatoxin testing and quantification are very expensive and requires a high level of expertise to operate. Many methods and equipment outlined in Table 3 are not commonly found in many laboratory centres, as such some laboratories take options of the available technology commonly accepted worldwide. Chromatographic method using HPLC is currently adopted because, regardless the high cost involved, it is fast, accurate with high sensitivity of detection (Gupta *et al*., 2014). Also it is the currently readily available service in many laboratories, with trained skilled technicians to assist researchers in eliminating the challenges of operation.

2.6.2 Standards for aflatoxins in food and feed

Different countries or group of countries have formulated their regulatory systems and set standards for maximum aflatoxin levels, particularly AFB1. Contamination of foods and feeds by aflatoxins in the world is generally high, but due to strict legislation for AFB1, foods and feeds exported to developed countries such as United Kingdom and other European countries found to contain very minimal to almost nil contamination of aflatoxins (Wu, 2006). Available data show that numbers of countries with regulatory strategies for mycotoxins aflatoxins in particular is increasing over years. The EAC partner countries have developed standards to monitor different issues in handling milk, dairy based products and feeds for animals and fish (Grace *et al*., 2015a). Tanzania has been using own previously developed standards under TBS that cover specific aspects of feeds, foods and many other products (Grace *et al.*, 2015a).

2.6.3 Enforcement of aflatoxin standards in feeds

Where aflatoxin regulations for feeds are fully enforced, the health and economic adverse impacts can be minimized tremendously, since the contaminated feeds and ingredients are taken out of consumption. But, this has an implication on trade, making many producers lose market access. The major negative consequence of the regulatory enforcement is the resulting scarcity of the regulated commodity (Grace *et al*., 2015a). Since it is not completely possible to eliminate aflatoxins from feeds using the available technologies, it is imperative to have level of aflatoxins that is safe, tolerable and widely acceptable. This will keep down the costs for ill health and economic losses, but also allow smooth trading. Various countries have regulatory authorities established by legal act for the purpose of enforcing the standards. For instance in USA responsible authority is Food and Drug Administration (FDA), India is

Bureau of India Standards (IBS) and in Tanzania is Tanzania Bureau of Standards (TBS). Some countries with their regulatory standards of aflatoxins in feeds are shown in Table 4.

Table 4: Aflatoxin standards for maximum limits in various countries

Country	Type of Feed	Aflatoxin regulated	Maximum Level (ppb)
EU	All feed materials	B1	20
	Complementary and complete feed	B1	10
USA	Corn and peanut products intended for finishing beef cattle	B1	300
	Cottonseed meal intended for beef cattle, swine, or poultry	B1	300
	Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	B1	100
	Corn, peanut products and other animal feeds and feed ingredients, excluding cottonseed meal, intended for immature animals	B1	20
	Corn, corn products, cottonseed meal, and other animal feeds and feed ingredients	B1	20
Japan	Corn meal	B1	20
	Formula feed for cattle (except dairy cattle and calves), pig (except piglet), domestic fowl (except chicken and broiler), quails	B1	20
	Formulated feed for dairy cattle	B1	10
China	Corn, peanut meal, cottonseed meal, rapeseed meal	B1	50
	Complementary, complete and concentrated feeding stuffs for fattening pigs, broilers, layers and quails	B1	20
	Supplementary feeding stuffs for dairy cattle	B1	10
	Groundnuts seed cake, Copra cake, Palm kernel seed cake, Cotton seed cake, maize	B1	50
	Other complete farm feeds	B1	10
Senegal	Straight feedstuffs, peanut products (all animals)	B1	50
	Feedstuff ingredients	B1	300
Brazil	All feeds (all animals	B1, B2, G1, G2	50
Canada	All feeds (all animals)	B1, B2, G1, G2	20
Egypt	All feeds (all animals)	B1	10
	All feeds (all animals)	B1, B2, G1, G2	20
South Africa	Complete feed for pigs and poultry	B1	50
	Other complete farm feeds	B1	10
Tanzania	Complete meal (all animals)	B1	5
	Complete meal (all animals)	B1, B2, G1, G2	10
EAC*	Complete mixed meals	B1	25
	Low risk feed ingredients	B1	20
	Corn, cottonseed, peanut and copra	B1	85

Egmond *et al*. (2004) and *Agag (2004) cited by Grace *et al*. (2015a)

2.7 Conclusion

Aflatoxins are noxious natural toxins scattered in foods and feeds, threatening life in many ways. In this review, apart from the serious health challenges of aflatoxins to animals and humans, there are negative economic impacts on livestock industry where livelihoods of majority in rural areas rely. It is imperative that aflatoxins are well addressed by developing strategies to mitigate their exposure to animals, first by determining the extent to which they are known by farmers and secondly designing practical, affordable and sustainable mechanisms for their mitigation. This review showed that efforts have been directed to getting information on the awareness of aflatoxins in relation to some specific aspects such as occurrence of the toxins in crops. Never-the-less, information on awareness of aflatoxin contamination of feeds, specifically in Tanzania is rare. Information showing a move to explore local materials with capacity to immobilize aflatoxins in feeds in the country is rare.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Socio-Economic factors influencing awareness of aflatoxins among farmers

3.1.1 Methods

(i) Description of the study area

The study was conducted in the coffee-banana belt, Meru district, situated within 3.0–3.4 °S and 36.3–37.0 °E and altitude between 1200 and 1600 m a. s. l. on the slopes of Mt. Meru in Arusha region of Tanzania. The district experiences average annual precipitation and temperature of 1200 mm and 25 °C respectively. The total population was 268 144, majority of them practicing mixed farming (Agwanda & Haidari, 2014). The district is one of the areas experiencing intensive livestock keeping in Tanzania, particularly dairy cattle raising (Swai & Karimuribo, 2011). Owing to the intensification, the animals seems be predisposed to aflatoxin hazards due to high feed supplementation with possible contaminated crop by-products (Grace *et al.*, 2015b).

(ii) Social survey for farmer' awareness of aflatoxins

A cross-sectional design using semi-structured questionnaire (Appendix 1) was adopted for data collection of socioeconomic characteristics of respondents, which included gender, age, level of education, field of specialization, employment categories (formal and informal), occupation categories (farming and non-farming), and length of time (experience) in keeping animals. In this study, two levels of education were considered, that is, below secondary education (<SE) and secondary education and above (≥SE). Also two categories of academic specialization were considered in comparison. Exposure to life/social sciences against the category exposed to any other studies (Arts/Social sciences, General/Engineering sciences). The listed factors were considered as potential variables to have influences on awareness of any social phenomenon.

Among the key question asked to respondent farmers focused on whether they had ever heard about aflatoxins and then about awareness in relation to the following items: (a) possibility of fungal toxins or aflatoxins to occur in feeds, (b) indicators for presence of fungal toxins or aflatoxins in feeds, (c) types of feed ingredients most prone to fungal toxin/aflatoxin

contamination, (d) possibility of natural toxins in feeds to affect health of animals, (e) possibility of fungal toxins transferred from feeds to foods of animal origin, (g) ability to identify/detect mould formation in feeds and (h) whether fungal toxins or aflatoxins in feeds can be detoxified to render the feeds safe. Direct physical assessment was done to ascertain some feed aspects in relation to farmer responses and views using detection indicators such as feed type, colour, odour and consistence. The questionnaire was first prepared in English to retain the required context and then translated into Swahili for smooth face-to-face interview. It was pretested, to check for its suitability by administering it to twenty-five respondents in an area outside the study area as suggested by Aswathappa (2003). Items noticed to be unclear in the questionnaire were legibly corrected.

(iii) Sampling design

Seven wards were purposively selected from thirty-five wards of the district based on the criteria of having higher population densities of livestock taking dairy cattle as reference.

Systematic random sampling technique was used to select households keeping livestock from the seven wards. The household sample size of 258 was determined by Yamane formula (Model 1) as applied by Ajay and Micah (2011):

$$n = N/(1+Ne^{2}) \text{..... Model 1,}$$

Where, N = the sampling frame for households keeping livestock and practicing feed supplementation in the wards, estimated to 725 from district database; e = the acceptable sampling error of 0.05 at the 95% confidence level.

Household head, spouse, or any household member/employee with sound mind aged eighteen and above who declared to participate in the household livestock activities and was ready to play the part of household spokesperson was interviewed. Candidate wards with selected proportionate sub-samples of households in brackets were: Ambureni (35), Imbaseny (39), Nkoaranga (34), Patandi (38), Poli (38), Seela-Sin'gisi (42) and Songoro (32). All the information on livestock population size and distribution by households was obtained from the Meru District Livestock Development Office.

(iv) Data analysis

Data were entered in EpiData 3.1 software for easy control of entry quality and then exported to IBM-Statistical Product for Service Solutions (SPSS version 20) software for analysis. Descriptive analysis was carried out to obtain descriptive results (frequency and percent distribution of the assessed variables). Bivariate regression analysis was preliminarily run to check for any crude association between the predictors and the outcome variables. Variables found to have any association were subjected to forward multivariate logistic regression to establish the actual significance and magnitude of association between the socio-economic factors and awareness of aflatoxin contamination of feeds. A value less than 5% was considered significant throughout the analyses conducted.

3.2 *In-vitro* evaluation of aflatoxin-binding capacity of clay and Ash-based materials

This evaluation was done to determine the aflatoxin-binding capacity of the selected clay and ash-based materials in binding aflatoxins spiked in *in-vitro* buffered solution.

3.2.1 Materials

(i) Test binding materials and their sources

Six test binding materials (TMs) were evaluated against a commercial binder, Mycobind® as a reference material (R). The TMs were four clays from Arusha (AC), Kilimanjaro (KC), Coast (CC) and Morogoro (MC) and two ashes named volcanic ash (VA) and rice-husk ash (RA). The nature, source and local use (ethno-utilization) of the TMs are shown in Table 5. An arbitrary amount of five kilograms of each of the AC, KC, MC and VA were purchased from respective local market places, and then the source was visited ascertain the originality. Similarly, same amount for the CC was directly taken from the mining site in Coast region. The samples were taken to the laboratory at the Geological Survey of Tanzania for cleaning, grinding, sieving and homogenization; then packed in zip bags for storage prior subsequent chemical analyses and evaluation for aflatoxin-adsorption capacity.

An amount of five kilograms of VA was purchased from local market, and the site of production was also visited to ascertain its originality and then, handled in the same manner as the clay samples. A representative sample of rice husks was taken from rice millers and incinerated in the laboratory furnace at the Nelson Mandela African Institution of Science

and Technology (NM-AIST) at a temperature of 550 °C for four hours, producing about five kilograms of ash.

Table 5: Physical appearance, sources and local uses of the test binding materials

Material ID	Physical Appearance	Source Region	Ethno-Utilization
Clays			Treatment of human skin infection and ailments
AC	Brick-red clogs	Arusha	
KC	Brownish-red blocks	Kilimanjaro	Geophagial satisfaction
CC	Shiny white granules	Coast	Stomach ailment treatment and for decorations
MC	Brownish-red granules	Morogoro	Geophagial satisfaction
Ashes			Food seasoning and tenderization in traditional cookery, feed additive
VA	Greyish Volcanic powder	Arusha	
RA	Greyish-white fine powder	Various places	Soil fertility improvement

In this and subsequent tables: AC = Arusha clay, KC = Kilimanjaro clay, CC = Coast clay, MC = Morogoro clay, VA = volcanic ash and RA = Rice-husk ash

(ii) The reference binder

For comparison of the binding capacity of the crude clays and ashes, a commercial mycotoxin detoxifier named Mycobind® (Evonik Industries AG) was purchased from Farmers Centre Limited in Dar es Salaam, Tanzania and employed in the study.

(iii) Aflatoxin solution

The stock solution of aflatoxins (Romer Labs, Inc., Washington, MO, USA) was donated by the then Tanzania Food and Drugs Authority (TFDA). Being a dangerous toxic solution any handling of the solution was done with much precautions with protective gears. All operations with this solution was done in the fume chambers and due to its sensitivity to light, amber vials and flasks were used in handling the solution.

3.2.2 Methods

(i) The *in-vitro* experiment

The experiment was conducted based the procedure suggested by Kong *et al.* (2014), simulating the gastrointestinal pH condition of pigs, also representing monogastric animals, which are more prone to aflatoxicosis. The experiment involved preparation of various

solutions, incubation of the binding materials with the solutions, centrifugation and determination of aflatoxin amount bound by the materials. The experiment was conducted at the then Tanzania Food and Drugs Authority (TFDA).

Preparation of the experimental solutions

- **Buffer solution**

The buffer solution was prepared from potassium chloride, potassium dihydrogen phosphate, anhydrous disodium hydrogen phosphate and sodium chloride in distilled water. The solution was used as pH buffer for the binding media.

- **Diluted aflatoxin solution**

The standard solution of combined aflatoxins AFB1, AFB2, AFG1 and AFG2 (250 ng/mL) in acetonitrile was diluted to 20 ng/mL using distilled water in an amber flask for spiking into some of test solutions.

- **Solutions of the binding materials (BMs) and their controls**

The test solutions contained components as shown in Table 6. Spiked solutions of the BMs (individual TMs and R) were prepared from suspensions containing 0.25% (w/v) of the BMs in the buffer solution spiked with 5 mL of diluted solution of aflatoxins. Non-spiked solution of the BMs, played a part of control for each of the spiked solutions of the BMs containing the suspensions of the BMs in the buffer solution. Positive control prepared from the buffer solution spiked with 5 mL of diluted solution of aflatoxins; and negative control containing the buffer solution only.

Table 6: Experimental solutions and composition

Solution samples	Composition	Number of samples	Replications	Total Units/Tubes
Spiked BMs	BMs in buffer solution and diluted aflatoxin solution	7	3	21
	Controls			
Non-spiked BMs	BMs and buffer solution	7	3	21
Positive control	Buffer solution spiked and diluted aflatoxin solution	1	3	3
Negative control	Buffer solution only	1	3	3

Setting of treatments

A sample of each BMs was prepared by weighing 0.025 g into 10 mL of phosphate buffer solution (0.1 M, pH 6.0) making a suspension of 0.25% (w/v). An aliquot of 2.5 mL suspension was pipetted into 25-mL centrifuge-tube; then, 5 mL of the diluted aflatoxin solution was added. Parallel with the solutions of the BMs, their respective negative controls (non-spiked with the diluted aflatoxin solution) were run. General positive and negative controls were included to eliminate the probable error effects such as due to aflatoxin impurities in the measuring/analysis system hardware and reagents. The positive control contained 2.5 mL of phosphate buffer, and five mL of the diluted aflatoxin solution, while the negative control contained five mL of the phosphate buffer solution only. Each solution sample was replicated thrice, and the pH in each centrifuge tube was adjusted to 2.0 by adding 1 M of HCl to simulate the pH in the stomach of pigs.

Incubation of the treatment samples

All of the samples were incubated at 39 °C in a shaking water bath for two hours; then, one millilitre of phosphate buffer (0.2 M, pH 6.8) was added to each tube. To simulate the conditions in the small intestine of pigs, the pH in all of the tubes was raised to 6.8 by adding 1 M of NaOH, followed by a second phase of incubation at 39 °C for four hours. After incubation, the mixture was centrifuged, and the supernatant was obtained for an analysis of the residual (unbound) aflatoxins B1, B2, G1 and G2 using high-performance liquid chromatography (HPLC).

Determination of binding capacity of the BMs

The pH of the clear supernatant was adjusted to about 7.4 using 0.1 M of NaOH. Unbound aflatoxin in the supernatant was determined by the procedure as suggested by Diaz and Smith (2005), where the clear supernatant was analysed for residual (unbound) aflatoxin without additional clean-up. The analysis employed a fluorescence detector connected to HPLC (Shimadzu Corp, Kyoto, Japan) at a mobile phase flow rate of 0.8 mL/min and a temperature of 28°C, through a stationary phase column (5 µm × 4.6 mm × 150 mm, Spherisorb ODS-1, Waters, Milford, MA, USA). Residual aflatoxins AFB_1, AFB2, AFG1 and AFG2 were quantified at 363-nm excitation filter and 440-nm cut-off emission filter wavelengths using the fluorescence detector (RF-10AXL SMN C20954406285, Knauer, Berlin, Germany).

