Assessment of Aflatoxin Awareness and Their Presence in Indigenous Chicken Products in Western Kenya

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Abstract

Aflatoxin contamination of chicken products can significantly impact food safety and health of consumers. A study was conducted to investigate presence of aflatoxins in meat and eggs of indigenous chicken and farmer awareness in Busia, Kakamega and Siaya Counties of Western Kenya. Purposive selection of farmers was carried out based on indigenous chicken population reared (above 10 chicken per farmer), production systems and how active the group is. A multi-stage sampling procedure was used involving: Counties, Sub-Counties, Divisions/Wards, Locations and Farmer groups. From each group, five members were interviewed using a questionnaire. A total of 180 farmers were interviewed on aspects such as production systems and mycotoxin awareness. An adult (>36 weeks old) and a young (12 - 16 weeks old) chicken was obtained from each identified farmer and slaughtered. Approximately 30g of the thigh and breast muscles, liver and kidney were obtained from each chicken together with two eggs collected, from farmer 1 and 5 of each group and tested for presence of aflatoxin. Samples were tested for aflatoxin by Enzyme-linked Immunosorbent Assay (ELISA). Generally, tissues and eggs from chicken reared under free range system had higher aflatoxin means in parts per billion (ppb) (Breast muscle, 3.143; Kidney, 2.157; Liver; 4.619; Thigh muscle, 3.371; Eggs, 0.078) than those reared in semi free range (Breast muscle, 3.753; Kidney, 1.926; Liver; 3.953; Thigh muscle, 2.276; Eggs, 0.066) The difference in levels of aflatoxin between tissues and eggs was significant at $p< 0.05$. This study confirms the presence of aflatoxins in chicken tissues and eggs. However, aflatoxin levels in tissues were lower than the KEBS, WHO/FAO safety levels of 10 and 20 ppb, respectively. There is need for preventive measures to be instituted to mitigate this challenge and farmers made aware of the effects of aflatoxicosis in poultry and poultry products.

Key words: Aflatoxin, Chicken tissues, Indigenous chicken, Production system

INTRODUCTION

Chicken meat and eggs are important sources of high quality animal proteins. Aflatoxin contamination occurs when chicken are fed on feed contaminated by toxigenic fungi like *Aspergillus flavus, Aspergillus. parasiticus* and *Aspergillus. nominus* which contaminate agricultural commodities by growing on them (Rahmani *et al.,* 2009). Aflatoxicosis affects all animal species including man. Animals are the most affected / susceptible due to their frequent exposure to contaminated feed. The tolerance levels in affected animals differ according to species, age, immunity against the toxins, amount and type of mycotoxins consumed and time of exposure.

According to Food and Agriculture Organization (FAO), 25% of agricultural products are contaminated with mycotoxins including aflatoxin (Reddy *et al*., 2010). Consequently, mycotoxins have become a serious problem worldwide causing significant economic loss to animal and crop producers (Bryden; 2012). In chicken, aflatoxin residues cause losses in meat and eggs due to rejection (Bintvihok *et al.*, 2002; Farombi, 2006). Among the mycotoxins, aflatoxin is the most studied due to its potential carcinogenic effect in man and animals. Aflatoxin occurs as AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2. AFB1 is the most toxic and abundant (Zain, 2011). According to Nemati *et al* (2014) and Monson *et al* (2015), aflatoxin intake compromises; weight gain, feed intake, feed conversion efficiency and reproductive performance of chicken. Chicken products are contaminated with aflatoxin as a result of ingestion of contaminated feeds and this poses a health hazard to consumers (Iheshiulor *et al.*, 2011) and chicken.

Previous studies have indicated the presence of aflatoxins in poultry feed and exposure to aflatoxin is more likely to occur where poor methods of feed handling and storage are common (Bennett and Klich, 2003; Bryden, 2012). Mycotoxins especially aflatoxin B1 is closely associated with liver cancer in humans (Saqer, 2013). Since research has revealed that most rural communities in Western Kenya value indigenous chicken as food and source of income (Munyasi *et al*., 2009), this research assessed production systems, feed handling and feeding of indigenous chicken through a baseline survey and determined aflatoxin levels in meat and eggs through laboratory analysis using Enzyme-Link Immunosorbent Assay (ELISA).

