THE EFFECTS OF AFLATOXIN CONTAMINATED FEED WITH OR WITHOUT A COMMERCIAL BINDER ON BROILER PERFORMANCE

THUITA FRIDA NJOKI

A thesis submitted to Graduate School in partial fulfillment for the requirements of the Master of Science Degree in Animal Nutrition of Egerton University

EGERTON UNIVERSITY

MAY, 2019

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not, wholly or in part, been presented for an award of a degree or diploma in this or any other institution known to me.

Signature: ..... Date: 30/05/2019

Frida Njoki Thuita

KM113/14462/15

Recommendation

This thesis has received our approval for submission as the official Egerton University supervisors.

Signature: ..... Date: 30.5.2019

Prof. James K. Tuitoek (PhD)

Department of Animal Sciences

Egerton University, Kenya

Signature: ..... Date: 30/5/2019

Dr. Anthony M. King'ori (PhD)

Department of Animal Sciences

Egerton University, Kenya

Signature: ..... Date: 30/05/19

Dr. Meshack A. O. Obonyo (PhD)

Department of Biochemistry & Molecular Biology

Egerton University, Kenya

COPYRIGHT

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to several people and institutions for their unending support throughout the project period. Firstly, I thank God Almighty for the provision in terms of good physical and mental health and financially. Secondly, I highly thank my parents Mr. and Mrs. Thuita Makumi for their great sacrifices, moral and financial support during my period of MSc. studies. Without all this, I might not be able to complete the studies properly.

Thirdly, I deeply thank my resourceful supervisors Prof. James K. Tuitoek and Dr. Anthony M. King'ori for their invariable technical advice, guidance and unwavering support. You have positively reinforced my attitude of scientific professionalism. Fourthly, I acknowledge the National Commission for Science, Technology and Innovation (NACOSTI) research grant (2016/2017) for funding this research. Fifthly, I appreciate Dr. Meshack A. O. Obonyo (PhD) and the Mycotoxin Research Laboratory, Egerton University funded by the Canadian Government for Aflatoxin Analysis for providing the required equipment and technical expertise required to carry out this research. Sixthly, I appreciate Prof Dr. A. K. Kahi and the center of Excellence in Livestock and business (CoELIB) in Egerton University for providing the broiler rearing facility and equipment.

I thank Egerton University's Graduate School and the Department of Animal Sciences for the opportunity to pursue postgraduate studies. Finally yet importantly, I cannot hesitate to thank my fellow postgraduate students for their moral support all through the MSc. Studies period.

SUMMARY

Agriculture plays a prominent role in Kenya's economy with livestock production contributing significantly to the gross domestic product (GDP). Among the challenges faced in this sector is the contamination of agricultural produce, mainly cereals, grains and their by-products, with mycotoxins, secondary fungal metabolites. This contamination could occur both pre and post-harvest depending on harvesting, storage and transportation methods. Aflatoxins (AFs) are mycotoxins produced by fungi of the genus *Aspergillus* (*A*), mainly *A. flavus* and *A. parasiticus* in agricultural produce when conditions are favorable. Poultry are the species most sensitive to the toxic effects of AFs. These effects are mainly on performance parameters, such as feed intake, growth rate and feed conversion efficiency, and health due to immunity suppression. Aflatoxins contamination of feeds is both of economic importance and a threat to public health as there is a risk of residual AFs in broiler tissues and organs. Aflatoxins are classified as number one cancer causing agents by the International Agency for Research on Cancer (IARC), they have also been reported to be immunosupressant, teratogenic and mutagenic. However, in Kenya there is inadequate data on the prevalence of AFs in commercial broiler feeds, effects of the AFs levels in commercial broiler feeds on broiler performance and digestibility and the if Mycosorb® AFs binder added in feeds is effective. Objective one was therefore a survey that determined the levels of total AFs in common commercial broiler feeds among feed manufacturers in Nakuru town and effects of these AFs levels in diet and AFs binder (Mycosorb®) inclusion in diets on broiler performance, organ weights and digestibility. The first objective was achieved by carrying out a survey in Nakuru town where forty commercial broiler feed samples were randomly collected from ten feed mill companies in two phases (April to May and June to July). Analysis for total aflatoxin levels in the feed samples was by Enzyme-linked immunosorbent assay (ELISA) technique following manufacturer's instructions (Helica Biosystems Inc). Thermo Scientific™ microtiter plate reader read the absorbance optical density (OD) of each microwell with at 450 nm and the data recorded. The OD data conversion into parts per billion (ppb) was by Graphpad prism 7 software. The ppb data were subjected to two way analysis of variance using the GLM procedures of Statistical Analysis System (SAS) (version 9.13). All the feeds collected contained aflatoxins with total AFs levels ranging from 1.07ppb to 41.01 ppb. Of the samples collected, 92.5% and 52.5% contained total aflatoxin levels that exceeded the World Health Organization (WHO) limits of 5 ppb in animal feeds and Food and Drug Administration (FDA) limits of 20 ppb in poultry

feeds respectively. For the second and third objectives, two feeding trial experiments were conducted in Completely Randomized Design. In both experiments, six treatments were tested. The treatments were own compounded broiler starter (fed from day 1 to day 21) and finisher (fed from day 22 to day 35 for experiment two and day 28 to 48 for experiment two), formulated to meet the nutritional requirement as recommended by NRC (1994). The treatments were three levels of AFs (6, 14 and 22 ppb) and two levels of Mycosorb® AFs binder addition for each AFs level (0 and 1 Kg/ tonne of feed). For the first experiment, two hundred one-day-old Arbo Acre broiler chicks purchased were randomly distributed into six experimental groups with four replicates each. Broilers watering and feeding was done daily, weighing on weekly basis to determine growth rate while, total feed given less the total left over feed determined daily feed intake. On day 36, eight broilers per treatment (two per replicate) were slaughtered and their liver, gizzard and heart weights recorded. For the second experiment, twelve broilers of uniform weight (0.8Kg± 0.05) were purchased at four weeks of age and the six treatments allocated randomly with replication. The broilers were allowed to acclimatize to the feeds for seven days then data collected for the next three days. The process was then repeated while ensuring that no broiler got the previously allocated diet. Daily feed intake was determined as feeds given less leftovers. Dry matter and aflatoxins digestibility were determined as; ((Total amount consumed less total amount in feaces) divided by total amount consumed) multiplied by 100. Data on mean separation was done using general linear model (GLM) procedure in SAS (version 9.0) at (p <0.05). Feed efficiency differed significantly (p<0.05) among the diets during the grower period. Diets had no significant effects ($p>0.05$) on feed intake, growth rate and organ weights. Moreover, inclusion of Mycosorb® AFs binder had no effect ($p>0.05$) in daily feed intake, daily growth, feed efficiency, organ weights and dry matter and AFs digestibility. In conclusion, feed conversion efficiency at grower stage was affected by AFs at 14.06 and 21.95 ppb in the treatment diets. Mycosorb® AFs binder was effective in binding the AFs in the treatments. Feed manufacturers should test for aflatoxins in the raw materials and avoid the fungal contamination in the broiler feeds at all stages of handling.

Key Words*: broiler, ELISA, feed, mycotoxin,*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AFs	Aflatoxins
AFB1 and AFB2	Aflatoxin B1 and Aflatoxin B2
AFM1 and AFM2	Aflatoxin M1 and Aflatoxin M2
DON	Deoxynivalenol
EAC	East African Community
ELISA	Enzyme-linked immunosorbent assay
FCE	Feed Conversion Efficiency
FDA	Food and Drug Administration
GDP	Gross Domestic Product
HACCP	Hazard Analysis Critical Control Point
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
KG	Kilo gram
NIRS	Near-Infrared Spectroscopy
NRC	National Research Council
OTA	Ochratoxin A
SAS	Statistical Analysis System
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization
ZEA	Zearalenone

CHAPTER ONE

INTRODUCTION

1.1 Background Information

About 10% of Kenya's gross domestic product (GDP) and more than 30% of its agricultural GDP comes from livestock production (Groote *et al.,* 2010). Broilers are the most important animal in intensive production systems in Kenya whereby, improved poultry breeds constitute about a quarter of all poultry while broilers constitute about 60% of the total commercial birds produced (Groote *et al.,* 2010). Commercial poultry population in Kenya is estimated at 8 million. This large population of commercially produced poultry rely on manufactured animal feeds. In Kenya, about 500,000 tons of animal feed is produced annually of which, roughly 70% is poultry feed (Atherstone *et al.,* 2016). The ingredients used for the manufacture of poultry feed is usually prone to mycotoxin contamination due to the environmental and storage conditions in the tropics (Bryden, 2012; Abrar and Anjum, 2013). Mycotoxins are toxic secondary metabolites of fungi occurring naturally in feeds and foods and are produced on agricultural materials when conditions are favourable for fungal growth (Herzallah, 2009). Where mycotoxins are produced by various fungi, AFs are toxins from members of the genus *Aspergillus*, mainly *Aspergillus flavus* and *Aspergillus parasiticus* in cereals and grains (Marchioro *et al*., 2013). There are 300–400 secondary fungal metabolites, with very different chemical configurations, that have been designated as mycotoxins (Bryden, 2012).

In broilers, AFs can cause economic losses, mainly because of their impact on performance parameters (Marchioro *et al.*, 2013) including; reducing daily feed intake, affecting feed efficiency, and average daily gain (Atherstone *et al.*, 2016). Atherstone *et al*. (2016) reported that growth rate in broilers is reduced by 5% for every 1000 ppb increase of aflatoxin in their diet. In addition to the negative effect on broiler performance, the aflatoxins can also be absorbed and end up in animal products (meat, eggs). Feeding poultry 3,000ppb AFs may result in 3ppb levels in meat with highest concentrations being found in the kidney, gizzard, and liver. Studies in which birds were fed contaminated feed containing 0.25-3.31 mg AFs/kg reported amounts of AFs in tissues varying between 0-0.003mg/kg (Grace *et al*., 2015). Presence of AFs residues in food of animal origin such as meat, milk, eggs and cheese may be as a result of feed contamination (Herzallah, 2009).

The limits of AFB1 and total AFs in foods are 2 and 4ppb in the European Union (EU) whilst they are 5 and 10ppb, respectively, in more than 75 countries around the world (Herzallah, 2009) and AFs limits for animals by WHO are 5ppb (Kajuna *et al*., 2013). AFs have mutagenic effects and are carcinogenic, teratogenic and hepatotoxic hence are a serious threat to public health (Herzallah, 2009). Kajuna *et al*. (2013) found that 91% of broiler feeds in Morogoro, Tanzania were contaminated with AFs and 73% of the contaminated feeds exceeded the FAO/WHO limit level of 5ppb. It is probable that Kenya has a similar scenario since Tanzania is one of the major exporters of raw materials for feeds to Kenya.

1.2 Statement of the Problem

Aflatoxins are a big threat to public health and the success of the agricultural sector. They are responsible for economic losses in terms of lost grains, cereals and animal feeds, lowered performance and increased mortality rates of livestock and compromising the quality of livestock products that could otherwise fetch high prices internationally. So far, only few studies have been carried out on the prevalence and concentration of AFs in broiler feeds in Kenya. As a result, there is low awareness on the prevalence and consequences of AFs in both the public and private sectors. In the manufacture of animal feeds, binders such as Mycosorb® have been used to mitigate against the detrimental effects of AFs but it is not known if they are effective or not.

1.3 Objectives

1.3.1 General objective

To contribute to better livelihoods and food security through reduction of aflatoxins in broiler feeds.

1.3.2 Specific objectives

i. To determine the prevalence and concentration of AFs in common commercial broiler feeds in Nakuru Town.
ii. To determine the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the performance of broilers and organ weights.
iii. To determine the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the digestibility of broilers.

1.4 Hypotheses

i. There are no detectable levels of AFs in common broiler feeds in Nakuru town.
ii. Aflatoxins contamination and use of commercial AFs binder (Mycosorb®) in AFs contaminated feed have no effects on performance of broilers and organ weights.
iii. Aflatoxins contamination and use of commercial AFs binder (Mycosorb®) in AFs contaminated feed have no effects on digestibility in broilers.

1.5 Justification

The production efficiency and profitability of the broiler industry in Kenya is hindered by AFs as they impact on performance parameters such as feed intake, daily weight gain and feed conversion efficiency hence causing economic losses. This can be attributed to utilization of contaminated (feed grade and off-colour) cereals and grains in broiler feed production. Moreover, there is inadequate data on the prevalence and concentration of AFs in broiler feeds in Kenya. Therefore, this study evaluated the prevalence and levels of AFs in feed in Nakuru, the effects of AFs contamination in broiler feed on broiler performance and the effectiveness of Mycosorb® as AFs binder in broiler feeds.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

Poultry and feed production in Kenya

The livestock sector plays a vital role in Kenya's economy in that, it contributes about 10% of the national GDP and above 30% of the agricultural GDP (Groote *et al.,* 2010). Livestock farmers are increasingly becoming commercially oriented, especially in pig and poultry production. Among a number of reasons that favour increased production of chicken is the relatively small space or land allocation that the enterprise demands as compared to the larger livestock types and crop enterprises (Abou-Zeid, 2015). Commercial poultry population in Kenya is estimated at 8 million (Atherstone *et al.,* 2016) while improved poultry breeds constitute about a quarter of all the poultry in Kenya, and broilers constitute about 60% of the total commercial poultry produced (Groote *et al*., 2010). Commercial poultry production in Kenya relies on the use of purchased feed, with very little feed produced on-farm.

About 500,000 tonnes of animal feed is produced in Kenya annually of which, roughly 70% is poultry feed (Atherstone *et al.,* 2016). These animal feeds are produced by several well established feed millers, mostly based in the major cities, using both local and imported ingredients. Maize, wheat and their by-products, such as bran, germ and pollard, are the most dominant raw materials used in the feed industry as energy sources while raw materials most used as protein sources include sunflower, soy bean and cotton seed meals (RTA and Nutrimix Limited, 2016). 100% of the cotton seed meal, wheat grain and maize germ, 75-80% of the maize grain, maize germ and wheat pollard and between 50-70% of the wheat bran, sunflower, maize germ bran and soy bean meals were reported to be locally sourced (RTA and Nutrimix Limited, 2016). On the other hand, 75% of the soy bean and sunflower cakes, and 57% of the cotton cake were imported. Several additives are added to the feeds in small quantities as per animal requirements. Such include vitamin and mineral premixes and amino acids whose source was reported to be 100% import (RTA and Nutrimix Limited, 2016). Other raw materials used for feed production include; sorghum, molasses, rice polish, copra/coconut meal and rice bran for energy and *Rastrineobola argentea* (omena), fish meal, peanuts and canola cake and meal. Raw materials for feeds in Kenya are imported mainly from Tanzania, Uganda and South Africa while the feed additives are from China (RTA and Nutrimix Limited, 2016).

Poultry feed production and costs are a major constraint faced by international as well as local industries due to competition for feed materials shared by animals and humans (Abidin *et al.*, 2011). The Kenyan feed industry highly depends on by-products from other industries such as breweries and food processors (ABS-TCM, 2013). Typically, lower quality raw materials likely to be contaminated with mycotoxins are used such as off colour and feed grade cereals and grains. Contaminants can arise from man-made organic chemical sources such as pesticides, from environmental sources of contamination, or from natural toxins present in plants (phytotoxins) and fungi (mycotoxins) (Spragg and Watts, 2013). Maize, cottonseed, copra, and peanuts are the animal feeds most prone to mycotoxins contamination, specifically, AFs contamination. However, if stored under inappropriate conditions, concentrates and supplements may also be affected (Grace *et al*., 2015).