The aflatoxin-binding capacity of a binding material was determined in percentage as proportion of AFB1, AFB2, AFG1 or AFG2 adsorbed. The percentage-binding capacity P_i of i^{th} BMs in binding j^{th} aflatoxin was determined using Model 3.

$$P_i = (IAT_{ij} - UAT_{ij})/IAT_{ij} \times 100 \text{ ……. Model 3}$$

where, IAT_{ij} (ng/mL) is the initial concentration of j^{th} aflatoxin in the test tube with i^{th} BM; and UAT_{ij} (ng/mL) is the residual (unbound) j^{th} aflatoxin in the test tube with i^{th} BM after the digestion period. The IAT_{ij} was considered to be the amount of aflatoxin recovered from the positive control adjusted by subtracting the value obtained for the negative control. The UAT_{ij} was adjusted by subtracting the residual aflatoxin amount that was obtained for the negative control of each of a BM from the concentration of residual aflatoxin in the supernatant of the solutions of BM spiked with diluted aflatoxin solution.

(ii) Data analysis

Statistical analyses

Data regarding the percent mean-binding capacity was analysed by the General Linear Model (GLM) programme of Statistical Analysis System (SAS) (Schabenberger, 2007) using the statistical expression of Model 4.

$$Y_{ij} = B_i + A_j + e_{ij} \text{ …… Model 4,}$$

where, Y_{ij} = response as binding capacity of the i^{th} BM in adsorbing the j^{th} aflatoxin

B_i = binding effect due to the capacity of the i^{th} BM in adsorbing the j^{th} aflatoxin; A_j = binding effect due to the ease with which the j^{th} aflatoxin is adsorbed to the i^{th} BM; e_{ij} = the error due observation on i^{th} BM and j^{th} aflatoxin. The mean separation was done by the Duncan Multiple Range procedure, and the significance was set at an alpha level of 0.05.

Determination of aflatoxin-binding capacity ratio of Mycobind® to the TMs

The aflatoxin-binding capacity of Mycobind® (CR, %) relative to the aflatoxin-binding capacity of a TM (CTM, %) as a ratio $\acute{R}$ was determined using Model 5 as $\acute{R}$.

$$\acute{R} = CR/CTM \text{ …… Model 5.}$$

3.3 Chemical properties influencing aflatoxin-binding capacity of the clay and Ash-based materials

3.3.1 Materials

The same binding materials (BMs) used in the *in-vitro* experiment were used in this evaluation to explore inherent chemical properties influencing aflatoxin-binding capacity of the material. These were further homogenized, ground and sieved through a one-millimetre sieve for the analyses of mineralogical composition, elemental content and cation exchange capacity (CEC).

3.3.2 Methods

(i) Mineralogical composition

Samples of the BMs were analysed for their mineralogical composition using non-destructive techniques that employed an X-ray diffraction (X-RD) analyser (BTX SN 231, Olympus Corporation, Tokyo, Japan) or a self-calibrated XRD analyser, depending on temperature as explained by Kahle *et al.* (2002). The samples were analysed at a temperature of −45 °C. About 15 mg of finely ground sample was sieved through a 150-µm sieve and loaded in the vibrating sample holder of the X-RD analyser for scanning. The results were X-RD spectrum patterns that were received on a screen of a computer connected to the analyser, showing peaks corresponding to each specific mineral present in the sample.

(ii) Elemental-oxide composition

The oxides in the BMs were quantified by Minipal-4, which was a high-performance bench top energy dispersive X-ray fluorescence spectrometer (PANalytical MINIPAL-4, EDXRF Spectrometer, Almelo, The Netherlands). The sample was ground into a fine powder; then, about 50 g of it was scanned by the spectrometer for metallic oxide composition at an energy dispersion of 30keV. The percent composition of the metallic oxides in each sample was determined and recorded as explained by Ahmad *et al.* (2009).

(iii) Cation exchange capacity

The cation exchange capacity (CEC) was determined by wet analysis employing the ammonium replacement method (Buchner funnels vacuum flasks), as explained by Brady and

Weil (2008) involving leaching of exchangeable cations in the binding materials (BMs) with ammonium acetate salt solution. The excess salt was removed by ethanol and followed by potassium chloride to leach NH_4^+, which initially replaced other various cations of the BMs. The amount of $NH4^+$ that was released and washed into a beaker beneath Buchner funnels was determined by the Kjeldahl distillation method as explained by Sikora & Moore (2014), and the CEC (meg/100 g) of BMs was computed using Model 2.

CEC = [(mg/L NH_4-N in leachate)*0.018*(100÷sample weight (g)] mg/L NH_4-N ……Model 2.

(vi) Determination of relationship of chemical properties of the BMs and their aflatoxin-binding capacity

The relations of inherent chemical properties of the BMs and their aflatoxin-binding capacity were determined by correlation analysis, using MS-Excel. The relationship sought were between (a) elemental oxide concentrations in the BMs and the CEC values, (b) elemental oxide concentrations in the BMs and their percent aflatoxin-binding capacity values and (c) CEC values and percent aflatoxin-binding capacity values.

3.4 Potential of the clay and Ash-based materials in reducing detrimental effects of dietary aflatoxins in animals

3.4.1 Materials

In this section materials used to study the potential of the clay and ash-based materials in reducing detrimental effects of dietary aflatoxins in animals are described:

(i) The test materials

*S*even binding materials (BMs) including clays from Arusha (AC), Kilimanjaro (KC), Coast (CC) and Morogoro (MC), volcanic ash (VA), rice-husk ash (RA) and reference binder (Mycobind®, R), as previously described in the *in-vitro* experiment in this study, were tested for their capacity to reduce detrimental effects in animals. A dietary treatment was formed by homogenizing 2% of one of the BMs in a separate portion of the basal diet, naturally contaminated with aflatoxins. The inclusion rate of the BMs was adopted from previous studies suggesting that that rate of binding materials such as clay in a diet can significantly reduce dietary aflatoxin biomarkers (Phillips *et al*., 2002). The materials AC, KC, CC, MC,

VA, RA, R in the basal diet and a control C, that is, basal diet alone, formed eight dietary treatments DAC, DKC, DCC, DMC, DVA, DRA, DR and DC respectively. Albino rats (*Rattus norvegicus*) were used for testing effect of including the BMs in diet, on the bioavailability impact of aflatoxins on the health of the animals.

(ii) Experimental animals and their handling

Initially, a total of 128 rats of mixed sex, aged 6 – 8 wk old were purchased from the unit of experimental animals of the College of Veterinary Medicine and Biomedical Sciences (CVMBS) of the Sokoine University of Agriculture (SUA), Tanzania. During acclimatization of the animals for one week some animals were eliminated as were found too weak and unfit for the experiment. Diet and water were offered ad-libitum to the animals once on daily basis in the morning around 08:00 am. The animals were inspected daily for general health appearance and behavioural changes.

(iii) Preparation of the basal diet

Prior to inclusion of the test binder, the aflatoxin-contaminated basal diet was prepared by incubating a broiler starter with contaminated peanut waste (at about 1:20) under wet condition, for five days, at room temperature (about 25 °C). The diet was then sterilized for 0.5h at about 121°C and dried at room temperature for 4 days under aeration as suggested by Salazar *et al.* (2012). The concentration of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) in the diet was determined by high-performance liquid chromatography (HPLC) adopting the method as explained by Khayoon *et al.* (2010). Concentration and relative proportion for each individual aflatoxin in the basal diets were obtained with and relative proportion (Table 7). This was for the purpose of gauging the level of the toxin in the diet to avoid unintended acute intoxication of the experimental rats.

Table 7: Individual aflatoxin concentration in the contaminated basal diets

Aflatoxins	**AFB1**	**AFB2**	**AFG1**	**AFG2**	**Total**
Concentration (ng/g)	209.8	3.2	8.0	0.2	221.2
Proportion of total (%)	95	1.5	3.6	<0.1	100

3.4.2 Methods

(i) Experimental design

A total of 109 rats of mixed sex in eight unbalanced groups in a complete randomized design experiment were used for the experiment. The animals were kept at room temperature in individual plastic cages, each fitted with a feeder and a drinker. The rats were randomly allocated into the eight treatments in a Complete Randomized Block Design (CRBD).

(ii) Measurement of physiological parameters

A number of assessments were carried to explore potential capacity of the proposed local materials in immobilizing toxic effects of dietary aflatoxin contamination in rats. These included total feed intake, growth rate, feed conversion efficiency, packed cell volume, concentration of serum components (total proteins, albumin and globulin and albumin/globulin ratio), relative weight of liver, kidney and spleen as well as histopathological examination of liver, kidney and spleen. It is widely known that aflatoxins impair digestion and absorption of nutrients in animal body (Nasrabadi *et al*., 2013), haematological processes and serum protein balance (Kaneko *et al.,* 2008 cited by Dónmez *et al*., 2012). In addition, liver, kidneys and spleen are internal organs immediately encounter effects of aflatoxins (Devendran *et al*., 2011).

(iii) Feed consumption and performance of the animals

- **Feed intake**

Daily feed intake (FI, g) for individual animal was determine as the amount (grams) of ration less amount (g) of refusal (g) per day as was applied by Vento *et al.* (2008). Value of FI for a treatment was determined as the mean FI of all animals on the treatment within 35 days of the experiment. Both daily ration and refusal were weighed using a sensitive electronic balance (CAMRY, ISO 9001: 2008, MODEL: EHE901) at two decimal placed.

- **Growth rate**

Daily growth rate (GR, g/d) for a treatment was determined as the mean body weight change per day for all animals on the treatment within five weeks of the experiment as was applied by Laaksonen *et al*. (2013) and Idoko *et al.* (2015). The animals were weighed in the

morning (around 8.00 am) before feeding. Weighing was done by a sensitive electronic balance (HT-CL Series Compact Scale, Model HT-500CL. A & D) at two decimal places.

- **Feed conversion efficiency**

Feed conversion efficiency (FCE) (in percentage) was determined as devised by as a ratio of the average weekly growth (GR) to the average weekly feed intake (AFI) per animal then as mean for a treatment using Model 6.

$$FCE = GR/AFI \times 100\% \quad \text{Model 6.}$$

(iv) Haematological parameters

- **Packed cell volume**

The Packed Cell Volume (PCV) of blood samples was determined by a haematocrit reader scale adoped from Kelani and Durotoye (2002). For each animal, blood sample was collected from orbital vein to fill about three quarters of heparanized glass capillary tube and one tip was sealed by dipping in seal wax. The capillaries were centrifuged at a speed of 3500 rpm for five minutes. The value for erythrocyte and buffy coat layers was read on haematocrit (Hawksley Micro-Haematocrit) reader scale and recorded in percentage.

- **Serum protein components**

Using a plain glass capillary, blood sample was collected from orbital vein into 1ml tube (Eppendorf International) per animal as explained by Parasuraman *et al.* (2010). Samples were left to clot at room temperature for thirty minutes and then centrifuged at 2000 rpm for ten minutes. Serum was then separated transferred to a new vial using micro-pipette and then stored at -20 °C for analysis of total serum proteins and albumin adopted from Rai and Vitzthum (2006).

- **Total proteins in serum**

Total serum proteins and serum albumins were determined by absorbance method and read using spectrophotometer (1100 RS spectrophotometer Cole Parmer^{R} Product of United Products & Instruments Inc.) according to the procedure applied by Zaia *et al.* (2005).

Conditions of the assay used were as follows: Absorbance wavelength was 540 nm (530-550), cuvette light path was 1cm, temperature range for the sample and standard solution was between 15 – 25 °C and the spectrometer instrument was adjusted to zero with distilled water. Amounts of 25 µL of the standard solution and sample were each pipetted into a separate vial of 1.5 mL. In each of the two vials and a third one with blank, 1 ml of the Biuret solution was added and mixed well. The mixture was incubated for ten minutes at room temperature. The absorbance (A) of the sample and standard was read against the blank. Concentration (C) of total protein was determined by Model 7.

$$C\ (g/dL) = A_s/A_{std} \times C_{std} \quad \text{..... Model 7,}$$

where, A_s = absorbance of sample, A_{std} = absorbance of standard and C_{std}= concentration of the standard = 7 g/dL.

- **Serum albumin component**

Serum albumin was determined a technique employing Bromocresol green a dye of the triphenylmethane family that highly bind to albumin as explained by Hill (1985) and Sabnis (2008). Conditions of the assay used were as follows: Absorbance wavelength was 630 nm (600-650), cuvette light path was 1.0 cm, temperature set for assay 15-25 °C conditions of the assay used were as follows: the instrument was adjusted to zero with distilled water. Exactly 5 µL of the standard solution and sample were pipetted into vials of 1.5 ml for each. In each of the vials with the standard, the sample and one with blank, 1.0 mL of the Bromocresol green solution was added and mixed well. The mixture was incubated for ten minutes at room temperature. The absorbance (A) of the sample and standard was read against the blank.

Concentration (C) of albumin was determined by Model 8:

$$C\ (g/dL) = A_s/A_{std} \times C_{std} \quad \text{.....Model 8,}$$

where, A_s = absorbance of sample, A_{std} = absorbance of standard and C_{std} = concentration of the standard = 5 g/dL.

- **Serum globulin component**

Serum globulins concentration was determined by difference between total proteins concentration and albumin concentration, with assumption that albumins and globulins almost complement to form the total proteins in serum as device by Busher (1990) and Nandedkar *et al.* (1986).

- **Albumin-globulin ratio in serum**

Albumin-Globulin Ratio (AGR) was determinate based on the procedure applied by Du *et al.* (2014) as determined according to Model 9;

AGR = Albumin concentration (g/dl) ÷ Globulin concentration (g/dL) …… .Model 9.

(v) Changes in liver, kidneys and spleen

- **Relative weights of liver, kidney and spleen**

The relative weight of the organs was determined as the ratio of the individual organ to the total body weight on sacrifice as adopted by Alimba *et al.* (2012). At the end of the experiment, the animals were weighed in the morning of necropsy day and then euthanized by ether asphyxiation. Using dissection kit the abdominal cavity was opened to expose internal organs. The liver, right kidney and spleen were carefully removed, weighed using sensitive electronic weighing balance (two decimal places). The final body weight (FBW) and weights of liver (LW), kidney (KW) and spleen (SW) were then recorded. Relative weight of individual organ RWO was determined by taking weight of the organ as percent of FBW (Model 10).

RWO =WO/FBW*100%, where WO =LW, KW or SW……...…. Model 10.

- **Histopathologal changes of the liver, kidney and spleen**

Tissue samples of liver, kidney and spleen were taken at necropsy, and fixed in 10% neutral buffered formalin for histopathological examinations through a standard as recommended by Anderson (2011). Briefly, the procedure involved dehydration with alcohol, clearing with xylene to remove the alcohol from the tissue and then blocking by molten wax to remove the xylene. The tissue was then sectioned into 4µm thick paraffin section using a rotary

microtome (Baird and Tatlock), then mounted on mounting bath (Electrothermal). Routine haematoxylin and eosin staining was done using haematoxylin and eosin dyes. The tissue was washed with water then dewaxed by xylene, treated by ethanol and then hydrated with water. The section was treated with Harris's haematoxylin, differentiated with 1% acid alcohol then blued in alkaline water made by saturated lithium carbonate. Eosin counter stain was applied then dehydrated by ethanol. The section was finally cleared by xylene, mounted with cover slip and then left to dry before examining under light microscope (Olympus BX41, Japan).

- **Gross appearance of the liver, kidney and spleen**

Liver, kidney and spleen from eviscerated rats were grossly examined using necked eyes to identify any conspicuous changes particularly colour then photo was taken using camera. Assessment was done by displaying the organ per the dietary groups of rats then identify colour change from grossly normal (deep red) to defective (pale red/yellowish-brown).

(vi) Data analysis

Quantitative data set of parameters (for total feed intake, growth rate, feed conversion efficiency, packed cell volume, concentration of serum components (total proteins, albumin, and globulin and albumin/globulin ratio), relative weight of liver, kidney and spleen) were analysed by the General linear model (GLM) programme of SAS for mean determination and mean separation Turkey procedure as explained by Schabenberger (2007). The diets, each formed by one of the BMs were the treatment term while sex made two blocks of the experiment.

The quantitative analysis was done based on the Model 11;

$$Y_{ij} = M + T_i + B_j + I_{ij} + e_{ij} \quad \text{Model 11}$$

Where, Y_{ij} = Over all response; M = baseline mean; T_i = i^{th} treatment effect; B_j = j^{th} block effect; I_{ij} = treatment and block interaction effect; e_{ij} = random error of the observation due the i^{th} treatment and j^{th} block.

