INVESTIGATION OF INDIGENOUS CHICKEN PRODUCTION AND AFLATOXIN CONTAMINATION OF EGGS AND MEAT IN WESTERN KENYA

JONATHAN KIPNG'ETICH TARUS

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DECLARATION

DECLARATION BY THE STUDENT

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MR. TARUS JONATHAN KIPNG'ETICH

_____ Date _____

AGR/PGA/002/14

DECLARATION BY THE SUPERVISORS

This thesis has been submitted with our approval as University supervisors.

_____ Date: _____

DR. RACHUONYO, HAROLD ANINDO.
Department of Animal Science,
University of Eldoret, Kenya

_____ Date: _____

DR. OMEGA, JOSEPH AMESA.
Department of Animal Science,
University of Eldoret, Kenya

_____ Date: _____

PROF. OCHUODHO, JULIUS ONYANGO.
Department of Seed, Crop and Horticultural Sciences,
University of Eldoret, Kenya

DEDICATION

I dedicate this work to God who has guided me through this demanding process of study. Also to my family who persevered with me even at times they needed me most as I engaged in the research.

ABSTRACT

Poultry plays an important economical role of developing nations and reports show that demand of poultry meat is on increase**.** Native chicken are valued in Kenya because they provide food and income, for domestic use, to rural households especially in Western Kenya. However, their production is low compared to exotic chicken; this could be due to ingestion of aflatoxins known to affect chicken productivity. The study investigated feed types, feeding regime and presence of aflatoxins in chicken products in Siaya, Busia and Kakamega Counties. A multi stage sampling was used and purposive selection entirely done based on indigenous chicken production among women and youth groups. In each of the 3 Counties, 3 sub Counties were selected; in each sub County, two Wards were identified and two locations were picked in each of them. Four farmer groups (two youth and two women groups) were identified in each of the picked locations and five group members each completed a questionnaire. A total of 180 farmers were interviewed, 260 feed samples, 60 egg samples and 240 tissue samples were obtained from sixty chicken slaughtered and from these chicken (30 young; 12-16 weeks old and 30 adults; >36 weeks old), Liver (n=60), kidney (n=60), breast (n=60) and thigh muscle (n=60). Nutrient composition of common chicken feeds was analyzed for percent crude protein (CP), dry matter (DM), and ash (ASH) using proximate method. Enzyme-linked Immunosorbent assay (ELISA) was used to detect presence of aflatoxins in products of indigenous chicken. Survey data was analyzed using SPSS Software version 25 while GENSTAT 14th Edition was used to analyze tissues, eggs and feed data. Farmer's age and education level determined type of production system practiced. Free range system was still popular than semi free range and intensive systems. Common feedstuffs were; maize, sorghum, cassava, groundnuts and commercial feeds. Cassava had the lowest crude protein at 2.4%, groundnuts had the highest crude protein at 20% as compared to other feedstuffs while Sorghum had higher CP at 10% than maize at 8%. Tissues had higher mean aflatoxin level (liver 4.19ppb, Breast muscle 3.57ppb, thigh muscle 2.66ppb and kidney 2.02ppb) but eggs had traces (0.081ppb). Chicken tissues had total aflatoxins, eggs had traces. This study informs on production systems, nutrient composition of indigenous chicken feeds and levels of aflatoxin in chicken products. Therefore the study recommends that strategies on minimizing future contamination and improving on production be put in place.

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ABBREVIATIONS AND ACRONYMS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFT	Total Aflatoxin
DON	Deoxynivalenol
FAO	Food and Agriculture Organization
FDA	Food and Drugs Administration
FUM	Fumonisin
GDP	Gross Domestic Product
IBD	Infectious Bronchitis Disease
KALRO	Kenya Agriculture and Livestock Research Organiztion
KEBS	Kenya Bureau of Standards
ND	Newcastle Disease
NGO	Non-governmental Organiztion
OTA	Ochratoxin A
RUFORUM	Regional Universities Forum
ZEN	Zearalenone

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

1 .1.1 Indigenous chicken production

Poultry industry, chicken in particular, plays an important role in the economy of developing nations and reports show that the demand for poultry meat is on increase (Dolberg, 2008**).** Besides this, chicken play socio-economic role globally, especially, in low income countries (Alders, 2004). The impact of village chicken is still felt particularly in the national economies of developing countries through improved nutrition, food security and livelihoods of smallholder households especially in Africa (Gondwe 2004; Abdelqader *et al*., 2007; Abubakar *et al*. 2007). This makes chicken products the most valuable and affordable proteins of animal origin. This is supported by the fact that indigenous chickens are wide spread in most rural Africa than any other livestock species and still very common in most African households despite the introduction of exotic and crossbred types of chicken.

Kenya's poultry population is estimated to be 31 million birds, of which 81% are indigenous chicken (*Gallus domesticus).* This sub-sector contributes 30% of agricultural gross domestic product (GDP), 55% of livestock sector and 3% of national GDP (GoK, 2016). These indigenous chicken are popular across all Counties of Western Kenya particularly Busia, Kakamega and Siaya Counties. These chicken provide food and employment for disadvantaged groups in most of the rural households (Guèye 2000; Kogunza *et al.,* 2008; Munyasi *et al.,* 2009). Predators, feed shortages, theft, poor marketing channels and housing are major challenges facing the industry. Chicken meat

and eggs forms an important source of essential animal proteins, a vast array of vitamins and minerals (Darwish *et al*., 2016). Chicken output may not be high in most families, but their great advantage is the frequent egg production, which provides nutrients of high nutritional value consumed by the vulnerable members of the household (FAO, 2014).

Poultry production is considered as one way of mitigating food insecurity if fully exploited and commercialized like the exotic commercial chicken. Although they are continuously exposed to diseases, inadequate health care and feeding, indigenous chicken are better adapted to scavenging. They are also known to survive weather conditions like cold and heat, wet and drought and management practices such as; sheltered and unsheltered outside or roosting in trees or in cages (Nhleko *et al*., 2003). These chicken survive largely on weed seeds, insects, feeds and kitchen wastes that would otherwise be a waste. Low productivity and lower growth rates of indigenous chicken can be minimized through protein supplementation (King'ori *et al*., 2007). To increase food security and income in the poor rural households, indigenous chicken production can be transformed to commercial production from the common subsistence production (Kyarisiima *et al* 2004). However, poultry faces expanded challenges like bacterial, viral, metabolic disorders and mycotoxicosis, in this century that must be met if the problems of food security, chicken production growth and employment are to be solved (McIntire, 2014; El-Yazeed, 2015).

Among the farmers, free range system seems to be preferred where chicken are let free in the morning to scavenge the rest of the day. Most farmers fed their chicken in the morning, before releasing them to scavenge, the rest of the day or till evening. This system requires low inputs in terms of feeds although it can be expensive when preventing them from diseases, predators and damaging the neighbours' property. The second in

popularity is the semi free range system (semi intensive) where chicken are kept in for some hours (commonly 6 hours) given feed supplements, then released to scavenge. Some farmers practiced this system only when feeds are available, after which they turn to free range system which seem easier to manage in terms of feeding.

Intensive system is not common with indigenous chicken farmers because it is minimally practiced as compared to free range and semi free range systems. This could be because it requires more inputs of feed and equipment and yet indigenous chicken growth rate is low. Unless indigenous chicken are improved genetically, intensive system can be uneconomical.

Chicken are exposed to major challenges such as adverse weather conditions, diseases, predators, feed shortages and theft. Lack of the required housing, management skills, training equipment and chance to effectively optimize their household indigenous chicken production are still major challenges to most communities (Mlozi *et al.,* 2003; Mangesha *et al*., 2011). Nongovernmental organizations (NGOs) and government agricultural extension agents have made efforts to minimize these challenges, but adoption rates are low in Western Kenya (Ochieng *et al*., 2013). Therefore there is an urgent need to modernize indigenous chicken production through farmer education, use of improved feeds (commercial feeds), housing and breeding. This will in turn improve indigenous chicken production and livelihoods of rural households especially women and youth.

1.1.2: Feed quality

Indigenous chicken are commonly fed on a variety of locally available ingredients such as maize, sorghum, millet, cassava, groundnuts, sweet potato tubers (boiled), bananas,

commercial feeds and kitchen leftovers. Designing ways to supply the required nutrients for scavenging chicken is paramount to attaining optimum production (Okitoi *et al*., 2009). The free rangers also feed on insects, vegetation and wild seeds. Feeds are known to improve chicken growth and productivity (Okitoi *et al*., 2006). Therefore it is necessary to quantify the nutrient content of available feedstuff so as to determine the feeding levels (diet) and disseminate the information to stakeholders at all levels for improved indigenous chicken production.

1.1.3: Aflatoxins

Aflatoxin is one of the most known and studied of all mycotoxins. Mycotoxin is obtained from a Greek word *mykes* for mould and latin word *toxicum* for poison thus mycotoxins are mould poisons (Rahmani *et al.,* 2009 ; Bullerman *et al*., 2011). Mycotoxins are toxic fungal metabolites that can contaminate a wide array of food and feed. In 1962, over 100,000 turkeys died in London due to consumption of peanuts which were contaminated with *Aspergillus flavus* a major producer of aflatoxins. Today there are over 500 known mycotoxins (Vetchick, 2010; Durali, 2014) although these can be in the range of 300,000 (Whitlow & Hagler, 2006), but the most common are aflatoxins. Mycotoxin problems are found in all regions of the world and mycotoxins are estimated to affect as much as 25 percent of the world's crops each year (Lawlor and Lynch, 2005). In Kenya aflatoxins are reported to be a major challenge to feed industry as leading contaminants (Kang'ethe & Lang'a., 2009).

Most common and significant mycotoxins in poultry industry, besides aflatoxins (**AF**) are: fumonisins (**FUM**), zearalenone (**ZEN**), ochratoxin A (**OTA**) and trichothecenes such as

deoxynivalenol (**DON**), and T-2 toxin and are also known to significantly impact the health and productivity of most poultry species (Murugesan *et al*., 2014)

Feed is the major contributor of aflatoxins into the poultry flocks and they exert their effects in poultry by; altering the nutrient content of the feed, its absorption and metabolism, affecting endocrine, exocrine and immune systems. The presence of aflatoxins in meat, edible tissues and eggs is of great concern since the residues can affect human health (Gareis &Wolff, 2000; Inheshiutor *et al.,* 2011). Miller (2008) reported that animal feeds containing ingredients such as peanut, oil seed and coconut cakes or corn germ often contain aflatoxin. Most of chicken feeds contain one or more of these ingredients particularly cotton seed cake, sunflower cake, soybean cake and maize germ. Indigenous chicken feeding involves the use of various types of locally available feedstuffs which are used by farmers for example maize grain, sorghum, millet, cassava tubers, boiled sweet potato tubers, kitchen remains and commercial feeds. These feeds can be contaminated in the field (pre-harvest, harvest and drying), during storage and processing; therefore, this makes it necessary to analyze and evaluate the presence of aflatoxins in chicken products due to carry-over to products.

Aflatoxin-contaminated feeds if consumed by poultry, will affect feed intake, feed conversion efficiency hence influencing the rate of weight gain and reproduction negatively (Nemati *et a*l., 2014). Consumption of large amounts of aflatoxins causes acute aflatoxicosis which almost always causes death while if consumed in small quantities for a long time causes chronic form which is associated with liver cancer. The residues of aflatoxins in feeds and food has led to key organizations (FAO, WHO, FDA and KEBS) to set up safety levels(10 and 20 ppb) of aflatoxin in products intended for both livestock

and human consumption so as to control and minimize effects of aflatoxins on consumers. Strict adherence to safe production and processing measures of feeds can lead to obtaining safe chicken products.

1.2: Statement of the Problem

Formulation of policies or strategies that will assist in prevention of future contaminations, reduction of use of poor quality feeds in chicken production and minimizing health hazards to humans and animals are needed. Indigenous chicken production in Kenya, particularly Western Kenya, faces the challenges of improving productivity and quality of products. Indigenous chicken farmers use a wide range of feeds for their poultry; including cereals (maize, sorghum, millet, groundnuts and beans), cassava, sweet potato tubers, homemade rations and commercial feeds (kienyeji mash, grower mash, layer mash and chick mash). The nutrient composition of these feeds is varied and should be determined for ration formulation of indigenous chicken. However, most rural communities lack the required training, skills, and opportunity to effectively improve their chicken production which has led to most rural chicken producers feeding their chicken only on one ingredient like maize, while expecting improved performance. On the other hand, aflatoxins are the major contaminants of feedstuffs and are reported to be a health risk to both man and animals if consumed. Indigenous chicken in this region, are reared under different production systems, like free range and semi free range, expose them to aflatoxin contamination. Humid and warm conditions, in Western Kenya, favour the growth of most mycotoxigenic fungi which can contaminate feedstuffs which if consumed by poultry will affect weight gain, feed intake, feed conversion efficiency and reproduction negatively. Thus, improper handling and storage conditions of feeds could

promote the problem of aflatoxicosis. Food and Agriculture Organization (FAO) reported that 25% of human foods and animal feeds are contaminated with mycotoxigenic fungi. In chicken, such feed compromise immune system and toxins are passed on to eggs and meat, subsequently affecting humans who consume them as food. Aflatoxicosis disease is one of the hindrances to chicken production in Kenya and the rest of the world. This has made study of aflatoxin a subject of international importance and thus, attracting worldwide attention due to significant economic losses, impact on human and animal health and trade. Scavenging indigenous chickens are exposed to various potential sources of aflatoxin contamination and there is therefore need to investigate its occurrence in chicken products which pose risks to human health.

1.3 Justification

Most of the developing nations, Kenya included, advocate for food security and poverty eradication strategies in their development agenda in order to minimize malnutrition and starvation. Poultry production is one of the considerations in mitigating the problem. Most communities in Kenya, Western Kenya in particular, view indigenous chicken production as a remedy to food insecurity and source of income (Munyasi *et al*., 2009). This could be because indigenous chicken are hardy, can thrive with minimal inputs and still produce and give products (Mailu *et al*., 2012). Aflatoxin occurrence is worldwide and levels are unpredictable due to geographical variations. Poor performance of indigenous chicken could be due to consumption of aflatoxin contaminated feeds during harvest, drying and storage. Little documented information on the presence of aflatoxins in chicken products is available, particularly for Western Kenya. This raised a need to determine the presence of aflatoxins, carried over from contaminated sources, to chicken

products which pose risks to human health and economic losses in trade. This study analyzed production systems of indigenous chicken, presence of aflatoxins in chicken tissues and eggs and nutrient composition of feeds.

1.4 Objectives

1.4.1 Broad Objective

To investigate indigenous chicken production systems, feed composition and determine aflatoxin contamination of indigenous chicken products for healthy product production in Western Kenya.

1.4.2 Specific Objectives

1. To identify types production systems, feeds and feeding through baseline survey in Western Kenya.

2. To determine nutrient composition of commonly used indigenous chicken feeds.

3. To determine levels of aflatoxins in indigenous chicken meat and eggs.

1.5 Hypotheses

H_o: There are different indigenous chicken types, production systems, feeds and feeding regimes

H_a: There are similar indigenous chicken types, production systems, feeds and feeding regimes

H_o: Nutritive value of feeds commonly fed to chicken is not varied

H_a: Nutritive value of feeds commonly fed to chicken is varied.