Poultry are suggested to be the species most sensitive to effects of mycotoxins (Denli and Okan, 2006) whereby broilers are reported to be more susceptible to AFs than layers (Atherstone *et al.,* 2016). Aflatoxins are a group of mycotoxins responsible for adverse effects on weight gain and sexual maturity as well as causing oxidative stress in chicken (Karaman *et al.,* 2010). Aflatoxins affect energy, nucleic acid and protein metabolism whereby, even small amounts of AFB1 may cause reductions in growth parameters, hatchability and also cause increased susceptibility to disease (Denli and Okan, 2006) hence hindering efficiency in broiler production.

2.2 Types of Mycotoxins found in feeds

The term mycotoxin was adopted in 1960s due to several incidences including; the death of approximately 100,000 turkey poults near London, England (Binder, 2007), a liver cancer outbreak in hatchery rainbow trout in United States (Almeida *et al*., 2012) and high incidences of liver disease in ducklings in Kenya (Semple *et al*., 2011). These disease outbreaks and deaths were linked to a peanut and cottonseed meal contamination with secondary metabolites from *Aspergillus flavus* and *Aspergillus parasiticus* (Binder, 2007; Semple *et al*., 2011; Almeida *et al*., 2012). The five most important naturally occurring Mycotoxins in agricultural products are shown in Table 2.1.

Table 2.1 List of common mycotoxins

	Mycotoxin	Producing Fungi
1	Aflatoxins	*Aspergillus flavus*
2	Ochratoxin	*Aspergillus ochraceus* and *Penicillium verrucosum*
3	Deoxynivalenol (DON)	*Fusarium graminearum*
4	Zearalenone (ZEA)	*Fusarium graminearum*
5	Fumonisin	*Fusarium verticillioides*

Source: Leslie *et al*. (2008)

The fungal species most often encountered with intoxications belong primarily to five genera: *Alternaria, Aspergillus, Cladosporium, Fusarium*, and *Penicillium*. Other genera including *Chaetomium, Claviceps, Diplodia, Myrothecium, Phoma, Phomopsis, Pithomyces* and *Strachybotrys* also contain mycotoxic fungi (Bryden, 2012). Mycotoxins have evolved as defenses against predation and competition and their contamination in forages, cereals and pulse crops mostly occurs in the field after infection of plants with pathogenic fungi or with symbiotic endophytes (Spragg and Watts, 2013). Mycotoxins production by fungi can also occur during processing and/or storage stages of harvested agricultural products when environmental conditions, particularly moisture and ambient temperature are appropriate for development of spoilage fungi (Spragg and Watts, 2013). Mycotoxins exists in many forms but amongst them, two are considered to be most important, that is, AFs because they represent one of the most potential carcinogenic substances known and are rated as Class 1 human carcinogens by the IARC (Binder, 2007) and ochratoxins (Abidin *et al*., 2011). When more than one fungal contaminant is present, toxicity within feed is increased due to additive synergistic interactions (Abidin *et al*., 2011). As the world population grows, the demand on the available food supply increases hence the threat from mycotoxins contamination is exacerbated in two ways; more fungus-damaged, potentially mycotoxin-containing foods and feeds are utilized rather than discarded and susceptibility to lower levels of foodborne Mycotoxins is increased due to malnutrition (Leslie *et al*., 2008). The most relevant mycotoxins in animal production are fumonisins, trichothecenes, zearalenone, ochratoxins and Aflatoxins.

2.2.1 Fumonisins

Fumonisins are a group of polar compounds that are based on a hydroxylated hydrocarbon chain with methyl and amino (or acetyl) substituents. Fumonisin B1 is the most common and toxic with B2 and B3 usually accompanying it but in much lower concentrations

(Spragg and Watts, 2013). Fumonisins appear to occur predominantly in maize, being produced by several *Fusarium spp*. that are associated with ear rot and stalk rot in maize globally with some reports also in sorghum (Spragg and Watts, 2013).

2.2.2 Trichothecenes

Deoxynivalenol (DON) and nivalenol (NIV) are trichothecenes (Spragg and Watts, 2013). Approximately 170 trichothecene mycotoxins have been identified to date, having a sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system (Binder, 2007). Type A trichothecenes including T-2 toxin produced by *F. sporotrichioides* and, *F. poeain* millet have been associated with the human disease, alimentary toxic aleukia that was first reported in Russia in the 19th century. Cold, moist grain storage appears to favour growth of these fungi (Spragg and Watts, 2013).

2.2.3 Zearalenone

Zearalenone (ZEA) is a substituted resorcyclic acid lactone. It is a non-steroidal estrogenic mycotoxin that has. It has not been proven to affect human health (Spragg and Watts, 2013). In maize, wheat, barley and triticale, ZEA is primarily produced by *F. graminearum*, a fungus responsible for causing ear and stalk rots of maize and head scab (head blight) of small grains (Spragg and Watts, 2013).

2.2.4 Ochratoxins

Ochratoxins are produced by seven species of *Aspergillus* and six species of *Penicillium* genera, *Aspergillus ochraceus* being the most important producer. There are different types of Ochratoxins: Ochratoxin A (OTA) being the most important (Abidin *et al.*, 2011). OTA is found in various cereals, grains, animal feeds, meats and human tissues all over the world *(Abidin et al*., 2011), it adversely affects the health of poultry and has been listed as a possible carcinogen to humans (group 2B) by IARC (Binder, 2007). The target organ for Ochratoxins is the kidneys (Bryden, 2012). Ochratoxins can be detected in an animal's blood, tissues and milk. They have been found to cause fatal human disease called Balkan endemic nephropathy (Binder, 2007).

2.2.5 Aflatoxins (AFs)

These toxins are named from the fungus producing them; that is "A" from the genus name *Aspergillus*, "fla" from one of the species name *flavus* added to toxin to give the name Aflatoxins (Kajuna *et al*., 2013). Aflatoxins are a group of chemically similar compounds that

fluoresce under ultraviolet light. Depending upon color of the fluorescence, AFs are divided into AFs B1 and B2 for blue, and G1 and G2 for green (Rawal *et al.*, 2010). AFs M1 and M2 (milk-AFs) are the metabolites of AFB1 and AFB2. AFsQ1 and aflatoxicol are AFB1 metabolites (Rawal *et al.*, 2010). The principal AFs are B1, B2, G1 and G2. Aflatoxins G1 and G2 are formed only by *A. parasiticus* while AFs M1 and M2 are found in milk when AFs B1 and G1 are ingested in feed (Semple et al., 2011). Aflatoxins are rated as Class 1 human carcinogens by the International Agency for Research on Cancer (IARC) (Binder, 2007) and they are also the most abundant mycotoxins hence were the main focus in this study.

2.3 Diagnosis of Mycotoxicosis

Mycotoxicosis is the disease caused by consumption of feeds containing harmful fungi metabolites. Mycotoxicosis should be suspected to reduce animal productivity or to cause disease if the outbreaks exhibit the following characteristics; the cause is not readily identifiable, the condition is not transmissible, syndromes may be associated with certain batches of feed, treatment with antibiotics or other drugs has little effect, and/or outbreaks may be seasonal as weather conditions may affect mould growth (Bryden, 2012). Diagnosis of mycotoxicosis can be made by observing gross lesions in organs, for instance; pale coloured liver with necrotized areas, which may be enlarged and fragile and swollen kidneys bulging out of their sockets with congestion (Abidin *et al.*, 2011).

It is necessary to demonstrate biologically effective concentrations of the toxin in the suspect feed so as to ascertain that a mycotoxin is the underlying cause of a field problem (Bryden, 2012). Biological and chemical methods can be used to determine mycotoxin levels in feed and animal carcass samples (Abidin *et al.*, 2011). Chemical methods are used to measure the levels of known toxins and are more appropriate because they are quicker, rapid, reproducible and low levels are also be detected. Two types of methods are used: chromatographic methods including open, column, thin layer, high performance liquid and gas liquid chromatography's and immunochemical methods including radioimmunoassay (RIA) and enzyme linked immuno-sorbent assay (Abidin *et al.*, 2011). The main challenge in relying on chemical feed analysis is obtaining a representative feed sample, this is because there exists 'hot spots' of fungal proliferation hence resulting in uneven distribution of toxins within the feed (Bryden, 2012). In the future, measurement of mycotoxin biomarkers should make it possible to determine mycotoxins intake by animals. This approach has been

successfully applied in determining the exposure of human populations to AFs by measuring AFs albumen adducts in serum (Bryden, 2012).

2.4 Techniques for Aflatoxins Detection and Analysis

Techniques used for AFs detection and analysis can be simply grouped into chromatographic methods and immunological detection and quantification methods. Chromatographic methods include; thin-layer chromatography (TLC), gas chromatography (GC) with electron capture detection (ECD) or mass selective detection (MS) as well as high-performance liquid chromatography (HPLC) with UV, fluorescence detection, also with (multiple) mass spectrometry (Binder, 2007). Results of sophisticated chromatographic procedures depend on the efficiency of the sample preparation, specifically on sampling, extraction and the further treatment of the extract, including any purification (Binder, 2007). Large number of interfering compounds present in samples may contaminate the primary sample extract, hence these components must be removed as completely as possible for most method applications (Binder, 2007).

Immunological detection and quantification methods include: immuno sorbent assays (ELISAs) or radio immune assays (RIAs), which usually require no further sample purification but have the major disadvantage that only one toxin can be determined by each test, referring to the specifity of the antibodies (Binder, 2007). ELISA test kits advantages are that they are the fastest and most cost effective antibody-based test systems, most effective in case of high sample throughput and quick results requirements, they have high throughput assays with low sample volume requirements, antibodies have high specifity and sensitivity to their mycotoxin target molecule, they are very user-friendly and that they have good detection limits of AFB1 (0.01–0.1 ng/mL) (Binder, 2007; Abrar and Anjum, 2013). Disadvantages of ELISA test kits are that compounds with similar chemical groups may also interact with the antibodies (that is, the matrix effect which is especially evident in cases of high complexity of the test material and can lead to overestimates, underestimates, or even false negative or positive results) and that the results of a certain material can be taken as trustworthy only if the kit is validated for the respective commodity. Examples of validated ELISA kits are the Agraquant® total AFs test and ochratoxin test (Binder, 2007).

For larger operations, HPLC could be feasible, though the time factor for clean-up, chromatography, and result calculation needs to be considered and in particular evaluated against the high costs for equipment involved, particularly when mycotoxin analysis is its sole

application (Binder, 2007). Advantage of HPLC is that the shortcoming of single analysis is overcome by parallel tests of the defined analytes and it also has good detection limits of AFB1 (0.01–0.1 ng/mL) (Binder, 2007 and Abrar and Anjum, 2013). Very often the same extracts or purified sample solution can be used for the determination of the most relevant toxins or toxin groups within one chromatographic procedure (Binder, 2007).

Near-Infrared Spectroscopy (NIRS) is an exceptional technique for a rapid and low cost method for the detection of AFB1 that uses an accelerated solvent extraction system followed by on-line Solid Phase Extraction-Liquid chromatography (SPE-LC) and is found appropriate for the determination of AFs (Abrar and Anjum, 2013). The on-line SPE-LC protocol mechanizes sample clean-up and AFs analysis, increasing throughput while decreasing labor. The technique is validated for method precision, linearity, recovery, accuracy, and system precision (Abrar and Anjum, 2013).

Table 2.2 Performance characteristics of different rapid methods for the detection of Aflatoxins in corn

Performance characteristics	**ELISA**	**Flow-through immunoassay**	**Lateral flow test**	**Fluorometric assay with IAC clean-up**	**Fluorometric assay with SPE clean-up**
Quantitative or semi-quantitative	Quantitative	Semi-quantitative	Semi-quantitative	Quantitative	Quantitative
Detection limits	2.5 ppb	20 ppb	4, 10 or 20 ppb	1 ppb	5 ppb
Recovery (%)	93.7–122.6%	NA	NA	105–123%	92–102%
Relative Standard Deviation for Repeatability (%)	4.8–15.9%	NA	NA	11.75–16.57%	8.8–19.6%
Assay time [a]	<25 min	<5 min	5 min	<15 min	<5 min
Equipment	ELISA reader	NA	NA	Fluorometer	Fluorometer

Source: Zheng *et al*. (2006)

where; Assay time [a] refers to the time needed to detect Mycotoxins in a single, pre-ground sample after extraction.

2.5 Occurrence of Aflatoxins

Aflatoxins prevalence is high in agricultural products such as cereals and grains, and hence animal feeds sourced from these agricultural products, is relatively higher in tropical and subtropical regions due to warm and humid weather which provides optimal conditions for the growth of the molds (Bryden, 2012; Abrar and Anjum, 2013). Moisture and temperature have a major influence on mould growth and mycotoxin production (Bryden, 2012). *A. parasiticus* growth temperature ranges from 6ºC to 46ºC with 25–35ºC being the optimal growth temperature range while at 36ºC growth is inhibited. *A. flavus* can produce AFs at a temperature range of between 12-34ºC with maximum production being between 28-30ºC while production is inhibited at 36ºC (Abrar and Anjum, 2013).

For pathogenic fungi to invade crops before harvest, higher moisture levels are required (200–250 g/kg) for infection than for fungi to proliferate during storage (130–180 g/kg) hence, most feedstuffs with moisture contents above 130 g/kg are susceptible to mould growth and mycotoxin formation (Bryden, 2012). Damage or stress to the crop also promote AFs contamination, this can be in terms of either drought prior to harvest, insect activity, poor timing of harvest, heavy rains at harvest and postharvest, or inadequate drying of the crop before storage. However, humidity, temperature, and aeration during drying and storage remain the most important factors (Abrar and Anjum, 2013).

According to a study conducted by FAO/WHO in 2013, where a total of 16,490 samples of corn (57.2%) and rice, sorghum and wheat (about 15.0% each) were analyzed for mycotoxins. 35.9% of the total sample was contaminated with AFs with sorghum having the highest contamination levels of 70.7%, followed by rice (53.7%), wheat (36.3%) and corn (23.2%). Aflatoxins levels on analyzed data ranged from 0.002 to 48,000ppb with the highest level found in a study that also analyzed samples during aflatoxicosis outbreaks in Kenya (FAO/WHO, 2013). Among the 2,193 contaminated corn samples analyzed in the studies 34.5% were from Africa. Contaminated rice, sorghum and wheat samples were mostly from Asia (78.5, 82.4 and 82.3% of the positive samples, respectively). Samples from Asia had the highest incidence of positive samples for all cereals. The lowest incidence of contamination was found in American samples, with no positive wheat samples reported (FAO/WHO, 2013). Aflatoxins and their metabolites are present in animal based foods and deserve studies in relation to the question of transmission of AFs to the East African Community (EAC) human population from animal food products (Grace *et al*., 2015). Kajuna *et al*. (2013) found that 91%

of broiler feeds in Morogoro, Tanzania were contaminated with AFs and 73% of the contaminated feeds exceeded the FAO/WHO limit level of 5ppb.

2.6 Legislations on Aflatoxins levels in Feeds and Foods

The limits of AFB1 and total AF in foods are 2 and 4ppb in the European Union (EU) whilst they are 5 and 10ppb, respectively, in more than 75 countries around the world (Herzallah, 2009). The United States Food and Drug Administration's current action level for AFB1 in poultry feed is 100 ppb for corn and peanut products and 300 ppb for cottonseed meal (Rawal *et al*., 2010). Factors, both scientific and socio-economic in nature, that influence the establishment of mycotoxin limits and regulations globally include availability of toxicological data, availability of data on the occurrence in different commodities, knowledge of the distribution of mycotoxins concentration within a lot, availability of analytical methods, national legislation, and need for sufficient food supply (Bryden, 2012).