Qualitative information for histopathological changes of liver, kidney and spleen were manually evaluated by content analysis.

(vii) Ethical consideration

The study protocol for the *in-vivo* study using rats was reviewed and approved (Ethical Clearance Reference No. SUA/NM-AIST/P120/T.13/1) by the Sokoine University of Agriculture Research Ethics Sub-Committee (SUA-RESC).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Socio-Economic factors influencing awareness of aflatoxins among farmers

4.1.1 Socio-economic characteristics of respondents

The targeted sample of household respondents required for the study was well attained. Briefing was done such that prospect respondents were motivated to participate in the interview leading to very few household refusals in answering the questionnaire. However, the refusals were handled by utilizing the advantage of the adopted systematic sampling technique, which allowed moving forward in selecting sampling units (households) until a required sample was obtained.

The socio-economic characteristics of the respondents shown in Table 8 indicate that livestock farming as an economic activity is done by people of various social groups as was also reported by Amimo *et al.* (2011) in a survey done in Western Kenya. A little more men than women participated in the interview, a phenomenon related to the tendency that majority of the household heads are men. Farmers aged above 45 had higher proportion against the younger ones since the latter are likely to be more active away from homes. The sample had a bit more farmers with education above secondary than those with education below secondary level. Probably, this could be due to the prevailing socioeconomic factors related to resource scarcity and mainly land, where majority endeavour to invest in knowledge capital for the sake of wider range of employment which is supported by Lehtimaki and Lehtimaki (2016).

About half of all the respondents were found to have been exposed to life/biological sciences based studies, a scenario also reported by Awuah *et al*. (2008) elsewhere in Ghana. More farmers were found under formal employment than those under informal probably due to the tendency that most of the farmers have dual employment, formal being primary. Similar analogy is explained for the occupation in terms of farming and non-farming. The proportion of farmers who had been keeping animals for ten years or less was a little more than those for over ten years. Possibly this is due to the tendency that more new people join the activity of animal keeping with time for sake of using the available market of animal products (Ngailo *et al*., 2001; Luyombya, 2014)

Table 8: Socioeconomic characteristics of the respondents

Socio-economic characteristics	Categories	Frequency (%)
Gender	Female	119(46)
	Male	139(54)
Age	≤45years	114(44)
	>45 years	144(56)
Education level	<SE	110(43)
	≤ SE	148(57)
Field of specialization	Life/Biological Sc.	132(51)
	Other studies	126(49)
Employment category	Formal	111(43)
	Informal	147(57)
Occupation type	Farming	124(48)
	Non-Farming	134(52)
Animal keeping experience	≤10 years	132(51)
	>10 years	126(49)

4.1.2 Description of the perception of respondents towards feed aflatoxins

The results on the descriptive analysis of the respondents' awareness of aflatoxin contamination of feeds are presented in Table 9. Only about a quarter of respondents had heard about the term aflatoxins. This level was relatively low as compared to the level of 93% reported by Marechera and Ndwiga (2014) in Kenya and a bit higher than the value of 20% reported by Kamala *et al.* (2016) in Kilosa district in Tanzania. The deviation may be due to time lag and locality attributes. For instance, in the Tanzanian cases, the study in Kilosa was conducted in 2010 and the current one was conducted in 2016 where different rates of awareness have been recorded. Other reasons may be due to factors such as nature of the study population. In Kenya, epidemiological events of aflatoxicosis that killed a number of people (Probst *et al.,* 2007) might have raised louder alarm on aflatoxins.

The fact that more than half of the respondents with awareness of aflatoxins got the information recently (≤1 year past) compared to about one-fifth who got it about two years ago implies that there has been an increase in knowledge of aflatoxins with time in the study

area. Over two-thirds of respondents who had ever heard about aflatoxins got the information from the mass media. The rest of respondents obtained the information through seminars and experts, neighbours and friends, and from written resources. Results show that just few farmers got information about aflatoxins through reading, probably indicating scarcity of written resources as information about aflatoxins, low reading motivation on the side of farmers, or else the materials being too technical for farmers. This implies that mass media may be the best way to sensitize livestock farmers, other key stakeholders and the general public about aflatoxins and means to alleviate their exposure. Mass media as mentioned by the farmers meaning radio, television and scantly newspapers which are the common and readily accessible sources of information. Of these, radio and television programs are considered the most appropriate sources of information especially for the Swahili-conducted programmes. Recently, there have been some initiatives to inform the public about aflatoxins in Tanzania through radio, television and newspapers (Nathaniels, 2014). Perhaps, the current level of awareness is the result of some initiatives to sensitize the general public about aflatoxins in the country through media.

Table 9: Respondent distribution based on socio-economic characteristics and awareness about aflatoxins

Socio-economic characteristics	Categories	Total frequency (n)	Heard about aflatoxins	
			Frequency	% of n
Gender	Female	119	39	33
	Male	139	32	23
Age	≤45years	114	35	30
	>45 years	144	36	25
Education level	<SE	110	12	11
	≥SE	148	59	40
Field of specialization	Life/Biological Sciences	132	55	42
	Other studies	126	16	13
Employment category	Informal	147	24	16
	Formal	111	47	42
Occupation	Farming	124	18	15
	Non-farming	134	53	40
Period of keeping animals.	≤10 years	132	44	33
	>10 years	126	27	21
Location (wards)	Ambureny	35	4	11
	Imbaseny	39	22	56
	Nkoaranga	34	13	38
	Patandi	38	9	24
	Poli	38	9	24
	Seela-Sing'isi	42	8	19
	Songoro	32	6	19

Farmers with education above secondary level, exposed to Life/Biological science based education, in formal employment and those dwelling is some specific locality such Imbaseny showed relatively higher knowledge of aflatoxins than their counterparts. The education level and field of specialization seemed to be the major socioeconomic factors governing farmers' awareness of aflatoxin contamination of feeds. Influence of higher education and academic exposure to life/biological based sciences on awareness of aflatoxins has been reported in other analogies (Dosman *et al.*, 2001; Magembe *et al.*, 2016; Ngoma *et al.*, 2017). The implication is that education level has direct and indirect (through other socioeconomic characteristics such as employment and occupation) positive influence on awareness of aflatoxin contamination.

Factors of gender/sex and age of respondents had no statistical influence on the awareness of aflatoxins among farmers. The finding that short period of keeping animals is associated with awareness about aflatoxins was not expected. Probably women are more engaged in managing livestock than men while young farmers are likely to be the recent ex-colleges, who are able to access information quickly. Short period in keeping animals may also be associated with young age which again enjoy easy access of information on various issues including animal keeping activity.

Wards such as Imbaseny and Nkoaranga showed relatively higher proportions of farmers with information about aflatoxins. Geographically these wards are enjoying close vicinity with a number of academic institutions. According to Meru District Council Socio-economic Profile (URT, 2017) existing institutions are the Nelson Mandela African Institution of Science and Technology (NM-AIST); Tumaini University Makumira (TUMA); University of Arusha (UA). Others are such as Tengeru Institute of Community Development (TICD); Livestock Training Agency - Tengeru (LITA – Tengeru) and National Artificial Insemination Centre (NAIC). These and huge number of other lower training centres and secondary schools are likely to favour awareness on many issues including aflatoxins.

4.1.3 Awareness of farmers on contamination of feeds by fungal toxins

The results of the descriptive statistics on the farmers' awareness on the general fungal toxins are presented in Table 10. About half of the respondents were aware that feeds may contain natural fungal toxins. Those found aware were further asked to mention by name any specific fungal toxins that may occur in feeds. Of these, only few (6%) managed to come out with the

term “aflatoxins” or its translation as “sumu-kuvu” in Swahili (the communication media used in the interview). Majority could not name any specific fungal toxins though they perceive that feeds may contain some inherent toxins on spoilage. About 20% managed to give at least miscellaneous and broader concepts as they perceive, such as mould, mould toxins/products, cancer causing toxins, diarrhoea-causing toxins, bloat-causing toxins, feed/food mould and toxins due to rotting/spoilage/rusting. In a similar study by Jelliffe *et al.* (2016) respondents had difficulty in naming the toxins occurring in groundnuts as “aflatoxins” instead they called them mould or bitter nuts.

The important fungal toxins known to occur in foods and feeds include aflatoxins, fumonisins, trichothecenes, zearalenone, citrinin, ergot alkaloids and ochratoxins-A, and patulin, however, aflatoxins is being recognized as the most hazardous toxins (Rocha *et al*., 2014). The results of the current study imply that livestock farmers have limited information about the fungal toxins and aflatoxins in particular. The low awareness and unclear concept about aflatoxins is common in many settings as reported in other studies (Rocha *et al.,* 2014; Gizachew *et al.,* 2015; Kiama *et al.,* 2016; Nleya *et al.*, 2017). The situation may allow high aflatoxin exposure level through contaminated feeds leading to health hazards in animals and humans.

Table 10: Awareness of farmers based of fungal toxin contamination of feeds

Respondents' perceptions on feed aflatoxins	Frequency (%)
Possible presence of fungal toxins in feeds (n=258)	
Yes	133(52)
No	111(43)
Not certain	14(5)
Specific probable fungal toxins in feeds (n=133)	
Aflatoxins	8(6)
Other toxin fungal names	26(20)
Do not know	99(74)
Feed ingredient susceptible to fungal toxin contamination (n=133)	
Maize bran	96(72)
Wheat feeds	3(2)
Wheat pollard	5(4)
Sunflower seed cake	1(1)
Cotton seed cake	1(1)
Other feed ingredients	4(3)
Do not know	23(17)
Possibility that fungal toxins in feeds affect animal health (n=133)	
Yes	113(84)
Not certain	18(14)
No	2(2)
Possibility that fungal toxins are transferred from feeds to foods of animal origin (n=133)	
Yes	21(16)
Not certain	11(8)
No	101(76)
Signs to suspect presence of fungal toxins in feeds (n=133)	
Abnormal colour	66(48)
Abnormal consistence	24(18)
Bad odour (rotten/soil smell)	47(36)
Insect/larva presence	3(2)
Impaired animal health/deaths	13(5)
Do not know any indicator	24(18)
Ability to detect mould in feeds (n=133)	
Yes	123(93)
No	9(7)
Not certain	1(1)
Possibility of detoxifying fungal toxins in feeds (n=133)	
Yes	83(62)
No	38(29)
Not certain	12(9)
Heard about aflatoxins (n=258)	
Yes	71(28)
No	187(72)
Means through which aflatoxins were heard (n=71)	
Reading	3(4)
Mass media (Radio/TV)	49(69)
Seminars/Experts	11(16)
Friends/Neighbours	8(11)
Time when heard about aflatoxins (n=71)	
≤ one year ago	40(56)
Two years ago	15(21)
>two years ago	16(23)

Majority of the farmers (Table 11) perceived that maize bran is the most susceptible feed ingredient to fungal toxin formation. This is supported by another report that maize is one of the most susceptible cereals to mycotoxin contaminations (Grace *et al.*, 2015b). Maize genome has genes that easily encode formation of some enzymes favouring fungal growth, sporulation and toxin production, additionally offer little environmental stress resistance which predisposes plants to toxigenic fungal invasion (Warburton *et al.*, 2013). In addition, bran as by-product of cereal grains is the major sink of mycotoxins initially carried in the whole grain (Nziramasanga *et al*., 2005). Farmers claimed that moisture in the maize bran due to water sprinkled into the maize grain prior to or during dehulling process favours further growth of the toxigenic mould with time in storage. One of the respondents, also a corn miller commented that water added in maize during dehulling and heat generated cause the bran spoilage and eventual toxin formation if quick drying of bran is not done. In Tanzania, where dry milling is a common practice, the farmers' perception that maize bran is the most susceptible to fungal contamination is valid.

Feed spoilage and contamination may occur due to relatively high postharvest moisture content, improper drying, delayed drying and storage with moisture above critical values for mould growth (Jay *et al.*, 2005; Kimanya *et al.*, 2010). This calls for prompt and proper drying of feeds particularly maize bran as a supportive measure in alleviating exposure to aflatoxin contamination of feeds.

A number of the respondents (Table 11) perceived that feeds with fungal toxin contamination have health hazards to animals. The respondents' perception has been empirically verified and reported (Sohooa *et al*., 2015). With acute levels such toxins may be fatal within a short time while chronic levels may cause death after a relatively long time through immuno-suppression, encouraging vulnerability and opportunistic diseases (Dhanasekaran *et al*., 2011). The fungal toxin contamination of feeds is also associated with animal production loss due to the impaired health leading to low production performance (Grace *et al*., 2015b).

Large proportion of the respondents (76%) had opinion that natural feed toxins cannot be transferred to animal tissues and ultimately to the foods of animal origin (Table 11). The findings concurred with another report (Kiama *et al*., 2016) which showed perception of some dairy farmers in Kenya that direct eating of mouldy food is harmful but eating products from animals fed mouldy feeds is safe. Some reports refute this perception (Grace *et al*., 2015b; Okoth, 2016). Their studies showed that fungal toxins consumed in feeds by animals

are assimilated into body tissues and then released into foods of animal origin as metabolites of the original toxins, which are also toxic to the secondary consumers. Studies have shown that AFB1 is metabolized to aflatoxin M1 (AFM1) in the liver and then transferred to milk, eggs and meat of animals ingested the toxin in feeds (Njugi *et al.*, 2018). Independent studies have been done by different researchers to validate this (Hussain *et al.*, 2016; Iqbal *et al*., 2014; Khan *et al*., 2013; Sassahara *et al*., 2005). Residues of aflatoxins were found in raw cow milk (Sassahara *et al*., 2005) eggs (Iqbal *et al*., 2014) and broiler meat (Khan *et al.*, 2013; Iqbal *et al*., 2014; Hussain *et al*., 2016). The amount of AFM1 in fresh milk may range from one to seven percent of the total amount of AFB1 ingested in a diet (Grace, 2013). In higher-yielding animals consuming large amounts of concentrates, the transfer rate from feeds to milk may be higher. Aflatoxin transfer to eggs and chicken meat has been found at rates of 0.1% and 0.01% respectively (Grace, 2013). These levels of aflatoxin transfer to foods of animal origin contribute to chronic intake of the toxins to human leading to great health risk. In practical sense these technicalities are beyond the knowledge capacities of many farmers and therefore, there is a need to simplify them into simpler expirations to suit all farmers. With this, farmers can comprehend the problem of these toxins in feed chain, on top of what they know about mouldy feeds and health hazards in animals. This will build care and habit among farmers to avoid feeding mouldy feeds to animals.

The respondents reported that they suspect presence of fungal toxins in feeds if the feeds look spoiled and may be tested by one or more of the indicators shown in Table 11. Feed abnormal colour such as brownish, blackish greenish, or bluish, rotten or soil smell, abnormal consistence such as clumps and fibrous forms, and presence of insect larvae were reported as key indicators for quick tests of feed spoilage. Other indicators to suspect presence of fungal toxins in feeds reported by the farmers were, for example, animal refusals of the feeds especially if associated with abnormal smell, general poor appetite of animals, abnormal milk taste, poor health, and animal deaths. Some of these indicators were also reported in an on-farm study as strategies to manage mould and fungal toxin formation in feeds (Golob, 2009). When strictly and carefully utilized, the indicative signs and symptoms may be helpful in detecting mouldy feeds that are likely to be contaminated with aflatoxins. However, it is worth noting that absence of these signs does not guarantee that the feeds are entirely free of the toxins and safe (Antony *et al*., 2012).

Studies have shown that it is feed contamination with fungal toxins under normal environment is inevitable (Grace, 2013). According to the results of the current study feed discoloration and off-smell are useful frontline indicative factors to suspect feed contamination and possibly presence of aflatoxins and other fungal toxins. Some respondents declared not knowing any indicator to suspect presence of these toxins in feeds. Inability to suspect and detect feed spoilage and contamination using quick test may allow exposure to aflatoxin contamination of feeds thus putting consumers into higher health risk.

Large proportion of the respondents (93 %) declared that they know and are able to detect mould formation in feeds (Table 11). This is because though fungal toxins in feeds are not visible, moulds growing on feeds are visible. The farmers reported that moulds often colour and affect the appearance of the feed on which they are growing (Golob *et al.*, 2009). Feeds invaded with mould take on an unappealing/off smell (Sim *et al.*, 2005) is well known that presence of mould in feeds is a good indicator of possible contamination with fungal toxins (Golob *et al*., 2009). This may help farmers to rule out that the feed is unfit for animals and discard it outright.