H_o: Aflatoxins consumed by chicken are not passed to eggs and meat of chicken.

H_a: Aflatoxins consumed by chicken are passed to eggs and meat of chicken

CHAPTER TWO

LITERATURE REVIEW

2.1 Indigenous chicken

Indigenous chicken (*Gallus domesticus)* are directly related to the Red Jungle Fowl (*Gallus gallus gallus*) as revealed by DNA study that was carried out (Dorji *et al*., 2012). Chicken are the most wide spread poultry species in the world and account for 90 percent of the poultry population. They provide nutrition for the family, some cash flow and meat for festivals or other need in some religious ceremonies, like bride price, cleansing, cursing and also for recreational activities, hence making a significant role of food security and poverty mitigation in many parts globally especially in developing countries (FAO., 2004; Dolberg, 2008; Nakkazi *et al*., 2014;). Indigenous chicken are now valued as part of income generation, and acts as an empowerment to rural women and youth (Ambre *et al*., 2016; Munyasi *et al.,* 2009*;* Dana *et al.,* 2010; Okeno *et al*., 2012). This is why chickens are probably the most important nutritionally among domestic animal species for the world's poor (Apuno *et al*., 2011). These indigenous chicken are still common in most African villages despite the introduction of exotic and crossbred types, because of high input requirement of the exotic or crossbred breeds. Keepers fear to invest in productive chicken and this could be the reason leading to popularity of poor scavenging indigenous chicken.

2.1.1 Present status.

According to Omiti (2011), indigenous chicken accounts for 81% of poultry population in Kenya and support livelihoods of over 21 million people especially in rural areas, mainly women and youth. *Gallus domesticus* have undergone several improvements in terms of

breeding and production which has led to production of “improved indigenous” or “improved Kienyeji’ chicken in Kenya (KARI, 2014).

2.1.2 Production systems

2.1.2.1 Free range system

Free range production system is the most frequently practiced by farmers followed by semi-free range in most parts of rural Africa (Sanka and Mbaga, 2014). Chicken are kept extensively for meat, eggs for household consumption, income and various socio-cultural activities (Njenga, 2005; Ochieng *et al*., 2013). Free-range chicken utilize such feed resources as various seeds, grass and insects (Birech., 2002). Scavenger chickens are usually self-reliant, hardy and are known to withstand the various weather conditions, minimal management and inadequate nutrition (Birech, 2002; Nakkazi *et al*., 2014). Supplementation with available feedstuffs is done once a day, morning or evening, depending on their availability (Nakkazi *et al*., 2014). In these rural set ups, water is freely available to the chicken all the time, using basins, sauce pans, container stuck in the ground, plates, cut jericans, commercial water troughs and other small tins (Nakkazi *et al*., 2014). Common housing models used in indigenous chicken production include; traditional raised houses, A-frame, brooding baskets, mud houses and iron sheet houses although majority of chickens roosts on trees (Tarwireyi and Fanadzo, 2013).

2.1.2.2 Semi free range (semi intensive)

In this system, chicken are fed and held in simple shelters to proper chicken houses for some hours, commonly 6 hrs and then they are let out to scavenge for the rest of the day.

King'ori *et al* (2010) reported that chicken are left to scavenge around homestead feeding on kitchen wastes, grass, insects, and other available feed ingredients.

2.1.2.3 Intensive Production System

Chicken are confined the whole day and fed on locally available and some commercial feeds. Indigenous chicken are reared under different production systems but to a lesser extent under intensive systems (Sanka and Mbaga, 2014). Under intensive system, indigenous chicken show low growth rates and poor feed conversion ratio than hybrid chickens (Mupeta, *et al.*, 2000). This indicates that indigenous birds' response to improved feeding management systems is low because they are not accustomed to confinement as compared to exotic birds (Sanka and Mbaga., 2014) mainly due to their genetic potential (Apuno *et al*., 2011) Through ingestion of aflatoxin contaminated feedstuffs, chicken are at a risk of being exposed to high concentrations that develop several health problems leading to large economical losses of meat and eggs in terms of quality and quantity (Bintvihok *et al*., 2002; Hall and Wild, 2003; Farombi, 2006).

2.1.3 Feeding and feeds

Birds under free range system are rarely confined, but if confined they are supplemented with maize, cassava and groundnuts (Plate 1), kitchen leftovers, any other available feed resource and water especially during cropping seasons (Khobondo *et al., 2015*). According to FAO (2012), 25% of human foods and animal feeds are contaminated with mycotoxigenic fungi. Hence, increased mortality of chicks has been associated with diets contaminated with aflatoxins (Oguz & Kutoglu, 2000). It is generally agreed that dietary aflatoxin reduces rate of weight gain, feed intake, and worsens feed efficiency (Chen *et*

al., 2013). Therefore, this could be an indication why indigenous chicken show a low weight gain.

A **B** **C**

Plate 1: Common indigenous chicken feedstuffs: Cassava (A), Groundnuts (B) and Maize (C) collected during the study (Source: Author, 2016)

2.1.4 Comparative advantage of indigenous chicken

Indigenous chicken are relatively adapted to living on the grounds where they naturally find their diet. Indigenous chicken are known to feed on pastures and grain or cereals. Scavenging village chicken products frequently fetch a higher price in the market because they are considered to be free of aflatoxin and other residues (FAO, 2014). Indigenous chicken are generally rated superior to the exotic hybrids because: they are more resistant to common diseases like coccidiosis, they can tolerate a wide range of environmental conditions (adverse temperatures, rains among others), their mothering ability, and defending of their chicks against everyday predators is high, can scavenge better, their meat and eggs preferred due to their taste and this enhances their market value. These characteristics make them more suitable to the village settings (King'ori *et al*., 2010; Bushra, 2012,; Nakkazi, *et al.*, 2014)

2.1.5 Diseases

Aflatoxins are known to affect immune system of poultry. Immune response can be impaired, in chickens, at levels that cannot affect growth rate (Chen *et al*., 2013). Experimental research done by Gabal and Azzam (1998) reported that feeding chicken with aflatoxin level of 200 ppb in feed and vaccinating them against most diseases lowered their immunity against successive experimental challenges. Besides inadequate feeding, housing and value chain organization, poultry health issues are some of the major constraints on family poultry production in Western Kenya. Although disease control is identified as a key factor in family chicken projects, disease control should be implemented alongside other appropriate management measures to ensure an optimum return on investments (Ahlers *et al*., 2009). Vaccines are available to prevent each of these diseases. Different vaccination models exist depending on the different ecological, economic and cultural environments (Alexander *et al*., 2004). According to Omiti *et al* (2005), aflatoxin contamination increased mortality to 35.6 percent as to compare with 3-21 percent in IBD and 0.03 percent mortality rate. Therefore, biosecurity practices must be tailored accordingly (FAO, 2008b).

2.1.5.1 Practices to prevent aflatoxin in chicken production

It is important to prevent diseases at all levels of production and the most important to consider are the biosecurity measures. According to FAO (2008b), biosecurity is implementation of measures that minimize the introduction and spread of pathogenic agents and this vary according to the production system involved. Health services for family poultry require attention from all stakeholders such as private sector, government ministries, local agencies, international organizations and donors to strengthen chicken

disease surveillance (FAO, 2008b). Prevention and control of contamination will make an important contribution to food security, poverty alleviation, early detection and control of zoonotic diseases including aflatoxicosis.

2.1.6 Marketing of chicken and products

Women and children are key players involved in chicken marketing. Their major markets are the nearest urban centers and farm gates. Both live chicken and egg marketing channels are more or less similar. Eggs are sold to food and retail shops, hotels and supermarkets in nearby towns (Fisseha *et al.,* 2010). Marketing of indigenous chicken and products is haphazard and involves price negotiation with buyers or as per market price (Nyanja, 2016).

2.1.7 Challenges and constraints to indigenous chicken production

Major limiting constraints of chicken production are: Diseases, predators, feed shortages; poor marketing system, management and lack of equipment (Hassen., 2007; Bogale., 2008; Fisseha., 2009; Addisu *et al*., 2013;**).** Poor genetic potential, low productivity, low egg hatchability, housing, lack of credit facilities, conflict with neighbours and high cost of commercial feeds are also challenges faced by indigenous poultry producers (Kabuage, 2010; Mutua, 2011; Ochieng, *et al*., 2013).

2.2 Mycotoxins

2.2.1 Prevalence of mycotoxins

All natural materials (grains, tubers, fruits, vegetation among others) and many man-made ones (like cakes, meals, germs, mashes) are subject to contamination by mycotoxins

under favorable conditions like temperature and high moisture content. Under these conditions, these fungi proliferate and produce mycotoxins (Vetchick, 2010). Most studies have indicated that mycotoxins are often invisible, tasteless, chemically stable and resistant to temperature and processing methods. Poorly stored (with high moisture) feed are contaminated with aflatoxins, hence posing danger to poultry, other animal and human health (Saqer, 2013). These feeds are a main source of aflatoxin contamination, of which, if fed to chicken can contaminate their products. Aflatoxin in layer feed can result in residues in eggs which make it very essential to control aflatoxins in feeds for this group of chicken (Oliveira *et al*, 2000). All species and life stages of birds are susceptible to aflatoxins to varying degrees (Durali *et al.,* 2014). If the daily feed given to chicken before being released is contaminated with low aflatoxin content, chronic consumption, poses a serious risk to the health of animals therefore rising susceptibility to infections or reducing efficacy of vaccines (Chen *et al*., 2013). Contaminated feed is the main source for aflatoxin infestation of farm animals. Feeds of cereal grain origin and nuts demonstrate the most susceptible commodities for mycotoxin contamination (Saqer, 2013). Oral intake of feed contaminated with fungal metabolites results into traces of residues in meat, edible tissues, milk and eggs (Gareis & Wolff, 2000). The capacity of fungi to produce toxins (mycotoxins) depends on the strain involved and the environmental conditions under which it grows (Kajuna *et al*., 2013). Studies have demonstrated that, aflatoxins are produced by certain fungi in/on foods and feeds and are probably the best known and most intensively studied mycotoxins in the world (Cornell University, 2015). Aflatoxins have received greater attention than any other mycotoxins

because of their demonstrated carcinogenic effect and are the most toxic (Cornell University, 2015; El-Yazeed, *et al.*, 2015).

2.2.2 Types of mycotoxins important in chicken production

2.2.2.1 Aflatoxin

Aflatoxins are a group of mycotoxins mainly produced by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nominus* (Cary *et al*. 2005) as they grow on their substrates. These toxins Aflatoxins are very stable compounds and are known to resist several food processing methods like roasting, extrusion, baking and cooking, therefore, they pose a severe health risk for humans and animals (Marin, *et al* 2013; Savard 2008; Richard 2007). The major ones are aflatoxins B1 (AFB_1), B2 (AFB_2), G1 (AFG_1) and G2 (AFG_2), thus named according to their fluorescence colour appearance such as blue and green light. Aflatoxin B1 and B2 fluorescent show blue light while G1 and G2 fluorescent show green light (Grace *et al*., 2015) during lab analysis. The most toxic and prevalent of these is aflatoxin B1 which is classified as a Group 1a carcinogen and associated with both toxicity and carcinogenicity in human and animal populations by the International Agency for Research on Cancer (IARC, 2002). It is known to infect crops at pre-harvest and post-harvest and even during processing. The most commonly infected feeds are: maize, groundnut, cassava, spices, oilseeds, beans, cotton, nuts and animal source feeds like dried fish. Aflatoxin cannot be detected by organoleptic tests like sight or smell in contaminated food or feed neither are they gotten rid of through cooking, or processing (Marin, *et al* 2013; Savard 2008). Carry-over of aflatoxins from feed to animal tissues depends on the amounts consumed; consequently, large amounts cause death, lowers

productivity and suppress the animal's body immunity. Of all mycotoxins, aflatoxin is the most toxic and rated as potential carcinogen in which AFB_1 is the most poisonous and the most occurring (Akande,*et al*., 2006). Aflatoxin contaminated feed, if consumed by poultry results into reduced weight gain, feed intake, feed conversion efficiency and reproductive performance (Nemati *et al*, 2014). As compared to other mycotoxins especially trichothecenes, elimination of aflatoxins out of the body is slower (Yunus *et al*., 2011). This is likely to be the reason for it being the most toxic as compared to other mycotoxins in terms of effects on consumers.

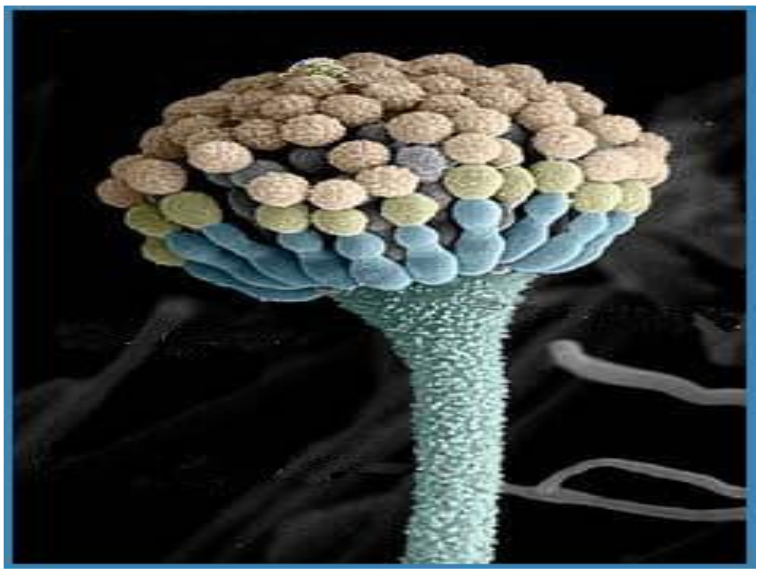


Plate 2 *Aspergillus flavus* responsible of producing Aflatoxins (B_1, B_2, G_1 and G_2) (Source: Biomin, 2017)