The Codex General Standard for Contaminants and Toxins in Food and Feed is concerned with hazards in feeds that could affect human health and sets out guidelines that apply to establishing maximum levels (MLs) in food and feeds. Principles for setting MLs for contaminants in food or feed are that they shall only be set for contaminants that present a significant risk to public health and trade, shall be set as low as reasonably achievable to protect the consumer, shall be set at a level slightly higher than the normal range of variation in levels in foods that are produced with current adequate technological methods in order to avoid undue disruptions of food production and trade, shall be based on data from various countries and sources, including the main production and processing areas of those products, and that they shall apply to representative samples per lot and where necessary sampling methods should be set out (Grace *et al*., 2015).

2.7 Metabolism of Aflatoxins in Animals and Humans

Metabolism of AFs in animals varies greatly depending on animal species, age and idiosyncrasy (Wu *et al*., 2009). However, the most important mechanism should be the metabolizing enzymes in the species (Dohnal *et al*., 2014). Once consumed, AFB_1 is rapidly absorbed from the small intestines into mesenteric venous blood after which it is extensively transformed into various metabolites (Dersjant-Li *et al*., 2003). For its toxicity to be exerted, AFB1 needs to be metabolically converted to its reactive and electrophilic exp-AFBO by cytochrome P450s (CYP450 family) enzyme in both human and animal liver (Rawal *et al*.,

2010; Dohnal *et al*., 2014). CYP1A2 and 3A4 in the human liver also play important roles in AFB1 activation where by, CYP1A2 and 3A4 isoforms mediate the activation of AFB1 hence producing cytotoxic and DNA-reactive intermediates (Dohnal *et al*., 2014). In the human lung, lipoxygenases (lOX) and prostaglandin H synthase (PHS) are important in AFB_1 biotransformation (Dohnal *et al*., 2014).

AFs consumed by animals and human beings are biotransformed in five main reactions; reduction, hydration, epoxidation, hydroxylation, and ortho-demethylation (Heidtmann-Bemvenuti *et al*., 2011) and are then distributed in tissues, biological fluids and milk (Wu *et al*., 2009). AFs metabolism occurs in stages where by phase 1 converts the original moecules into more hydrophilic compounds mainly using enzymaitic hydrolytic and oxidative reduction while phase 2 involves conjugation of either the original molecules or its metabolites with nucleoplilic molecules like; glutathione, glucuronides, and sulfonides (Dohnal *et al*., 2014). Conjugation with glutathione catalyzed by family of GST is the principal route detoxification (Dohnal *et al*., 2014). Not all of these reactions occur in a single species; thus, there are interspecies differences in aflatoxins metabolism.

There are four pathways for AFB_1 metabolism in animals, that is, O-dealkylation to AFP_1, ketoreduction to AFL, epoxidation to AFB_1-8,9-epoxide (a highly toxic, carcinogenic and mutagenic compound) and hydroxylation to AFM_1 (highly toxic), AFP_1, AFQ or AFB_{2a} (all relatively non-toxic) (Wu *et al*., 2009). The electrophilic metabolite exp-AFBO react with cellular nucleophiles and can induce mutations by alkylating DNA, principally at the N^7 position of guanine forming the 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB1 (Rawal *et al*., 2010). AFBO is also capable of binding to proteins and other critical cellular nucleophiles. Most of these AFB_1 metabolites can be transformed to further metabolites, for instance, AFQ_1 to AFH_1 in the liver, while AFB_1-8,9-epoxide can undergo three further metabolic pathways. These are; hydrolysis to form AFB_1-8,9-dihydrodiol, conjugation to form AFB_1-8,9-dihydro-8-(N^7-guanyl)-3-hydroxy (or AFB_1-N^7-Gua) and conjugation with soluble nucleophilic molecules, e.g. glutathione, where glutathione S-transferase (GST) is the catalyst (Wu *et al*., 2009).

Excretion of AFB_1 and its metabolites in poultry and pigs is reported to be slow and it occurs primarily with bile and to a lesser extent with urine. A small percentage of the consumed AFs can be excreted unchanged in several animal species (Dersjant-Li *et al*., 2003).

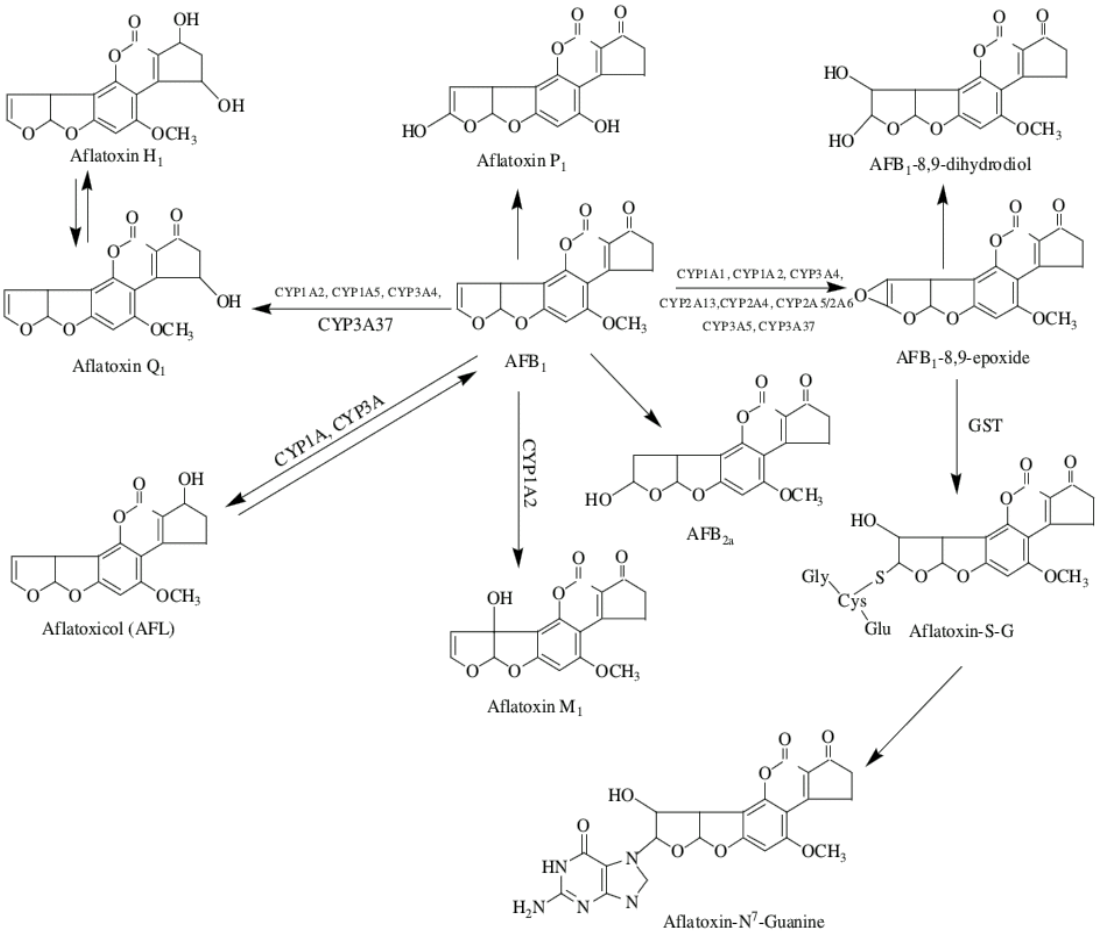

Figure 2.1 The major metabolic pathways and key metabolizing enzymes of Aflatoxin B1 in animals and human beings

Source: Dohnal *et al.* (2014)

2.8 Effects of Aflatoxins on Livestock and Human Beings

2.8.1 Effects of Aflatoxins on human health

The ingested AFB1 and AFB2 are metabolized by livestock into AFM1 and AFM2, respectively, with estimated conversion ratio of 1–3% between AFB1 and AFM1 (Herzallah, 2009). The International Agency for Research on Cancer (IARC) evaluated AFs B1as a Group 1 carcinogen, i.e., carcinogenic to humans *(*Leslie *et al*., 2008; Semple *et al*., 2011). AFB1 is an effective hepatotoxin and hepatocarcinogen with the liver being its primary target *(*Leslie *et al*., 2008 and Abrar and Anjum, 2013). Chronic AFs exposure is associated with the development of hepatocellular carcinoma in humans, especially in those infected with hepatitis B virus. Thus, chronic AFs exposure is a major and significant public health problem *(*Leslie *et al*., 2008).

Toxins including AFs and DON largely influence reproduction efficiency indirectly through reduced feed intake and impairment of metabolic function, especially of the liver. Importantly there is evidence of AFs transfer in utero to the developing foetus in both pigs and humans (Bryden, 2012; Abrar and Anjum, 2013). AFs-induced liver damage may make children suffering from the disease less able to cope with the high protein diets usually recommended as the cure for kwashiorkor (Semple *et al*., 2011). Numerous studies have been published since the late 1960s on the effects of AFs on the immune system of animals (in vivo studies) and on animal and human immune cells *in vitro (*Leslie *et al*., 2008).

Children are more susceptible to toxicants than adults, including an increased sensitivity to genotoxic carcinogens. Aflatoxins are well-known mutagenic agents. Their effects are mainly due to adduct formation with DNA, RNA, and protein and also cause lipid peroxidation as well as oxidative damage to DNA (Abrar and Anjum, 2013).

The danger of consuming foodstuffs contaminated with AFs at levels above the regulatory limit was again demonstrated in 2004 in Kenya where 125 people died following the consumption of homegrown maize containing high levels of AFs *(*Leslie *et al*., 2008). AFs may be a factor in Reye's syndrome, a common cause of death in South East Asian children. Significant levels of AFs (1-4 g/kg) were found in livers of 23 Thai children who had died of Reye's syndrome (Semple *et al*., 2011).

2.8.2 Effects of Aflatoxins on animals

Chronic mycotoxicosis is first indicated by growth depression, resulting from reduced feed intake, impaired nutrient utilization, changes in feed quality or toxicity. It was estimated that with each mg/kg increase of AFs in the diet, the growth rate would be depressed by 16% for pigs and 5% for broilers (Bryden, 2012).

Mycotoxin ingestion cause reduced feed conversion efficiency hence causing impaired nutrient utilization which could be as a result of iterations in nutrient content of heavily moulded grains. Aflatoxins ingestion significantly reduce the energetic efficiency of bodyweight gain, the utilization of AME for tissue energy gain and increase heat production of broilers. Avian aflatoxicosis reduce iron absorption by 54%, circulating calcium levels by 20% and together with OTA cause mal-absorption hence impaired absorption and decreased circulating concentrations of vitamins E and C and carotenoid levels in tissues. Mycotoxins promote free radical formation in the intestines which in

turn results in antioxidant depletion, oxidative stress, apoptosis which all contribute to the development of mal-absorption.

Aflatoxins and DON largely influence reproduction efficiency indirectly through reduced feed intake and impairment of metabolic function, especially of the liver. Importantly there is evidence of AFs transfer in utero to the developing foetus in both pigs and humans (Bryden, 2012). Aflatoxicosis can cause a drop in egg production to 5% and lead to decreased egg size but have no effects on egg shells. In controlled feeding trials, AFs have been shown to cause a delayed decrease in egg production in layers and broiler breeders and to reduce egg size. The reduction in hatchability may be due deposition of AFs into eggs . AFs can cause chicks of broiler breeder hens to have immune dysfunction implying increased susceptibility to disease due to humoral and cellular immunity suppression.

Aflatoxins can cause increased susceptibility of animals and birds to infectious disease such as salmonellosis in chicken, turkeys and pigs, candidiasis, coccidiosis and Marek's disease in chicken, pasteurellosis in turkey and erysipelas in pigs (Bryden, 2012). Following aflatoxicosis, broilers have a decreased dressed weight; the carcass contains less fat and protein and there is a decrease in the yield of breast meat but an increase in the yield of parts or cuts with a smaller meat-to-bone.

Transfer of AFs into milk and ochratoxin A into meat have been the issues of most concern. Available evidence suggests that tissue accumulation of AFs or its metabolites is very low and that residues are excreted in a few days . Animals are effective toxin eliminators with milk, the animal product most likely to contain AFs residues. The hydroxylated metabolite of AFs B1, AFM1 is excreted into milk from 1 to 6% of dietary intake (Bryden, 2012). There are significant differences among pig and poultry tissue deposition studies and this is presumably due to differences in absorption and metabolism of the toxin. It has been shown that the half-life of ochratoxin A in pigs and chickens is 180–140 h and approximately 4 h, respectively. Caution should be exercised when extrapolating or predicting tissue residues as there is presently insufficient data on which to anticipate the outcome of any field toxicosis.

Table 2.3 Summary of Aflatoxin levels fed to poultry and their effects

Class of Poultry	Levels of AFs fed	Effects	References
Broilers	2000 ppb	Lowered body weights, depressed feed intake, increased FCR	(Girish & Devegowda, 2006)
Cherry valley ducks	20 and 40 ppb AFB_1	Decreased body weight gain, decreased feed intake, increased feed to gain ratio, lowered protein digestibility, increased digestive enzyme activities of duodenum contents	(Han *et al*., 2008)
Broilers	700ppb, 1700ppb and 2800ppb AFs	Lowered body weights, depressed feed intake, increased FCR, pancreatic activity of lipase and a-amylase were significantly increased in treatments with 1700ppb and 2800ppb, while the specific activity of trypsin was only affected during treatment with 2800ppb	(Marchioro *et al*., 2013)
Broilers	40ppb and 80ppb AFB_1	80ppb resulted in lower body weight gain and feed efficiency, alteration in total protein concentration in the serum and increased liver weights	(Denli and Okan, 2006)
Broilers	150ppb and 300 ppb AFs	Enlarged livers and kidneys especially in groups fed 300ppb AFs and varying degrees of atrophy in the bursa of Fabricius was observed in some animals	(Karaman *et al*., 2010)
Broilers	1000ppb AFB_1	Lowered growth rate, reduced serum immunoglobulin contents, negative alteration on serum biochemical contents, and enzyme activities, and induced histopathological lesion in the liver	(Shahid *et al*., 2017)
Broilers	14.06 and 21.95 ppb	Lowered feed conversion efficiency	Current experiment

2.9 Economic Impact of Aflatoxins

The Council for Agricultural Science and Technology reported, crops contamination with AFs to be a global problem with approximately 25% of world's food supply being contaminated with mycotoxins annually and the United States poultry industry losing more than $143 million annually due to AFs (Rawal *et al*., 2010). Economic losses to the poultry industry due to AFs are experienced in terms of reductions in growth rate, hatchability, feed efficiency and immunity towards diseases. Dietary AFs (2.5 mg/kg) can significantly reduce the feed intake by 9–11% of poultry among all age groups (7–280 days old) while dietary exposure of broiler hens to AF (10 mg/kg) can result in embryonic mortality and lowered immunity in the progeny chicks. Embryonic exposure with AFs can lead to long-term depression of the immune function in chicks with the liver being most severely affected organ in poultry, primary consequences being hepatotoxicity and carcinogenicity (Rawal *et al*., 2010). Implementation of a new European Union (EU) AFs standard which is lower (4 ng/g AFs B1) than the internationally accepted Codex Alimentarius standard would reduce health risks by 2.3 deaths per billion people per year. The potential annual cost of contamination of food and feed crops in the United States with three mycotoxins (AFs, fumonisin and deoxynivalenol) is estimated to be US$ 946 million on average (Leslie *et al.*, 2008). In addition, the costs of mycotoxin management, including research and monitoring, are estimated at between US$ 500 million and US$ 1.5 billion (Leslie *et al.*, 2008)

2.10 Prevention and control of Aflatoxins

Aflatoxins Control at Farm Level

Integrated understanding of crop biology, agronomy, fungal ecology, harvesting methods, storage conditions, feed processing and detoxification strategies is required to reduce the occurrence and impact of mycotoxins (Bryden, 2012). Risk of AFs contamination can be minimized by adopting various protective measures at early stages in the farm such as good agronomic practices, growing resistant varieties and early harvesting (Abrar and Anjum, 2013). High moisture content is likewise a risk factor for mycotoxin infestation hence rapid drying to 150 g moisture content per kilogram is suitable (Binder, 2007; Abrar and Anjum, 2013).