About two-thirds of the respondents perceived that fungal toxins already formed in feeds can be detoxified to render the feed safe for animal. The respondents reported that possibly soda-ash, plant ashes, charcoal, salt and some herbs may reduce the fungal toxins if fed with feed resources suspected to be contaminated. Ashes are used in treating animal feeds for other purposes such as reducing ant-nutritional factors (e.g. tannins) in feeds for monogastric animals (Kyarisiima *et al*., 2004) and roughage-fibre digestibility improvement in ruminants (Laswai *et al.*, 2007). Also has been studied and fit as possible source of minerals for livestock (Ndlovu, 2007). Nixtamalization a traditional process of preparing cereal foods, particularly corn in Mexico and Central America employs a similar alkali media of lime (Albores *et al.,* 2004). The process has been proved to reduce aflatoxins in a traditional food named “tortilla” prepared by nixtamalization. Upon nixtamalization, aflatoxins is assumed be physically removed during steeping and washing, degraded, modified, or released/bound in the matrix of high pH (Schaarschmidt *et al.*, 2019). It is from these analogies ash-based materials have been employed in the subsequent parts of the current study.

Farmers also showed to have been using some herbs to counteract digestive disorders in animals assumed to be due to ingestion of toxic materials. Some compounds in form of antioxidants from plant sources have counteractive effects against the oxidative stress induced by aflatoxin in animal body after absorption (Ameen, 2011).

Life science oriented individuals are likely to be sensitive to function and interactions of living organisms and their environments unlike other groups in community. Educational background and interest may cause significant variation in levels of awareness of aflatoxin contamination of feeds among livestock farmers. About forty-two percent of the farmers who attained higher education and studied Life/Biological sciences were found aware about aflatoxins while only about twenty-five percent of those who attained higher education and studied other fields were aware of aflatoxins (Table 11). This disparity may be due to the effect of academic specialization which is likely to favour or disfavour interest and curiosity towards issues such as contamination of feeds and associated hazards.

Table 11: Awareness of aflatoxins based on education level, specialization of respondents

Level of education	Specialization/Means of accessing information	Total frequency	Heard about aflatoxins
			Frequency (% of n)
	Specialization		
<SE	Life/Biological sciences	0	0(0)
	Other fields	110	12(11)
≥SE	Life/Biological sciences	132	55(42)
	Other fields	16	4(25)
Total		258	71(28)
	Means of accessing information		
<SE	Reading	3	1(33)
	Mass media (Radio/TV)	69	6(9)
	Seminars/Experts	11	1(9)
	Friends/Neighbours	8	3(37)
≥SE	Reading	3	2(67)
	Mass media (Radio/TV)	69	43(91)
	Seminars/Experts	11	10(91)
	Friends/Neighbours	8	5(63)

(n=258)

4.1.4 Association of socio-economic characteristics and awareness of aflatoxin

Tables 12 and 13 show crude and adjusted associations between some socioeconomic characteristics and awareness of aflatoxin in contaminated feeds respectively. Exposure to life/biological sciences based training had significant ($p<0.05$) effect on the awareness about aflatoxins. The results show that likelihood of having heard about aflatoxins was six times higher for farmers got exposure in life/biological sciences compared to those with those studied other fields. The findings matched to another report by Awuah *et al.* (2008) which found a similar analogy of aflatoxin awareness menace in Ghana. Probably individuals with exposure in these studied capable of recalling and accessing information related to microbiology/mycology in which fungal products are studied, though not done real mycotoxicological studies.

Farmers with higher level of education (≥SE) were twice more aware that aflatoxins do occur in feeds than those with lower education. This result concurs with finding of other studies showing that people with higher education have higher chances to be informed and more aware of risky factors in food than people with less education (Dosman *et al*., 2001; Magembe *et al.*, 2016; Ngoma *et al*., 2017). In another similar analogy (Nyangaga, 2014) found that people with secondary and tertiary education were more aware about aflatoxins in foods and feeds than those of lower education. This may be linked to the general high reasoning capacity of the learned people.

Farmers under formal employment were five times likely to be able to detect mould formation in feeds than those under informal employment. The reason may be due to the tendency that majority of the individuals under formal employment are those with higher education. Additionally they are likely to have close contact and wider chance of sharing information and experience with each other on various issues that may include news on aflatoxins. Likelihood of knowing that aflatoxins contamination of feeds is detoxifiable was three times higher among farmers who kept animals for ten years or less compared to those in livestock industry for over ten years. The relationship between short time of keeping animals and more awareness may be linked to young age status of the farmers. Young individuals are likely to be learned with broader reasoning capacity that potentially supports the perception. This is also supported by the observation that higher proportion of young farmers had heard about aflatoxins compared to the older ones as similarly reported elsewhere (Lee *et al*.,

2017). These farmers suspected some local materials such as wood ash to have capacity to detoxify aflatoxins and other toxins in feeds.

Table 12: Crude association between socio-economic factors and aflatoxin awareness variables

Socio-economic characteristics/factors	Ever heard about aflatoxin(s)		Occurrence of aflatoxins in feeds		Ability to detect mould in feeds		Aflatoxins in feeds are detoxifiable	
	COR(95%CI	p-Value	COR(95%CI	p-Value	COR(95%CI	p-Value	COR(95%CI	p-Value
Gender								
Female	2.1(1.1-4.2)	0.04	1.0(0.6-1.7)	0.87	1.2(1.3-4.2)	0.82	2.1(1.0-4.3)	0.42
Male (r)								
Respondents' age								
≤45 yrs	1.1(0.6-2.1)	0.81	1.5(0.9-2.4)	0.12	4.6(1.0-22.7)	0.06	1.(0.9-3.7)	0.10
>45yrs (r)								
Level of education								
High	6.4(2.8-14.9)	0.00	2.0(1.2-3.3)	0.01	2.2(0.5-11.0)	0.32	1.4(0.7-2.9)	0.40
Low (r)								
Stage of education								
≤Secondary (r)								
Secondary	3.3(1.0-10.7)	0.05	2.0(1.2-3.4)	0.01	2.9(0.6-14.3)	0.19	1.6(0.7-3.5)	0.24
Tertiary	7.7(3.2-18.6)	0.00	1.7(1.1-2.8)	0.03	4.1(0.8-19.9)	0.08	1.7(0.8-3.4)	0.15
Field of specialization								
Life/Social Sc.	5.8(2.7-12.60	0.00	1.8(1.1-2.9)	0.03	1.8(0.4-7.2)	0.44	1.3(0.6-2.7)	0.46
None/Other fields (r)								
Employment category								
Formal	4.7(2.2-9.7)	0.00	1.5(0.9-2.5)	0.09	4.8(1.0-23.5)	0.05	2.5(1.2-5.1)	0.14
Informal (r)								
Occupation								
Farming (r)								
Non-farming	4.3(2.0-9.1)	0.00	1.6(1.0-2.7)	0.05	3.1(0.6-15.4)	0.16	2.3(1.1-4.8)	0.03
Animal keeping experience								
≤10yrs	1.4(0.7-2.8)	0.35	1.9(1.1-3.1)	0.01	1.7(0.4-6.9)	0.45	2.9(1.4-6.3)	0.01
>10yrs (r)								
Location (wards)								
Ambureni (r)								
Imbaseny	7.0(2.0-25.0)	0.00	0.2(0.1-0.55)	0.00	2.8(0.3-22.8)	0.34	1.7(0.4-7.0)	0.48

COR: Crude Odds Ratio; (r): Reference

Table 13: Association between socio-economic factors and aflatoxin awareness variables

Socio-economic characteristics/factors	Ever heard about aflatoxin(s)		Occurrence of aflatoxins in feeds		Ability to detect mould in feeds		Aflatoxins in feeds are detoxifiable	
	AOR(95%CI)	p-Value	AOR(95%CI)	p-Value	AOR(95%CI)	p-Value	AOR(95%CI)	p-Value
Gender								
Female	1.6(0.7-3.5)	0.27	1.1(0.6-1.8)	0.82	1.1(0.3-4.3)	0.93	1.6(0.7-3.5)	0.23
Male (r)								
Respondents' age								
≤45yrs	1.6(0.6-4.1)	0.36	1.1(0.6-2.0)	0.74	4.1(0.6-27.6)	0.15	1.1(0.4-2.7)	0.83
>45yrs(r)								
Education level								
High	1.5(0.3-8.2)	0.65	2.0(1.2-3.3)	0.01	1.4(0.6-3.2)	0.50	1.4(0.2-8.0)	0.72
Low (r)								
Education stage								
≤Secondary (r)								
Secondary	1.6(0.3-7.7)	0.53	2.4(0.6-10.0)	0.21	1.0(0.01-93.5	0.93	3.2(0.3-33.6)	0.32
Tertiary	2.4(0.4-17.0)	0.37	1.1(0.4-3.3)	0.85	1.9(0.8-4.5)	0.13	2.3(0.3-16.0)	0.38
Field of specialization								
Life/Social Sc.	5.8(2.7-12.6)	0.00	1.3(0.4-4.0)	0.66	1.4(0.1-1.5)	0.77	1.3(0.3-6.0)	0.71
None/Other fields (r)								
Employment category								
Formal	1.2(0.2-6.8)	0.81	1.3(0.4-3.9)	0.62	4.8(1.0-23.5)	0.05	5.8(0.8-43.7)	0.09
Informal (r)								
Occupation category								
Farming (r)								
Non farming	1.9(0.5-6.8)	0.33	1.1(0.5-2.50	0.79	1.2(0.1-22.5)	0.91	1.0(0.3-3.8)	0.94
Animal keeping experience								
≤10yrs	1.4(0.5-3.8)	0.50	1.5(0.8-2.8)	0.16	1.6(0.3-10.3)	0.60	2.9(1.4-6.3)	0.01
>10yrs (r)								
Location (wards)								
Ambureni (r)								
Imbaseny	6.0(1.4-25.5)	0.01	0.3(0.1-1.2)	0.09	0.4(0.1-2.5)	0.31	1.9(0.4-7.0)	0.48

AOR = Adjusted Odds ratio; (R) = reference

4.2 Evaluation of *In-vitro* aflatoxin-binding capacity of the clay and Ash-based materials

Results of the percentage of aflatoxin-binding capacity of the BMs are presented in Table 14 (across the columns) and Appendix 1. Percentage binding capacity of the TMs ranged from a minimum value of 40 (CC) to a maximum value of 85 (RA), while the reference binder had percentage capacity of 98. The mean proportions of aflatoxins as adsorbed by the BMs are also shown on Table 15 (across the rows). The proportions of aflatoxins that were adsorbed were relatively high for AFB1and AFG1 and low for AFG2 and AFB2. Since AFB1and AFG1 are more toxic than AFG2 and AFB2 (Rocha *et al*., 2014), it is likely that the former are also more reactive and so highly adsorbed to the TMs than the later.

The results of the aflatoxin-binding capacity evaluation of the BMs concurred with the results of other previous related *in-vitro* studies in which the binding capacity levels of clay-based binders such as bentonites (about 90%) have been reported (Kong *et al*., 2014; Manafi *et al*., 2009). The Mycobind® employed as a reference aflatoxin-binding material in this study, could bind about 98% of the total aflatoxins subjected to it. A similar product that was evaluated in Kenya, Agrolite-Mycobind®, showed an aflatoxin-binding capacity of 95% (PASITO, 2017). Regarding the minimum experimental set-up standards as suggested by Emanuele (2006) and disregarding the slightly higher capacity of the reference binder in this study, the two product match in aflatoxin-binding capacity. This match can validates the status of the Mycobind® as a reference binder for the test aflatoxin-binding materials studies in this study.

Table 14: *In-vitro* binding capacity of test binding materials and the reference binder

BMs	Mean percent of bound individual aflatoxin				Mean percent of total aflatoxin bound	SEM
	AFB1	AFB2	AFG1	AFG2		
AC	97.9	60.6	99.9	32.2	72.6^{ab}	32.5
KC	95.4	40.1	96.1	14.5	61.5^{bc}	40.9
CC	96.6	14.4	31.3	17.3	39.9^{c}	38.5
MC	95.6	32.6	94.6	25.3	62.0^{bc}	38.3
VA	97.9	28.9	71.5	30.7	57.3^{bc}	33.5
RA	94.6	79.8	91.5	72.7	84.7^{ab}	10.2
R	97.7	99.2	98.8	96.4	98.1^{a}	1.3
Mean	96.5^{a}	50.8^{b}	83.4^{a}	41.3^{b}		
SEM	1.4	30.4	24.9	31.0		

SEM = Standard error of the means; Means with similar superscripts do not differ significantly

Results of the aflatoxin-binding capacity of Mycobind® (R) relative to the TMs are shown in Table 15. The binding capacity of R was one fold that of the clay from Arusha (AC) and rice husk ash (RA), twice that of the clays from Kilimanjaro (KC) and Morogoro (MC) and volcanic ash (VA), and thrice that of the clay from the Coast (CC).

The binding capacity ratio of R to the TMs as was observed in this study conversably implied that AC and RA bind an equivalent of 100%, KC, MC and VA bind 50%, and CC binds 33.3% of the total aflatoxins in solution. The prominent advantage is that all of the TMs showed high capacity to bind AFB1which according to Udomkun *et al.* (2017) and Feddern *et al.* (2013) it is the most potent type of toxin occurring naturally in feeds and foods. Generally, the TMs sequestered AFB1 nearly 100% as much as what R adsorbed.

This indicates that although they adsorbed aflatoxins at varying levels, the locally available crude TMs had potential to adsorb aflatoxins in solution media, and can possibly reduce the aflatoxin contamination of feeds.

Table 15: Aflatoxin-binding capacity of Mycobind® relative to the TMs

Aflatoxins	Test binding materials (TMs)					
	AC	KC	CC	MC	VA	RA
AFB1	1.0	1.0	1.0	1.0	1.0	1.0
AFG1	1.0	1.0	3.2	1.0	1.4	1.1
AFB2	1.6	2.5	6.9	3.0	3.4	1.2
AFG2	3.0	6.6	5.6	3.8	3.1	1.3
Overall	1.0	2.0	3.0	2.0	2.0	1.0

4.3 Chemical Properties of the clay and Ash-based materials influencing their aflatoxin-binding capacity

The study on these particular material is the first one ever conducted. Therefore there is no documented information concerning their chemical composition and properties is available for comparison and reference.

4.3.1 Mineralogical composition of the binder materials

The major minerals contained in the BMs are shown in Table 16 and the X-RD analysis results in Appendix 3. The muscovite mineral was observed in clays from Arusha (AC) and

Kilimanjaro (KC), kaolinite was observed in the clays from Coast (CC) and Morogoro (MC), leucite was observed in the clay from Morogoro (MC), microline and ephicite were observed in volcanic ash (VA), albite and terranovite were observed in rice husk ash (RA), while metanatrolite and phlogopite were observed in R.

The BMs contained different minerals that seem to occur in solitary manner in one material. Silicate and aluminium components were observed in most of the minerals. All of the BM contained these components, indicating that they form the backbone of the chemical structure of each of the materials as was previously reported for other similar materials (Anjos *et al.*, 2016; Karnland, 2010; Tebandeke *et al.*, 2015). Presence of these minerals in the BMs just give preliminary picture of the content nature of materials, but no indication in relation with the capacity to bind aflatoxins.