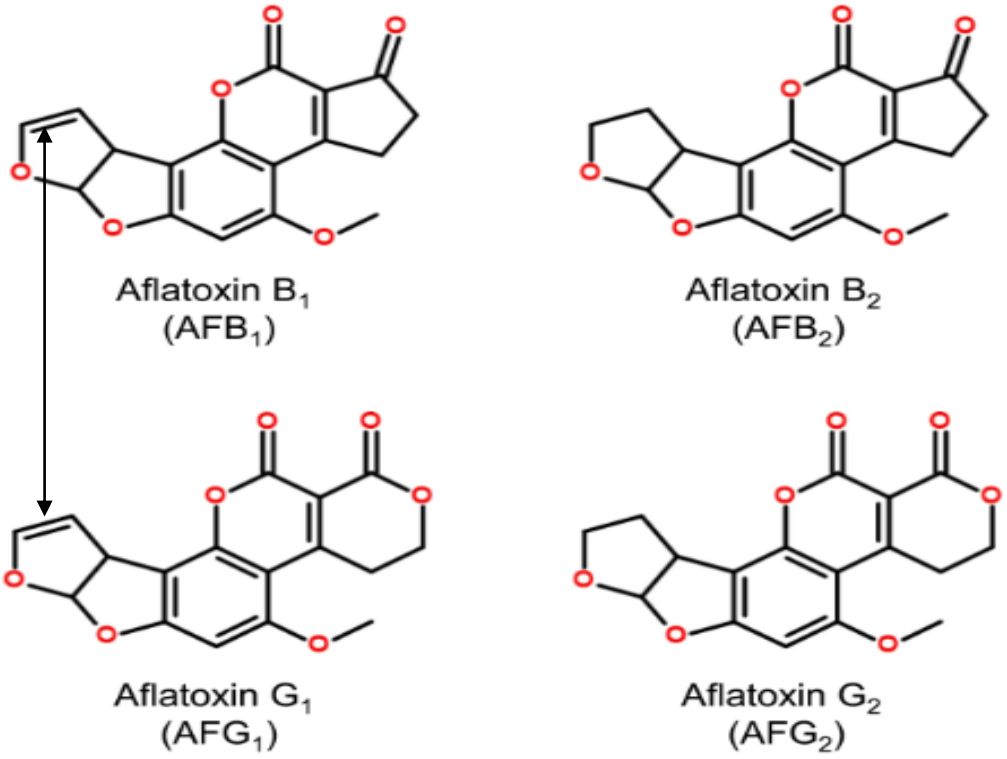


Figure 1 Chemical structure of AFB1, AFB2, AFGI, AFG2 (Melissa *et al*., 2015).
Notice the difference in one of the benzene rings (as shown by the arrow); the double bond in AFB1 and AFG1 is an indication of high toxicity

2.2.2.2 Zearalenone

It is produced by *Fusarium graminaerum* and *fusarium culmorum* and commonly occurs in warm and temperate climates on a variety of cereal crops. Zearalenones cause estrogenic effects in various animals especially cattle and sheep. Zearalenone appears to be well tolerated by poultry (Cortyl, 2008). Allen *et al*, (1981) reported that up to 800 ppm of ZON in feed fed to chicks from 6-9 weeks of age does not affect performance of broiler chicken.

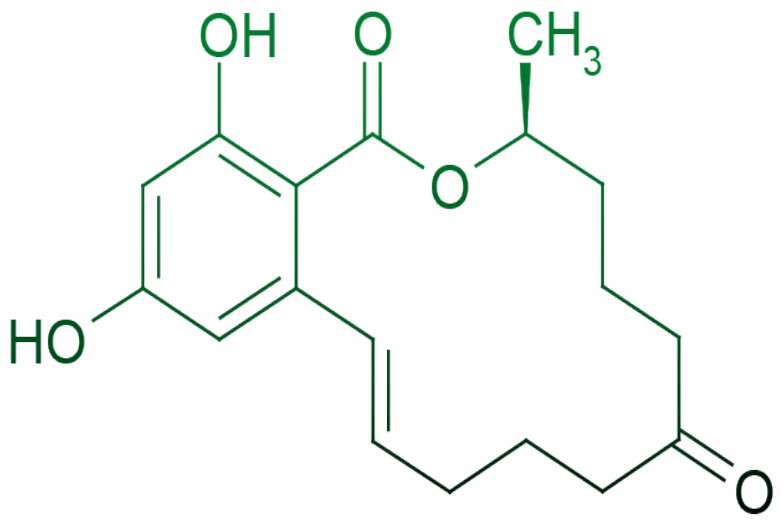


Figure 2 Chemical structure of zearalenone (Biomin , 2017)

2.2.2.3 Ochratoxin A

It is a metabolite of some species of *Aspergillus* and *Penecillium.* Ochratoxin A is a major contaminant of cereals such as maize, barley, wheat, oats, soy beans and peanuts in some parts of the world. It is responsible for embryonic death hence poor hatchability and also impairs immune system.

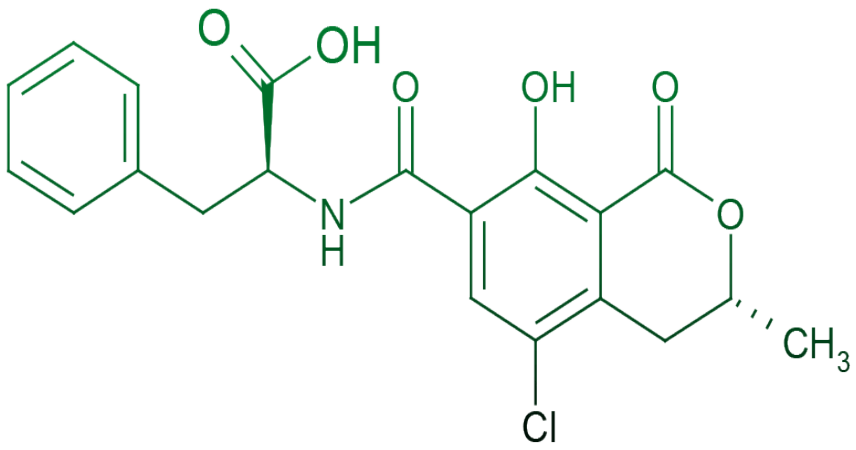


Figure 3 Chemical structure of Ochratoxin A (Biomin , 2017)

2.2.2.4 Fumonisin

Fumonisins are produced by fungi of the genera *Alternaria* and *Fusarium*, mainly by *F. moniliforme*, found majorly in corn throughout the world and have been detected in grain and grain products (Scott, 2011). Fumonisins with highest occurrence and toxicological significance in the form FB_1 and FB_2 (Mallmann & Dilkin, 2007) are considered to have cancer promoting activity (Bacon and Nelson 1994).

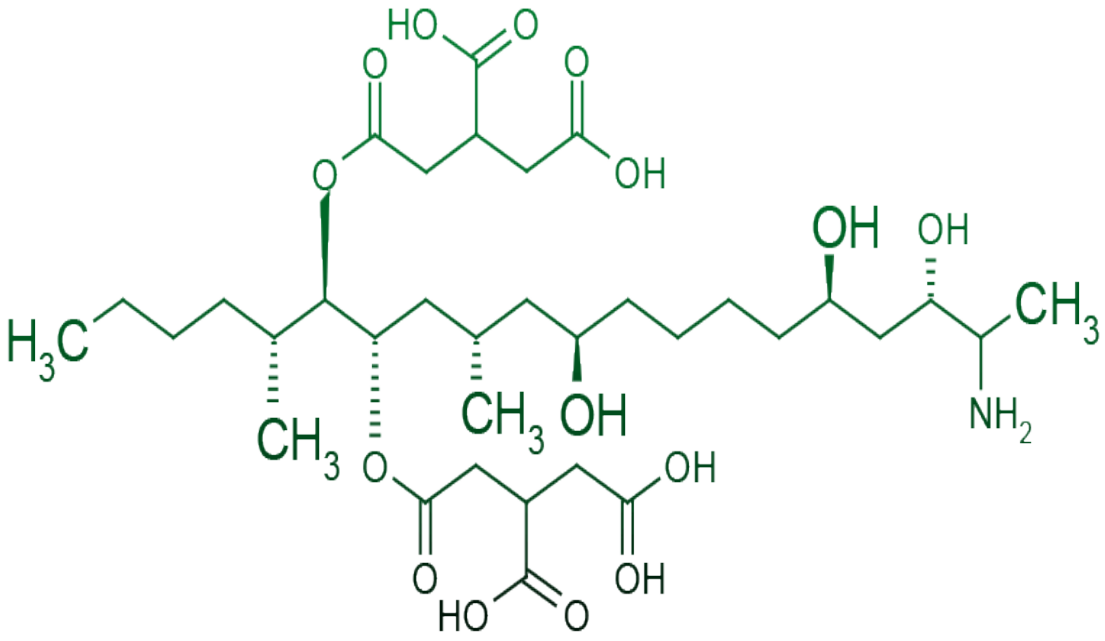


Figure 4 Chemical structure of Fumonisin (Biomin , 2017)

2.2.2.5 Trichothecenes

Trichothecenes are known to be the most harmful small molecule which inhibit protein synthesis with main toxic effect synthesis of DNA and RNA at the cellular level (Rocha *et al.,* 2005). Trichothecenes toxins show symptoms such as; decreased egg production and poor egg shell quality, oral lesions, growth retardation, feathers easily pull out,

regression of the gland- bursa of Fabricius, slows blood coagulation, leucopoenia and proteinemia, peroxidative changes in liver and immunosuppression (Danicke, 2002).

2.2.3 Aflatoxin biotransformation/metabolism in the liver

Biotransformation is the process whereby a chemical substance is changed from one chemical form to another (transformed) by a series of enzymatic or chemical reaction(s) within the body and the eventual excretion of the byproducts or metabolites mainly through renal excretion (Bbosa *et al*., 2013). Aflatoxins are not toxic *per se*, but they undergo biotransformation in the liver by CYP1A2 and CYP3A4 isoenzymes (or the cytochrome P450 family of enzymes) by the initial two electron transfer oxidation reactions to become an active metabolite; *exo*-AFB1-8, 9-epoxide (AFBO) and exert its toxicity (Chen *et al*., 2013; Wild *et al*., 2002; Vondracek, *et al.*, 2001). Aflatoxins (AFBs), in the liver, mainly AFB1 are bio-transformed to various metabolites which interact with biomolecules/macromolecules including nucleic acids, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) lead to metabolic activation. The binding of 8,9 epoxide of aflatoxin with DNA is formed with the N7 of guanine, to form adducts Af-N7-guanine in the target cell (Bbosa *et al*.,2013). Aflatoxicol is produced which is less toxic as compared to AFB_1 but the conversion is reversible and the aflatoxicol can serve as a reservoir toxicity of AFB1 in the intracellular space (Bbosa *et al*.,2013; Bemvenuti *et al*., 2011)

Toxigenic moulds are known to produce one or more of the toxic secondary metabolites. It is well established that not all moulds are toxigenic and not all secondary metabolites from molds are toxic (H-Bemvenuti *et al*., 2011). There are five mechanisms responsible

for the biotransformation of Aflatoxin (AFB1) such as reduction, epoxidation, hydration, hydroxylation and ortho-demethylation as shown in Figure 5.

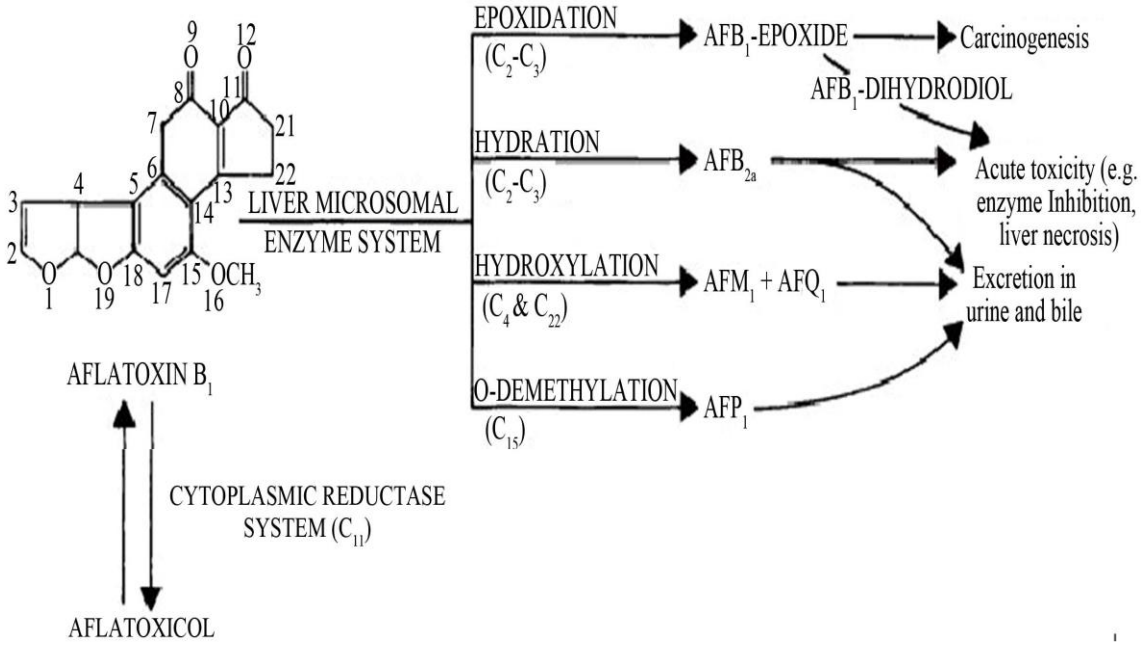


Figure 5: Metabolism of aflatoxin in liver (Source; Dhanasekaran *et al*., 2011). Metabolites are known to accumulate in various tissues of chicken such as liver, kidney, adipose (fat), muscles,(like thigh, breast) and eggs (Dhanasekaran *et al*., 2011; H-Bemvenuti *et al*., 2011; Radmila, *et.al.*, 2009). After absorption, 80-90 percent of aflatoxins consumed are absorbed in the upper part of the small intestine (Chen *et al*., 2013)

2.2.4 General effects of mycotoxins on chicken, man and other animals

The occurrence and incidence of Aflatoxins, Ochratoxin A and Zearalenon in chicken meat and eggs are alarming and may cause health hazards and raising the need for continuous monitoring of there control (Shazad *et al*., 2014). Blood *et al* (1999) reported that *Aspergillus, fusarium* and *penicillium* are common species of fungi infecting stored feeds and those growing in the field. The ability of fungi to produce toxic metabolites depends on the strain involved (Kajuna *et al.,* 2013). These compounds have adverse

health effects such as kidney and liver damage, mutagenic and teratogenic effects, birth defects, and cancerous effects that result in symptoms ranging from skin irritation to immuno-suppression, neurotoxicity, and death (Bennett and Klich 2003). Studies have shown that oral intake of fungal metabolites with feed, by animals, results in traces of residues in meat, edible tissues and eggs (Gareis *et al*., 2000). Ochratoxin has been found to reduce egg production, egg weight and weight gain. Trichothecenes causes decreased resistance to environmental and microbial stressors and increased susceptibility to disease, anorexia, egg shell quality decreases and impaired feed conversion ratio (Nutriad, 2014). Biomin (2017) reported that contamination of feedstuffs with aflatoxin toxins poses a serious threat to the health and productivity of animals especially low fertility, immune suppression and even death. Reduced protein synthesis results in reduced production of essential metabolic enzymes and structural proteins for growth. Relatively high dosages (200ppb) of aflatoxins in chicks result in hepatocellular necrosis; prolonged low dosages result in reduced growth rate, immunosuppression, and liver enlargement (Osweiler, 2014). Dietary aflatoxin reduces weight gain and feed intake and worsens feed efficiency (Chen *et al*., 2013). These will depend on; the toxins (type, level consumed and duration of intake), animals (species, sex, age, breed, health, immune status and nutrition) and Environment (management, temperature, hygiene).