The hazard analysis critical control point (HACCP) system can be used to manage the risk of mycotoxins throughout the production chain. At pre-harvest stage, use of antifungal peptides in crops is the most promising effort to combat AFs contamination in feeds (Abidin

et al., 2011; Abrar and Anjum, 2013). There are three main methods to control mycotoxins; Physical, chemical and biological. Common physical methods employed include; mechanical separation of broken kernels, density segregation, color sorting, and screening. Simple washing procedures using water or sodium carbonate solution results in some reduction of mycotoxins in maize and other grains (Binder, 2007; Abidin *et al*., 2011; Abrar and Anjum, 2013). The use of mould inhibitors or preservation by acids can only reduce the amount of mould but does not influence the content of mycotoxins generated prior to treatment. If mycotoxins have been produced earlier they will not be affected in any form by mould inhibitors or acid mixtures, as they are very stable compounds (Binder, 2007; Abrar and Anjum, 2013).

Chemical methods include treatment of feed with ozone acidic and alkaline substances, ammonia and antioxidants. Ozone, ammonia and organic acids have been shown to be effective against AFs and ochratoxins (Abidin *et al*., 2011). Antioxidants like butylated hydoxyanisole (BHA), butylated hydroxytoluene (BHT), praben (PP) and their mixtures have a negative impact on AFs and Ochratoxins (Abidin *et al.*, 2011) . Aqueous extracts of some traditional medicinal plants, including *Carum carvi* seeds, *Camellia sinensis* leaves, *Boswellia serrata* resins, *Alpinia galangal* rhizomes, and *Cenchona Sofficinalis* bark, have been suggested to have therapeutic effects beneficial in modulating the alterations induced in kidney and heart under the toxic effects of AFB1 (Abrar and Anjum, 2013).

Biological methods significantly reduce AFs in all the vulnerable crops in a cost-effective manner and over a broad geographic area (Abrar and Anjum, 2013). They include use of microorganisms (probiotics) against mycotoxins present in feed. These probiotics form a chelated complex with mycotoxins and prevent absorption of mycotoxins by the body (Abidin *et al*., 2011). Probiotic bacteria such as *Lactobacillus rhamnosus* and *Nocardiacoryne bacteroides* can be used to reduce the lesions caused by AFs and other mycotoxins in broilers (Abidin *et al.*, 2011).

2.11 Remedies to Aflatoxicosis

Removal or decontamination is recommended if mycotoxins levels are high in feed, however, removal of the contaminated portion of feed is difficult (Abidin *et al*., 2011). Ammoniation has been suggested as a method for decontaminating cereals but there is no method to detoxify the toxins present in forages. Chemical, biological and physical methods of decontamination of diet are available and a de-epoxidising enzyme isolated from pure strain of bacteria has also been identified (Abidin *et al.*, 2011).

Mycotoxin binders prevent adverse effects of mycotoxins by binding to the toxins, thus preventing their adsorption within the digestive system. The use of binders is considered to be a preventative than a therapeutic approach (Abidin *et al*., 2011). Different binders are available, and include activated carbon, complex indigestible carbohydrates (e.g. cellulose, glucomannans, and peptidoglycans), and synthetic polymers such as polyvinylpyrrolidone, cholestryamine and alluminosilicates such as bentonite, clay, zeolite, montmorillonite and phyllosillicates. Activated carbon has excellent quality of adsorption, due to its large surface area, and is considered to be a general binder for a variety of toxicities of digestive system. Activated charcoal is associated with reducing the levels of AFs residues within cow's milk, however it is needed in higher quantities to facilitate this (Abidin *et al.*, 2011). Silicate is another class of mycotoxin binders whose mechanism of action is to chelate the β-dicarboxyl moiety of AFs with free metal ions present in clay material. Sodium bentonite can be used to bind AFB1 in broiler feed and has been shown to reduce the symptoms produced due to aflatoxicosis.

Use of Silymarin, L-Carnitine and Vitamins: Products like Silymarin, L-carnitine and vitamins can combat the harmful effects of mycotoxins and can improve the immune system of birds. L-carnitine (LC) is a quaternary ammonium (small water soluble) compound that is naturally synthesized from the amino acids methionine and lysine (Abidin *et al.,* 2011). The basic role of LC is to generate energy during lipid breakdown, whereby it transports fatty acids from the cell cytosol to the mitochondria. It has antioxidant qualities and protects against lipid peroxidation. L-carnitine has the ability to bind AFB1, thereby limiting toxin interactions with tissue proteins and DNA. The compound may improve other metabolic disorders of broilers. Silymarin is an extract of Silybummarianum (milk thistle plant or lady's thistle plant). Silymarin has been used as remedy for the treatments of cancers and hepatic disorders in humans (Abidin *et al.,* 2011). It has various beneficial effects including promoting DNA and RNA synthesis and regeneration of liver tissue. Silymarin can help negate the toxic effects produced by AFB1 in broilers (Abidin *et al*., 2011). Vitamin E is an excellent antioxidant with a major role in preventing lipid peroxidation. It has the ability to enhance the immune status of the birds which are immune-suppressed by mycotoxin exposure. This vitamin improves the overall performance of the birds.

CHAPTER THREE

PREVALENCE OF AFLATOXINS CONTAMINATION IN COMMERCIAL BROILER FEEDS IN NAKURU, KENYA

3.1 Abstract

Aflatoxin contamination of broiler feed is a major barrier to sustained agricultural productivity and trade. Aflatoxins are a type of mycotoxins (secondary fungal metabolites) produced by fungi of the genus Aspergillus (A), mainly *A. flavus* and *A. parasiticus*, in agricultural produce when conditions are favorable. This survey determined the levels of total aflatoxins (AFs) in common commercial broiler feeds among feed manufacturers in Nakuru town, Kenya. Forty compounded broiler feed samples were randomly collected from ten feed mill companies in Nakuru town (ten broiler starter and ten broiler finisher feed samples per company) in two phases. Each collection phase was determined by the frequency of purchase of raw materials by the individual milling companies. The total aflatoxin levels in the feed were analyzed using the ELISA technique in the Mycotoxin Research Laboratory at Egerton University. The data was subjected to SAS procedures using two way analysis of variance. All the feeds collected contained aflatoxins within a range of 1.07- 41.01 ppb. The samples (92.5%) contained total aflatoxin levels which exceeded the WHO limits of 5 ppb in animal feeds. Of the samples collected, 52.5% exceeded the FDA limits of 20 ppb in poultry feeds. To avoid high levels of AFs in broiler feeds, feed manufacturers should test for aflatoxins in the raw materials and avoid the fungal contamination in the broiler feeds at all stages of handling.

Key words: Aflatoxins, broiler, ELISA, feed

3.2 Introduction

Livestock production contributes approximately 10 and 30% of the national and agricultural GDP respectively (FAO, 2013). In Kenya, food ingredients rejected for human consumption (off-color and feed grade cereals and grains) are normally utilized for feed production. These off-color cereals and grains are often contaminated with mycotoxins and there are risks that contamination may exceed acceptable standards. Poultry have been reported to be very sensitive to the effects of mycotoxins (Denli and Okan, 2006). Mycotoxins are secondary fungal metabolites with very different chemical configurations (Bryden, 2012). Aflatoxins are produced by members of the genus Aspergillus (A), mainly *A. flavus* and *A. parasiticus*, in cereals and grains when moisture and ambient temperature are favorable

(Bryden, 2012; Marchioro *et al.,* 2013; Spragg and Watts, 2013). The poultry sector therefore is at risk of economic losses due to the negative impact of AFs on their performance (Marchioro *et al.,* 2013; Atherstone *et al.,* 2016).

Aflatoxins may cause oxidative stress, reductions in growth parameters, increased susceptibility to disease and adversely affect sexual maturity and hatchability in chicken (Denli and Okan, 2006; Karaman *et al.,* 2010). In addition to the negative effects of AFs in poultry feed on the performance of broilers, contamination of feeds with aflatoxins may result in the presence of AFs residues in foods of animal origin such as broiler meat posing a serious threat to public health (Herzallah, 2009). AFs can cause mutagenic effects, are carcinogenic, teratogenic and hepatotoxic and they also depress immunity in both human beings and animals (Leslie *et al.,* 2008 ; Herzallah, 2009 ; Abrar *et al.,* 2013). The limits of AFB1 and total AFs in foods are 2 and 4 ppb respectively in the European Union whilst they are 5 and 10 ppb, respectively, in more than 75 countries around the world (Herzallah, 2009). WHO has set AFs limits for animal feeds at 5 ppb (Kajuna *et al.,* 2013) and FDA at 20 ppb (Reddy and Raghavender, 2007). The aim of this survey was to determine the prevalence and levels of AFs in commercial broiler feeds manufactured in Nakuru town, Kenya.

3.3 Materials and methods

3.3.1 Survey sites and sampling procedure

This survey was carried out in Nakuru and Egerton University, Kenya. Broiler feed samples were collected from ten randomly selected millers out of the twenty feed milling companies in the town that manufacture broiler feeds. A total of forty compounded broiler feed samples were collected randomly. Ten broiler starter and ten broiler finisher feed samples were collected per feed miller in two phases, the first phase was April and May, 2017 and the second phase between June and July, 2017. The phases of collection were determined by the frequency of purchase of raw material by the individual milling companies. The samples were collected in different khaki bags, labelled, transported to the Mycotoxin Research Laboratory in Egerton University and stored at 4^{o}C as per (Nemati *et al.,* 2014) awaiting analysis. For each collected sample, one kilogram composite sample was collected by picking random portions of samples from the same feed batch and mixing thoroughly to form a homogeneous sample (Rodrigues *et al.,* 2011). The samples were blended into a homogenous sample then, subjected to ELISA technique to determine the concentration of total aflatoxins.

3.3.2 Analysis of samples

Extraction of Aflatoxins from the samples

Extraction Solution (70% Methanol) was prepared by adding 30ml of distilled water to 70ml of methanol (reagent grade) for each sample to be tested. Representative feed samples were individually finely ground such that 95% passed through a 20 mm mesh screen. A 20g ground portion of the sample was weighed and 100ml of the Extraction Solvent (70% methanol) added. They were mixed by shaking in a sealed container or in a blender for a minimum of 2 minutes. Particulate matter was allowed to settle, then filtered about 10ml of the extract through a Whatman #1 filter paper and filtrate collected to be tested.

Assay Procedure

The levels of total AFs in each sample was determined using ELISA technique following manufacturer's instructions (Helica Biosystems Inc) as described here in. All the reagents were brought to room temperature before use. One Dilution Well was placed in a microwell holder for each Standard and Sample to be tested. An equal number of Antibody Coated Microtiter Wells were placed in another microwell holder. 200µl of the Conjugate was dispensed into each Dilution Well. A new pipette tip for each was used to add 100µl of each Standard and Sample to appropriate Dilution Well containing Conjugate. Mixing was achieved by priming pipettor at least 3 times. The location of each Standard and Sample throughout test was recorded. Using a new pipette tip for each, 100µl of contents was transfered from each Dilution Well to a corresponding Antibody Coated Microtiter Well. They were incubated at room temperature for 15 minutes. The contents from microwells were decanted into a discard basin. The microwells were washed by filling each with distilled, then decanted the water into a discard basin. Wash was repeated for a total of 5 washes. Microwells were tapped face down on a layer of absorbent towels to remove residual water. The required volume of Substrate Reagent (1 ml/strip or 120 µl/well) was measured and placed in a separate container. Each microwell was added 100µl and incubated at room temperature for 5 minutes. The required volume of Stop Solution (1 ml/strip or 120 µl/well) was measured and placed in a separate container. Added 100µl in the same sequence and at the same pace as the Substrate was added. Optical density (OD) of each microwell was read with a Thermo Scientific™ microtiter plate reader at 450 nm. The optical density (OD) of each microwell was recorded.

3.3.3 Statistical data analysis

Graph pad prism 7 software was used to convert the optical density (OD) data to ppb. The data were then subjected to a two way analysis of variance using the GLM procedures of SAS (version 9.13) and the means were separated using the paired *t*-test. In addition, frequencies and percentages were also calculated. The model was as described below.

$Yijk = \alpha i + \beta j + \alpha\beta ij + \varepsilon k$

where; Yijk represents AFs levels the samples,

αi =the feed type i.e. either broiler starter or finisher

βj =phase of sample collection i.e. either phase 1 or phase 2

$\alpha\beta ij$ =the interaction between feed type and phase and,

εk =the error term.

3.4 Results

The results of these analyses are summarized in Tables 3.1 and 3.2. All the compounded broiler feed samples collected in Nakuru town contained total AFs levels ranging between 1.07 - 41.01 ppb. The range for broiler starter feed was 1.07- 41.01 ppb whereas that for broiler finisher was 4.69-35.76 ppb. Of the samples, 92.5% (90% broiler starter and 95% broiler finisher) exceeded the WHO recommended level of 5ppb total AFs limits in animal feeds. Whilst 52.5% of the samples (50% broiler starter and 55% broiler finisher) exceeded the FDA 20ppb total AFs limits in poultry feeds. The mean total AFs levels for the broiler starter and broiler finisher feed samples were 19.37 ± 2.45 and 19.86 ± 2.21 ppb respectively. On the other hand, the mean total AFs levels for feed samples collected in phase 1 and phase 2 were 18.00 ± 2.03 and 21.22 ± 2.54 ppb respectively. The aflatoxin levels in the starter and finisher samples were not different (p=0.88). Aflatoxin levels in samples collected in phase 1 were not different from those collected in phase 2 of sample collection (p=0.34). There was no interaction between the feed type and sample collection phase (p=0.57).

Table 3.1 The level of total Aflatoxins (ppb) in commercial broiler feeds according to feed type and collection phase in Nakuru town, Kenya

Broiler feed type	n	Minimum	Maximum	Mean	SEM	*p*-Value
Starter	20.00	1.07	41.00	19.40	2.44	0.88
Finisher	20.00	4.69	35.80	19.90	2.20	
Phase 1 (April-May)	20.00	1.07	29.20	18.00	2.03	0.34
Phase 2 (June-July)	20.00	3.76	41.00	21.20	2.54	

p-value >0.05 means that the aflatoxin levels in samples are not different.

Table 3.2 The proportion (%) of commercial broiler feeds containing various concentrations of total Aflatoxins (N=40)

Level of Aflatoxin (ppb)	Type of Feed			
	Broiler Starter	Broiler Finisher	% proportion-all samples	Cumulative % proportion
Undetected	0.0	0.0	0.0	0.0
< 5	10.0	5.0	7.5	7.5
5-10	15.0	15.0	15.0	22.5
10.001-20	25.0	25.0	25.0	47.5
20.001-30	35.0	45.0	40.0	87.5
30.001-40	10.0	10.0	10.0	97.5
40.001-50	5.0	0.0	2.5	100.0

3.5 Discussion

The results corroborate previous studies which reported that compounded animal feeds, specifically poultry feeds, had both high prevalence and concentration levels of aflatoxins. In a study conducted by Kajuna *et al.* (2013) in Tanzania, 78.1% of all the compounded feed samples collected were contaminated with aflatoxins with broiler feeds having the highest contamination percentage (91.7%). Kenya imports broiler feed ingredients from neighbouring countries such as Tanzania hence the risk of similar AFs contamination levels. A study

conducted for a three-year period in Kenya reported that all animal feed samples were contaminated with AFs, ninety five percent (95%) of samples exceeding 10 ppb and while 35% exceeded 100 ppb and AFs levels ranging from 5.13 -1123 ppb (Okoth and Kola, 2012). In another study, 324 samples of grains, finished animal feeds and other feed commodities were collected from thirteen countries in the Middle East and Africa and tested for various mycotoxins including aflatoxins (Rodrigues *et al.,* 2011). Fumonisins were the main contaminant per country in all the samples collected except for samples from Nigeria and Kenya which had AFs as the main contaminant. The prevalence and level were 94 and 78%, 115 and 52 ppb respectively for Nigeria and Kenya (Rodrigues *et al.,* 2011). AFs prevalence in agricultural products such as cereals and grains, and hence animal feeds compounded from these agricultural products, is relatively higher in tropical and subtropical regions due to warm and humid weather conditions which provide optimal conditions for the growth of the moulds (Bryden, 2012; Abrar *et al.,* 2013). Poultry feed production and costs are a major issue faced by international as well as local industries due to competition for feed materials by animals and humans (Abidin *et al.,* 2011). Typically lower quality raw materials are used in poultry feed production (Abidin *et al.,* 2011) such as off-color and feed grade cereals and grains. Overall, 92.5% of broiler feeds manufactured in Nakuru town contained AFs levels higher than accepted by WHO standard. These levels may negatively impact on the performance of broilers and may end up in broiler meat and the human food with disastrous consequences to human health.