Table 16: Mineralogical and chemical formula of the binding materials

BMs	Prominent Minerals	Chemical formula
AC	Muscovite	$KAl_2(AlSi_3O_{10})(F,OH)_2$
	Hematite-proto	$Fe_{1.9}H_{0.06}O_3$
KC	Quartz	SiO_2
	Muscovite	$KAl_2(AlSi_3O_{10})(F,OH)_2$
	Lizardite	$Mg_3Si_2O_5(OH)_4$
CC	Kaolinite	$Al_2Si_2O_5(OH)_4$
MC	Kaolinite	$Al_2Si_2O_5(OH)_4$
	Leucite	$K[AlSi_2O_6]$
	Lizardite	$Mg_3Si_2O_5(OH)_4$
VA	Pigeonite	$(Ca, Mg, Fe)\ (Mg, Fe)Si_2O_6$
	Microcline	$KAlSi_3O_8$
	Ephesite	$NaLiAl_2\ (Al_2Si_2)O_{10}(OH)_2$
RA	Albite	$NaAlSi_3O_8$ or $Na_{1.0–0.9}Ca_{0.0}$
	Terranovaite	$NaCaAl_3Si_{17}O_{40}8H_2O$
	Sepiolite	$Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$
R	Metanatrolite	$Na_2Al_2Si_3O_{10}$
	Phlogopite	$KMg_3(AlSi_3O_{10})(F,OH)_2$
	Andradite /Melanite	$Ca_3Fe_2(SiO_4)_3$

4.3.2 Elemental oxide composition of the BMs

Results in Table 17 show the elemental (oxide) composition of the BMs. All of the samples of the BMs contained aluminum and silicon elements as the backbone of the minerals. Other important elements that were observed as parts of the chemical formula of the prominent minerals in the BMs were iron in AC, VA and R; calcium in VA, RA and R, and potassium in all of the materials except CC and RA. The VA and R had minerals containing all of the main elements; aluminum, silicon, iron, calcium and potassium. The RA showed the lowest content of aluminum oxide (alumina) of 0.5%; all of the other BMs had content above that of R (5.1%). Percent silicon oxide (silica) contents in CC and RA were above that of R, while the other BMs had contents from 22–32.8%, which was lower than that of R (49%). The VA and RA had percent contents of potassium oxide a little bit higher than that of R. The VA had calcium oxide content that was a bit higher than that of R, while the rest of the BMs had contents below that of R. The AC and RA had the highest and the lowest contents of iron oxide, respectively. Except for RA and CC, which had lower percent of iron oxide contents, AC, KC, MC and VA had values above that of R.

The chemical composition of the binding materials (BMs) observed in this study was comparable to that of aluminosilicate-based binders reported in other related studies. For instance, the alumina content of the materials was within the range reported in other studies of 0.45–21.7% (Karnland, 2010) and 13.2% (Anjos *et al.,* 2016), except for clay from the Coast (CC), which contained higher level of alumina, at about 33%. Except for the RA, which showed much higher content of silica, the other BMs had content comparable to the reported values for clay materials, ranging from 1.1–69.0% (mean of 59.6%) (Karnland *et al*., 2010) and 44.3–71.0% (mean of 55.3%) (Tebandeke *et al.,* 2015). Similar to Mycobind®, volcanic ash (VA) and RA had potassium oxide content above the previously reported range of 0.1–3.3% (Karnland *et al.,* 2010) and 0.1–2.6% (Tebandeke *et al*., 2015) and 0.1% (Anjos *et al*., 2016) for high aflatoxin binding. The 0.01% potassium oxide content of clay from Kilimanjaro (KC) was below the reported levels. The calcium oxide content in all of the BMs were found to be within the previously reported range of 0.1–31.4% (Anjos *et al.*, 2016; Karnland, 2010; Tebandeke *et al.,* 2015) for clay materials. Except for the CC and RA, the rest of the BMs showed iron oxide content above the previously reported range of 0.2–14.8% for binders (Anjos *et al.*, 2016; Karnland, 2010; Tebandeke *et al.*, 2015).

Composition of the tested materials was comparable to that of aluminosilicate clays, including those previously studied and proved to bind aflatoxins. Aluminosilicate-based materials are reported to exhibit CEC (meq/100 g) values ranging from 10 (kaolinite mineral) to 100 (illite and smectite minerals) and medium values are found around the value of 25 (Leal *et al.*, 2019). From the results all the of the TMs had CEC values within the previously documented range, as were observed from 7 meq/100g for clay from Morogoro (MC) to 38.9 meq/100g for R.

4.3.3 Cation exchange capacity of the clay and ash-based materials

The values of CEC for the BMs are also shown in Table 17. The values of CEC for the TMs ranged from a minimum of 7 meq/100 g (CC) to a maximum of 27.2 meq/100 g for (RA). All of the TMs had lower values of cation exchange capacity (CEC) compared to that of Mycobind® (38.9 meq/100 g).

Table 17: The major elemental-oxide composition of the binding materials

BMs	Elemental-oxide composition of the BMs (%)					CEC
	Al_2O_3	SiO_2	K_2O	CaO	Fe_2O_3	(meq/100g)
AC	18.0	26.0	0.22	0.79	45.31	27.2
KC	25.0	31.0	0.01	0.24	39.73	18.8
CC	32.8	61.3	0.63	0.49	2.14	7.0
MC	24.0	34.8	0.52	0.54	36.1	15.4
VA	15.0	22.0	8.78	14.9	26.2	25.4
RA	0.5	75.7	9.54	1.71	0.59	27.2
R	5.1	49.0	6.99	13.4	19.8	38.9

In this and subsequent tables, BMs = TMs and R, TMs=Test binding materials, R=Reference materials and CEC = Cation exchange capacity

4.3.4 Relationship of the chemical composition of the BMs and their aflatoxin binding capacity

Among the evaluated binding materials, RA and AC had outstanding higher aflatoxin-binding capacity comparable to that of the R, particularly in binding AFB_1 and AFG1, which are the most toxic types (Feddern *et al*., 2013). Probably, high binding capacity of these materials was due to their high CEC values as reported by Vekiru *et al*. (2015). The CEC values of both RA and AC were 27.2 meq/100 g of each of the materials, high next to that of R (38.9 meq/100g). High CEC values of aflatoxin-binding materials have been reported to have positive influence on their binding capacity (Vekiru *et al.*, 2015). Relatively high cationic

values of calcium (Ca^{2+}) and potassium (K^{+}) in the aluminosilicate minerals of the evaluated materials showed higher positive correlation with CEC of the BMs in general. Calcium and potassium seemed to promote the CEC values of the materials. Studies have shown that concentrations of Ca^{2+} and K^{+} ions make a great contribution to CEC levels in aluminosilicate materials (Brady & Weil, 2008; Rayment & Higginson, 1992). The presence of silicon (Si^{4+}), aluminum (Al^{3+}), and iron (Fe^{3+}) seemed to have low or negative influence on the CEC values of the BMs. According to Brady and Weil (2008), values of CEC increase with decreasing acidity and vice versa. Furthermore, the authors showed that ions Si^{4+}, Al^{3+} and Fe^{3+} promote the acidity of materials in solution, unlike Ca^{2+} and K^{+}, hence, negatively influencing the CEC values of the BMs, and subsequently their capacity to bind aflatoxins in solution.

Disregarding other factors such as the structural effect of a material, it is probable that materials such as CC showed a low capacity for aflatoxin binding partly due to their higher concentration of Al^{3+} and Si^{4+} and partly due to their relatively higher content of a kaolinite type of mineral, which has a low CEC (Leal e*t al.*, 2019). Furthermore, KC and MC could not bind aflatoxins efficiently, which was possibly due to their relatively higher concentration of Al^{3+} and Fe^{3+}.

X-ray diffraction (X-RD) analysis displayed minerals with potential elemental components that can influence aflatoxin-binding capacity of the BMs. The results showed that comparably to the R, RA and AC contained major minerals such as andranite/melanite, terranovite, and albite; all of these contained calcium and phlogopite, as well as muscovite, which contains potassium. It is possible that these components rendered RA and AC relatively superior to others in binding aflatoxins. In aflatoxin-binding ions, Ca^{2+} in particular synchronously bonds to two aflatoxin carbonyls, and at the same time binds to the four oxygen atoms of the Si–O ring on the clay binder surface (Kang *et al*., 2016). However, AC had low Ca^{2+} and K^{+} cations, yet its CEC value was relatively high enough to favour high aflatoxin-binding capacity. Seemingly, the way that active cations such as calcium and potassium are incorporated in different structures of the BMs, and their associations with other structural elements, may affect the adsorptive potential of the BMs.

Results of the relationship of elemental (oxide) concentration in the BMs and their respective cation exchange capacity (CEC) are presented in Table 18. The relationship as correlation coefficients was positive and relatively higher with CaO (0.63), K_2O (0.59) and Fe_2O_3 (0.11),

and negative with SiO_2 (−0.06) and Al_2O_3 (−0.86). Similarly, the relationship between the elemental (oxide) concentration in the BMs and their respective capacity to bind total aflatoxins, was positive and relatively higher with K_2O (0.51), CaO (0.34), SiO_2 (0.21), Fe_2O_3 (0) and negative with Al_2O (−0.88). The relationship between the CEC of the BMs and their capacity to bind total aflatoxins was relatively high, with a correlation coefficient of 0.90 (Table 18).

The AFB1 and AFG1 were highly adsorbed into the BMs as compared to AFB2 and AFG2. This is due to the fact that unlike AFB2 and AFG2, the AFB1 and AFG1 have a higher polarity of the β-dicarbonyl group, which is a key functional group of the aflatoxins (Grant *et al*., 1998). With respect to the polarity, AFB1 was rendered the most adsorbed by the TMs, followed by AFG_1. This was an advantageous since the adsorption tendency of types of aflatoxin commensurate toxicity tendency of the aflatoxins. The efficacy of aflatoxin-binding capacity of the materials subjected to the *in-vitro* test evaluated materials, can be further tested for confirmation using an *in-vivo* test where the dietary and animal's gastrointestinal tract factors are automatically accommodated. However, since exported binders are costly to farmers in low-income countries, the material can be fairly utilized in feeds to reduce the hazardous effects of aflatoxins on animals. Traditionally, farmers have been using an array of such materials for various intentions, including uses in animal feeds.

It has been observed that wild animals and birds are less affected by many natural toxins, which probably include aflatoxins, owing to their geophagial instincts (Diamond, 1999; Mahaney & Krishnamani, 2003). Essentially, these animals and birds fetch and eat clayey soil, which renders them safe from the inherent food toxins (Brightsmith *et al.*, 2008). Clays have been proved to bind aflatoxins and render then less toxic (Denli *et al.*, 2009; Kaoud, 2012; Phillips *et al*., 2002). Harnessing this natural phenomenon may be economically helpful to farmers as one of the strategies for lowering aflatoxin menace, which is difficult to avoid in feeds. In the *in-vitro* test each of the TMs showed certain capacity to bind aflatoxins, but RA and AC showed outstanding higher capacity, promising to be useful materials in combating aflatoxins in feeds.

Table 18: Relation of elemental oxide content in the BMs with their aflatoxin-binding capacity

BMs		Elemental-oxide composition of the BMs (%)					CEC (meq/100 g)	MTAB (%)
		Al_2O_3	SiO_2	K_2O	CaO	Fe_2O_3		
AC		18.0	26.0	0.22	0.79	45.31	27.2	72.6
KC		25.0	31.0	0.01	0.24	39.73	18.8	61.5
CC		32.8	61.3	0.63	0.49	2.14	7.0	39.9
MC		24.0	34.8	0.52	0.54	36.1	15.4	62.0
VA		15.0	22.0	8.78	14.9	26.2	25.4	57.3
RA		0.5	75.7	9.54	1.71	0.59	27.2	84.7
R		5.1	49.0	6.99	13.4	19.8	38.9	98.1
Correlation coefficients	EC with CEC	-086	-0.06	0.59	0.63	0.11		
	EC with AF-BC	-0.88	0.21	0.51	0.34	0.00		
	CEC with AF-BC						0.90	0.90

BMs = test binding materials (TMs and R), TMs = test binding materials, R= reference binder, CEC = cation exchange capacity, EC = elemental-oxide concentration, PBC= Percent binding capacity, MTAB = mean total aflatoxin-binding capacity of BMs

4.4 Potential of the clay and Ash-based materials in reducing detrimental effects of dietary aflatoxins in animals

Preliminary data analysis showed that block effect that is, animal sex and interaction of sex and dietary treatments were not significant in all the parameters assessed. For that matter, data for male and female were pooled together to increase replications of treatment in the experiment.

4.4.1 Feed intake, growth rate and feed conversion efficiency of the rats

Mean values of daily feed intake (FI, g/d), daily growth rate (GR, g/d) and feed conversion efficiency (FCE, %) for the experimental animals are presented in Table 19 and Appendix 4. Animals in all dietary treatments had statistically equal mean FI. The average GR of animals fed on diet DKC was significantly ($p<0.05$) higher than that of animals on control diet (1.5 g/d) and those fed on diets with the other test binding materials, but statistically equal to those on DR. Similarly, the FCE by the animals on DKC was significantly ($p<0.05$) higher compared to animals on the other test treatments and control, but statistically equal to that of animals on the DR. The FCE by animals on control treatments was significantly ($p<0.05$) lower than that of animals fed on diets with the test binding materials and DR.

Table 19: Mean values of feed utilization parameter of the rats

Dietary Treatments	FI (g/d)	GR (g/d)	FCE (%)
DAC	11.7	1.9^{ab}	16.1^{ab}
DKC	12.6	2.1^{a}	16.6^{a}
DCC	11.4	1.8^{ab}	16.2^{ab}
DMC	11.7	1.8^{ab}	15.3^{ab}
DVA	11.0	1.8^{ab}	16.4^{ab}
DRA	12.2	1.9^{ab}	15.4^{ab}
DR	12.3	2.1^{a}	17.5^{a}
DC	11.7	1.5^{c}	12.9^{b}
SEM	0.4	0.1	0.9
p-values	NS	$p<0.05$	$p<0.05$

In this and subsequent tables DAC, DKC, DCC, DMC, DVA, DRA, DR and DC mean treatments of Arusha Clay, Kilimanjaro Clay, Coast Clay, Morogoro Clay, Volcanic Ash, Rice-husk Ash, Reference binder and Control respectively. The SEM is Standard Error of the Means while NS is Non-significant. Means with similar superscripts are not significantly different.

Mean feed intake (g/d) and growth rate (g/d) of the rats were comparable to those previously reported elsewhere (Hofler *et al*., 2016; NRC, 1995; NRC, 2010). Statistically, all animals in all treatments had similar FI probably implying that effect of aflatoxin on feed intake is not so strong. The observed mean daily growth rate (GR) and feed conversion efficiency (FCE) by the animals in the present study agreed well with the reported values of 2.1 g/day (Salifu *et al.*, 2016) and 12.3% respectively (Abu *et al*., 2013). Mean GR and FCE differed significantly among treatments, implying possibly varying potential of the binding materials in the different treatments to bind dietary aflatoxins. Dietary aflatoxins impair GR and FCE as was observed in broilers (Yang *et al*., 2012), white shrimps (Salazar *et al*., 2012) and quails (Mahmood *et al.*, 2017).

Probably, additional function of the test binding materials and the Mycobind was to counteract the adverse effects of aflatoxins on some biochemical processes in animals, rather than direct binding the toxins. Aflatoxins reduce GR and FCE through reduced activities of specific enzymes responsible for digestion and absorption of nutrients mainly carbohydrates, proteins, lipids and other essential nutrients (Grenier & Applegate, 2013). In addition, aflatoxins impair absorption of nutrients through negative modification in morphology of gastro-intestinal tract (Nasrabadi *et al*., 2013; Yang *et al*., 2012). Based on the results of the

present study, the binding materials could have immobilized the dietary aflatoxins differently. Treatment DKC comparable to DR appeared superior in counteracting suppressive effects of the dietary aflatoxins on GR and FCE of the rats. Animals on the other treatments also showed significant higher GR and FCE compared to those fed on DC. This implies that each binding material was potential in immobilizing the adverse effect of dietary aflatoxins in varying capacities. The ultimate parameters in evaluating capacity of binders to immobilize dietary aflatoxins in animals, could be FCE, making DKC the best treatment when considering feed intake and utilization.

4.4.2 Packed cell volume and serum proteins of the rats

Mean values of haematological parameters: packed cell volume (PCV), concentration of total serum proteins (TP), serum albumins, serum globulins and albumin/globulin ratio (AGR) of the experimental animals are shown in Table 20 and Appendix 4. All the treated animals showed values of PCV within the normal range of 37.6 - 54.3% as reported by Delaney (1996); Giknis and Clifford (2008) and Sampathkumar *et al.* (2018). Animals fed on treatment DCC had significantly ($p<0.05$) higher serum (6.5 g/dl) than animals in the other groups (Table 20). Animals on treatments DAC, DMC and DRA had serum TP statistically equal to that of animal in control (6.3 g/dl), but significantly ($p<0.05$) higher than that of animals in the DR (6.0 g/dL). Animals on treatments DKA and DVA had TP equal to that of animals on DR (6.03 g/dL) but significantly ($p<0.05$) lower than that of animals in control and the rest of the test treatments. The mean PCV values of animals on dietary treatments DKC and DMC were significantly higher ($p<0.05$) than that of animals on DR and the other test treatments. The control treatment had the lowest PVC value.