Most studies have shown that chickens consuming diets contaminated with aflatoxin show renal lesions, lower growth rate, diminished immune performance and lower survival rates than birds fed contaminant-free diets. Poultry species are the most susceptible food animals to AFB1 and even small amounts of AFB1 in feed, where upon consumption results in significant adverse health effects, growth retardation,

immunological alterations and histological changes in the liver which may result into death (Devreese, *et al*., 2013). When aflatoxins are present in feed and food, they are potentially hazardous to the health of the animals and human beings due to their carcinogenic and mutagenic effects (Shazad, *et. al.*, 2014). Poultry products (meat and eggs) derived from chicken consuming contaminated diets are crucial in safe food chain, hence special attention should be directed towards determining and preventing possible contamination in the chain (Radmila, *et al.*, 2009). When feed contain more than one complex and diverse mycotoxins, poultry will show various responses associated with toxins responsible. (Anonymous, 2015). It is also reported that mycotoxins may act independently or interact with genetic, hormonal and age factors (Nutriad, 2015). Interaction of mycotoxins in the animal often leads to a more adverse mycotoxicosis and alter clinical manifestations of individual mycotoxins hence complicating field diagnosis (Durali., 2014). Aflatoxins are known to be carcinogenic, mutagenic and teratogenic in several species of animals. The target organ in aflatoxicosis is the liver but some studies have reported a high level of aflatoxicosis in such organs as the brain, kidney, myocardium and the lung and causes cirrhosis and acute necrosis of the liver (Gulyas, 2013; *http://www.mycotoxins.org/ 2017).* Clinical signs of mycotoxin poisoning are either classified into acute, sub acute, or chronic poisoning. Acute poisonings have a violent course and dramatic reaction in the body of the organism. Chronic and sub-acute poisoning are caused by low doses of aflatoxin consumed for a long time, and these may cause liver cancer (aflatoxins) or disorders of the kidney associated with ochratoxins as reported by Lazicka *et al* (2010).

2.2.5 Specific effects of Aflatoxins

2.2.5.1 Effects of aflatoxins on eggs

Contamination of the diet with mycotoxins increase late mortality of embryos, increase the number of infertile eggs and impair hatchability (Nutriad, 2015). Aflatoxins reduce carotinoid content, which also prevent the oxidation and destruction of fragile, vital substances such as vitamins in egg yolk and elevated plasma uric acid level (Nutriad, 2015). Mallmann *et al.,* (2011) reported that embryo mortality in eggs intoxicated with aflatoxins happens because these substances, after being biotransformed in the liver, is eliminated from the body through the egg yolk especially aflatoxin M_1. Aflatoxin B_1 and aflatoxicol is found in the yolk within 24 hours after ingestion hence contaminating the yolk, egg white and egg shell. The shell becomes thicker, thus affecting hatchability due to reduction in gas exchange, by reducing the number of air spores on the egg shell, between the embryo and the environment (Mallmann *et al*., 2011). Besides reducing the production of eggs, aflatoxicosis also induces a reduction in egg size, as well as the proportional reduction in the size of the yolk, due to the damage caused in the lipid and protein synthesis (Mallman *et al*., 2011). In layer hens feed intake and egg production can be reduced and irregular pigmentation may occur on brown eggs (Durali *et al.,* 2014). Oliveira *et al* (2000) reported that AF in laying hen feeds can result in an AF residue in the eggs with a feed to egg AFB1 transmission ratio of approximately 5000:1); therefore it is very important to control AF concentrations in feeds for laying hens.

2.2.5.2 Effects of aflatoxins on meat

Mould contamination of frozen chicken meat and giblets may lead to their spoilage and production

of mycotoxins with potential health hazards to human due to their carcinogenic effects, liver diseases and organ damage (Darwish, *et al*., 2014).

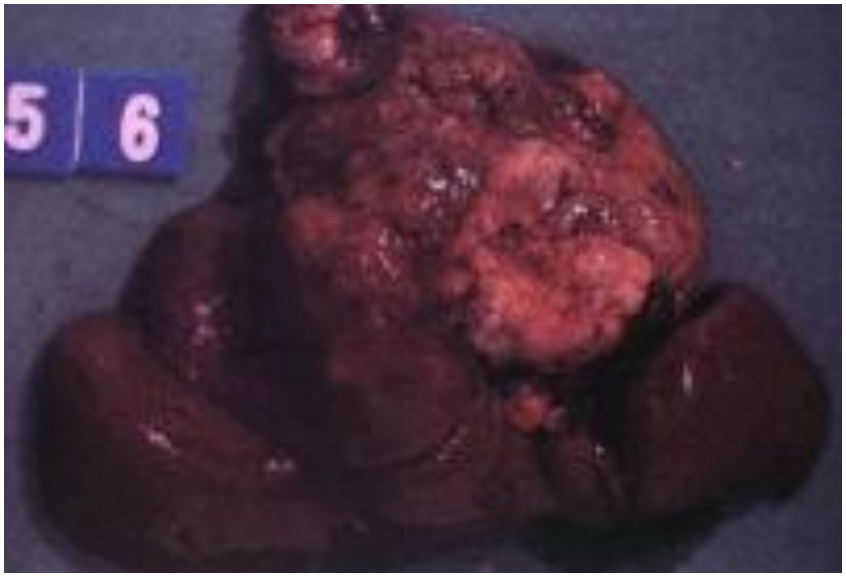


Plate 3 A tumorous liver of a rat fed on aflatoxin B1 (*http//www.ansci.cornell.edu, 2015*)

2.2.5.3 Effects of aflatoxins on immunity of poultry

Trichothecenes causes decreased resistance to environmental and microbial stressors and increased susceptibility to disease, anorexia, egg shell quality decreases, impairs feed conversion ratio (Nutriad., 2015). Acute form of aflatoxin poisoning in poultry is rare because they are considerably resistant to it (Radmila *et al.*, 2009). All animal species are susceptible to aflatoxin, but outbreak occurs mostly in pigs, sheep and cattle (Radostits *et al*., 2000). Aflatoxin can cause oncogenesis, chronic toxicity or peracute signs depending on the species, age of animal, dose and duration of aflatoxin exposure (Smith, 2002). Low

or undetectable levels of aflatoxins are responsible for reduced production and increased susceptibility to infectious diseases (Nutriad, 2015). Mycotoxic effects include mutagenesis due to alkylation of nuclear DNA, carcinogenesis, teratogenesis, reduced protein synthesis, and immunosuppression. Aflatoxin has the greatest potential effects and can to bind to DNA and RNA and inhibit synthesis of macromolecules through interference of transcription and aspects of protein synthesis (Murugesan, *et al.*, 2015). Immunosuppression in chickens can be favoured by several factors such as nutritional, management, diseases and stress which suppress immune functions and may decrease resistance to other infections (Biomin, 2017).

Epidemiological data of recent studies has indicated that there's a high correlation between Newcastle disease outbreaks and aflatoxin contamination of broiler rations (Yunus *et al.*, 2011). Aflatoxin is an inhibitor of protein synthesis and represses thymus gland development and the weight of the bursa of Fabricius, resulting in serious deficiencies in cellular and antibody responsiveness of chicken immune system (Celik *et al.*, 2000). Inhibited macrophage functions, T lymphocyte activity and or cytokine expression by aflatoxin, causes vaccine pathogen persistence which leads to low immunoglobulin production (Verma *et al*, 2004; Yunus *et al*, 2011). Evidence has shown that immunosuppression aflatoxins results in disease outbreaks, poor antibody titres and vaccination failures (Devegowda *et al*, 2005).

2.2.5.4 Effects of aflatoxins on economy

According to the Food and Agriculture Organization (FAO), more than 25% of the world's agricultural production is contaminated with mycotoxins, resulting in economic

losses in the grain industry (Bullerman *et al.,* 2011; Lawlor and Lynch, 2005). Animals are considered the most exposed to high concentration of aflatoxins through feedstuffs that resulting into several health problems resulting into large economic losses. These losses are well pronounced in meat and eggs in terms of quality and quantity as a result of contamination with aflatoxins residues (Bintvihok *et al*., 2002; Hall and Wild, 2003; Farombi, 2006). Aflatoxin losses to poultry producers from aflatoxin-contaminated feeds include death and immune suppression, reduced growth rates, and losses in feed efficiency hence negatively affecting productivity (Cornell University, 2015). Aflatoxins have a significant impact on poultry health, thus affecting international trade (Zhang *et al*., 2013). Low levels of residual AFB1 in poultry feeds can cause reduction in growth, feed conversion, egg production and compromised immune functions, resulting in significant economic costs to producers (Melissa *et al*., 2015). Discovery and isolation of aflatoxin back in 1960, as Turkey-X disease, when several thousands of turkey poults in the United Kingdom died (Mallmann *et al.,* 2011). Aflatoxin contamination remains a threat to the chicken production and results in economic losses to producers due to sub-lethal toxic effects (Chen *et al*., 2013). The carcinogenic effect and related diseases in humans given their seemingly unavoidable occurrence in foods and feeds make the prevention and detoxification of these aflatoxins one of the most challenging toxicological issues of present time (Cornell University, 2015)

2.3 Feeds

Masked mycotoxins are chemically modified by specific biochemical reaction in which mycotoxins can be bound to certain ingredients in feeds for instance glycosides, fatty acid esters and proteins This makes them undetectable by conventional methods of analysis but

are unmasked during digestion process; thus affect the animal (*http://www.mycotoxins.info/myco-info/ani*, 2015). Broad variety of information on the effects of individual mycotoxins in various animal species, are offered but concurrent exposure to multiple mycotoxins is more likely in livestock industry and thus lowers production (Zaki *et al*, 2012). Along with primary contamination of crops, aflatoxins can be transferred to meat and eggs of poultry fed on these toxins (Melissa *et al*., 2015). The economic impact of aflatoxins on poultry production is related to direct effects on health and grain (feed) rejection (Antonissen *et al*, 2016).

Effects of climate change are a threat to food and feed security in many regions of the globe, including sub-Saharan Africa (Wheeler and Von Braun, 2013). Previous studies have indicated the presence of mycotoxins in poultry feed (Bryden, 2012; Astoreca *et a*l., 2011). They mainly contaminate cereals and grains such as rice, maize, sorghum, millet, groundnuts and dried cassava during the storage and poor processing conditions (Bbosa *et al*., 2013). Aflaoxins are produced during stages such as: production, harvest, transportation, storage and food processing (Murphy *et al*., 2006). Miller (1994) reported that animal feeds with ingredients such as oil seed cakes, peanuts and coconut cake or corn grits often contain aflatoxins. A global survey on mycotoxin done in 2013 revealed that 81% of 3,000 grain and feed samples that were analyzed had at least one type of mycotoxin (Murugesan, *et al.,* 2015). Mycotoxins are highly resistant toxins and therefore, their destruction by conventional food processing is difficult (Murphy *et al*., 2006). *Fusarium* species are the main contaminants of poultry feed hence are responsible for reduced performance and increased susceptibility to infections (Nutriad, 2014). The fungi grow and proliferate well in cereals, especially maize, wheat and sorghum, where

they find a highly nutritious substrate for their development. The fungal growth and aflatoxin production in cereals may occur at different stages of development, maturation, harvesting, transporting, processing or storage of grain. Therefore, the reduction of grain moisture by drying is important for the reduction of the levels of contamination (Mallmann & Dilkin, 2007).

Nutritive value of infected grains and cereals drops after contamination by moulds (Iheshiulor, 2011) and fungi are known to consume fats, protein and carbohydrates making them unavailable to the animal (Zaki *et al.*,2011). Mycotoxins also affect the alimentary value and organoleptic characteristic of feed and hence a risk of toxicosis (Inheshiulor *et al*, 2011).

2.3.1 Effects of contaminated feed on chicken productivity

Several studies have shown that animals consuming contaminated hay or feed, there's increase disease incidence and reduced production efficiency. Some of the gross effects of mycotoxins can include: 1) Reduce feed intake and feed refusal 2) Reduction in nutrient absorption and metabolism 3) Digestive disorders including haemorrhage and necrosis 4) Tissue and organ damage 5) Gangrene of the extremities 6) Endocrine effects and skin lesions 7) Reproductive disorders, embryonic death, abortions and oral lesions 8) Nervous disorders, tremors and poor coordination 9) Suppression of the immune system 10) Death (Prairie View A & M University, 2012). Andretta *et al.*, (2011) and Chen *et al* .,(2013) observed that an average aflatoxin concentration of 0.95 mg/kg reduced both feed intake and daily weight gain by 11 percent and worsened feed conversion by 6 percent.

2.3.2 Effects of aflatoxins on human beings

Aflatoxins B1 are human carcinogens while fumonisins, like AFB1, are carcinogenic and may contribute to neural tube defects (Han *et al*., 2008). Aflatoxin B_1 is the most prevalent and toxic form for humans due to its strong carcinogenic, mutagenic and teratogenic effects (Santacroce *et al*., 2008 & Han *et al*., 2008). It has also been demonstrated that AFB1 is associated with a high incidence of human cancers of the liver, breast, prostate, and gastrointestinal tract; protein-energy malnutrition in children as well is linked to progression of HIV infection, especially in low-income countries (Cardwell, 2001; Turner *et al*., 2007; Wild *et al*., 2010; Wu *et al*., 2011).The body excretes these toxins and research has shown that elimination of Aflatoxins especially AFB_1 from tissues is rapid in older poults than in younger ones (Yunus *et al*., 2011).

Table 1 Effects of aflatoxin on human health

Organs/ Systems affected	Aflatoxin effects
Nervous system	Abnormal Behavior, depression
Reproductive System	neonatal outcomes-low birth weight
Growth	Reduced growth rate
Gene and Gene Expression	Teratogenic effect (birth defect) due to base transversion, mutation and glutathione alterations
Gene and Gene Suppression	Carcinogenic effect—higher incidence of cancer
Immunosuppression	Decreased resistance and susceptibility to, HIV, TB, and other opportunistic infections due to effect on lymphocytes and T-cells

(Source: Dr Subroto Mukherjee USAID/East Africa, 2015)

2.4 Predisposing factors to mycotoxicosis

Approximately 4.5 billion of the world's population is exposed to aflatoxins (Williams *et al*., 2004) due to its worldwide presence. Factors that determine aflatoxin contamination in feed include; climate of the region, the genotype of the crop grown, soil type, minimum and maximum daily temperatures and daily net evaporation (Strosnider *et al*., 2006). Studies have shown that, aflatoxin contamination is promoted by stress or damage to crops before and after harvest, due to drought, insect activity, poor timing of harvest, heavy rains during and after harvest, inadequate drying of the crop before storage and levels of humidity, temperature and aeration during storage (Cotty & Jaime-García, 2007; Paterson & Lima, 2010; *www.intechopen.com*). Temperatures above 27°C (80°F), humidity levels greater than 62% and moisture levels in the feed above 14% enhances the synthesis of aflatoxins in feeds (Royes and Yanong, 2002). Environmental conditions such as heat, water, and insect damage cause plant stress and predispose plants in the field to aflatoxin contamination (Prairie View A & M University, 2012).