3.6 Conclusion

i. All the feed samples collected in Nakuru town were contaminated with AFs.
ii. 92.5% of the feed samples collected exceeded the maximum AFs limits set by WHO while 52.5% exceeded the FDA standards.

3.7 Recommendations

i. Feed millers should test for AFs levels in raw materials for broiler feeds.
ii. Aflatoxin contaminated feed ingredients should be avoided in broiler feed formulation.

CHAPTER FOUR

THE EFFECTS OF LOW AFLATOXIN LEVEL AND COMMERCIAL BINDER ON DIGESTIBILITY AND BROILER PERFORMANCE

4.1 Abstract

This experiment evaluated the effects of low AFs doses and commercial AFs binder (Mycosorb®) addition in feed on digestibility and broiler performance. The basal diet was formulated using soybean meal, maize grain, *Rastrineobola argentea* (omena), corn oil, dicalcium phosphate, lime, salt and broiler mineral premix to meet the National Research Council (NRC) recommendations for growing and finishing broilers. Six treatments were formulated by use of AFs contaminated maize to end up with 6, 14 and 22ppb in treatments with or without a AFs binder (Mycosorb®). The experimental design for both experiments was completely randomized design (CRD). In the first phase of this experiment, two hundred one-day-old Arbo Acre broiler chicks were purchased from Kenchick Limited. Vaccination against Gumboro and New Castle disease were administered at the hatchery. The day old broilers were randomly distributed into the six experimental groups (as treatments) with four replicates each. Broilers were weighed on a weekly basis henceforth to determine daily growth rate as, current weight less the previous week's weight divided by seven. Feed intake was determined daily by subtracting amounts of left over feed from the total feed given. In the second phase, twelve broilers of uniform weight (0.8Kg±0.05) were purchased at four weeks of age and distributed randomly each into its own cage and six experimental treatments (as described in the first experiment) allocated randomly with replication. The broilers were allowed to acclimatize to the feeds for seven days then data collected for the next three days. The process was then repeated by randomly reallocating the treatments and ensuring that no bird got the previous treatment diet. Daily feed intake was determined as total feeds given less the leftovers. Dry matter and aflatoxins digestibility was determined by weighing the fecal material on dry matter basis and analyzing the AFs levels in the fecal material then calculated as,

$$\frac{Total\ amount\ consumed - total\ amount\ in\ feaces}{Total\ amount\ consumed} \times 100$$

Feed efficiency differed significantly (p<0.05) among the diets during the grower period. There was a significant difference (p <0.05) between the control diets and the diets spiked with AFs at 14.06 and 21.95 ppb in feed efficiency for the grower period and overall. There was no

significant difference ($p>0.05$) in daily feed intake, weight gain and organ weights (hearts, livers and gizzards) among the diets. There was no significant difference ($p >0.05$) in dry matter and AFs digestibility when control diets were compared with the other diets. Moreover, there was no significant difference ($p>0.05$) in dry matter and AFs digestibility between diets with AFs binder and those without the binder. There was no significant difference ($p >0.05$) in daily feed intake, daily growth, feed efficiency and organ weights between the diets with Mycosorb® binder and those without the binder. Mycosorb® AFs binder was inefficient in binding the AFs in the feeds. In conclusion, feed efficiency at grower stage was affected by AFs at 14.06 and 21.95 ppb in the diets.

Keywords: Aflatoxins, effective, ELISA, treatments

4.2 Introduction

Mycotoxins are toxic secondary metabolites of various fungi occurring naturally in feeds and foods (Herzallah, 2009). So far, about 300–400 mycotoxins with very different chemical configurations have been identified (Bryden, 2012). Of most concern among the mycotoxins are aflatoxin B1 (AFB1) and Ochratoxin A (OTA) because of their toxicological effects (Hassan *et al*., 2012). Aflatoxins (AFs) are group toxic fungal metabolites produced mainly by *A. flavus* and *A. parasiticus*. Although AFs B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) are the major naturally occurring analogues of AFs, the most common analogues in agricultural commodities are AFB1 and AFB2 (Rossi *et al*., 2012). AFB1 are the most toxic AFs and they mainly target the liver (Denli and Okan, 2006; Mogilnaya *et al*., 2010). They can occur in agricultural produce, such as; cereals, grains, compounded poultry feeds and feed ingredients, before and after harvest, during storage, processing and manufacturing hence causing great economic losses (Osweiler *et al*., 2004; Suganthi *et al*., 2011).

The factors that have most influence on fungi growth and production of AFs in commodities are moisture and temperatures (Denli and Okan, 2006; Bryden, 2012; Abrar and Anjum, 2013). When more than one fungal contaminant is present, toxicity within feed is increased due to additive synergistic interactions (Abidin *et al*., 2011). Aflatoxins can manifest a variety of biological and health effects on animal species (Karaman, *et al*., 2010) for example, immune suppression, lowered growth rate and productivity, liver damage and death in severe cases. Their toxicity depends on several factors, such as concentration in the feed, duration of exposure, species involved, gender, age, and animal's health status (Denli and Okan, 2006;

Marchioro *et al*., 2013). Aflatoxins binders have been reported to prevent the absorption of aflatoxins by binding them in the gastrointestinal tract, although a universally effective inactivating agent for the 6-10 most common known mycotoxins is lacking (Osweiler et al., 2004). This experiments evaluated the effects of low aflatoxin levels in feed on broiler performance. Additionally, the effect of a common commercial toxin binder (Mycosorb®) was evaluated.

4.3 Experiment 1: Effects of low level Aflatoxins contamination and binder (Mycosorb®) on broiler performance

4.3.1 Materials and Methods

Management of Broilers

Two hundred unsexed day-old Arbo Acre broiler chicks were purchased from a large commercial hatchery in Kenya (Kenchic Limited). All the required vaccination procedures against Gumboro and New Castle diseases were carried out in the hatchery before supply. Upon arrival at Egerton University, the chicks' individual weights were taken then, they were randomly distributed into six experimental groups with four replicates each of 8 chicks. The chicks were artificially brooded for four weeks in a deep litter system separated by cardboards and provided lighting 24 hrs a day. The temperatures in the rooms were manipulated using infrared bulbs whereby, they were kept at about 34^{o}C during the first 3 days and gradually decreased by 2^{o}C weekly down to 24^{o}C (Abou-Zeid, 2015). A starter ration was formulated and provided at all times until 21days and then the feed was changed to a broiler finisher fed until day 35. Fresh feed and water was available to the chicks at all times.

Experimental diets

The experimental diets were formulated to meet the nutritional requirements of broilers at growing and finishing phases as recommended by NRC (1994). The basal diet were formulated using soy bean meal (42% CP, analyzed value), maize grain, *Rastrineobola argentea* (omena) (60% CP, analyzed value), corn oil, dicalcium phosphate, lime, salt and broiler mineral premix. The composition of the treatments were as shown on Tables 4.1 and 4.2 below. Six treatments were formulated in total based on the levels of AFs that were found in common commercial broiler feeds in Nakuru town in a survey described by (Thuita *et al*., 2019). The six experimental diets had three levels of AFs, with or without Mycosorb® AFs binder.

Source of Aflatoxin

For feed contamination, AFs were inoculated in maize by mixing good and AFs contaminated maize, soaking it in water for about 30 minutes then allowing the free water to drain. The moist maize was then incubated at 35^{o}C for a week. Enzyme-linked immunosorbent assay technique was used to determine the Aflatoxins levels in the maize before formulating the diets 3, 4, 5 and 6. AFs levels for each diet was determined using ELISA technique. In total, six diets were compounded: three with Mycosorb® AFs binder incorporated and three without Mycosorb® AFs binder (Tables 4.1 and 4.2).

Table 4.1 Ingredient composition of experimental diets (on as fed basis) during the grower period (1 to 21 days)

Ingredient (%)	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6
Maize	63.1	63.1	63.1	63.1	63.1	63.1
Soy bean meal	22.0	22.0	22.0	22.0	22.0	22.0
Omena[1]	11.5	11.5	11.5	11.5	11.5	11.5
Corn oil	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium Phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
Mineral and Vitamin premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Mycosorb®[3]	0.0	0.1	0	0.1	0	0.1
Calculated CP	20.6	20.6	20.6	20.6	20.6	20.6
Analyzed AF content (ppb)	6.1	6.1	14.1	14.1	22.0	22.0

[1]Scientific name: *Rastrineobola argentea*, common name; silver cyprinid, and it's also called the Lake Victoria sardine or mukene.

[2]Supplied the following per 2Kg of diet: Each 2Kg contains: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 10,000 IU; Vitamin K3, 2,000 mg; Vitamin B2, 6,000 mg; Vitamin B3, 25,000 mg; Vitamin B5, 11,000 mg; Vitamin B6, 500 mg; Vitamin B9, 350 mg; Vitamin B12, 10 mg; Chlorine chloride, 250,000 mg; Antioxidant, 125,000 mg; Fe,

25,000 mg; Mn, 80,000 mg; Zn, 50,000 mg; Cu, 2,000 mg; I, 1,200 mg; Co, 200 mg; Se, 100 mg.

[3] A patented broad-spectrum mycotoxin binding feed supplement derived from yeast.

Table 4.2 Ingredient composition of experimental diets (on as fed basis) during the finisher period (22 to 35 days)

Ingredient (%)	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6
Maize	66.4	66.3	66.4	66.3	66.4	66.3
Soy beanmeal	19.2	19.2	19.2	19.2	19.2	19.2
Omena[1]	10.0	10.0	10.0	10.0	10.0	10.0
Corn Oil	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium Phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
Mineral and Vitamin Premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Mycosorb®[3]	0.0	0.1	0.0	0.1	0.0	0.1
Calculated CP	18.8	18.8	18.8	18.8	18.8	18.8
Analyzed AF content (ppb)	6.1	6.1	14.1	14.1	22.0	22.0

[1]Scientific name: *Rastrineobola argentea*, common name; silver cyprinid, and it's also called the Lake Victoria sardine or mukene.

[2]Supplied the following per 2Kg of diet: Each 2Kg contains: Vitamin A, 8,500,000 IU; Vitamin D3, 1,600,000 IU; Vitamin E, 4,000 IU; Vitamin K3, 2,000 mg; Vitamin B2, 5,000 mg; Vitamin B3, 20,000 mg; Vitamin B5, 8,800 mg; Vitamin B6, 1,200 mg; Vitamin B9, 00 mg; Vitamin B12, 8 mg; Chlorine chloride, 200,000 mg; Antioxidant, 125,000 mg; Fe, 5,000 mg; Mn, 80,000 mg; Zn, 50,000 mg; Cu, 2,000 mg; I, 1,200 mg; Co, 200 mg; Se, 100 mg.

[3] A patented broad-spectrum mycotoxin binding feed supplement derived from yeast.

Measurements

Several tests were conducted in this experiment and data generated for analysis. These tests included; samples of the compounded feeds were collected and analyzed for AFs concentration using the ELISA technique. Secondly, broilers were weighed individually on arrival and on weekly basis henceforth and this data used in determination of daily weight gain as $current\ wt\ less\ previous\ week\ wt/7$. Thirdly, total feeds given and leftover feeds were weighed daily and the data used in determination of average daily feed intake as, total feed given less leftover feed. Feed efficiency was determined as $Daily\ weight\ gain/\ Daily\ feed\ intake$. Fourth, eight broilers per diet (two per replicate) were slaughtered on day 36. All the hearts, livers and gizzards obtained from the slaughtered broilers were weighed and their mean weight calculated.

Statistical analysis

All the data collected on average daily gain, average daily feed intake, feed efficiency, dry matter and AFs digestibility were subjected to a two way analysis of variance using the GLM procedures of SAS (version 9.13) at (p<0.05). Means were separated using the t-test (LSD) test at (p<0.05). Frequencies and percentages were also calculated. The model was as described below.

Experimental design

The experimental design used was Completely Randomized Design (CRD).

The model; $Y_{ij=}\mu + t_i+\varepsilon_{ij}$

where; Y_{ij}= Overall observation of the i^{th} diet and j^{th} replication

μ= overall mean

t_i= the diet effect of the AFs

ε_{ij}= random error term

4.3.2 Results

The mortality data of the broilers over the experimental period are in table 4.3, while the effects of treatments on average daily feed intake, average daily gain, feed efficiency and organ weights are in tables 4.4, 4.5, 4.6 and 4.7.

Table 4.3 Mortality rate of the broilers throughout the experimental period

Treatment	Mortalities	(%)
1	0	0.0
2	0	0.0
3	1	3.0
4	0	0.0
5	1	2.8
6	2	5.6
Total	**4**	**2**

Table 4.4 Effects of diets on average daily feed intake during grower, finisher and overall experimental

	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Pooled SEM	*p* Value
[1]**ADFIg**	53.93	51.39	52.10	51.16	57.12	47.47	3.41	0.5762
[2]**ADFIf**	105.58	99.71	108.33	104.62	116.74	99.86	7.02	0.6664
[3]**ADFIo**	79.75	75.56	80.21	77.89	86.93	73.66	5.03	0.6514

All the means were not significantly different (*p>0.05*)

[1]ADFIg represents Average Daily Feed Intake for the grower phase

[2]$ADFI_f$ represents Average Daily Feed Intake for the finisher phase

[3]ADFIo represents Overall Average Daily Feed

The treatments had no significant effect (*p >0.05*) on average daily feed intake during grower, finisher and overall experimental period.

Table 4.5 Effects of diets on average daily weight gain during grower, finisher and overall experimental

	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Pooled SEM	*p* Value
[1]**ADGg**	24.11	26.03	23.83	23.21	25.07	21.67	1.98	0.7985
[2]**ADGf**	49.45	46.05	48.39	50.99	45.68	46.49	4.38	0.8686
[3]**ADGo**	36.78	36.04	36.11	37.10	35.38	34.08	2.79	0.9327

All the means were not significantly different (*p>0.05*)

[1]ADGg represents Average Daily Gain for the grower phase

[2]ADG_f represents Average Daily Gain for the finisher phase

[3]ADGo represents Overall Average Daily Gain

The treatments had no significant effect (*p >0.05*) on average daily gain during grower, finisher and overall experimental period.