Treatments with clay from Kilimanjaro (DKC) and Morogoro (DMC) exhibited higher and better levels of PCV within the normal range. This might be indicator of capacity to normal blood level against suppressive effect of aflatoxins. However, PCV may not be better indicator as the binders' chemical properties may be assumed to affect haemoglobin level and slightly mask the aflatoxin binding effect.

Albumin in the serum of animals on treatment DAC (3.11g/dL) was significantly ($p<0.05$) higher than that of animals on other test treatments, but equal to that of animals in DR (3.08 g/dl). Except for animals on treatment DVA which showed lower albumin of 2.77 g/dL, animals in all the other test treatments had albumin significantly ($p<0.05$) higher than animals

in control (2.77 g/dL) but, lower than animals in DR. Serum globulin concentration of animals in all test treatments was significantly ($p<0.05$) lower than that of animals in control but higher than that of animals on DR.

The treatments DAC and DVA exhibited favourable level of serum albumin, similar to those shown by the animals on DR. However, animals on DAC did not manifest higher level of AGR as those on DVA and DR owing to the relatively high level of globulin shown by animals on DAC. Low level of albumin relative to globulin in serum is a manifestation of poor albumin synthesis in the cell (Bernardi *et al.*, 2012; Dhanasekaran *et al.*, 2011).

The ratio of albumins to globulins (AGR) in the serum of animals on treatment DVA was significantly ($p<0.05$) higher than that of animals in the other test treatments, but statistically equal to that of animals on DR. Animals in all test treatments showed significant ($p<0.05$) higher AGR than animals in control, but lower than that of animals on DR.

The observed values of PCV and serum proteins, that is, TP, Albumin and AGR in rats were in agreement to those reported by other workers. For instance the values of PCV were in agreement to the reported ranges of 37.6 - 50.6% by Delaney (1996), 43.3 - 45.0% by Giknis and Clifford (2008) and 34.5 - 54.3% by Sampathkumar *et al*. (2018). The normal concentration of TP, albumin, globulin and AGR in serum of rats are reported to range from 5.2 -10.4 g/dL, 3.4 - 5.8 g/dL, 1.5 - 2.5 g/dL and 1.5 - 3.07 respectively (Delaney, 1996).

Dietary aflatoxin contamination suppresses normal level of PCV and AGR. (Kaneko *et al.*, 2008 cited by Dónmez *et al*., 2012). Thus, the observed high values of PVC in rats fed on DKC and DMC may reveal higher capacity of these diets to maintain favourable haematological status of the animals against detrimental effects of aflatoxins. The level of serum TP may mean various outcomes following changes in the status of animal health revealed by serum protein status. For instance, high concentration of serum TP may result due to body fighting against some infections or other health impairments (O'Connell *et al,* 2005). Concentration of serum TP may be low when there is less production of the protein by the liver or when there is increased loss or degradation of the proteins (He *et al*., 2017; O'Connell *et al.*, 2005).

Abnormal low concentration of TP is a symptom of many conditions. The AGR in serum is known to be more informative factor for health status of animals whereby relatively high ratio within the normal range indicates better serum protein balance (He *et al*., 2017). The

AGR reflects whether a change in protein concentrations is due to changes in either albumin or globulin. Low level of albumin production, as in impaired liver function, automatically leads to low AGR, since albumin is synthesized solely by liver cells (Bernardi *et al.*, 2012). This implies that if aflatoxins impair function of liver cells will result into reduced albumin production and low AGR.

Animals fed on DVA and those fed on DR had relatively higher AGR than those on the other test treatments, hence seemed less affected by the dietary aflatoxins. However, all animals fed on diets treated with the other binding materials exhibited higher mean values of AGR than those fed on DC. Having the significantly higher value of AGR for the group fed DVA similar to those fed DR, makes VA to be more efficacious material in immobilizing dietary aflatoxins.

Table 20: Mean haematological parameters of the rats

Treatments	Means				AGR
	PCV (%)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	
DAC	46.2^{bc}	6.19^{ab}	3.11^{a}	2.84^{bc}	1.11^{ab}
DKC	49.2^{a}	6.03^{b}	3.06^{ab}	2.85^{bc}	1.10^{ab}
DCC	47.6^{ab}	6.47^{a}	3.04^{b}	3.26^{ab}	1.08^{ab}
DMC	49.2^{a}	6.26^{ab}	2.96^{bc}	3.27^{ab}	1.17^{ab}
DVA	48.6^{ab}	5.98^{b}	2.77^{c}	2.93^{abc}	1.21^{a}
DRA	48.1^{ab}	6.22^{ab}	2.84^{bc}	3.18^{abc}	1.01^{ab}
DR	48.5^{ab}	6.03^{b}	3.08^{a}	2.78^{c}	1.21^{a}
DC	44.6^{c}	6.31^{ab}	2.77^{c}	3.36^{a}	0.88^{b}
SEM	0.64	0.09	0.06	0.10	0.07
p-values	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

4.4.3 Changes in relative weight of liver, kidney and spleen of the rats

Mean values of relative weights of liver (RWL), kidney (RWK) and spleen (RWS) on sacrifice point of the rats in the experiment are presented in Table 21 and Appendix 4. Animals on treatment DRA had RWL favourably low and equal ($p<0.05$) to those on DR (3.71%), but lower than those on the other test treatments and control. Except for the animals on DAC which showed a bit larger RWK, the rest of the animals in test treatments did not differ significantly ($p>0.05$) with control, but they had RWK significantly ($p<0.05$) larger

than those on the DR. The RSW of animals on all treatments of the experiment did not differ statistically.

Relative weights of organs have been taken as a way of assessing the toxic effects of some chemical agents and could be the most sensitive indicator of testing the toxic effects of the local materials used in the present study. This is because significant differences in relative organ weights of animals on the treated diets against those on the control (untreated) may occur without apparent morphological and histological changes of the organs (Bailey *et al*., 2018).

Overall mean relative weight of liver of rats in the present study was within the range of 2.16 – 4.30% reported in other studies (Aniagu *et al*., 2005; Piao *et al.*, 2013). The relative weight of kidneys in the present study was fairly lower than the reported values (0.63 – 0.88%) by other workers (Aniagu *et al*., 2005; Piao *et al*., 2013). The same authors reported normal values of relative weight of spleen ranging from 0.17 – 0.42%, values which are in agreement to the mean values observed in the present study.

Animals fed on diets treated with rice-husk ash (DRA) had favourably lower relative weight of liver similar to those fed on the DR, indicating it to be the most protective binding material against effect of dietary aflatoxins on the liver. All test diets were alike in maintaining favourable kidney relative weight, but inferior to DR. They were similarly alike in terms of the relative spleen weight. The present results showed liver to be the most sensitive to the effects of aflatoxins compared to kidneys and spleen, where the tested binding materials seemed to protect it with different capacities. All treatments showed relatively lower relative weight of kidney than the reported normal value of 0.63 – 0.88% (Aniagu *et al*., 2005; Piao *et al.*, 2013). This may indicate that the dietary level of aflatoxins had no apparent effect on the kidneys. Similarly, no treatment made any significant difference on relative weight of spleen and all values were within the reported values (0.17 – 0.42%), indicating insignificant effect of the dietary aflatoxins on spleen. The results of the relative weights of organs were also supported by the results of histological evaluation of the liver, kidneys and spleen from the same experimental animals. Seemingly, spleen was easily protected than kidney and kidney than liver. On the other side, it is likely that spleen is less affected by aflatoxin as compared to liver and kidneys.

Table 21: Mean values of relative weight of liver, kidney and spleen of the rats

Dietary Treatments	Relative weight (%)		
	Liver	**Kidney**	**Spleen**
DAC	4.18^{abc}	0.41^{a}	0.25
DKC	4.37^{a}	0.39^{ab}	0.23
DCC	4.33^{ab}	0.37^{ab}	0.23
DMC	3.94^{abc}	0.39^{ab}	0.24
DVA	3.85^{bc}	0.37^{ab}	0.23
DRA	3.78^{c}	0.36^{ab}	0.28
DR	3.71^{c}	0.34^{b}	0.22
DC	3.94^{abc}	0.37^{ab}	0.28
SEM	**0.16**	**0.02**	**0.03**
p-values	**p<0.05**	**p<0.05**	**NS**

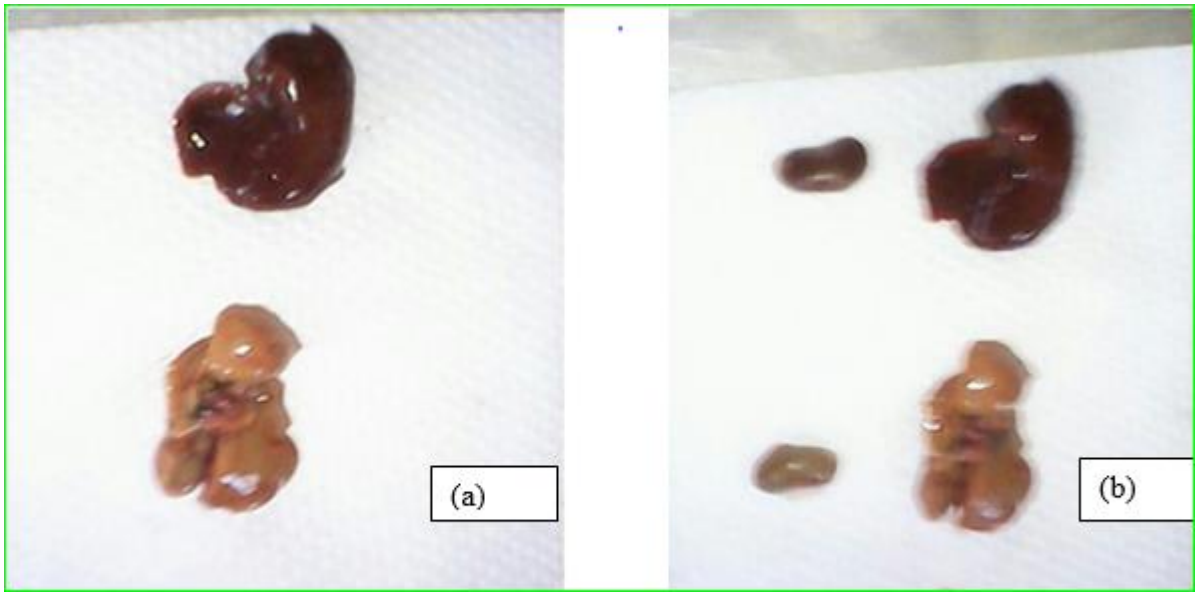
4.4.4 Histopathologal assessment of liver, kidney and spleen of the rats

Results of the histopathologal assessment of liver, kidney and spleen of experimental animals are shown in Table 22. Unlike all the other treatments, animals on DVA showed normal liver. Animals on DMC like those on the DR had normal kidneys with mild congestion, while those on the rest of the test treatments exhibited normal kidneys. Almost all the treatments exhibited normal spleen for the animals. Potential capacity of DVA in immobilizing dietary aflatoxin contaminations was further manifested in maintaining normal histological status of liver, kidney and spleen. Animals on DVA showed no signs of fatty change in the liver tissue unlike those on the other treatments. One of the adverse effects of aflatoxins, particularly AFB1 is impairment of lipids and accumulation in the cells of the liver (Dhanasekaran *et al.*, 2011).

4.4.5 Gross appearance of liver and kidney of the experimental rats

Results of the gross appearance of internal organs (liver and kidney) from sample animals are shown in Fig. 5 and further demonstrated in histological assessment of liver, kidney and spleen of the experimental rats in Table 22. Animals fed on DVA showed normal liver, while those on all the other treatments, including the DR exhibited some sort of fatty changes. In some clear observed sampled cases such as in Fig. 5a show normal liver (deep red) among animal group fed on DRA against the abnormal liver (pale-red) from a sampled animal among those fed on DC. Figure 5b is showing abnormal kidneys and livers from sample animals fed on diets DVA and DC respectively. Effects of potentially active binders against

aflatoxins can also easily seen in the gross appearance of internal organs such as liver, kidney and spleen. The present observation commensurate with the report of Zhao *et al*. (2010) which reported a normal liver in broilers fed on aflatoxin-contaminated diet when treated with hydrated sodium calcium aluminosilicate and abnormal liver of the control group.



(a) Normal coloured liver (top) from a group fed on diet DRA against pale-fried liver (bottom) from a rat-group fed on the control diet DC. (b) Normal-coloured (top) and discoloured (bottom) kidneys and livers of rats fed on DVA and DC respectively

Figure 5: Liver and kidneys from rats fed on diets with/without aflatoxin-binding materials

Table 22: Histological appearance of liver, kidney and spleen from the sample rats

Treatment	Liver	Kidney	Spleen
DAC	Mild Fatty Change	Normal	Normal
DKC	Mild Fatty Change	Normal	Artefactual
DCC	Fatty Change	Normal	Normal
DMC	Fatty Change	Normal and Congestion	Normal
DVA	Normal	Normal	Normal
DRA	Mild Fatty Change	Normal	Normal
DR	Mild Fatty Change	Normal and Congestion	Artefactual
DC	Fatty Change	Congestion/haemorrhage and tabular congestion.	Normal

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Awareness about aflatoxins among farmers was low that may render animals kept and managed at household level at high risk of aflatoxin exposure. This may also put humans consuming foods of animal origins from those animals at risk of chronic aflatoxin exposure. Improved exposure of education especially in biological or life science oriented fields seemed to be key factor in raising level of awareness among farmers and the public at large. Some farmers had some ideas on possibility of detoxifying aflatoxins, suggesting use of materials such as ash. Though at varying levels, all of the tested materials had potential capacity to immobilize aflatoxins, where all had capacity to bind greater percent of aflatoxin B1, the most potent type of these toxins. Rice-husk ash and clay from Arusha seemed to be the best materials in immobilizing aflatoxin in *in-vitro* buffered solution. Based on the *in-vitro* test, high CEC values of the binders, contributed by high contents of Ca^{2+} and K^{+} seem to enhance aflatoxin-binding capacity of the clay and ash-based materials. Based on the *in-vivo* test, rice-husk ash, volcanic ash and clay from Kilimanjaro appeared as the best materials in reducing detrimental effects of dietary aflatoxins in animals. Based on both *in-vitro* and *in-vivo* tests, the rice-husk ash appeared to be the best material in combating aflatoxin challenges in feeds.

5.2 Recommendations

5.2.1 Developmental purposes

(i) Government authorities such as Ministries, TBS, local authorities, health facilities (hospitals and health centres) and academic institutions should endeavour to sensitise stakeholders such as famers, dealers of animal feeds and the public in general on issues of aflatoxins. Emphasis should be on hazards and control measures of these toxins.

(ii) Integrating various interventions that involve farmers who can willingly use the developed measures such as local binders in feeds to reduce exposure of aflatoxin-contamination feeds is advised.

(iii) Refining the more potential local aflatoxin-binding materials for scaling up into industrial focus is suggested.

(iv) Testing more varieties materials (clays and ashes) and establish the country's database is a crucial strategy for expanding scope to control aflatoxins in feeds.

(v) Laying down strategies to control exhaustion (take) of clays found efficacious in binding aflatoxins in the country is advocated.

5.2.2 Suggestions for further studies

(i) Further verification of the capacity of the clay and ash based materials in binding aflatoxins to reduce bioavailability using blood and urine biomarkers.

(ii) Determining the most suitable inclusion rate of each of the clay and ash based materials into feeds and also checking for the aflatoxin amount bound and pass out in faeces.

(iii) Verifying whether the clay and ash based materials have negative effects on the physiological activities of animals such as binding some micro-nutrients and interference of haemoglobin synthesis.

(iv) Exploring the actual model of actions of the clay and ash based materials in aflatoxin immobilization, where each of the tested material seemed to offer different protective effect to the animals. Probably, there are more actions of the materials, other than simply binding of aflatoxins.

(v) Advancing to test for synergistic effect of combining two or more materials in binding aflatoxins. For sound results, subjecting all of the materials in the evaluation is recommended, as each of them may have a salient unique potential. Mathematically, fifteen binary combinations of the materials are suggested, that is, including about other eight non-binary (>2 materials) combinations, a total of twenty three combinations/samples are estimated. A cost of 6 000.00 – 7 000.00 USD is estimated for the study, where the other suggested gaps may also be covered.