2.5 Food safety levels

Aflatoxic compounds can contaminate food and feedstuffs and these contaminated materials may be pathogenic for animals and humans; therefore, one of the most effective measures to protect the public health is to establish reasonable regulatory limits of these mycotoxins. Consequently, guidelines regarding the allowed levels of mycotoxins present in food and feed products and in raw materials have been established by the FAO (2001). Commercially accessible multiple mycotoxin analysis assist in greater understanding of overall risk to animals as well as allowing for development of improved mycotoxin management solutions (Durali *et al*., 2014). World Health Organization (WHO) classifies

AFB1 as a human carcinogen and proposes no safe dose (El-Yazeed, *et al.*, 2015). However the objectives of food and feed safety measures are to guarantee a high level of protection of human health and of consumers' interests while also taking into account animal health and welfare, plant health and the environment (Rentokil, 2013)

Table 2: U.S. Food and Drug Administration (FDA) action levels for total aflatoxins in food and feed

Feedstuff/ Products	Concentration (ppb)
All products, except milk, designated for humans	20
Corn for immature animals and dairy cattle	20
Corn and peanut products for breeding beef cattle, swine, and mature poultry	100
Corn and peanut products for finishing swine (>100 lbs.)	200
Corn and peanut products for finishing beef cattle	300
Cottonseed meal (as a feed ingredient)	300
Milk	0.5

Most of these set levels /standards are accepted in most parts of the world (Dohlman, 2004). In Kenya, these levels have been lowered, by KEBS, to 10ppb in feedstuffs and animal products except for milk. These safety levels (Table 2) have been set to control levels of mycotoxin/aflatoxin so as to minimize their effects on man and animals who consume the feeds and products. Some organizations, like FDA, are in the forefront to ensure these standards are adhered to in most parts of the world. In each country, there is a legislative framework which regulates the levels of toxins, like aflatoxins,

contaminating food and feedstuffs especially those deemed toxic to human and animal health. These safety regulatory standards have implications for both international and local trade of cereal crops which can result in barriers for the export or import of commodities and products from most parts of the world (Chen *et al*., 2013).

Tolerance levels: Dietary levels of aflatoxin (ppb) generally tolerated are ≤50 in young poultry, ≤100 in adult poultry, ≤50 in weaner pigs, ≤200 in finishing pigs, <50 in dogs, <100 in calves, and <300 in cattle (Osweiler, 2014).

2.5.1 Known organizations / commissions concerned with mycotoxins

Organization	**Reference**
Codex Alimentarius Commission (Codex)	*(www.codexalimentarius.org/)*
FAO - Food and Agriculture Organization	*(www.fao.org)*
FDA- Food and Drug Administration (USA)	*(www.fda.gov)*
KEBS- Kenya Bureau of Standards	*(https/www.kebs.org/#)*
PACA- Partnership for Aflatoxin Control in Africa	*(https/www.aflatoxinpartnership.org/)*
WHO - World Health Organization	(FAO/WHO, 2001)

2.5.2 Methods of preventing and controlling mycotoxins

Mycotoxicoses are generally not successfully treated with medical approach but preventive approach is preferred- this approach deals with recognition of risk factors and avoiding or reducing exposure (Osweiler, 2014). Aflatoxins accumulation often occur during storage and therefore post-harvest control measures aims to minimize fungal growth and aflatoxin production (Eva *et al.*, 2011). Best management practices are aimed

at prevention of the occurrence of mycotoxins during harvesting and storage, inactivation of the preformed toxin in grain or feed and in the GI tract (Osweiler., 2014). Control of mycotoxins during growing seasons and storage is a crop management problem and food technology consideration respectively (Tumbleson, *et al.*, 2006). Other preventive measures include: Testing of suspect grain at harvest, maintaining clean and dry storage facilities, using acid additives to control mould growth in storage and reducing storage time of prepared feeds (Osweiler, 2014). Controlling moisture in feed materials from which the feed is prepared, complete elimination of all moldy feed from all feed manufacturing and handling equipment, use of chemical mold inhibitors such as: (1) individual or combinations of organic acids (propionic, sorbic, benzoic, and acetic acids), (2) salts of organic acids (calcium propionate and potassium sorbate) and (3) copper sulfate, use of probiotics and use of resistant domestic poultry (Melissa *et al.*, 2015). Biologically, use of different microorganisms such as *lactobacillus pentosus* and *lactobacillus brevis* is considered another way of reducing aflatoxin in livestock feed (Hasheminya and Dehghannya., 2013). Prevention and control will make a significant contribution to poverty alleviation, food security and the early detection and control of zoonotic diseases (FAO, 2008b).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

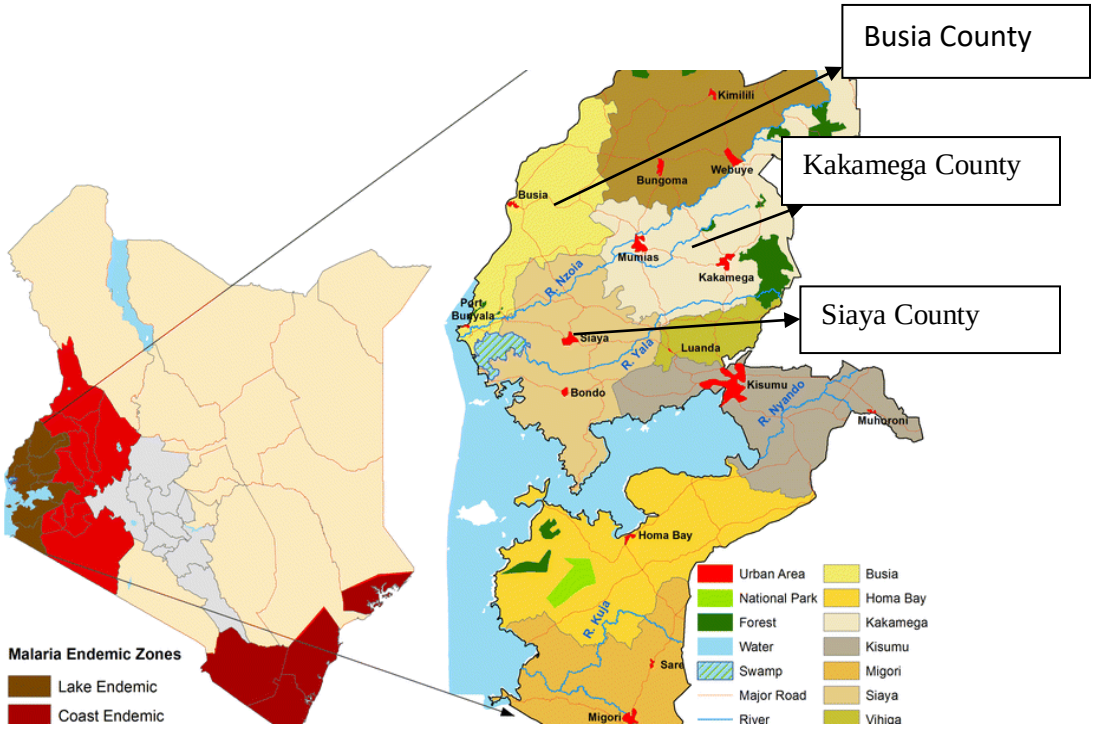


Figure 6: Map of area of study

Coordinates (GPS): Busia: Latitude: $0^0$25'59.99N; Longitude: $34^0$08'60.00E. Elevation: 1198m *(http//latitude.to/articles-bycounty/ke/Kenya/41241/Busia-County);* Kakamega: Latitude: $0^0$16'60.00N; Longitude: $34^0$45'0.00E. Elevation: 1240m-2000m *(http//latitude.to/articles-by-county/ke/Kenya/40817/Kakamega-county);* Siaya: Lat: $0^0$04'60.00N; Long: $34^0$14'60.00E. Elevation 1319m (4327 feet) *(http//latitude.to/articles-by-county/ke/Kenya/39121/Siaya- County)*

A baseline survey was carried out (Appendix 5) in three Counties of Western Kenya namely; Siaya, Busia and Kakamega. Three sub counties per County were selected, viz: Siaya (Gem, Alego and Ugenya.), Busia (Teso South, Matayos and Nambale) and in Kakamega (Lurambi , Lugari and Navakholo).

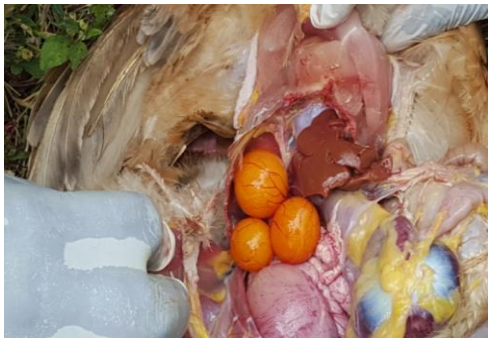
3.2 Sampling of farms and sample collection

This formula was used to determine sample size; $n = \frac{Z^2 p(1-p)}{1^2}$ (Fischer, 1998)

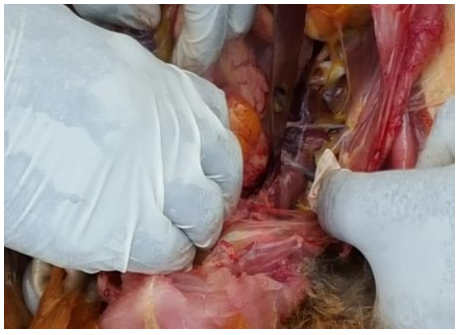
Where; **n** - sample size of population>10,000; **Z** -Normal deviation at desired confidence interval (95%); **P** - proportion of population with desired traits; $\mathbf{1^2}$ - degree of precision

A total of 180 farms were selected, sixty (60) in each County. A multi- stage sampling technique was used. Counties were purposively selected based on indigenous chicken population and activeness (still engaged actively in indigenous chicken production) of the poultry farmer groups. In every County, 3 sub Counties were purposively selected and in each these sub Counties, two Wards were identified. In each Ward two locations were identified where two farmer groups (a youth and a women group) were selected and from each group, five members were purposively selected to complete a questionnaire (Appendix 6). These members had mixed flock as per age (12-16weeks & >36 weeks old), 10 or more chicken (population) and layer(s). Together with two (2) University of Eldoret students at masters' level and two (2) undergraduate interns under training and frontline field Extension Officers, the survey was conducted from late February (21/02) to mid-March (12/03) 2016. Feed Samples were collected from the first, third and fifth farmer in each group. Egg samples were collected from the first and fifth farmers while chicken were obtained from the first identified farmer in each group. This farmer gave two chicken one young (12-16 weeks old) and one adult (> 36 weeks old). A total of 60 eggs were collected and 60 chicken were sacrificed and from each chicken approximately 30g of liver, breast muscle, thigh muscles and 15g of kidney (they are smaller) samples (Appendix 2) were collected for laboratory analysis, (according to analyzing lab recommendations; 10-30g per sample) giving a total of 240 tissue samples. A total of 260 feed samples were collected and 60 of them were sub-sampled (from these samples) and

submitted for proximate laboratory analysis to establish nutrient composition. Tissue samples were collected and put in well labeled zip-lock bags and kept in a cooler box with ice packs (Plate 4; c & d) and later stored in a freezer (model: No RLFF 13242; SERIAL NO: 2G0341.RF/136) at-15º C. (Appendix 1). Feed samples for aflatoxin analysis was done by another researcher.



a



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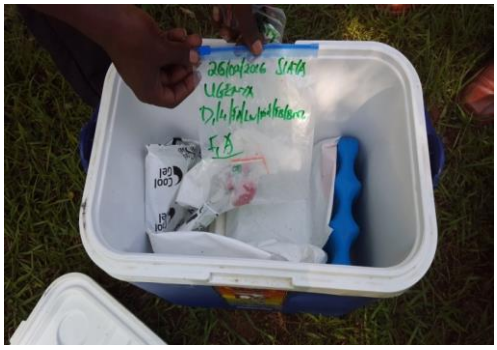

d

Plate 4: Chicken tissue samples (a & b) are packed (c) and preserved (d)
(Source: Author, 2016)

3.3: Sample analysis

3.3.1: Feed sample analysis

Unground feed samples (60) were submitted to the Laboratory (KALRO-Naivasha Laboratory) which was then ground to pass through a 1mm sieve before analysis. Feed

samples were weighed on a weighing machine – (OHAUS^{TM}), PA413 (OHAUS Corporation USA; *www.ohaus.com* and were subjected to proximate analysis method (AOAC, 2000) to determine dry matter (DM) (%), crude protein (CP) (%) and ash (%).*)*. DM content was determined by drying samples at 105^{o}C for 20 h. Crude protein was determined by Kjeldahl method using a digester block Gerhardt^{R} Kieldatherm Digester (Kjedal) and was distilled in a distillation machine – (Kjeltec 8200 Auto Distillation, Type 10014901; Yr, 2011. Serial No.91708870, FOSS). Crude protein value was achieved by multiplying the nitrogen by correction factor 6.25 which is based on the fact that most protein contains 16% nitrogen (AOAC, 2000). Ashing was carried out in a muffle furnace - Heraeus Hanau, Type MR 170, Holland (WC Heraeus GMBH, Hanau) for 3 h at 550^{o}C.

3.3.2: Egg and tissue sample analysis for total aflatoxins

Eggs samples were submitted to the laboratory (Appendix 3) at room temperature (Plate 5: 1). Eggs were broken and the albumin and yolk poured out into a beaker (Plate 5: 2 & 3). Egg samples were analyzed using competitive ELISA method (Zhang *et al*., 2011) where 70% Methanol (to extract aflatoxins) was added to 20g of sample, stirred for 10 minutes and filtered in a whattmans paper (Plate 5: 3). Conjugate, substrate and antibody were mixed in microwells (Appendix 4) before inserting into the aflatoxin kit (***helica***– Total Aflatoxin Assay, Cat. No.941AFL01M-96, USA) where analysis was done and the results are released in a sheet of paper (Plate 5: 4 & 5)

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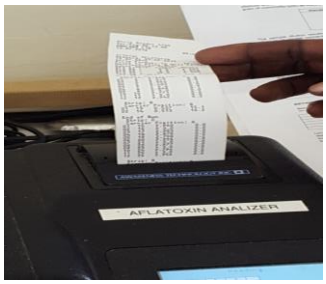

5

Plate 5: Egg samples are stored well in cold chamber of the fridge (1), then broken (2), contents are poured in a beaker, stirred and filtered (3). Microwells are inserted into microwell-holder in the analyzer (4) and the results are printed in a sheet of paper (5).