Table 4. 6 Effects of diets on feed efficiency during grower, finisher and overall experimental

	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Pooled SEM	*p* Value
[1]**FEg**	0.53^{a}	0.53^{a}	0.41^{b}	0.47^{ab}	0.42^{b}	0.42^{b}	0.02	0.0083
[2]**FEf**	0.48	0.50	0.46	0.46	0.45	0.39	0.04	0.4351
[3]**FEo**	0.50	0.51	0.44	0.46	0.44	0.40	0.03	0.0904

$^{a, b, c}$ *Means in the same row with different superscripts differ significantly (p< 0.05)*

The results are reported as Mean ± SEM (standard error of means)

[1]IntakeFEg represents Feed Efficiency for the grower phase

[2]FE_f represents Feed Efficiency for the finisher phase

[3]FEo represents Overall Feed Efficiency

Treatments had a significant effect ($p<0.05$) on feed efficiency during grower period but not in the finisher and overall experiment period (*p>0.05*)

Table 4.7 Effects of diets on liver, gizzard and heart weights

Parameter, % of body weight (kg)	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Pooled SEM	*p* Value
Liver weight	25.90	27.04	27.92	28.83	32.93	28.00	2.44	0.4679
Gizzard weight	36.83	32.87	28.79	30.06	27.52	29.09	2.46	0.1602
Heart weight	6.14	6.43	6.23	5.67	7.49	7.07	0.61	0.3650

All the means were not significantly different (*p>0.05*)

Treatments had no significant effect (*p>0.05*) on gizzard, liver and heart weights. Aflatoxins binder (Mycosorb®) had no significant effect (*p>0.05*) on both feed efficiency and liver, gizzard and heart weights per kilo gram (Kg) body weight.

Upon data analysis on the effects of treatments on broiler performance showing that commercial AFs binder (Mycosorb®) had no significant effect (*p>0.05*) on average daily feed intake, average daily gain, feed efficiency and organ (liver, gizzard and heart) weight, means were pooled as per AFs levels in treatment. The three treatments data were analyzed whereby, treatment 1 had 6.1 ppb AFs, treatment 2 had 14.1 ppb AFs and treatment 3, 22.0 ppb AFs. The pooled data were analyzed and the results were as shown in appendix 3. The treatments had no significant effect (*p >0.05*) on average daily feed intake and average weight gain during grower, finisher and overall experimental period. Treatments had a significant effect ($p<0.05$) on feed efficiency during grower period but not in the finisher and overall experiment period (*p>0.05*)

4.4 Experiment 2: Effects of diets on dry matter and Aflatoxins digestibility

4.4.1 Materials and methods

Experimental Set Up

Twelve broilers at four weeks of age were used in this experiment. The broilers were in good health and of uniform weight (0.8Kg±0.05) at purchase. All the required vaccinations against New Castle and Gumboro diseases had been carried out on day 7 and 21 and day 14 respectively. The broilers were distributed randomly each into its own cage and six treatments (as described in experiment 1 above) allocated randomly with two replications each. The treatments were as in experiment 1 above in terms of nutrient composition, AFs levels and AFs binder (Mycosorb®) inclusion. The broilers were allowed to acclimatize to the feeds for seven

days then data collected for the next three days. The diets were then reallocated to the broilers randomly ensuring that no bird got the same diet as it had previously been on. Another seven-day acclimatization period was allowed for the broilers and data collected for the next three days.

Measurements

First, daily feed intake data was determined as, total feed given less left over feed. Second, digestibility data was generated by collection of fecal material from each bird and determining its weight on dry matter (DM) basis. Total AFs levels were determined using ELISA technique. Dry matter and AFs digestibility were calculated as;

$$\frac{Total\ amount\ consumed - total\ amount\ in\ feaces}{Total\ amount\ consumed} \times 100$$

Experimental design:

The experimental design used was Completely Randomized Design (CRD).

The model; $Y_{ij=}\mu + t_i+\varepsilon_{ij}$

where; Y_{ij}= Overall observation of the i^{th} diet and j^{th} replication

μ= overall mean

t_i= the diet effect of the AFs

ε_{ij}= random error term

4.4.2 Results

Table 4.8 The effect of diets on the digestibility of dry matter and Aflatoxins

	Trt 1	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**Trt 6**	**Pooled SEM**	***p* Value**
Dry Matter Digestibility	79.74	81.73	79.80	77.28	83.90	81.86	3.25	0.7774
AFs Digestibility	98.63	97.57	98.64	98.29	99.15	98.91	0.37	0.0907

All the means were not significantly different (*p>0.05*)

Treatment diets had no significant effect (*p>0.05*) on dry matter and AFs digestibility. Aflatoxins binder (Mycosorb®) had no significant effect (*p>0.05*) on dry matter and AFs digestibility.

4.5 Discussion

The findings of this research are consistent with previous studies showing that AFs in feeds have a negative effect on the feed conversion efficiency (Marchioro *et al*., 2013; Atherstone *et al*., 2016). These negative effects can be associated with poor digestibility of the diet and utilization of protein and energy due to interference of AFs over several enzymatic systems (Osweiler *et al*., 2004; Denli and Okan, 2006; Karaman *et al*., 2010; Marchioro *et al*., 2013). However, in this experiment the daily feed intake, growth rate and DM digestibility were not affected. Feed conversion efficiency differed significantly ($p<0.05$) among the treatment diets with diets 1 and 2 having the highest feed conversion efficiency while diet 6 had the lowest. Orthogonal contrast analysis also revealed a significant difference ($p<0.05$) between the control diets and the diets spiked with AFs at 14.06 and 21.95 ppb in feed conversion efficiency for the grower and overall period. In a similar research conducted on ducks, broilers fed on diets with 20 and 40 ppb AFs had significantly reduced body weights and higher feed conversion ratio when compared to those on control diets (Han *et al*., 2008). Contrary to the findings of Denli and Okan, (2006), where diets with 40 and 80ppb AFB1 significantly decreased body weight gain ($p< 0.05$) as compared to the control group, there was no significant difference ($p>0.05$) in daily weight gain among the diets for the grower and finisher phases. This difference could be due to the lower doses of AFs administered in this experiment. There was no significant difference ($p>0.05$) in dry matter and AFs digestibility among the diets and furthermore, when control diets were compared with the other diets, there was no significant difference ($p>0.05$) in dry matter and AFs digestibility.

Saxena *et al*. (2009) in an *in vitro* study evaluating the efficiency of various binding agents in combination with different carboxylic acid additives, Mycosorb® and yeast had the least AFs binding efficiency when compared to sodium bentonite and Ultrasil (HSCAS). Binding efficiency was however reported to increase when different binding agents were used in combination. The current findings corroborate previous studies showing no significant difference ($p>0.05$) in average daily feed intake, average daily growth and feed conversion efficiency between the diets with Mycosorb® binder and those without the binder for the grower and finisher periods. There was also no significant difference ($p>0.05$) in dry matter and AFs digestibility between diets with AFs binder and those without the binder. However, although Mycosorb® AFs binder had no significant effect on AFs digestibility ($p=0.0907$), it tends to have a slight effect on the AFs absorption (Table 4.8).

The main effects of AFs are related to liver damage hence increased liver weight is a classic symptom of aflatoxicosis (Denli and Okan, 2006; Karaman *et al*., 2010). Results from a previous study showed that the liver weights were significantly higher ($p< 0.05$) in chicks consuming AFB1 at 80 µgAFB1/kg feed than without any adsorbents (Denli and Okan, 2006). In yet another study, all the diets exposed to AFs also showed an increase in liver weight (Osweiler *et al*., 2004). Contrary to these studies, there was no significant difference ($p>0.05$) in liver, gizzard and heart weights expressed per Kg body weight among the diets. There was no significant difference ($p>0.05$) in liver, gizzard and heart weights/ Kg body weight between the diets with Mycosorb® binder and those without the binder either. This contradiction could be because of the low AFs doses administered in this experiment hence there was no liver damage caused to the broilers. The gizzard weights/Kg body weight of the control diets differed significantly from those with 14.06ppb and 21.95ppb ($p<0.05$). In a research aimed at determining the level of tissue deposition of AFs in broilers, highest levels were found in gizzards, liver and kidneys (Chen *et al*., 1984). Han *et al*., (2008), found AFs (20 and 40 ppb) to significantly increase the relative liver, kidney and pancreas weights in ducks when compared to the control diet but the heart weight was not affected.

4.6 Conclusion

i. The results from this experiment demonstrate that feed efficiency of broilers at grower stage is affected by AFs levels as low as 14.06 ppb in diets which may in turn influence the efficiency and profitability of commercial broiler enterprises.
ii. In addition, inclusion of Mycosorb® AFs binder at the rate recommended by the manufacturer was ineffective in binding AFs in diets to prevent the broilers from absorbing them.

4.7 Recommendations

i. Aflatoxin contaminated feed ingredients should be avoided in broiler feed formulation.
ii. There is need to carry out more research on effective ways of detoxifying contaminated feed materials.

CHAPTER FIVE
GENERAL DISCUSSION AND CONCLUSIONS

5.1 Aim of the study

As the world population grows, the pressure on the available food supply rises hence the threat from mycotoxins contamination is aggravated in two ways; more fungus-damaged, potentially mycotoxin-containing foods and feeds are utilized rather than discarded and susceptibility to lower levels of foodborne Mycotoxins is increased due to malnutrition (Leslie *et al*., 2008). The overall goal of this study was to contribute to better livelihoods and food security through reduction of aflatoxins in broiler feeds. The specific objectives were: i.)to determine the prevalence and concentration of AFs in common commercial broiler feeds in Nakuru Town, ii.) to determine the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the performance of broilers and organ weights and, iii.) To determine the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the digestibility of broilers.

The thesis addresses three major research objectives; i.) there are no detectable levels of AFs in common broiler feeds in Nakuru town, ii.) Aflatoxins contamination and use of commercial AFs binder (Mycosorb®) in AFs contaminated feed have no effects on performance of broilers and organ weights and, iii.) Aflatoxins contamination and use of commercial AFs binder (Mycosorb®) in AFs contaminated feed have no effects on digestibility in broilers.

5.2 Study methodology

The first objective determined the prevalence and concentration of AFs in common commercial broiler feeds in Nakuru Town. This was achieved by carrying out a survey in Nakuru town. Ten broiler feed milling companies were randomly selected in total. Fourty broiler feed samples collected from them in two phases, first phase was between April and May and June to July and twenty starter and finisher mash samples collected in total. Samples were stored at 4^{o}C at Egerton University and then tested for AFs prevalence and concentration using ELISA technique following manufacturer's instructions (Helica Biosystems Inc).

The second objective determined the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the performance of broilers and organ weights. Basal treatment was compounded using maize grain, soybean meal (42% CP, analyzed), *Rastrineobola argentea* (omena) (60% CP, analyzed), corn oil, dicalcium phosphate, mineral

premix and salt to meet the NRC recommendations for growing and finishing broilers. Six treatments were formulated using AFs contaminated maize, with or without AFs binder. The treatments were three levels of AFs (6, 14 and 22 ppb) and two levels of Mycosorb® AFs binder addition for each AFs level (0 and 1 Kg/ tonne of feed). Two hundred one-day-old Arbo Acre broiler chicks purchased were randomly distributed into six experimental groups with four replicates each. The feeding trial experiment was conducted in Completely Randomized Design. Broilers vaccinations against Gumboro and New Castle diseases had been carried out at the hatchery, watering and feeding was done daily. Broilers weighing was on weekly basis to determine growth rate. Daily feed intake determined as total feed given less the left overs. On day thirty six, eight broilers per treatment (two per replicate) were slaughtered and their liver, gizzard and heart weights recorded.

Determination of the effects of AFs and addition of a commercial AFs binder (Mycosorb®) in contaminated feed on the digestibility of broilers was carried out in the second experiment. Twelve broilers of uniform weight (0.8Kg± 0.05) were purchased at four weeks of age. Vaccination against New castle and Gumboro diseases were carried out at day 7 and 21 and 14 respectively before purchase. Treatments were as described for experiment two above and the experiment was conducted in Completely Randomized Design. Upon arrival, broilers were weighed and randomly housed in individual cages. The six treatments were then allocated randomly with replication. The broilers were allowed to acclimatize to the feeds for seven days then data collected for the next three days. The process was then repeated while ensuring that no broiler got the previously allocated diet. Daily feed intake was determined as feeds given less leftovers. Dry matter and aflatoxins digestibility were determined as; ((Total amount consumed less total amount in feaces) divided by total amount consumed) multiplied by 100.

5.3 General discussion

The prevalence of AFs in agricultural products such as cereals and grains, and hence animal feeds compounded from these agricultural products, is relatively higher in tropical and subtropical regions due to warm and humid weather conditions which provide optimal conditions for the growth of the moulds (Bryden 2012; Abrar *et al*.,2013). Due to the use of AFs contaminated ingredients (cereals and oil cakes) the commercial poultry feeds may contain AFs concentrations which are beyond regulatory limits. Previous studies in Kenya, have reported that compounded animal feeds, specifically poultry feeds, have both high prevalence and concentration levels of aflatoxins. Okoth and Kola (2012), reported that all animal feed

samples collected were contaminated with AFs with levels ranging from 5.13 -1123 ppb, 95% of the samples exceeding 10 ppb and 35% exceeded 100 ppb. Kajuna *et al*, (2013) conducted a study in Tanzania and found that broiler feeds had the highest AFs contamination percentage (91.7%) the prevalence and levels being 94 and 78%, 115 and 52 ppb respectively for Nigeria and Kenya (Rodrigues *et al*., 2011). In yet another study where grains, finished animal feeds and other feed commodities were sampled and tested for various mycotoxins, AFs were the major contaminant in samples from Nigeria and Kenya, unlike in samples from other African and Asian countries where fumonisins were the major contaminant (Rodrigues *et al*., 2011). In the current survey, broiler feeds manufactured in Nakuru town contained AFs levels higher than accepted by WHO. This finding confirms that despite the level of awareness on the effects of AFs in public health, it continues to be common in broiler feeds in Kenya.

Previous studies have shown that AFs in feeds have a negative effect on the feed conversion efficiency (Marchioro *et al*., 2013; Atherstone *et al*., 2016) which could be due to AFs interference over several enzymatic systems hence hindering digestibility of the diet and utilization of protein and energy (Osweiler *et al*., 2004; Denli and Okan, 2006; Karaman *et al*., 2010; Marchioro *et al*., 2013). Control diets (1 and 2) had the highest feed conversion efficiency while diet 6 had the lowest ($p<0.05$). Similar research conducted on ducks, birds fed on diets with 20 and 40 ppb AFs had significantly higher feed conversion ratio when compared to those on control diets (Han *et al*., 2008). Contrary to the findings of Denli and Okan, (2006), where diets with 40 and 80 ppb AFB1 significantly decreased body weight gain ($p< 0.05$) as compared to the control group, there was no significant difference ($p>0.05$) in daily weight gain among the diets for the grower and finisher phases. This difference could be due to the lower doses of AFs administered in this experiment. There was no significant difference ($p>0.05$) in dry matter and AFs digestibility among the diets. Furthermore, when control diets were compared with the other diets and diets with Mycosorb® compared to those without, there was no significant difference ($p>0.05$) in dry matter and AFs digestibility. An *In vitro* study evaluating the efficiency of various binding agents in combination with different carboxylic acid additives, Mycosorb® and yeast had the least AFs binding efficiency when compared to sodium bentonite and Ultrasil (HSCAS) (Saxena *et al*., 2009). The current findings corroborate previous studies showing inclusion of Mycosorb® in diet had no significant effect ($p>0.05$) on the average daily feed intake, average daily growth and feed conversion efficiency. The main effects of AFs are related to liver damage hence increased liver weight is a classic symptom of aflatoxicosis

(Denli and Okan, 2006; Karaman *et al*., 2010). In yet another study, all the diets exposed to AFs also showed an increase in liver weight *(Osweiler et al*., 2004). Contrary to these studies, there was no significant difference ($p>0.05$) in liver, gizzard and heart weights expressed per Kg body weight among the diets and when diets with Mycosorb® were compared with those without. This contradiction could be because of the low AFs doses administered in this experiment hence there was no liver damage caused to the broilers. The gizzard weights/Kg body weight of the control diets differed significantly from those with 14.06ppb and 21.95ppb ($p<0.05$). Han *et al*., (2008), found AFs (20 and 40 ppb) to significantly increase the relative liver, kidney and pancreas weights in ducks when compared to the control diet but the heart weight was not affected. AFs levels in diet may negatively impact on the performance of broilers and may end up in broiler meat and the human food with detrimental consequences to human health.