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APPENDICES

Appendix 1: Questionnaire for socio-economic survey on farmers' awareness of aflatoxin contamination of feeds in Meru District, Arusha, Tanzania

QN	QUESTIONS	RESPONSES
1	Name	
2	Place of residence	Village…….. Ward……..
3	Sex	Female ( ) Male ( )
4	Age	
5	Education	P/School ( ) S/school ( ) college ( ) University./Tertiary ( )
6	(If beyond P/school) Education base/field	Biological/Life Sciences. ( ) General Sciences./Engineering ( ) Arts/social Sciences ( )
7	Occupation	Formal employment ( ) Informal employment ( )
8	Main occupational activity	
9	What type of livestock do you keep and number?	D/Cattle ( )…… D/Goats ( )……Sheep ( )…...Pigs ( )…. Local chickens ( ). Commercial layers ( )…. Commercial broilers ( )…… Others …….( )…
10	For how long have you been keeping the animals?	D/Cattle….. D/Goats …… Sheep…..Pigs…. Local chickens ….. Commercial layers….. Commercial broilers…… Others ……
11	Do you supplement your ruminant animals?	Yes ( ) No ( )
12	What supplements do you offer?	
13	How much (kg) of each supplement do you offer per day?	
14	What is the source of the supplements?	
15	Do you include/mix any additive in the feeds?	Yes ( ) No ( )
16	What additives do you include/mix in the feeds?	
17	What is the source of the feed additives?	
18	Why do you include/mix the additives?	
19	How much additive do you include/mix in feeds?	
20	How did you know about the additive use?	From: Elders ( ) Own intuition ( ) Reading ( ) media ( ) Seminar ( ) Peer/Friend ( ) any other (specify)( )
21	What response do you observe in your animals when you include the additives?	
22	What symptoms do you observe in your animals when the additives are not included/mixed?	
23	Do you think animal feeds can contain natural toxins?	Yes ( ) No ( )
24	(If Yes) What can be a possible natural toxin in feeds?	
25	From your experience what do you think cause(s) the natural toxins in feeds?	
26	From your experience what feeds are susceptible to the occurrence of the natural toxins?	
27	Do you think the natural toxins in feeds can harm animals?	Yes ( ) No ( )
28	(If Yes) How did you know?	From: Elders ( ) Own intuition ( ) Reading ( ) media ( ) Seminar ( ) Peer/Friend ( ) any other (specify)( )

29	Do you think natural toxin in feeds may be transferred to animal products (milk, eggs and meat)?	Yes ( ) No ( )
30	(If Yes) How did you know?	From: Elders ( ) Own intuition ( ) Reading ( ) media ( ) Seminar ( ) Peer/Friend ( ) any other (specify)( )
31	What signs do you see in feeds/grain so as to suspect presence of natural toxins in feeds	
32	Have you ever heard of Aflatoxins (in Swahili *sumukuvu*)?	Yes ( ) No ( )
33	(if yes) When did you hear it for the first time?	This year ( ) Last year ( ) more than two years ago ( )
34	Where did you get its information?	Reading ( ) media ( ) Seminar ( ) Peer/Friend ( ) any other (specify) ( )
35	Do you think it can occur in animal feeds?	Yes ( ) No ( )
36	(If Yes) How can you suspect its presence in feeds?	
37	Have you ever seen some mould (in Swahili kuvu/*ukungu*) in feeds?	Yes ( ) No ( )
38	(If Yes) Which feeds are mostly likely to show mould growth?	
39	What do you think cause(s) the mould?	
40	What do you do with mouldy supplementary feeds?	
41	What do you do with cereal grains which apparently appear to run bad?	
42	What do you think can be done to prevent occurrence of natural toxins in grains/animal feeds?	
43	From experience do you think natural toxins/aflatoxins in grains/feeds can be rendered harmless?	Yes ( ) No ( )
44	What do you think can be added in feeds to render natural toxins harmless?	
45	What is your general comment on the safety of animal feeds?	

Appendix 2: Data of the *in-vitro* test of Tanzanian crude clay and ash-based materials tested in binding aflatoxins in solution

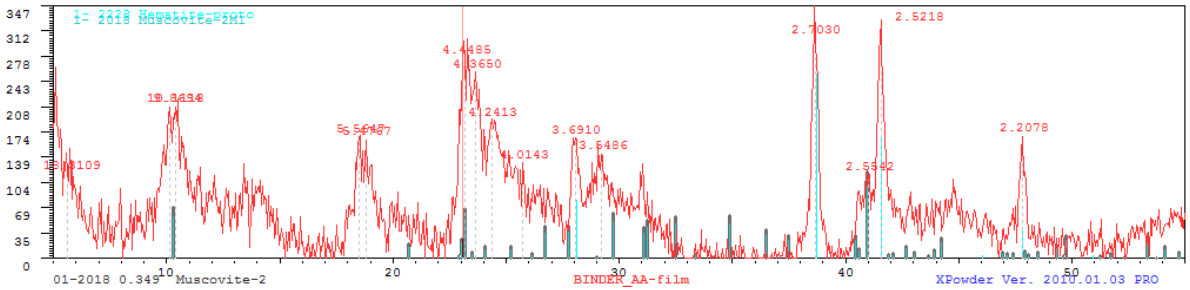
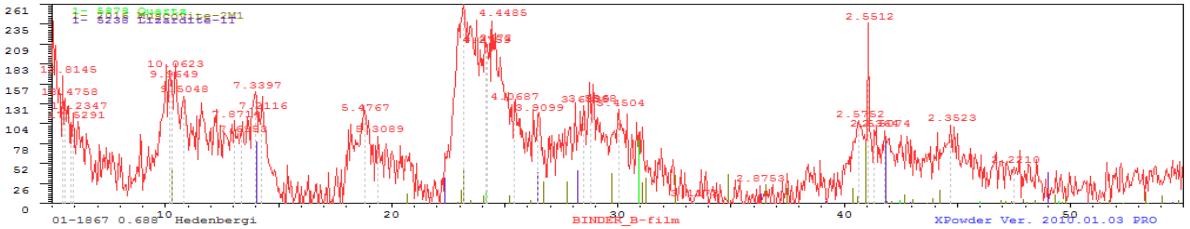
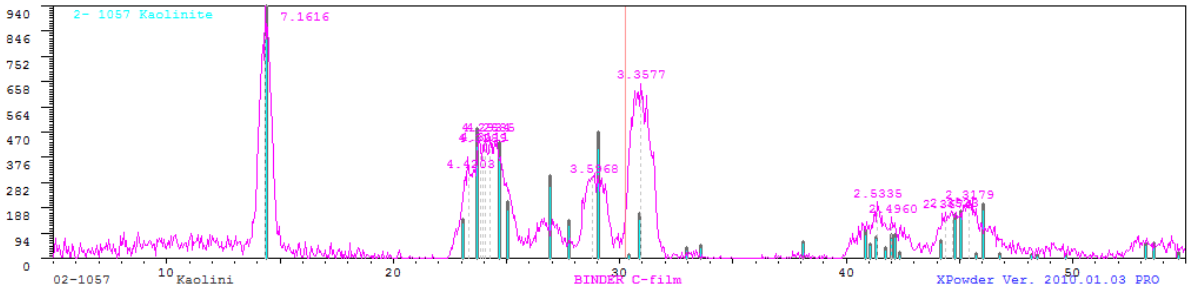
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AC	G2	4.14	0.03	4.11	2.77	2.86	2.80	0.02	2.75	2.84	2.77	1.36	1.27	1.34	33.08	30.91	32.49	32.16	1.12
	G1	2.72	0.05	2.68	0.01	0.01	0.01	0.01	0.00	0.00	0.00	2.68	2.68	2.68	99.91	99.91	99.91	99.91	0.00
	B2	4.13	0.07	4.06	1.67	1.68	1.68	0.07	1.60	1.61	1.60	2.47	2.46	2.46	60.69	60.47	60.54	60.57	0.12
	B1	2.98	0.30	2.68	0.08	0.11	0.08	0.03	0.05	0.08	0.05	2.63	2.60	2.63	98.24	97.16	98.24	97.88	0.62
KC	G2	4.14	0.03	4.11	3.54	3.61	3.50	0.04	3.51	3.57	3.46	0.60	0.54	0.65	14.61	13.04	15.72	14.46	1.35
	G1	2.72	0.05	2.68	0.15	0.15	0.15	0.04	0.11	0.11	0.11	2.57	2.57	2.57	96.06	96.06	96.06	96.06	0.00
	B2	4.13	0.07	4.06	2.50	2.51	2.50	0.07	2.43	2.44	2.43	1.64	1.63	1.63	40.25	40.00	40.16	40.14	0.13
	B1	2.98	0.30	2.68	0.15	0.15	0.15	0.03	0.12	0.12	0.12	2.56	2.56	2.56	95.40	95.40	95.40	95.40	0.00
CC	G2	4.14	0.03	4.11	3.48	3.47	3.40	0.05	3.43	3.42	3.35	0.68	0.69	0.76	16.61	16.83	18.47	17.31	1.01
	G1	2.72	0.05	2.68	1.91	1.91	1.85	0.05	1.86	1.86	1.80	0.82	0.82	0.88	30.48	30.55	32.71	31.25	1.27
	B2	4.13	0.07	4.06	3.61	3.49	3.54	0.07	3.54	3.42	3.47	0.52	0.64	0.59	12.87	15.75	14.61	14.41	1.45
	B1	2.98	0.30	2.68	0.46	0.36	0.34	0.29	0.16	0.07	0.05	2.52	2.61	2.63	93.94	97.51	98.22	96.55	2.29
MC	G2	4.14	0.03	4.11	3.13	3.07	3.13	0.04	3.09	3.03	3.09	1.02	1.08	1.02	24.76	26.30	24.76	25.27	0.89
	G1	2.72	0.05	2.68	0.19	0.17	0.20	0.04	0.15	0.13	0.16	2.53	2.55	2.52	94.52	95.10	94.04	94.55	0.53
	B2	4.13	0.07	4.06	2.82	2.82	2.81	0.08	2.74	2.74	2.73	1.32	1.32	1.33	32.46	32.55	32.72	32.58	0.13
	B1	2.98	0.30	2.68	0.15	0.15	0.15	0.03	0.12	0.12	0.12	2.56	2.56	2.56	95.57	95.57	95.57	95.57	0.00
VA	G2	4.14	0.03	4.11	2.92	2.86	2.91	0.05	2.87	2.81	2.86	1.24	1.30	1.25	30.19	31.69	30.35	30.74	0.82
	G1	2.72	0.05	2.68	0.86	0.77	0.79	0.04	0.82	0.72	0.75	1.86	1.95	1.93	69.40	73.00	72.01	71.47	1.86
	B2	4.13	0.07	4.06	2.95	3.00	2.93	0.07	2.88	2.93	2.86	1.18	1.14	1.21	29.10	27.95	29.71	28.92	0.89
	B1	2.98	0.30	2.68	0.35	0.34	0.36	0.30	0.06	0.04	0.07	2.62	2.64	2.62	97.85	98.38	97.57	97.93	0.41
RA	G2	4.14	0.03	4.11	1.15	1.16	1.13	0.02	1.12	1.14	1.10	2.98	2.97	3.01	72.64	72.29	73.16	72.70	0.43
	G1	2.72	0.05	2.68	0.26	0.26	0.28	0.04	0.22	0.22	0.24	2.46	2.46	2.44	91.74	91.74	91.11	91.53	0.36
	B2	4.13	0.07	4.06	0.90	0.89	0.89	0.07	0.83	0.82	0.82	3.23	3.24	3.24	79.64	79.81	79.80	79.75	0.09
	B1	2.98	0.30	2.68	0.19	0.16	0.18	0.03	0.16	0.13	0.15	2.52	2.55	2.53	94.19	95.31	94.29	94.60	0.62
R	G2	4.14	0.03	4.11	0.16	0.20	0.17	0.03	0.13	0.17	0.14	3.98	3.94	3.97	96.83	95.86	96.60	96.43	0.51
	G1	2.72	0.05	2.68	0.05	0.03	0.03	0.00	0.04	0.03	0.03	2.64	2.65	2.65	98.43	99.03	99.03	98.83	0.35
	B2	4.13	0.07	4.06	0.04	0.04	0.04	0.01	0.03	0.03	0.03	4.03	4.03	4.03	99.24	99.24	99.24	99.24	0.00
	B1	2.98	0.30	2.68	0.11	0.08	0.08	0.03	0.08	0.05	0.05	2.60	2.63	2.63	97.02	98.07	98.07	97.72	0.61

AC=Arusha clay; KC=Kilimanjaro clay; CC=Coastal clay; MC=Morogoro clay; VA=Volcanic ash; RA=Rice-husk ash; R=Reference binder

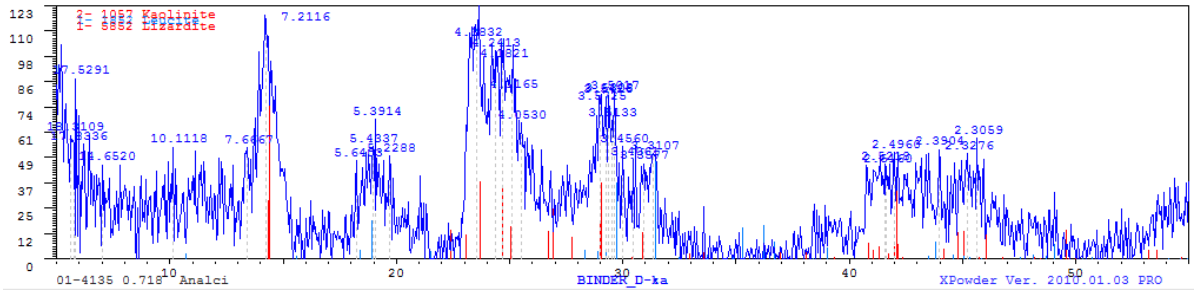
Description of the columns

1: Types of binding materials; 2: Types of aflatoxin in the test-tube; 3: Amount of AF in the test-tube with positive control (buffered solution spiked with solution of AF) after incubation; 4: Residual AF (as impurities) in the test-tube with negative control (buffers solution without AF spiking) - an average of triplicate samples; 5: Actual amount of AF recovered for positive control (3-4); 6, 7 and 8: Amount of AF in the test-tube with suspension of binding material in buffer solution spiked with AF solution for triplicate samples 1, 2 and 3, respectively; 9: Residual AF (as impurities) in the test-tube with suspension of binding material in buffer solution without AF spiking (blank); 10, 11 and 12: Actual amount of AF in the test-tube with suspension of binding material spiked with AF solution, that is, 6-9, 7-9 and 8-9, respectively; 13, 114 and 15: Actual amount of AF bound by binding material after incubation (triplicates), that is, 5-12, 5-13 and 5-14, respectively; 16, 17 and 18: Percent adsorption capacity of the materials in binding AF in buffered solution (triplicates), that is, 13/5*100, 14/5*100 and 15/5*100, respectively; 19: Average percent adsorption capacity of the binding materials in binding AF in buffered solution calculated as mean of the triplicates that is, (16+17+18)/3; 20: Mean standard deviation of 16, 17 and 18.