METHODOLOGY

Study area

The study was carried out in three Counties of Western Kenya namely; Siaya, Busia and Kakamega. Three Sub-Counties were covered in each County: Siaya (Gem, Alego and Ugenya.), Busia (Teso South, Matayos and Nambale) and in Kakamega (Lurambi, Lugari and Navakholo) sub-Counties**.** These areas were purposively selected because they had organized indigenous chicken farmer groups (involving youth and women), higher number of indigenous chicken population, aflatoxin awareness and varied indigenous chicken production systems.

Sampling technique, sample size and data collection

Farmers' groups and sample size determination

This study was conducted for three weeks (from late February to mid-March 2016) targeting the active farmer groups in the area of study. A total of 180 farmers participated, 60 in each County. A multistage sampling technique was used which involved Counties (n=3), sub counties (n=9), wards/divisions (n=18), locations (n=36); 2 locations per ward, farmer groups (youth and women groups; n=36); 1 farmer group per location and 5 members per group in that order. Selection of farmer groups was based on production system, how active the farmer group was, population of indigenous chicken (above 10 chicken per farmer) and composition of the flock (young; 12-16weeks old and adult > 36 weeks). The identified group members filled questionnaires from which information such as; production systems, mycotoxin awareness among others were obtained.

Collection of chicken samples (Tissues and Eggs)

Tissues

A total of 60 chickens, 30 adults (>36 weeks old) and 30 young (12-16 weeks old) were obtained from the first farmer in each selected group and location. A total of 20 chicken were obtained from each County. Each chicken was slaughtered and samples were obtained; approximately 30g of the thigh and breast muscle and liver and 10g of kidney were obtained totalling to 240 tissues. Each sample was placed in appropriately labelled zip-lock bags then packed in a cool box and transported to the laboratory where they were stored at -15º C until determination of aflatoxin levels was done.

Eggs

A total of 60 eggs were obtained, 20 eggs from each county. These samples were collected from the first and fifth farmers in each group and stored in appropriate egg trays and kept at 4º C in a cold chamber until use.

Determination of total aflatoxins in chicken tissues

Eggs

All the 60 egg samples were analyzed for Total aflatoxin levels using ELISA (Zhang *et al*., 2011) where each egg shell was broken, 20 g of mixture of albumin and yolk poured out into a clean beaker to which 100 ml of 70% methanol was added and stirred thoroughly for 10 minutes. The mixture was then passed through a Whatman filter and 5 ml of the filtrate was collected into a sterile test tube. 100 µL of the filtrate was then mixed with 100 µL aflatoxin-enzyme conjugate. This mixture was added to 200 µL microplate wells pre-coated with anti-aflatoxin antibody and left for 15 minutes at room temperature. Unbound material was washed off three times using washing buffer before the substrate was added to the wells for colour development. Incubation was done in a dark chamber for 5 minutes (to induce reaction). The plates were finally fitted into the aflatoxin ELISA reader (***helica***–Total Aflatoxin Assay, Cat. No.941AFL01M-96,USA) for analysis. All the results were automatically recorded in a sheet of paper.

Tissues

Each chicken tissue sample was ground in a mince or grinder and homogenized using a blender. To 10g of homogenized sample, 50ml of 70% methanol was added before mixing them thoroughly for 20 minutes on a shaker. The sample was then centrifuged for 10 minutes at 4000 x g at room temperature and 300 µL of the resultant supernatant was diluted with 1ml of distilled water. 50 µL of each diluted sample was placed in Total Aflatoxin ELISA Test Kit, USA, 2008 microwell plates pre-coated with anti-aflatoxin antibody. The plates were then analyzed on the ELISA Reader (1055-04, MaxSignal®, Bioo Scientific Corporation; *www.biooscientific.com*). The results were automatically recorded in a sheet of paper from the aflatoxin reader. All results were recorded, both positive and negative and interpreted.

Data Analysis

Descriptive data analysis of results was done at $p< 0.05$ using GENSTAT 14^{TH} EDITION Software for both total aflatoxin and survey data.