(Source: Author, 2016)

Chicken tissue samples were submitted to the laboratory when frozen and subjected to competitive ELISA method (Zhang *et al*., 2011). Each tissue sample weighing 10g was ground and 50ml of 70% methanol was added and shaken for 20 minutes using a shaker. The sample was then centrifuged for about 10 minutes at 4000 rpm and the resultant fluid (supernatant) is mixed with 1ml of distilled water. The content was then poured into microwells (containing conjugate, substrate and antibody) at 50µl/well. The wells were then inserted into the analyzer (Model: 1055-04, MaxSignal®, Bioo Scientific Corporation; Total Aflatoxin ELISA Test Kit, USA, 2008; *www.biooscientific.com*) and the results are read.

3.4 Data analysis

Survey data collected was based on; indigenous chicken production and production systems. The survey data collected was analyzed by use of Statistical Package for Social Sciences (SPSS) Version 25. Feed data was analyzed based on, Dry matter %, Crude protein % and Ash % using Genstat 14th Edition. Tissue and egg data were analyzed based on aflatoxin levels on; age of chicken (adult and young), group type (women and youth), Counties and sub Counties using Genstat 14th Edition.

CHAPTER FOUR

RESULTS

4.1 Indigenous chicken and production system

Types of chicken reared, among rural households, in this region included indigenous, improved indigenous (genetically improved for traits like growth rate, broodiness and weight gain), crosses and others (exotic chicken, turkeys, ducks & geese). Indigenous chicken were common among other types (Table 3).

Table 3: Chicken types and their number in Siaya, Busia and Kakamega Counties in 180 households

County	Types and number of chicken			
	Indigenous	**Improved indigenous**	**Crosses**	**Others**
Siaya	1768	455	78	37
Busia	2547	57	11	10
Kakamega	1882	861	57	80
Total	**6197**	**1373**	**146**	**127**
Percentage (%)	**79.01**	**17.51**	**1.86**	**1.62**

Free range and semi free range systems were the most preferred in the three Counties; Farmers practicing free range production system were 60%, 58% and 29% in Kakamega, Busia and Siaya Counties, respectively. Semi-free range production system was practiced by 35%, 42% and 70% of farmers in Kakamega, Busia and Siaya Counties, respectively while Intensive production system was minimally practiced in Kakamega (5%), Busia (0%) and Siaya (2%), (Table 4).

Table 4 Chicken Production Systems in Siaya, Busia and Kakamega Counties of Western Kenya

Counties	Production systems (%)		
	Free range	Semi free range	Intensive
Siaya	28.8	69.5	1.7
Busia	58.3	41.7	0.0
Kakamega	60.0	35.0	5.0

Table 5 Proportion of farmers practicing FR, SFR and Intensive systems in Siaya, Busia and Kakamega Counties

		Production System (%)		
County	**Sub-County**	**FR**	**SFR**	**Intensive**
Busia	Teso South	65.0	35.0	0.0
	Matayos	50.0	50.0	0.0
	Nambale	80.0	20.0	0.0
Kakamega	Lurambi	40.0	55.0	5.0
	Lugari	70.0	30.0	0.0
	Navakholo	70.0	25.0	5.0
	Gem	45.0	50.0	5.0

Siaya	Alego Usonga	45.0	55.0	0.0
	Ugenya	65.0	35.0	0.0

FR-free range; SFR-semi free range

Most of the chicken farmers in Nambale , Lugari and Navakholo sub-Counties practiced free range system at 80% , 70% and 70%, respectively, while semi free range system was equally practiced in Alego , Lurambi , Matayos and Gem at 55%, 55%, 50% and 50%, respectively. Less than 5% of the farmers practiced intensive system across the sub-Counties as shown in Table 5. This was an indication that intensive system is not popular among the poultry farmers particularly in rural areas where there's still space for chicken to scavenge.

Table 6 Duration of farmers' involvement in different chicken production systems

Production System %	**Duration of keeping chicken (Years)**			
	<5	**5-10**	**11-15**	**>15**
Free range	38.6	23.9	11.4	26.1
Semi free range	58.0	17.0	4.5	20.5
Intensive	75.0	25.0	0.0	0.0

Most farmers (75%) who practiced intensive and semi free range (58%) systems had done so for less than 5 years while those who practiced free range for the same period of time were only 38.6%. However, 26.1% and 20.5% of the farmers had practiced free range and semi free range, respectively, for over 15 years (Table 6).



1 2 3

Plate 6 Indigenous chicken kept under free range (1), semi free range (2) and Intensive (3) systems of production taken during data collection (Source: Author, 2016)

Table 7 Reasons of keeping indigenous chicken in Busia, Kakamega and Siaya Counties

County	Reason for poultry keeping (%)				
	Economic	Nutritional	Cultural	Health	Other
SIAYA	40.0	16.7	3.3	36.7	3.3
BUSIA	31.7	26.7	6.7	30.0	5.0
KAKAMEGA	38.3	21.7	3.3	26.7	10.0
MEAN	**36.7**	**21.7**	**4.4**	**31.13**	**6.1**

Indigenous chicken were kept for reasons such as; economic, health, nutritional and cultural values. Indigenous chicken are kept majorly for economic (36.7%) and for health (31.13%) reasons. Cultural reasons (4.4%) and other reasons (6.1%), like breeding, sports, hobby, were not valued much during the study as shown in Table 7.

Table 8: Influence of famer age on production system in chicken production

	Age Category			
	0-19 (young youth)	**20-40 (Mature youth)**	**41-60 (Adults)**	**>60 (Elders)**
Free range	0.9	55.7	33.0	10.4
Semi free range	1.4	45.1	42.3	11.3
Intensive	0.0	0.0	66.7	33.3
Mean	**0.7**	**33.6**	**47.3**	**18.4**

NB The age grouping is applied for the purpose of this research only.

Most youths (55.7%) aged between 20-40 years preferred keeping poultry under free range system compared to adults aged between 41-60 years (66.7%) who preferred intensive system. Most indigenous chicken farmers (47.3%) were of age category 41-60 years but only 0.7% was of age category 0-19 years (Table 8).

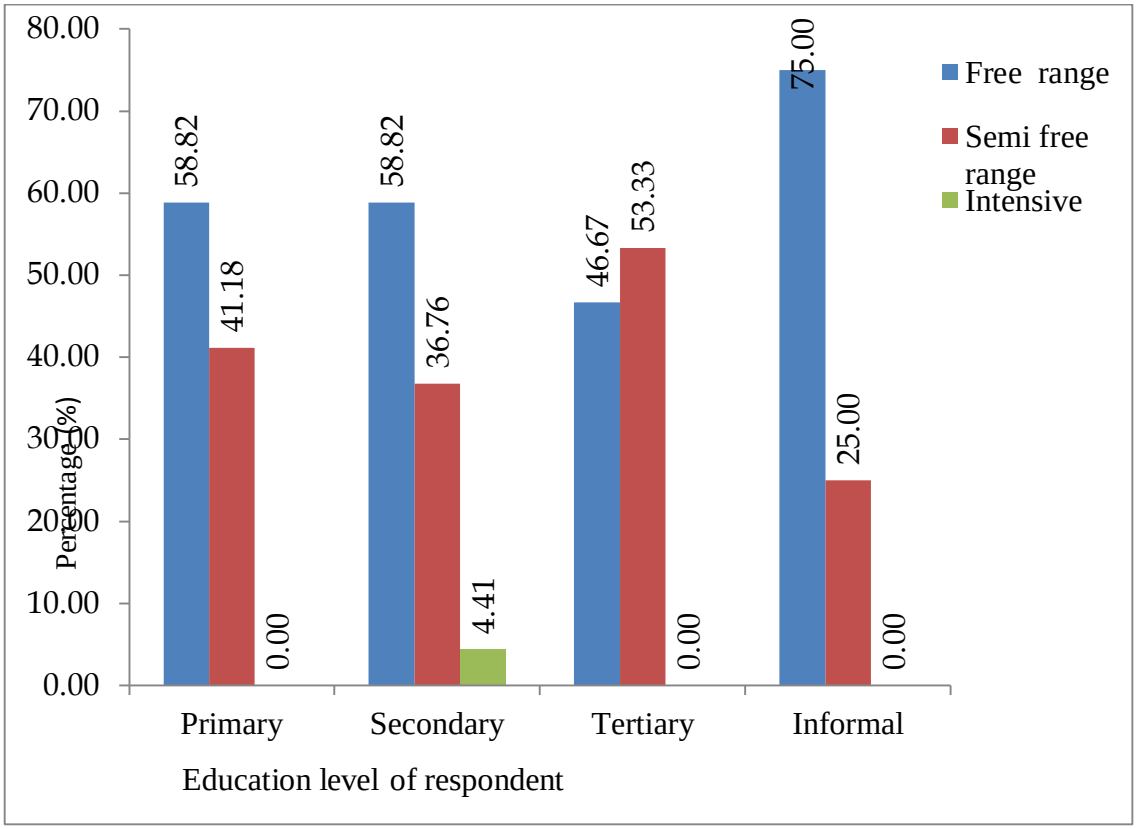

Figure 7 Education level versus production system

Education level of respondents (primary, secondary, tertiary and informal) influenced the kind of production system engaged in. Most (75%) of the respondents with informal education engaged in the free range, those with secondary level (4.41%) of education tried the intensive system of production. On the other hand those with tertiary level of education (53.33%) preferred semi free range system as shown in Figure 7.

4.2 Feeds

Proximate analysis was carried out to determine DM%, CP% and ASH% as shown in Table 9. Maize and sorghum (Plate 1) was the most common feed ingredient, across the

study area, thus it is necessary to compare their nutrient composition as per the regions so as to form a basis of feed rationing. More comparison are shown in Table 9

Table 9: Mean nutrient composition of maize and sorghum as indigenous chicken feed ingredients

Nutrient (%)	Counties		
	Busia	**Kakamega**	**Siaya**
		Maize	
DM	89.67±0.19 •	88.75±0.19	88.94±0.20
CP	7.56±0.20	7.64±0.30	8.90±0.40
ASH	0.90±0.11	0.82±0.10	1.33±0.10
		Sorghum	
DM	90.12±0.30	89.56±0.03	89.13±0.15
CP	10.68±0.60	9.77	9.82±2.0
ASH	1.99±0.25	2.64±0.06	2.0±0.11

•Mean ± SEM (standard error of mean)

Cassava, fed to chicken broken, ground or boiled, was common as a chicken feed in Busia and Siaya counties. Therefore the study sought to establish its quality as shown in Table 10. Cassava from Siaya was high in ash content as compared to that from Busia.

Table 10 Mean nutrient composition of cassava as feed ingredient for indigenous chicken

	Cassava in counties	
	Busia	**Siaya**
DM	89.2±0.19 •	89.6±0.23
CP	2.60±0.33	2.23±0.32
ASH	2.17±0.09	3.05±0.48

•Mean ± SEM (standard error of mean)

Groundnuts were common in Kakamega and Siaya counties and their nutrient quality is shown in Table 11. Groundnuts from Kakamega County have high ash content than those from Siaya County but the CP% is not significantly different between the two areas.

Table 11 Mean nutrient content of groundnuts (%) as feed ingredient for indigenous chickens

Nutrient (%)	**Counties**	
	Kakamega	**Siaya**
DM	93.91±0.63 •	94.08±0.04
CP	20.75±2.24	19.07±1.4
ASH	6.23±2.57	3.4±0.17

•Mean ± SEM (standard error of mean)

Some farmers made their own rations (home-made-rations) using available feed ingredients while others used commercial feeds and analysis became necessary to determine their quality as shown in Table 12. Homemade rations from Siaya had the lowest ash content while that from Kakamega was high in CP content. Commercial feeds from Siaya were high in percent CP and ASH as compared to those from Busia. There is no difference in CP content between the two feedstuffs; however, ASH content was

different with commercial feeds having high values across the Counties as shown in Table 12.

Table 12 Mean nutrient (%) of home-made rations (HMR) and commercial poultry feeds.

Nutrients	Counties		
	Busia	**Kakamega**	**Siaya**
		HMR •	
DM	91.6±0.82 •	91.5	89.6±0.23
CP	10.3±1.77	17.21	7.16±1.18
ASH	16.5±10.15	17.8	4.9±1.83
		Commercial Feeds	
DM	92.23±0.36	92.11±1.03	92.47±0.03
CP	7.58±0.92	11.88±0.61	13.72±0.47
ASH	15.0±2.89	18.99±13.02	21.44±5.25

•Mean ± SEM (standard error of mean), HMR: Home-made rations

The study sought to analyze specific commonly used commercial feeds like layers mash which had high CP% and ASH% content as compared to chick mash and Kienyeji mash as shown in Table 13.

Table 13 Mean nutrient content of specific commercial feeds of indigenous chicken

Feed type	Nutrient levels (%)		
	DM	CP	ASH
Kienyeji mash	91.5 ±0.4 •	9.57 ±2.91	13.84 ±4.05
Layers mash	92.8 ±0.32	12.73 ±1.46	27.45 ±0.76
Growers mash	92.52 ±0.08	10.88 ±2.38	14.16 ±2.04

•Mean ± SEM (standard error of mean)

Feedstuffs used as chicken feed was also compared for quality. Groundnuts have higher CP %, Commercial feeds have the highest ASH% content while maize have the lowest ASH%. The DM% had no difference among the feedstuffs as shown in figure 8. High ash content of layers mash is an indication of minerals incorporated in feed for bone development and egg development for layers.

4.3 Aflatoxin Residues in chicken eggs and tissues

A proportion of farmers in Siaya (68%), Busia (73%) and Kakamega (65%) were well aware of aflatoxins contamination but not the effects on chicken and feed quality. Egg samples in all the three Counties of study area were found to contain traces of aflatoxin as shown in Figure 9.