Appendix 3: X-Ray diffraction analysis of clay and ash-based materials

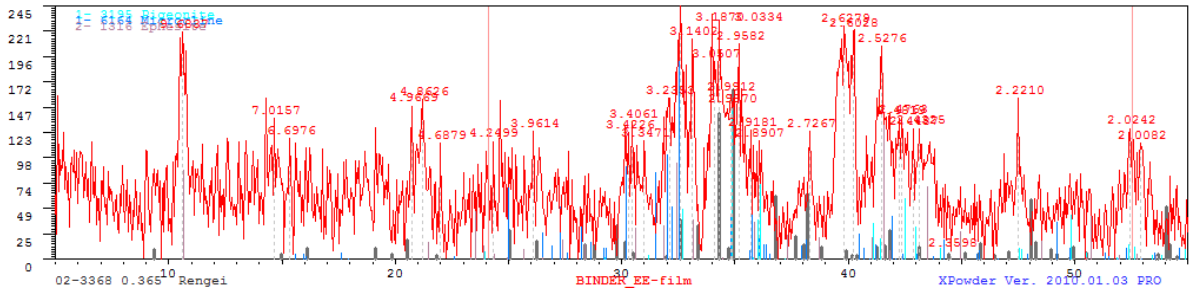
Test-binding materials	X-RD pattern of the test-binding materials	Mineral content of the test-binding materials	
		Names	Chemical formula
AC		Muscovite Hematite-proto	$KAl_2(AlSi_3O_{10})(F,OH)_2$ $Fe_{1.9}H_{0.06}O_3$
KC		Quartz Muscovite Lizardite	SiO_2 $KAl_2(AlSi_3O_{10})(F,OH)_2$ $Mg_3Si_2O_5(OH)_4$
CC		Kaolinite	$Al_2Si_2O_5(OH)_4$

MC



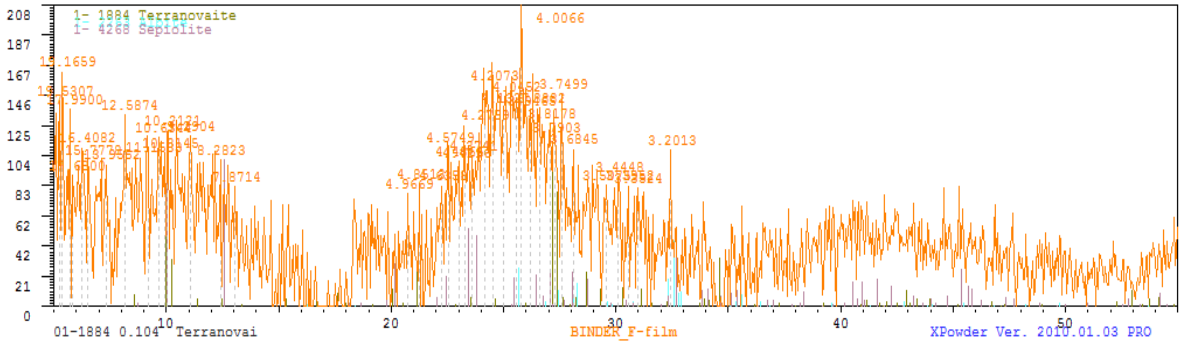
Kaolinite	$Al_2Si_2O_5(OH)_4$
Leucite	$K[AlSi_2O_6]$
Lizardite	$Mg_3Si_2O_5(OH)_4$

VA



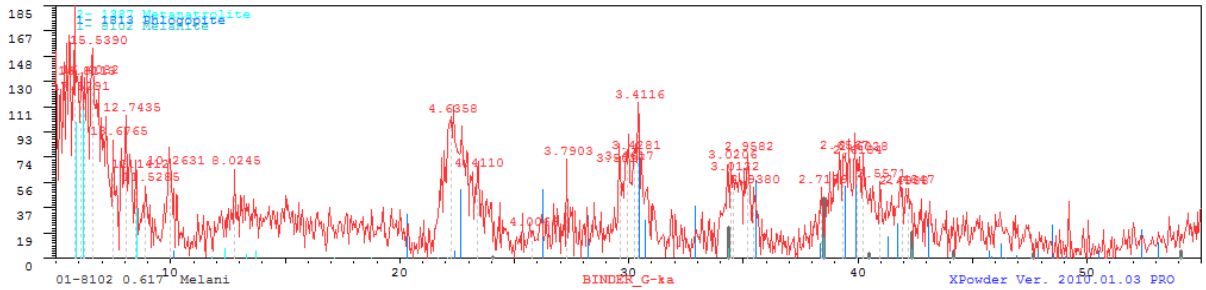
Pigeonite	(Ca, Mg, Fe) (Mg, Fe)Si_2O_6
Microcline	$KAlSi_3O_8$
Ephesite	$NaLiAl_2(Al_2Si_2)O_{10}(OH)_2$

RA



Albite	$NaAlSi_3O_8$ or $Na_{1.0-0.9}Ca_{0.0}$
Terranovaite	$NaCaAl_3Si_{17}O_{40}8H_2O$
Sepiolite	$Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$

Appendix 3 (continue)

Reference binder	X-RD Pattern	Name	Chemical formula
R		Metanatrolite Phlogopite Andradite /Melanite	$Na_2Al_2Si_3O_{10}$ $KMg_3(AlSi_3O_{10})(F,OH)_2$ $Ca_3Fe_2(SiO_4)_3$

Appendix 4: Data of effects of basal diet with/without the BMs on biomarkers of rats

BMs	FI (g/d)	GR (g/d)	FCE (%)	PCV (%)	TP (g/dL)	Al (g/dL)	Glb (g/dL)	AGR	RWL (%)	RWK (%)	RWS (%)
DAC	12.68	1.87	14.76	47	5.91	3.39	2.52	1.44	3.62	0.40	0.26
	14.23	1.75	12.28	44	5.96	3.16	2.80	1.05	3.93	0.42	0.19
	13.47	1.99	14.78	48	6.04	3.18	2.86	1.06	4.76	0.46	0.27
	10.79	1.06	9.86	44	6.37	2.87	3.50	0.73	3.42	0.34	0.18
	11.84	1.65	13.97	41	5.92	2.94	2.97	0.98	4.10	0.43	0.24
	9.73	1.55	15.91	48	5.72	2.88	2.84	1.00	4.83	0.40	0.32
	12.54	1.40	11.18	47	5.83	2.95	2.88	1.08	4.18	0.37	0.30
	12.36	2.20	17.81	50	6.20	3.20	3.00	1.08	3.84	0.39	0.21
	12.00	2.15	17.88	46	5.79	2.98	2.81	1.03	4.60	0.36	0.20
	11.24	2.71	24.13	44	6.00	3.33	2.67	1.24	4.76	0.67	0.26
	10.48	1.59	15.17	50	6.00	3.49	2.51	1.45	4.10	0.37	0.23
	10.26	1.91	18.58	47	6.10	3.09	3.01	0.96	4.74	0.45	0.28
	11.46	2.25	19.63	47	5.66	2.87	2.79	0.99	4.49	0.40	0.26
	11.09	2.09	18.82	44	5.80	3.14	2.66	1.41	3.17	0.34	0.29
DKC	15.16	2.70	17.81	47	5.70	3.15	2.55	1.16	4.77	0.38	0.24
	13.43	2.39	17.76	49	5.48	2.93	2.55	1.07	4.67	0.40	0.23
	11.00	1.59	14.45	48	5.58	2.71	2.87	0.99	3.68	0.36	0.28
	12.75	1.48	11.63	50	6.04	2.95	3.09	0.95	4.09	0.45	0.24
	12.53	1.77	14.12	46	5.44	3.13	2.31	1.28	5.03	0.42	0.25
	10.72	1.96	18.29	47	6.25	3.12	3.13	1.06	5.01	0.48	0.28
	14.42	2.15	14.89	50	5.78	3.20	2.58	1.15	3.98	0.33	0.21
	12.95	2.62	20.24	49	5.58	3.19	2.39	1.53	4.77	0.39	0.21
	13.29	1.63	12.29	53	6.27	3.01	3.25	0.92	3.95	0.37	0.19
	11.60	1.99	17.16	47	6.37	3.06	3.31	0.99	4.53	0.42	0.22
	14.27	2.68	18.80	54	6.06	3.16	2.91	1.11	4.35	0.39	0.20
	12.23	2.11	17.27	50	5.90	3.06	2.85	1.05	4.13	0.35	0.26
	9.81	2.05	20.92	50	6.42	3.11	3.30	1.02	3.83	0.37	0.21
DC	12.76	1.67	13.11	48	6.03	3.63	2.40	1.76	3.77	0.30	0.21

C											
	12.53	2.31	18.44	45	6.38	3.20	3.18	1.03	4.70	0.39	0.21
	10.84	2.36	21.74	47	6.64	3.30	3.35	1.13	4.36	0.40	0.21
	11.37	1.78	15.66	47	6.36	3.16	3.20	0.95	3.97	0.35	0.26
	9.51	1.27	13.37	50	6.04	3.05	2.99	1.10	4.15	0.44	0.22
	10.55	1.59	15.07	48	6.12	2.91	3.21	0.92	4.52	0.35	0.28
	14.48	1.22	8.44	47	6.32	3.51	2.82	1.65	4.54	0.36	0.20
	10.27	1.75	17.00	48	6.54	2.81	3.73	0.83	3.81	0.34	0.23
	11.53	1.72	14.95	48	5.67	2.96	2.71	1.29	4.79	0.41	0.30
	9.87	1.94	19.68	48	6.78	3.32	3.46	1.02	4.54	0.37	0.19
	14.69	2.07	14.08	48	7.30	2.77	4.53	0.63	4.95	0.42	0.24
	10.78	1.78	16.47	47	5.93	2.92	3.01	1.19	4.19	0.39	0.24
	10.60	1.84	17.31	49	5.37	2.23	3.14	0.77	4.98	0.38	0.21
	10.21	2.18	21.37	47	6.71	2.75	3.96	0.79	3.40	0.31	0.23
D											
M											
C	12.87	2.06	15.97	48	6.30	2.75	3.56	0.81	3.91	0.46	0.20
	11.40	1.59	13.96	52	5.54	2.84	2.70	1.28	4.27	0.36	0.22
	15.22	1.64	10.79	49	5.77	2.92	2.85	1.90	3.55	0.36	0.20
	11.71	1.65	14.09	49	6.18	2.71	3.48	0.86	4.12	0.40	0.24
	11.64	1.59	13.63	52	6.33	3.07	3.26	1.17	3.94	0.38	0.28
	9.95	1.62	16.27	51	6.34	3.22	3.11	1.26	4.03	0.39	0.26
	14.29	1.77	12.41	50	5.81	3.42	2.40	1.66	4.34	0.34	0.20
	11.20	2.04	18.19	46	6.47	3.10	3.37	1.10	3.52	0.34	0.21
	10.31	1.92	18.61	49	6.83	2.81	4.03	0.74	4.06	0.38	0.21
	11.97	2.17	18.09	49	6.15	2.85	3.30	1.06	3.95	0.39	0.31
	11.97	1.59	13.28	47	6.15	3.15	3.00	1.43	3.90	0.43	0.25
	9.37	1.79	19.06	49	6.41	2.89	3.52	0.91	4.06	0.43	0.31
	10.14	1.53	15.12	48	6.67	2.76	3.90	1.06	3.55	0.34	0.23
D											
V											
A	12.30	1.62	13.19	51	5.81	2.82	2.99	0.90	3.87	0.36	0.21
	14.04	2.44	17.36	53	5.71	2.96	2.75	2.39	4.19	0.41	0.33

	13.19	2.66	20.13	48	5.93	2.79	3.14	1.19	4.17	0.37	0.24
	11.27	1.42	12.64	48	5.77	2.84	2.93	1.07	4.06	0.37	0.24
	11.92	1.57	13.14	51	6.10	2.68	3.42	0.94	4.30	0.41	0.21
	12.24	2.16	17.64	49	6.09	2.98	3.10	1.18	3.88	0.35	0.24
	10.31	1.55	15.00	39	5.10	2.65	2.44	1.48	3.19	0.36	0.22
	10.04	1.20	11.99	49	5.42	2.80	2.62	1.33	3.55	0.32	0.24
	10.89	1.94	17.77	50	5.62	2.72	2.90	1.03	3.30	0.31	0.19
	9.57	2.29	23.97	50	5.62	2.76	2.85	1.09	4.24	0.38	0.23
	9.42	1.57	16.63	46	5.68	2.68	3.00	1.06	3.38	0.36	0.21
	9.50	1.29	13.62	50	5.73	2.62	3.10	0.99	3.80	0.40	0.23
	10.66	2.21	20.74	46	5.36	2.69	2.67	1.25	4.11	0.34	0.20
	9.26	1.47	15.82	50	5.99	2.85	3.13	1.07	3.86	0.41	0.24
DR											
A	14.76	2.29	15.48	50	6.28	3.15	3.13	1.05	3.38	0.34	0.21
	12.47	2.01	16.11	50	6.10	2.77	3.32	1.02	3.60	0.36	0.25
	13.21	2.26	17.14	48	5.61	2.65	2.96	1.02	4.49	0.34	0.20
	11.91	1.60	13.48	45	5.67	2.92	2.75	1.20	3.86	0.34	0.23
	11.25	1.36	12.11	48	6.21	2.70	3.52	0.79	3.22	0.37	0.24
	12.92	2.12	16.42	49	5.73	2.84	2.89	1.06	3.67	0.27	0.25
	12.37	1.63	13.14	47	5.83	2.64	3.19	0.97	3.21	0.29	0.24
	14.19	1.88	13.22	50	6.02	2.59	3.43	0.82	3.21	0.32	0.20
	11.21	1.58	14.09	50	5.97	2.54	3.43	0.75	3.98	0.42	0.38
	11.23	2.28	20.35	51	5.83	2.83	3.00	1.01	5.22	0.58	0.68
	10.60	1.51	14.20	49	6.53	2.94	3.59	0.91	3.72	0.33	0.21
	13.14	2.42	18.44	49	5.86	3.33	2.54	1.63	3.30	0.32	0.24
	9.77	1.68	17.24	43	6.07	2.78	3.29	0.86	3.58	0.39	0.26
	12.23	1.81	14.80	44	6.45	3.04	3.41	1.00	4.46	0.39	0.26
DR	13.82	2.21	15.96	50	6.22	3.07	3.15	1.00	3.19	0.31	0.22
	12.41	2.46	19.80	52	5.83	3.08	2.75	1.19	3.80	0.39	0.21
	12.26	1.38	11.23	45	6.10	3.32	2.78	1.36	3.89	0.31	0.24
	11.63	1.73	14.90	49	6.11	3.10	3.01	1.05	3.92	0.33	0.22
	14.05	1.92	13.64	48	5.66	3.08	2.58	1.18	4.05	0.36	0.30
	10.52	2.23	21.23	45	5.50	3.13	2.37	1.61	4.24	0.40	0.20
	12.08	1.69	13.98	49	5.74	2.99	2.75	1.19	3.62	0.34	0.21

	14.80	2.85	19.23	50	5.59	2.95	2.64	1.20	3.99	0.36	0.19
	13.91	1.98	14.23	51	5.92	2.99	2.93	1.15	3.85	0.30	0.27
	11.97	2.46	20.54	50	6.05	2.96	3.09	0.97	3.40	0.32	0.23
	10.23	2.13	20.77	49	6.11	3.20	2.91	1.17	3.14	0.31	0.24
	10.36	2.82	27.25	47	5.57	3.12	2.45	1.24	3.42	0.32	0.24
	11.27	1.59	14.14	46	5.82	3.06	2.76	1.37	3.71	0.30	0.15
DC	11.57	1.37	11.81	40	6.80	3.21	3.59	0.75	4.46	0.47	0.76
	14.69	1.42	9.64	48	6.43	2.69	3.74	0.72	4.17	0.37	0.19
	10.92	1.49	13.64	48	5.80	2.56	3.24	0.97	3.99	0.30	0.23
	10.56	1.09	10.28	43	6.03	2.85	3.17	0.98	3.72	0.33	0.26
	11.73	1.63	13.86	42	6.22	2.93	3.29	0.79	3.70	0.31	0.22
	12.93	1.34	10.35	46	5.42	2.51	2.91	0.88	3.87	0.34	0.24
	11.84	1.08	9.11	43	6.69	2.79	3.90	0.87	4.03	0.44	0.22
	13.24	1.61	12.15	45	5.85	3.01	2.84	1.17	4.00	0.39	0.31
	9.79	1.55	15.79	45	6.24	2.66	3.58	0.75	4.02	0.36	0.21
	12.33	1.97	15.95	46	6.26	2.68	3.58	0.88	3.61	0.37	0.26
	12.08	1.73	14.31	46	5.90	2.76	3.14	0.99	3.57	0.39	0.24
	11.20	1.42	12.69	44	6.24	2.43	3.81	0.62	3.66	0.40	0.26
	11.18	1.82	16.29	43	5.88	2.75	3.13	0.96	4.40	0.39	0.24
	9.45	1.39	14.71	45	6.08	2.96	3.12	1.01	3.90	0.32	0.27

DAC, DKC, DCC, DMC,DVA, DRA,D R and DC are diets with Arusha clay, Kilimanjaro clay, Coast clay, Morogoro clay, Volcanic ash, Mycobind® and Control, respectively.

FI: Daily feed intake, GR: Daily growth rate, FCE: Feed conversion efficiency, PCV: Packed cell volume, TP: Total protein of serum, Alb: Albumin, Glb: Globulin, RWL: Liver relative weight, RWK: Kidney relative weight, RWS: Spleen relative weight.