5.4 Conclusions

i. More than 50% of the broliler feed samples collected exceeded the maximum limits set by WHO, 5 ppb in animal feeds and FDA, 20 ppb in poultry feeds.
ii. Feed conversion efficiency of broilers at grower stage was affected by AFs levels as low as 14.06ppb in diets
iii. Inclusion of Mycosorb® AFs binder at the rate recommended by the manufacturer was not effective in binding AFs in diets

5.5 Recommendations

i. Feed manufacturers should test for AFs in the raw materials.
ii. Broiler feed manufacturers should avoid the fungal contamination in the broiler feeds at all stages of handling
iii. Contaminated feed ingredients should be avoided in feed formulation.
iv. There is need to carry out more research on effective ways of detoxifying contaminated feed materials by AFs.

5.6 Research Gaps

Further research is required on the following,

i. Effects of commercial AFs binders on young broiler birds (growing phase)
ii. Residual effects of low level AFs contamination in feeds on broiler tissues and organs
iii. Effective AFs decontamination methods in animal feeds.

REFERENCES

Abidin Z., Khatoon A. and Numan M. (2011). Mycotoxins in Broilers : Pathological Alterations Induced by Aflatoxins and Ochratoxins , Diagnosis and Determination , Treatment and Control of Mycotoxicosis. *World's Poultry Science Journal*, *67*(September), 485–496. Retrieved from http://doi.org/10.1017/S0043933911000535

Abrar M., Anjum F. M., Butt M. S., Pasha I., Randhawa M. A. and Farhan S. K. (2013). Aflatoxins: Biosynthesis , Occurrence , Toxicity , and Remedies. *Food Science and Nutrition*, *874*(53), 862–874. Retrieved from http://doi.org/10.1080/10408398.2011.563154

Abou-Zeid A.E., El-Damarawy S.Z. and El-Rayes T.K. (2015). Broiler Fed Aflatoxin B1 Contaminated Diet Treated. *Egyptian J. Nutrition and Feeds*, *18*(2), 409–420.

ABS-TCM. (2013). Study on the Kenyan Animal Feed and Fodder Sub-sectors: Kenya Feed Industry Policy and Regulatory Issues (Sub-report III). Part of the "Kenya Market-led Dairy Programme" (KMDP) of SNV/Kenya Netherlands Development Organisation. Retrieved from https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.dialogkenya.info/docs/RES-KenyaFeedIndustryPolicyRegulatoryIssues--2013.pdf&ved=2ahUKEwjzwqKTsqXiAhVIRBoKHe8FCVYQFjAAegQ1AxAB&usg=AOvVaw18HLNDrO6etKqus2ApfUgU

Ahamad, D. B., Vairamuthu, S., George, V. T., Balachandran, C., Manohar, B. M., and Balasubramaniam, G. A. (2009). Occurrence of Aflatoxin B1 in the Compounded Feeds and Feed Ingredients of Poultry Ration. *The Indian Veterinary Journal*, *86*(20), 894–895.

Almeida, A., Viegas, C., and Carolino, E. (2012). Occupational Exposure to Aflatoxin (Afb 1) in Poultry Production. *Journal of Toxicology and Environmental Health*, *75*, 1330–1340.

Atherstone C., Grace D., Lindahl J. F., Kang'ethe E. K. and Nelson F. (2016). Assessing The Impact of Aflatoxin Consumption. *African Journal of Food, Agriculture Consumption on Animal Health and Productivity*, *16*(3), 10949–10966.

Binder E. M. (2007). Managing the Risk of Mycotoxins in Modern Feed Production. *Animal Feed Science and Technology*, *133*, 149–166. Retrieved from http://doi.org/10.1016/j.anifeedsci.2006.08.008

Bryden W. L. (2012). Mycotoxin Contamination of the Feed Supply Chain : Implications for Animal Productivity and Feed Security. *Animal Feed Science and Technology*, *173*(1–2),

134–158 Retrieved from http://doi.org/10.1016/j.anifeedsci.2011.12.014

Chen, C., Pearson, A. M., Coleman, T. H., Gray, J. I., Pestka, J. J., Aust, S. D., … Afgi, A. F. M. (1984). Tissue Deposition and Clearance of Aflatoxins from Broiler Chickens Fed a Contaminated Diet. *Food and Chemical Toxicology*. *22*(6), 447-415

Denli M. and Okan F. (2006). Efficacy of different adsorbents in reducing the toxic effects of aflatoxin B 1 in broiler diets. *South African Journal of Animal Science*, *36*(4), 222–228.

Dersjant-li, Y., Verstegen, M. W. A., and Gerrits, W. J. J. (2003). The impact of low concentrations of aflatoxin , deoxynivalenol or fumonisin in diets on growing pigs and poultry. *Nutrition Research Reviews*, *16*, 223–239. Retrieved from http://doi.org/10.1079/NRR200368

FAO/WHO (2013). Joint FAO/WHO Food Standards Programme-Codex Committee On Contaminants In Foods: discussion paper on aflatoxins in cereals, 1–17. *www.**fao**.org/**fao**...**codex**alimentarius/.../es/?...**1**...**fao**.org%252Fsites%252F**codex**%25...*

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

Grace, D., Kang'ethe, E., Lindahl, J., Atherstone, C., Nelson, F., and Wesonga, T. (2015). Building an Aflatoxin Safe East African Community Technical Policy Paper 4 Aflatoxin : Impact on Animal Health and Productivity. *https://cgspace.cgiar.org/handle/10568/75536* accessed on 8/15/16.

Grace, D., Lindahl, J., Atherstone, C., Kang'ethe, E., Nelson, F., Wesonga, T., and Manyong, V. (2015). Building an Aflatoxin Safe East African Community Technical Policy Paper 7: Aflatoxin Standards for Feed. *https://cgspace.cgiar.org/handle/10568/75535*.

Han, X., Huang, Q., Li, W., Jiang, J., & Xu, Z. (2008). Changes in Growth Performance , Digestive Enzyme Activities and Nutrient Digestibility of Cherry Valley Ducks in Response to Aflatoxin B 1 Levels. *Livestock Science*, *119*(1–3), 216–220. http://doi.org/10.1016/j.livsci.2008.04.006

Hassan, Z. U., Khan, M. Z., Khan, A., & Hussain, Z. (2012). Effects of Individual and Combined Administration of Ochratoxin A and Aflatoxin B1 in Tissues and Eggs of White Leghorn Breeder Hens. *J of Sci Food Agric*, *92*(July 2011), 1540–1544. http://doi.org/10.1002/jsfa.4740

Herzallah S. M. (2009). Determination of Aflatoxins in Eggs , Milk , Meat and Meat Products Using HPLC Fluorescent and UV Detectors. *Food Chemistry*, *114*(3), 1141–1146 Retrieved from http://doi.org/10.1016/j.foodchem.2008.10.077

Hugo De Groote, T. N. and Wahome, R. (2010). Quality Protein Maize for the Feed Industry in Kenya. *Outlook on Agriculture*, *39*, 291–298.

Kajuna F. F., Temba B. A. and Mosha R. D. (2013). Surveillance of aflatoxin B1 contamination in chicken commercial feeds in Morogoro, Tanzania. *Livestock Research for Rural Development*, 25, 51.

Karaman M., Ozen H., Tuzcu M., Cigremis Y., F. O. and K. O. (2010). Pathological , biochemical and haematological investigations on the protective effect of α -lipoic acid in experimental aflatoxin toxicosis in chicks. *British Poultry Science*, *51*(1), 132–141. http://doi.org/10.1080/00071660903401839.

Leslie, J. F., Bandyopadhyay, R. and Visconti, A. (2008). Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. *Research for Food Safety in Global Systems*. Retrieved from dx.doi.org/10.1079/9781845930820.0000.

Marchioro, A. A., Mallmann, A. O., Diel, A., Dilkin, P., Rauber, R. H., Blazquez, F. J. H., … Oliveira, C. M. G. A. (2013). Effects of Aflatoxins on Performance and Exocrine Pancreas of Broiler Chickens Effects of Aflatoxins on Performance and Exocrine Pancreas of Broiler Chickens. *American Association of Avian Pathologists*, *57*(2), 280–284.

Macdonald, S., Chan, D., Scott, K. L. H., and Randall, L. (2016). Research Review No . 85 Mycotoxin contamination : assessment of risk in livestock systems. *AHDB Cereals & Oilseeds*, *85*, 1–116.

Mogilnaya, O. A., Puzyr' A. P. and Bondar, Vladimir. (2010). Growth and bioluminescence of luminous bacteria under the action of aflatoxin B1 before and after its treatment with nanodiamonds. *Applied Biochemistry and Microbiology*. 46. 33-37. 10.1134/S0003683810010059.

Naing, L., Winn, T., and Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. Archives of Orofacial Sciences, 1, 9–14.

Nemati Z., Janmohammadi H,. Taghizadeh A., Nejad H. M., Mogaddam G. and Arzanlou M. (2014). Occurrence of Aflatoxins in Poultry Feed and Feed Ingredients from Northwestern Iran. *European Journal of Zoological Research*, *3*(3), 56–60

Okoth S. A. and Kola M. A. (2012). Market samples as a source of chronic aflatoxin exposure

in kenya 1. *African Journal of Health Sciences*, *20*(1), 56–61

Osweiler, G. D., Jagannatha, S., Trampel, D. W., Imerman, P. M., Ensley, S. M., Yoon, I., and Moore, D. T. (2004). Evaluation of XPC and Prototypes on Aflatoxin-Challenged Broilers, 1887–1893. http://doi.org/10.3382/ps.2010-00773

Rajarajan, P. N., Rajasekaran, K. M., and Devi, N. K. A. (2013). Isolation and Quantification of Aflatoxin from *Aspergillus flavus* Infected Stored Peanuts. *Indian Journal of Pharmaceutical and Biological Research*, *1*(4), 76–80.

Rawal, S., Kim, J. E., and Jr, R. C. (2010). Research in Veterinary Science Aflatoxin B 1 in poultry : Toxicology , metabolism and prevention. *Research in Veterinary Science*, *89*(3), 325–331. http://doi.org/10.1016/j.rvsc.2010.04.011.

Reddy B. N. and Raghavender C. R. (2007). Outbreaks of Aflatoxicoses in India. Volume 7 No. 5 2007. *African Journal of Food Agriculture Nutrition and Development*, *7*(5), 1–15

Right Track Africa (RTA) and Nutrimix Limited. (2016). Mapping Animal Feed Manufacturers and Ingredient Suppliers in Kenya. www.kenyamarkets.org.

Rodrigues I., Handl J. and Binder E. M. (2011). Food Additives and Contaminants : Part B : Surveillance Mycotoxin Occurrence in Commodities , Feeds and Feed Ingredients Sourced in the Middle East and Africa. *Food Additives and Contaminants: Part B*, *4*(3), 168–179 Retrieved from http://doi.org/10.1080/19393210.2011.589034

Rossi, C. N., A, Takabayashi, C. R., B, Ono, M. A., C, … Elisabete Yurie Sataque Ono. (2012). Immunoassay Based on Monoclonal Antibody for Aflatoxin Detection in Poultry Feed. *Food Chemistry*, *132*(4), 2211–2216. http://doi.org/10.1016/j.foodchem.2011.12.067

Saxena, N., Kumar, P., Madan, J., and Lal, D. (2009). Evaluation of Various Binding Agents in Detoxifying Aflatoxins in Animal Feeds. *The Indian Veterinary Journal*, *86*(29), 1123–1126.

Semple, R. L., Frio, A. S., Hicks, P. A., and Lozare, J. V. (2011). Mycotoxin Prevention and Control in Foodgrains. *Food and Agriculture Organization of the United States.* 1–299. www.fao.org/docrep/x5036e/x5036e00.HTM.

Shahid Ali Rajput 1 ID , Lvhui Sun 1, Niya Zhang 1, Mahmoud Mohamed Khalil 1,2 ID , Xin Gao 1 ID , Zhao Ling 1, Luoyi Zhu 1, Farhan Anwar Khan 3, Jiacai Zhang 1 and Desheng Qi 1,. Ameliorative Effects of Grape Seed Proanthocyanidin Liver Histopathology and Aflatoxin Residues in Broilers Exposed to Aflatoxin B 1. *Toxins*, 9, 371 http://doi.org/10.3390/toxins9110371

Spragg J. F. and Watts R. D. (2013). A Review of Potential Contaminants in Australian Livestock Feeds and Proposed Guidance Levels for Feed. *Animal Production Science, 53*, 181–208

Suganthi, R. U., Suresh, K. P., and Parvatham, R. (2011). Effect of Aflatoxin on Feed Conversion Ratio in Broilers : *Journal of Animal Science*, *24*(12), 1757–1762.

Sumit Rawal, Ji Eun Kim and Roger Coulombe Jr. (2010). Research in Veterinary Science Aflatoxin B 1 in poultry : Toxicology , metabolism and prevention. *Research in Veterinary Science*, *89*(3), 325–331.

Thu Hyunh, Chritina Ly, Peter Knight and Wondu Wolde-Mariam. (2012). Quantitation of Aflatoxin B 1 by ELISA in Commodities that Pose a Matrix Effect. *www.helica.com/assets/lowmatrixaflatoxin.pdf* accessed on 11/4/16.

Thuita F. N., Tuitoek J. K., King'ori A. M. and Obonyo M. A. (2019). Prevalence of aflatoxins contamination in commercial broiler feeds in Kenya. Livestock Research for Rural Development, 31, 1. Retrieved from www.lrrd.org/lrrd31/1/jtuit31003.html%0A%0A

Wu, Q., Jezkova, A., Yuan, Z., Pavlikova, L., and Dohnal, V. (2009). Biological degradation of aflatoxins. *Drug Metabolism Reviews*, *41*(October 2008), 1–7. http://doi.org/10.1080/03602530802563850

Zheng, M. Z., Richard, J. L., and Binder, J. (2006). A Review of Rapid Methods for the Analysis of Mycotoxins. *Mycopathologia*, *161*, 261–273. https://doi.org/10.1007/s11046-006-0215-6

APPENDICES

Appendix 1: Nakuru Feed Samples data analysis output

The SAS System 11:42 Friday, March 5, 2018 35

The GLM Procedure

Class Level Information

Class	Levels	Values
PHASE	2	1 2
FEEDTYPE	2	1 2

Number of observations 40

The SAS System 11:42 Friday, March 5, 2018 36

The GLM Procedure

Dependent Variable: OD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	142.530315	47.510105	0.43	0.7336
Error	36	3989.431575	110.817544		
Corrected Total	39	4131.961890			

R-Square	Coeff Var	Root MSE	OD Mean
0.034495	53.67628	10.52699	19.61200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PHASE	1	103.4908900	103.4908900	0.93	0.3403
FEEDTYPE	1	2.3863225	2.3863225	0.02	0.8842
PHASE*FEEDTYPE	1	36.6531025	36.6531025	0.33	0.5688

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PHASE	1	103.4908900	103.4908900	0.93	0.3403
FEEDTYPE	1	2.3863225	2.3863225	0.02	0.8842
PHASE*FEEDTYPE	1	36.6531025	36.6531025	0.33	0.5688

The SAS System 11:42 Friday, March 5, 2018 37

The GLM Procedure

t Tests (LSD) for OD

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	110.8175
Critical Value of t	2.02809
Least Significant Difference	6.7514

Means with the same letter are not significantly different.

t Grouping	Mean	N	PHASE
A	21.221	20	2
A			
A	18.004	20	1

The SAS System 11:42 Friday, March 5, 2018 38

The GLM Procedure

t Tests (LSD) for OD

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	110.8175
Critical Value of t	2.02809
Least Significant Difference	6.7514