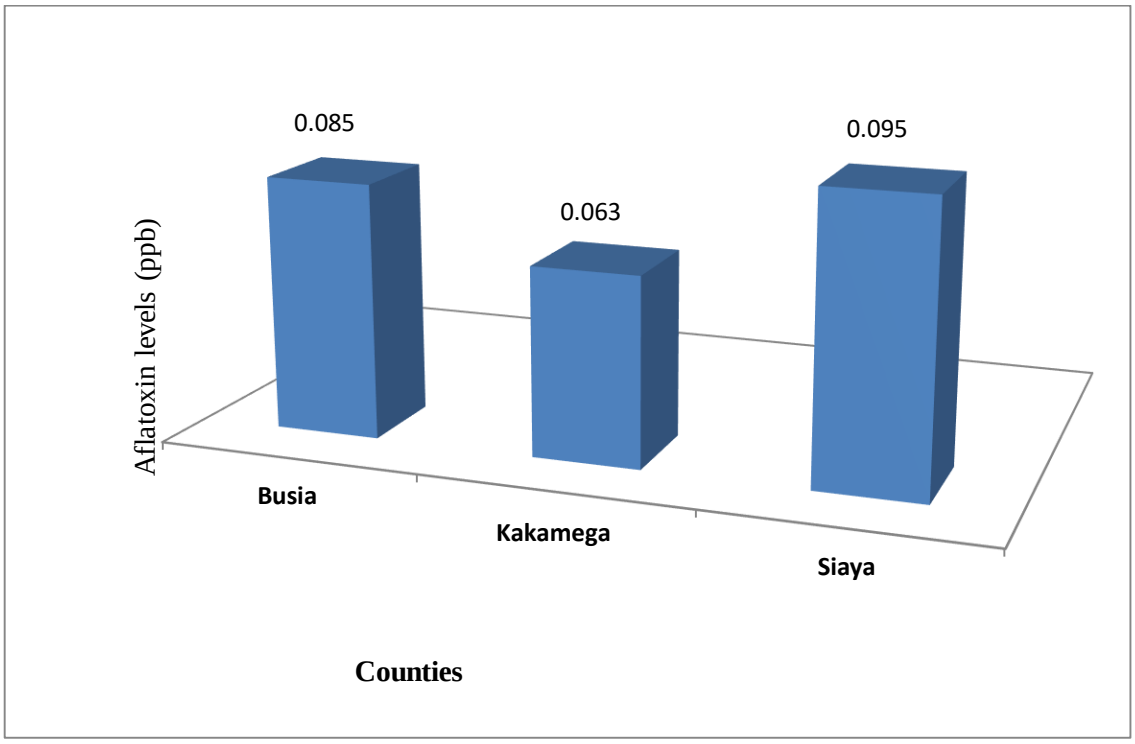

Figure 8 Mean aflatoxin levels (Ppb) in indigenous chicken eggs in Busia, Kakamega and Siaya

Method of handling (or production system) chicken in relation to aflatoxin exposure was one of the objectives of this study, the results revealed that there is no difference in aflatoxin levels in tissues of chicken under free range and semi free range production systems except for thigh muscle (TM) which have high levels in free rangers than those of semi free rangers as shown in table 14

Table 14 Mean aflatoxin levels (ppb) in tissues of chicken kept under two different production systems.

Production system	Tissue	Mean
Semi free range	BM	3.753±0.439 •
	K	1.926±0.387
	L	3.953±0.520
	TM	2.276± 0.511
Free range	BM	3.143±0.677
	K	2.157± 0.771
	L	4.619± 0.857
	TM	3.371± 1.106

•Mean ± SEM (Standard error of mean), BM-Breast muscle, K-Kidney, L-Liver, TM-Thigh muscle

Among the tissues, the liver had high mean level as compared to the rest of the tissues. In Busia, Kakamega and Siaya, liver had 5.24, 2.68 and 4.67 ppb, respectively with a mean of 4.19 ppb. The breast muscle showed a mean of 3.57 ppb. More information is shown in Table 15.

Table 15 Mean aflatoxin levels (ppb) in chicken tissues from three Counties of Western Kenya

County	Chicken Tissues			
	Breast muscle	**Kidney**	**Liver**	**Thigh muscle**
Busia	4.96±0.51 •	0.83±0.40	5.24±0.66	1.76±0.60
Kakamega	2.74±0.84	1.91±0.75	2.68±0.85	3.85±1.17
Siaya	3.01±0.41	3.34±0.54	4.67±0.73	2.38±0.76
Mean	**3.57±0.59**	**2.02±0.56**	**4.19±0.75**	**2.66±0.84**

•Mean ± SEM (standard error of mean)

a

b

Plate 7 Chicken fed on the ground (a &b) are exposed to aflatoxin contamination which in turn contaminate their products. Aflatoxins are present in the soils (Cary *et al*., 2005) and chicken can pick them with feed on the ground (Source: Author, 2016)

Aflatoxin levels in chicken tissues from Busia County were compared. Matayos and Teso South Sub-counties have the highest levels of aflatoxin in breast muscle and liver respectively as shown in table 16.

Table16 Mean aflatoxin levels (Ppb) in chicken Tissues in Busia County

Factors	**Tissues**			
County Level	**Breast muscle**	**Kidney**	**Liver**	**Thigh muscle**

Mean	4.96±0.51 •	0.83±0.40	5.24±0.66	1.76±0.60
S d	2.30	2.15	2.94	2.67
Sub County levels				
Matayos				
Mean	6.28±0.66	1.57±1.37	4.37±1.39	2.33±1.28
S d	1.60	3.35	3.40	3.13
Nambale				
Mean	2.75±1.15	0.95±0.91	4.1±0.82	1.95±1.25
S d	2.81	2.23	2.01	3.07
Teso South				
Mean	5.63±0.30	0.18±0.05	6.75±1.00	1.18±0.79
S d	0.86	0.15	2.83	2.23

•Mean ± SEM (standard error of mean), S d- standard deviation

Women and youth group means had no significant difference including that of age of chicken (young and adult). However, mean aflatoxin level in the kidney from women group is higher than that of youth group while AF mean of thigh muscle of young chicken is relatively high as compared to that of adult as shown in Table 17

Table 17 Mean aflatoxin levels (ppb) in chicken tissues and age of chicken (12-16 weeks and >36 weeks) among women and youth groups in Busia County

	Tissues			
	Breast muscle	**Kidney**	**Liver**	**Thigh muscle**
Women group				
Mean	5.38±0.65 •	1.3±0.78	5.62±0.82	1.6±0.69
S d	2.24	2.71	2.85	2.39
Youth group				
Mean	4.33±0.85	0.11±0.05	4.68±1.12	1.99±1.13

S d	2.39	0.14	3.17	3.21
Age of chicken				
Adult				
Mean	5.65±2.04	0.98±2.61	5.14±2.91	1.33±2.01
S d	0.65	0.83	0.92	0.64
Young				
Mean	4.27±0.77	0.67±0.54	5.34±0.99	2.18±1.03
S d	2.44	1.70	3.13	3.26

•Mean ± SEM (standard error of mean), S d- standard deviation

Levels of aflatoxin (ppb) in chicken tissues from Kakamega County are shown in table 18. AF levels in the BM from Lugari and TM from Lurambi are high while that of Liver from Lugari and BM from Navakholo are low.

Table 18 Mean aflatoxin levels (ppb) in chicken tissues in Kakamega County

Factors	**Tissues**			
County level	**Breast muscle**	**Kidney**	**Liver**	**Thigh muscle**
Mean	2.74±0.84•	1.91±0.75	2.68±0.85	3.85±1.17
S d	3.66	3.34	3.81	5.21
Sub County level				
Lugari				
Mean	4.32±1.87	2.1±1.68	1.88±1.29	4.22±1.80
S d	4.58	4.10	3.18	4.40
Lurambi				
Mean	2.75±1.34	1.23±0.77	3.08±1.52	5.37±3.22
S d	3.79	1.88	3.73	7.88
Navakholo				

Mean	0.86±0.41	2.28±1.38	2.98±1.64	2.43±1.18
S d	0.91	3.89	4.63	3.34

•Mean ± SEM (standard error of mean), S d- standard deviation

The results revealed that mean total aflatoxin (AFT) levels of youth group is higher than those of women groups in all tissues except for the liver which is not different. On the age of chicken, thigh muscle from the young have low mean AFT as compared to that of adult chicken as shown in table 19

Table 19 Mean aflatoxin levels (ppb) in chicken tissues among women and youth groups and age of chicken in Kakamega County

Parameter	Tissues			
	Breast muscle	**Kidney**	**Liver**	**Thigh muscle**
Women group				
Mean	2.02±0.86	0.5±0.15 •	2.78±1.11	3.66±1.13
S d	2.96	0.53	3.83	3.91
Youth group				
Mean	4±1.74	4.03±1.63	2.58±1.42	4.13±2.49
S d	4.61	4.62	4.02	7.02
Age of chicken				
Adult				
Mean	2.44±1.01	1.84±1.06	3.11±1.31	4.99±2.00
S d	3.04	3.35	4.13	6.34
Young				
Mean	3.02±1.36	1.98±1.11	2.25±1.14	2.90±1.23
S d	4.29	3.51	3.62	3.90

•Mean ± SEM (standard error of mean), Sd- standard deviation

Mean AFT of breast muscle from Gem Sub County was low while that of the thigh muscle and liver was high as compared to other sub Counties. Aflatoxin levels in thigh muscle and liver from Alego sub County and that of kidney from Ugenya Sub County were low. Gem sub County had high mean aflatoxin as compared to Alego and Ugenya sub Counties as shown in Table 20

Table 20 Mean aflatoxin levels (ppb) in chicken tissues in Siaya County

Factors	Tissues			
County Level	**Breast muscle**	**Kidney**	**Liver**	**Thigh muscle**
Mean	3.01±0.41 •	3.34±0.54	4.67±0.73	2.38±0.76
S d	1.77	2.34	3.19	3.33
Sub County Level				
Alego				
Mean	3.73±0.43	3.0±0.58	2.921.24±	0.09±0.44
S d	1.05	1.42	3.05	1.07
Gem				
Mean	2.33±0.76	4.61±0.88	5.85±1.06	4.61±1.45
S d	3.33	2.50	2.99	4.15
Ugenya				
Mean	3.24±1.07	1.78±1.07	4.90±1.49	0.58±0.46
S d	2.39	2.17	3.34	1.02

•Mean ± SEM (standard error of mean), S d- standard deviation

Plate 8 Chicken fed together regardless of their ages as captured during the research. Exposure to aflatoxins can be similar but accumulation in tissues can differ. (Source: Author, 2016)

Women and youth groups including the age of chicken were the target in the study in Counties such as Siaya. Mean AFT level of thigh muscle among women group was lower than that of the youth while liver among youth group was high. Adult kidney had high mean AFT level as compared to the young as shown in table 21

Table 21 Mean aflatoxin levels (ppb) in chicken tissues among women and youth group and within chicken age sets in Siaya County

	Tissues			
	Breast muscle	**Kidney**	**Liver**	**Thigh muscle**
Women group				
Mean	3.28±0.40 •	2.96±0.60	3.8±0.96	1.32±0.75
S d	1.33	2.00	3.17	2.50
Youth group				
Mean	2.64±0.81	3.90±0.99	5.88±1.06	3.85±1.38
S d	2.29	2.80	3.00	3.91
Age of chicken				
Adult				

	Mean	3.1±0.72	4.34±0.48	4.61±0.92	2.91±1.13
	S d	2.17	1.45	2.76	3.88
Young					
	Mean	2.93±0.45	2.47±0.85	4.73±1.17	1.91±1.07
	S d	1.44	2.69	3.69	3.83

•Mean ± SEM (standard error of mean), S d- standard deviation

Women and youth were major players of indigenous chicken production in the study area, thus tissues of chicken obtained from these groups were analyzed. AFT levels in kidney and thigh muscle of women group was relatively low as compared to that of the youth as shown in figure 10

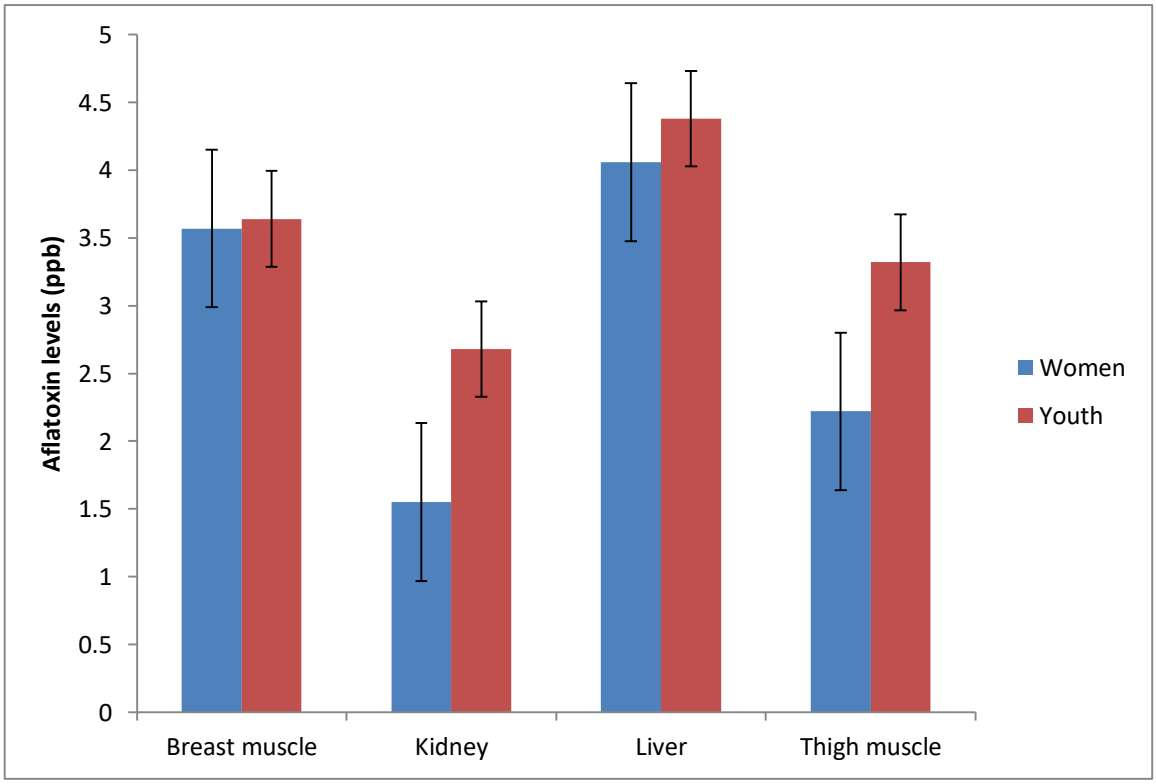

Figure 9 Mean aflatoxin level (ppb) of chicken tissues from women and youth groups across the three counties

CHAPTER FIVE

DISCUSSION

5.1: Indigenous chicken production

The breed of chicken kept in Western Kenya comprised of; Indigenous (79.01%), improved indigenous (17.51%), crosses (1.86%) and others (exotic chicken, ducks, turkeys and quails) (1.62%).This showed that indigenous chicken were popular in this region and regular improvement has been done by use of genetically improved cocks from KALRO Kakamega and Naivasha. Crosses were not common; these are chicken which have been crossed with other common types of chicken like the naked neck. Other breeds especially exotic breeds like brown leghorns were less popular because they prefer intensive system of production and therefore costly to keep. Indigenous chicken are kept under three major production systems; free range, semi free range and intensive systems. Free range was more popular in Kakamega (60%) and Busia (58.3%) as compared to Siaya where Semi free range system (69.5%) was the most popular. Intensive system was not popular with indigenous chicken production although some farmers (5%) practiced it in Kakamega County. This was similar to Sanka and Mbaga (2014) who reported that local chickens were reared under different production systems, mainly scavenging, semi-intensive system and to a lesser extent intensive systems. This could be due to closer attention and feeding required according to the respondents who stated that free range is easy to manage. This is also in agreement with Alders *et al (*2009); Abdelqader (2007); Abubakar *et al*. (2007) who reported that backyard poultry require low inputs /investment to start and maintain as compared to commercial poultry.

Production of indigenous chicken was categorized in terms of years of practice or duration of keeping chicken. It was revealed that most farmers had engaged in indigenous chicken production for less than 5yrs; intensive (75%), semi free range (58%) and Free range (38.6%). This means that intensive system is less popular since it had only been practiced for less than 5 years as compared to free range and semi free range which had been practiced in the last 15 years. This also shows that semi free range is now gaining popularity among indigenous chicken farmers.