Means with the same letter are not significantly different.

t Grouping	Mean	N	FEEDTYPE
A	19.856	20	2
A			
A	19.368	20	1

Appendix 2: Data analysis on the effects of treatments on broiler performance

Average feed Intake

The SAS System 18:08 Saturday, May 15, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	6	1 2 3 4 5 6

Number of observations 24

The GLM Procedure

Dependent Variable: IntakeGP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	225.721278	37.620213	0.81	0.5762
Error	17	789.362418	46.433083		
Corrected Total	23	1015.083696			

R-Square	Coeff Var	Root MSE	IntakeGP Mean
0.222367	13.05576	6.814183	52.19292

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	205.0791708	41.0158342	0.88	0.5133
Rep	1	20.6421075	20.6421075	0.44	0.5139

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	205.0791708	41.0158342	0.88	0.5133
Rep	1	20.6421075	20.6421075	0.44	0.5139

The GLM Procedure

Dependent Variable: IntakeFP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	806.709847	134.451641	0.68	0.6664
Error	17	3350.595737	197.093867		
Corrected Total	23	4157.305583			

R-Square	Coeff Var	Root MSE	IntakeFP Mean
0.194046	13.26887	14.03901	105.8042

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	799.7786333	159.9557267	0.81	0.5575
Rep	1	6.9312133	6.9312133	0.04	0.8535

Source	DF	Type III SS	Mean Square	F Value	Pr >
Treatment	5	799.7786333	159.9557267	0.81	0.5575
Rep	1	6.9312133	6.9312133	0.04	0.8535

The GLM Procedure

Dependent Variable: IntakeO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	427.125403	71.187567	0.70	0.6514
Error	17	1721.584247	101.269662		
Corrected Total	23	2148.709650			

R-Square	Coeff Var	Root MSE	IntakeO Mean
0.198782	12.73874	10.06328	78.99750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	426.2031500	85.2406300	0.84	0.5386
Rep	1	0.9222533	0.9222533	0.01	0.9251

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	426.2031500	85.2406300	0.84	0.5386
Rep	1	0.9222533	0.9222533	0.01	0.9251

The GLM Procedure

Least Squares Means

Treatment	IntakeGP LSMEAN	Standard Error	Pr > |t|
1	53.9325000	3.4070913	<.0001
2	51.3850000	3.4070913	<.0001
3	52.0950000	3.4070913	<.0001

4	51.1575000	3.4070913	<.0001
5	57.1150000	3.4070913	<.0001
6	47.4725000	3.4070913	<.0001

IntakeFP Standard

Treatment	LSMEAN	Error	Pr > |t|
1	105.580000	7.019506	<.0001
2	99.705000	7.019506	<.0001
3	108.330000	7.019506	<.0001
4	104.615000	7.019506	<.0001
5	116.737500	7.019506	<.0001
6	99.857500	7.019506	<.0001

IntakeO Standard

Treatment	LSMEAN	Error	Pr > |t|
1	79.7525000	5.0316414	<.0001
2	75.5450000	5.0316414	<.0001
3	80.2125000	5.0316414	<.0001
4	77.8850000	5.0316414	<.0001
5	86.9275000	5.0316414	<.0001
6	73.6625000	5.0316414	<.0001

The GLM Procedure

t Tests (LSD) for IntakeGP

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	46.43308
Critical Value of t	2.10982
Least Significant Difference	10.166

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	57.115	4	5
A			
A	53.933	4	1
A			
A	52.095	4	3
A			
A	51.385	4	2
A			
A	51.158	4	4
A			
A	47.473	4	6

The GLM Procedure

t Tests (LSD) for IntakeFP

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	197.0939
Critical Value of t	2.10982
Least Significant Difference	20.944

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	116.738	4	5
A			
A	108.330	4	3
A			
A	105.580	4	1
A			
A	104.615	4	4
A			

A	99.858	4	6
A			
A	99.705	4	2

The SAS System 18:08 Saturday, May 15, 2019 8

The GLM Procedure

t Tests (LSD) for IntakeO

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	101.2697
Critical Value of t	2.10982
Least Significant Difference	15.013

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	86.928	4	5
A			
A	80.213	4	3
A			
A	79.753	4	1
A			
A	77.885	4	4
A			
A	75.545	4	2
A			
A	73.663	4	6

Average Daily Growth

The SAS System 18:04 Saturday, May 15, 2019 15

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	6	1 2 3 4 5 6

Number of observations 24

The GLM Procedure

Dependent Variable: GainGP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	47.3863117	7.8977186	0.50	0.7985
Error	17	267.6206842	15.7423932		
Corrected Total	23	315.0069958			

R-Square	Coeff Var	Root MSE	GainGP Mean
0.150429	16.54200	3.967668	23.98542

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	45.37647083	9.07529417	0.58	0.7174
Rep	1	2.00984083	2.00984083	0.13	0.7253

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	45.37647083	9.07529417	0.58	0.7174
Rep	1	2.00984083	2.00984083	0.13	0.7253

The GLM Procedure

Dependent Variable: GainFP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	184.600020	30.766670	0.40	0.8686
Error	17	1306.708030	76.865178		
Corrected Total	23	1491.308050			

R-Square	Coeff Var	Root MSE	GainFP Mean
0.123784	18.32529	8.767279	47.84250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	89.86815000	17.97363000	0.23	0.9423

Rep	1	94.73187000	94.73187000	1.23	0.2824

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	89.86815000	17.97363000	0.23	0.9423
Rep	1	94.73187000	94.73187000	1.23	0.2824

The GLM Procedure

Dependent Variable: GainO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	54.5573117	9.0928853	0.29	0.9327
Error	17	530.0102842	31.1770755		
Corrected Total	23	584.5675958			

R-Square	Coeff Var	Root MSE	GainO Mean
0.093329	15.54701	5.583644	35.91458

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	23.41667083	4.68333417	0.15	0.9772
Rep	1	31.14064083	31.14064083	1.00	0.3316

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	23.41667083	4.68333417	0.15	0.9772
Rep	1	31.14064083	31.14064083	1.00	0.3316

The GLM Procedure

Least Squares Means

Treatment	GainGP LSMEAN	Standard Error	Pr > |t|
1	24.1075000	1.9838342	<.0001
2	26.0275000	1.9838342	<.0001
3	23.8300000	1.9838342	<.0001
4	23.2050000	1.9838342	<.0001
5	25.0700000	1.9838342	<.0001
6	21.6725000	1.9838342	<.0001

Treatment	GainFP LSMEAN	Standard Error	Pr > |t|
1	49.4500000	4.3836394	<.0001
2	46.0525000	4.3836394	<.0001
3	48.3900000	4.3836394	<.0001
4	50.9875000	4.3836394	<.0001
5	45.6825000	4.3836394	<.0001
6	46.4925000	4.3836394	<.0001

Treatment	GainO LSMEAN	Standard Error	Pr > |t|
1	36.7825000	2.7918218	<.0001
2	36.0400000	2.7918218	<.0001
3	36.1100000	2.7918218	<.0001
4	37.0975000	2.7918218	<.0001
5	35.3750000	2.7918218	<.0001
6	34.0825000	2.7918218	<.0001

The GLM Procedure

t Tests (LSD) for GainGP

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.74239
Critical Value of t	2.10982
Least Significant Difference	5.9192

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	26.028	4	2
A			
A	25.070	4	5
A			

A	24.108	4	1
A			
A	23.830	4	3
A			
A	23.205	4	4
A			
A	21.673	4	6

The GLM Procedure

t Tests (LSD) for GainFP

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	76.86518
Critical Value of t	2.10982
Least Significant Difference	13.08

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	50.988	4	4
A			
A	49.450	4	1
A			
A	48.390	4	3
A			
A	46.493	4	6
A			
A	46.053	4	2
A			
A	45.683	4	5

The GLM Procedure

t Tests (LSD) for GainO

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	31.17708
Critical Value of t	2.10982
Least Significant Difference	8.33

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	37.098	4	4
A			
A	36.783	4	1
A			
A	36.110	4	3
A			
A	36.040	4	2
A			
A	35.375	4	5
A			
A	34.083	4	6

Feed Efficiency

The SAS System 18:12 Saturday, May 15, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	6	1 2 3 4 5 6

Number of observations 24

The SAS System 18:12 Saturday, May 15, 2019 2

The GLM Procedure

Dependent Variable: FEg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.06089167	0.01014861	4.28	0.0083
Error	17	0.04030417	0.00237083		
Corrected Total	23	0.10119583			

R-Square	Coeff Var	Root MSE	FEg Mean
0.601721	10.53732	0.048691	0.462083

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	0.06087083	0.01217417	5.13	0.0047
Rep	1	0.00002083	0.00002083	0.01	0.9264

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	0.06087083	0.01217417	5.13	0.0047
Rep	1	0.00002083	0.00002083	0.01	0.9264

The GLM Procedure

Dependent Variable: FEf

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.03228667	0.00538111	1.04	0.4351
Error	17	0.08809667	0.00518216		
Corrected Total	23	0.12038333			

R-Square	Coeff Var	Root MSE	FEf Mean
0.268199	15.79244	0.071987	0.455833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	0.02668333	0.00533667	1.03	0.4315
Rep	1	0.00560333	0.00560333	1.08	0.3130

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	0.02668333	0.00533667	1.03	0.4315
Rep	1	0.00560333	0.00560333	1.08	0.3130

The GLM Procedure

Dependent Variable: FEo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.03426333	0.00571056	2.23	0.0904
Error	17	0.04352000	0.00256000		
Corrected Total	23	0.07778333			

R-Square	Coeff Var	Root MSE	FEo Mean
0.440497	11.09977	0.050596	0.455833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	0.03183333	0.00636667	2.49	0.0727
Rep	1	0.00243000	0.00243000	0.95	0.3436

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	0.03183333	0.00636667	2.49	0.0727
Rep	1	0.00243000	0.00243000	0.95	0.3436

The GLM Procedure

Least Squares Means

Treatment	FEg LSMEAN	Standard Error	Pr > |t|
1	0.53000000	0.02434560	<.0001
2	0.52750000	0.02434560	<.0001
3	0.40750000	0.02434560	<.0001
4	0.46500000	0.02434560	<.0001
5	0.42250000	0.02434560	<.0001
6	0.42000000	0.02434560	<.0001

Treatment	FEf LSMEAN	Standard Error	Pr > |t|
1	0.47750000	0.03599360	<.0001
2	0.49750000	0.03599360	<.0001
3	0.46000000	0.03599360	<.0001
4	0.46250000	0.03599360	<.0001
5	0.44750000	0.03599360	<.0001
6	0.39000000	0.03599360	<.0001

Treatment	FEo LSMEAN	Standard Error	Pr > |t|
1	0.49750000	0.02529822	<.0001
2	0.50500000	0.02529822	<.0001
3	0.44000000	0.02529822	<.0001
4	0.45750000	0.02529822	<.0001
5	0.43500000	0.02529822	<.0001
6	0.40000000	0.02529822	<.0001

The GLM Procedure

t Tests (LSD) for FEg

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	0.002371
Critical Value of t	2.10982
Least Significant Difference	0.0726

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	0.53000	4	1
A			
A	0.52750	4	2
A			
B A	0.46500	4	4
B			
B	0.42250	4	5
B			
B	0.42000	4	6
B			
B	0.40750	4	3

The GLM Procedure

t Tests (LSD) for FEf

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	0.005182
Critical Value of t	2.10982
Least Significant Difference	0.1074

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	0.49750	4	2
A			
B A	0.47750	4	1
B A			
B A	0.46250	4	4
B A			
B A	0.46000	4	3
B A			
B A	0.44750	4	5
B			
B	0.39000	4	6

The GLM Procedure

t Tests (LSD) for FEo

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	0.00256
Critical Value of t	2.10982
Least Significant Difference	0.0755

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	0.50500	4	2
A			
A	0.49750	4	1
A			
B A	0.45750	4	4
B A			
B A	0.44000	4	3
B A			
B A	0.43500	4	5
B			
B	0.40000	4	6

Appendix 3: Results on pooled data

Table 1: Effects of treatments on average daily feed intake, average daily gain and feed efficiency using pooled means

	Trt 1	Trt 2	Trt 3	Pooled SEM	*p* Value
[1]**ADFIg**	52.66	51.63	52.29	2.45	0.9555
[2]**ADFIf**	102.64	106.47	108.30	4.89	0.7103
[3]**ADFIo**	77.65	79.05	80.30	3.55	0.8712
[1]**ADGg**	25.07	23.51	23.37	1.33	0.6175
[2]**ADGf**	47.75	49.69	46.09	2.93	0.6890
[3]**ADGo**	36.41	36.60	34.73	1.83	0.7332
[1]**FEg**	0.53^{a}	0.44^{b}	0.42^{b}	0.02	0.0003
[2]**FEf**	0.49	0.46	0.42	0.02	0.1603
[3]**FEo**	0.50^{a}	0.45^{b}	0.42^{b}	0.02	0.0080

[a, b] Means in the same row with different superscripts differ significantly (p< 0.05)

The results are reported as Mean ± SEM (standard error of means)

[1]ADFIg represents Average Daily Feed Intake for the grower phase

[2]$ADFI_f$ represents Average Daily Feed Intake for the finisher phase

[3]ADFIo represents Overall Average Daily Feed

[1]ADGg represents Average Daily Gain for the grower phase

[2]ADG_f represents Average Daily Gain for the finisher phase

[3]ADGo represents Overall Average Daily Gain

[1]IntakeFEg represents Feed Efficiency for the grower phase

[2]FE_f represents Feed Efficiency for the finisher phase

[3]FEo represents Overall Feed Efficiency

Appendix 4: Publication abstract

Prevalence of aflatoxins contamination in commercial broiler feeds in Kenya

F N Thuita, J K Tuitoek, A M King'ori and M A Obonyo[1]

Department of Animal Sciences, Egerton University, P O Box 536-20115, Egerton, Kenya

jtuitoek@egerton.ac.ke

[1]Department of Biochemistry and Molecular Biology, Egerton University, P O Box 536 -20115 Egerton, Kenya.

Abstract

Aflatoxin contamination of broiler feed is a major barrier to sustained agricultural productivity and trade. Aflatoxins are a type of mycotoxins (secondary fungal metabolites) produced by fungi of the genus Aspergillus (A), mainly A. flavus and A. parasiticus, in cereals and grains when conditions are favorable. The aim of this survey was to determine the levels of total aflatoxins (AFs) in common commercial broiler feeds among feed manufacturers in Nakuru town, Kenya. Forty compounded broiler feed samples were randomly collected from ten feed mill companies in Nakuru town (ten broiler starter and ten broiler finisher feed samples per company) in two phases. Each collection phase was determined by the frequency of purchase of raw materials by the individual milling companies. The total aflatoxin levels in the feed were analyzed using the ELISA technique in the Mycotoxin Research Laboratory in Egerton University. The data was subjected to SAS procedures using two way analysis of variance. All the feeds collected contained aflatoxins within a range of 1.07- 41.01 µg/kg. The samples (92.5%) contained total aflatoxin levels which exceeded the WHO limits of 5 µg/kg in animal feeds. Of the samples collected, 52.5% exceeded the FDA limits of 20 µg/kg in poultry feeds. To avoid high levels of AFs in broiler feeds, feed manufacturers should test for aflatoxins in the raw materials and avoid the fungal contamination in the broiler feeds at all stages of handling.

Key Words: ELISA, mycotoxins

Appendix 5: National Commission for Science, Technology and Innovation Research permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349,3310571,2219420
Fax: +254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/19/24844/30148** Date: **6th May 2019**

Frida Njoki Thuita
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effects of aflatoxin contamination and aflatoxin binder incorporation in broiler feed on broiler performance and product safety."*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **3rd May, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

CHARITY MUSEMBI
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.