This study also revealed that indigenous chicken were kept for various reasons such as; economic (36.7%) where farmers sell live chicken and eggs for school requirements and other domestic chores like food, clothes and utensils. For health reasons (31.13%), respondents claimed that white meat of indigenous chicken, is safer in terms of cancer and allergies. Respondents also indicated that they kept chicken for nutritional reasons (21.7%) and claimed that meat and eggs of indigenous chickens are tastier and nutrient rich compared to those of exotic breeds hence fetched a better price in the market. This is in agreement with the findings of Paresh *et al* (2016). The present study is not in agreement with the findings of. Tarwireyi and Fanadzo, (2013) on their part reported that live indigenous chickens could not compete with the commercial breeds in the market because they are regarded as of poor quality in KwaZulu-Natal, South Africa.

These chicken were also kept for cultural purposes (4.4%); as tokens for visitors, family members and during ceremonies especially in Busia. The study also revealed that age of respondent influenced the type of production system to engage in. Free range was common among 20-40 year old age category (55.7%) while intensive system was common among 41-60 year old age category (66.7%). Semi free range was common

among the 20-40 and 41-60 year old categories at the rate of 45.1% and 42.3% respectively. This can be attributed to the fact that people are realizing the important role of chicken to curb unemployment among the rural poor especially after they leave school or retirement.

Education level of respondents was also shown to influence the production system they practiced. Those with tertiary level of education preferred semi free range (53.33%) as compared to free range (46.67%) while those with informal education preferred free range system (75%).

5.2: Nutritive value of chicken feeds

Indigenous chicken production involved the use of various types of feeds ranging from whole grains to compounded ones that included chick mash, layer mash, grower mash and kienyeji mash. Most farmers fed their chicken on locally available feeds including kitchen waste. Feed quality is an integral part of chicken performance; therefore it is important to know their nutrient composition through laboratory analysis so as to determine daily nutrient intake.

Maize from Siaya County has higher percentage of crude protein as compared to those from Busia and Kakamega Counties. Sorghum from Kakamega was high in ash content as compared to those from Busia and Siaya Counties. Maize was basically lower in CP% and ASH% as compared to sorghum. Cassava had the lowest crude protein content (2.4%) therefore chicken fed largely on cassava diets are likely to run into hypo-protein related conditions like poor growth rate. Groundnuts (peanuts) had the highest mean level of crude protein at 20% as compared to feedstuffs like sorghum, maize and cassava. Thus mixing cassava and groundnuts, according to the birds' daily demand, can take care of

hypo-protein related problems of cassava. Okitoi (2009) reported that locally available feedstuffs, if properly formulated, can provide 21.2% CP which was the best performing level in terms of growth and productivity of indigenous chicken in Western Kenya.

Sorghum had high CP of 10% as compared to maize's 8% which was similar to the findings of Abdo *et al* (2015). Sorghum or maize can substitute each other as part of ingredients in chicken feeds but sorghum is known to contain high levels of tannins (Tahirou *et al*., 2006) which are an anti-nutritional factor that suppress feed intake. Home-made rations from different study areas had different levels of CP like Busia 10.3%, Kakamega 17.21% and Siaya 7.1%. This was largely affected by the type of ingredients locally available and the amount incorporated in the feed. Maize was the most popular ingredient of the homemade rations due to its availability.

Ash% was high in commercial feeds at 18.3% as compared to 13.0% in HMR. This was attributed to use of ingredients targeted to certain type or group of chicken, for example layers, by millers. Among the commercial feeds, layers mash was high in CP at 12.73% and ASH at 27.45%. These feeds were formulated to suit the daily demand of the type of chicken.

However, scanty information is available on nutritional requirements of native chickens or strains for sustainable low input rural poultry production (Raju *et al* 2004; Mandal *et al*., 2005 and Okitoi *et al*., 2006). The information required was largely depending on the purpose of the chicken kept; like for breeding, egg or meat production and age of chicken.

5.3: Aflatoxin residues in chicken products

Aflatoxins in feed consumed by chicken have been shown to pass to eggs and tissues causing contamination of products as expected (Gareis *et al*., 2000). Tissues and eggs of

indigenous chicken contained aflatoxin residues with the latter showing only trace amounts. This could be attributed to the fact that cytochrome P450 enzymes are responsible for detoxification of AFB1 by formation of AFBO in the liver (Tulayakul *et al*., 2005) in chicken. This could suggest why eggs contained only traces of aflatoxins and that eggs take only 24 hours to be laid; while tissues act as deposition (muscles especially the breast muscle) and detoxification sites (liver and kidney) of these toxins. Method of handling chicken (production system) influenced the levels of aflatoxins in different tissues. Liver from chicken kept under free range system was higher (4.619±0.857) than those kept under semi free range (3.953±0.520); this could be due to exposure of chicken to aflatoxins while in the field (free ranging). Chicken consume contaminated waste grains, vegetation, soils, wild seeds, among others, in the field since it is hard to control what they consume. Free scavenging chicken are not supplemented or if any it is minimal, therefore they will consume more in the field. Under semi free range, chicken feeding is controlled because they are fed in the morning and released for a few hours to free range hence less exposed to aflatoxicosis. Among the tissues the liver had the highest mean aflatoxin level across the Counties. These results are in agreement with Darwish *et al* (2016) who analyzed chicken tissues for total aflatoxin in Egypt and found that liver had higher total aflatoxin level compared to other parts of chicken. Research has shown that liver is the target organ of aflatoxin as the site of detoxication thus residues are more often found here (Braese *et al*., 2007). AFT levels in the thigh muscles were different between the two production systems; semi free range (2.276± 0.511) and free range (3.371± 1.106). This muscle is known to be one of the deposition sites of aflatoxins, therefore those under free range move a lot causing high nutrient and or other substance

supply in the muscle. Aflatoxins are also supplied to the tissues and get deposited there after biotransformation in the liver (Bbosa *et al*., 2013). The presence of aflatoxins in chicken tissues regardless of production system is an indication of aflatoxin status of chicken feeds, which shows that the feeds are contaminated with aflatoxins from the farm level. Since these feed ingredients were also human food, there was a tendency of feeding chicken with wasted/rotten grains and the clean ones reserved as human food.

Although there is no safe level (El-Yazeed *et al*., 2015), WHO/FAO have set permitted amounts of 20ppb and KEBS of 10ppb so as to control exposure to higher levels which can be significant to public health. Continuous consumption of aflatoxins in small quantities for a long time (chronic aflatoxicosis) causes cancer especially that of the liver, while those taken in large quantities within a short time (acute aflatoxicosis) cause death. According to findings by Cornell University (2015), 10% of aflatoxins consumed were eliminated from the body and 90% were retained. Thus, long time exposure to aflatoxins is significantly important to public health. This is in agreement with reports by Hussain *et al*. (2010) who found that aflatoxin in flesh/meat of broilers is of significance in public health especially after accumulation of toxins in the tissues. AFT level in different ages of chicken was not different, which could be because these chickens consume the same types of feed materials. As indicated by respondents, chicken were fed together regardless of age (Plate 8), therefore exposure to aflatoxins is relatively similar unlike the exotic ones which are fed according to ages with differently formulated rations.

Busia County showed the highest AF levels in liver and breast muscle while Siaya County had the highest level in kidney; Kakamega County indicated higher level in thigh muscle. These variations could be due to type of feed (levels of contamination, ground or intact

grain, insect infested or not) where insect infested and ground grains are more susceptible to aflatoxin contamination than intact or uninfested feeds. Aflatoxins have been shown to get deposited in the adipose tissues; therefore, parts with fat seem to show high level of aflatoxin during analysis (Saqer, 2013).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) This study has revealed that different production systems and improvement programs are practiced in poultry farms in Western Kenya region and locally available feed materials are used. Most chicken producers practiced free range system of production.

ii) Nutritive value of indigenous chicken feed is not known to the farmers leading to poor formulation of the homemade rations.

iii) Chicken tissues and eggs had aflatoxin residues; the liver and breast muscle had the highest levels at 4.19ppb and 3.57ppb respectively while eggs had traces.

6.2 Recommendations

i) The levels of aflatoxin contamination of chicken products especially meat and organs, as reported in this study, should be a wakeup call for stringent monitoring of aflatoxins residues in eggs and meat so as to prevent their effects in humans.

ii) There is need to commercialize indigenous poultry production and encourage cottage factories to compound the feeds at village level so as to improve feed quality.

iii) Frequent farmer education programs should be organized at farm level so as to increase knowledge on prevention measures and minimize aflatoxin contamination of chicken products.

iv) More research should be emphasized towards determining aflatoxin levels in chicken products from chicken kept under intensive system in comparison with those under free range system; breed comparisons can also be done

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APPENDICES

Appendix I: Data information from the study area (Source: Author, 2016)

County		**Busia**	**Kakamega**	**Siaya**	**Total**
Sub-counties		3	3	3	**9**
Wards		6	6	6	**18**
Locations		12	12	12	**36**
Farmer groups		12	12	12	**36**
Feed samples		20	20	20	**60**
Egg samples		20	20	20	**60**
Chicken Sacrificed	Adult (>36 weeks old)	10	10	10	**60**
	Young (12-16 weeks old)	10	10	10	
Tissues samples obtained (4 samples from each chicken; liver, kidney, breast & thigh muscles)		80	80	80	**240**

Appendix II: Removing a thigh muscle sample from chicken for mycotoxin test in the lab. This muscle is an active muscle and popular on the table (Source: Author, 2016).

Appendix III: Egg samples in the lab ready for analysis by ELISA method (Source: Author, 2016)

Appendix IV: Microwells with filtrate, substrate and antibody ready to be inserted into the analyzer for AFT analysis (Source: Author, 2016)

Appendix V: Information is collected during baseline survey from individual farmers in three selected Counties of Western Kenya namely: Busia, Kakamega and Siaya Counties (Source: Author, 2016).

Appendix VI: Sample of questionnaire used to collect information during baseline survey (Source: Author, 2016)

BASELINE SURVEY QUESTIONNAIRE ON MYCOTOXINS IN FEEDS AND EVALUATION OF THEIR EFFECTS ON INDIGENOUS CHICKEN PRODUCTION

FARMER'S BIO-DATA

Code [ ]
Date……../……..…../2016

Enumerator Name	Education level
Name of farmer	Survey refer
Farmer's Contact	County
Age	Sub-county
Family size	Division
Gender	Location
Farmer's group	GPS ref: Latitude Altitude

SECTION A
FEEDS, FEED HANDLING AND STORAGE METHODS/FACILITIES

1. Feed types, source and storage.

Type of feed	**Source**	**Storage method**	**Storage condition**	**Storage length**
Maize				
Sorghum				
Cassava				
Millet				
Peanuts				
Kitchen remains				
Commercial				
Others (list).....				

2. What is the condition of the grain feeds given to your chicken? a) Clean b) Broken c) Rotten d) Other,
   Specify.....

.....
.....

3. Where do you store your chicken feeds? a) In the house b) Modern store c) Traditional granary d) Others (specify)…….....
4. Do you dry the grains, how?
   .....
5. Any sorting prior to storage; (Yes) or (No)

If yes, how is sorting done: Manually (Hand picking) Other, specify…… What criteria do you put in place when sorting: a) size shape b) Colour c) insect infested d) diseased e) others (specify)……..……

6. How do you preserve feeds?
   .....
7. How do you prepare your land before planting?
   ….....
   .....
   .....
8. Do you practice mixed farming? (Yes) or (No)

If yes, what crops? a)……
b)…….
c)……

9. How do you harvest your crops? a) Hand b) machinery c) other, specify…….
   ……
   ……

10. At what stage do you harvest these crops? a) Dry b) partially dry c) green d) other, specify
    ……
    ……

11. Did you notice any diseases in your farm prior to harvesting? (Yes) or (No)

If yes, what were the symptoms?
……
.

.....
.....
.....
.....

12. What are the major challenges you encounter in indigenous chicken feeds?

.....
.....
.....

SECTION B

INDIGENOUS CHICKEN PRODUCTION

1. What breeds of chicken are kept on your farm and how many?

Indigenous	Improved	Crosses	Others specify
No. =	No. =	No. =	No. =

2. a) How long have you been involved in keeping indigenous chicken?

Less than 5 years	5-10 years	11-15 years	Over 15 years

b) Why do you prefer indigenous chicken to others? a) Hardiness b) resistant to diseases c) delicious e) easy to manage f) profitability g) cultural purposes h) other,
specify.....
.....

c) Kindly state the age groups and numbers of the following categories of chicken that you have.

Chicken type	**Age group**	**Numbers**
Chicks		
Growers		
Layers		

Others, specify		

3. Feeding system and production index
   i. Feeding System

Production system; Free Range (FR), Deep Litter (DL)	
Other, specify	
Amount of feed given per chicken per day	
Supplementation Yes/No	
Type of feed supplements	
Amount of feed supplements given per chicken per day	

ii. Indigenous Chicken Production Index

No. of Layers		Eggs per day		Difference	
Eggs provided for hatching per hen		No. of chicks hatched		Difference	
Eggs sold per day/week/month					
No of chickens sold per day/week/month					
Age at first laying					
Total no. of eggs during a laying period					
Time from hatching to laying (open period)					
Any abnormalities of the egg observed (shell, yolk, other.....)					
Age of chicken disposed off for meat					
Weight of chicken disposed off for meat					
Other reasons for disposal					

4 Diseases and Conditions

Common diseases/conditions at the	**Common clinical signs observed**

farm/in the area i.e. from vet records	

i) Most recent outbreak
   a. When
      _____
   b. Name of Disease
      _____

ii) Mode of treatment/control of diseases
   a. Drugs _____ Ethno-veterinary
      _____
   b. Vaccinations
      _____
   c. De-flocking
      _____
   d. Nothing done/not aware
      _____

iii) Deaths and Losses

Disease outbreaks	No. of Birds before the outbreak	Deaths	Difference
Disease outbreaks	No. of eggs before outbreak	Eggs produced during disease	Difference

5 What are the major challenges you encounter in indigenous chicken production?

i) Feeding.....
.....
.....
.....

ii) Egg production.....
.....
.....
.....

iii) Chicken production.....
.....
.....

iv) Diseases/Conditions.....
.....
.....
.....

v) Others, specify.....
.....
.....

SECTION C

MYCOTOXIN AWARENESS

1. Do you know the food/feeds can be unsafe? (Yes) (No)

2. Have you heard of mycotoxins (aflatoxins) contamination? (Yes) (No)

Where did you hear of it?
.....

Do you know what it is and the diseases or conditions it causes?
.....

.....
.....

What practices are you putting in place to reduce/ eliminate mycotoxins contamination of your food/chicken feeds?

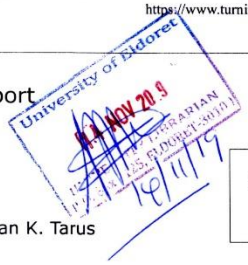
.....

.....

.....

ANY OTHER OBSERVATIONS

APPENDIX VII: SIMILARITY REPORT



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