RESULTS AND DISCUSSIONS

Survey

Results indicate that the level of awareness on aflatoxin in chicken and eggs in Siaya, Busia and Kakamega counties were above 60% in all Counties as shown in Table 1

Table 1: Aflatoxin awareness by respondents

	Aflatoxin Awareness %	
County	Yes	No
Siaya	73.3	26.7
Busia	68.3	31.7
Kakamega	65	35
Mean	68.9	31.1

As indicated by results, 68.9% of the respondents were aware of aflatoxin contamination in chicken feeds only, but were not aware of any contamination in products while 31.1% were not aware of aflatoxin contamination in any way. The levels of awareness amongst the respondents were high in Siaya (73.3%), Busia (68.3%) and Kakamega (65%) counties. This might have been due to extension services offered by both government and Non-Governmental Organizations active in these areas. These organizations prepared seminars, workshops and field days where farmers got information on aflatoxin contamination of chicken feed and health impact on human and chicken. As indicated by respondents, media and educational institutions also gave information on aflatoxins. Farmers therefore tried to handle the feeds to avoid contamination by drying and storing of grain properly in dry and well aerated conditions/places so as to avoid losses. Respondents also indicated that they were not aware of aflatoxins contaminating eggs and meat, therefore this study was important to them.

Tissues of chicken raised under ***free range*** system had higher total aflatoxins (AFT) levels than those raised under ***semi free range system*** (Table 2).

Table 2: Mean aflatoxin levels (ppb) in chicken tissues under different production systems where?

Production system	Tissue	Mean±S.E*	Standard deviation	Coefficient of variation
Semi free range	BM	3.753 ± 0.439	2.706	72.10
	K	1.926 ± 0.387	2.387	123.9
	L	3.953 ± 0.520	3.205	81.09
	TM	2.276 ± 0.511	3.148	138.3
Free range	BM	3.143 ± 0.677	3.101	98.68
	K	2.157 ± 0.771	3.532	163.8
	L	4.619 ± 0.857	3.929	85.07
	TM	3.371 ± 1.106	5.069	150.4

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

As observed, aflatoxin was present in flesh from chicken fed with different types of feed ingredients (maize, sorghum, cassava, groundnuts and mashes) under different management systems in Western Kenya. Just like in this study, Darwish *et al.,* (2016) found that, the liver had the highest levels of aflatoxins, followed by breast muscle and thigh muscle. That aflatoxin levels were higher in the liver than other tissues could be explained by the fact that biotransformation of toxins takes place in the liver (Bbosa *et al*., 2013), which by default receives most of the toxins in the body. This leads to accumulation of the toxins in the liver which in the long run destroy liver cells causing hepatocarcinoma as shown in most of the affected consumers (FAO, 2007)

Total aflatoxins in tissues from free range chicken were higher than that of semi-free range due to exposure of chicken to aflatoxin contaminated feed materials in the field. Free range chickens are rarely supplemented with feed as compared to semi free range chickens which are fed before being released out to scavenge. Therefore, free range chickens are likely to pick on any feed material in the field, including contaminated materials, so as to satisfy their metabolic needs. Indigenous chicken are also known to feed on wild seeds in the field which could be one of the major sources of contamination since they pick them from grounds (soils) which harbour mycotoxigenic fungi, hence raising total aflatoxin levels in products (Salle *et al*.; 2001; Iqbal *et al*., 2014). Grains and pastures are the major sources of contamination to free rangers especially during harvest season where a lot of contaminated grains (Owiro *et al*., 2019), are found scattered all over the field particularly in Busia, Kakamega and Siaya Counties. This could be an indication that chicken are exposed more to aflatoxin as they scavenge the whole day than when they are restrained for some time then released (Semi free range system). This is in agreement with Feddern *et al* (2013) who fed chicken with aflatoxin that might approximate contamination in the field and also by Hussain *et al* (2010) who concluded that aflatoxin levels in flesh/meat (including chicken) could affect health of those who consume them as food, therefore raises a public health concern.

It is clear that even one type of tissue (like breast muscle, thigh muscle and kidney) under the same type of production system and even from the same chicken, showed variations in aflatoxin levels. These variations, in production systems, could indicate that contamination levels differ from farm to farm or household to household. Difference in aflatoxin levels in tissues from the same bird occur due to their metabolic functions such as elimination of wastes (kidney), deposition (muscles) and detoxification (liver). Liver will contain a higher level than other parts of the body, like muscles, because some toxins were eliminated from the body by the kidney which is one of the routes of toxin elimination (Bbosa, *et al*., 2013). These could also be attributed to levels of contamination of commonly used feed ingredients, the amount of feed consumed by chicken and duration of exposure of chicken to aflatoxins (Iqbal *et al*., 2014).

Egg yolk and albumin of eggs, from different regions and production systems were analyzed for total aflatoxins and the results indicated that only traces were found in them and varied according to regions and production system as shown in Figure 1.

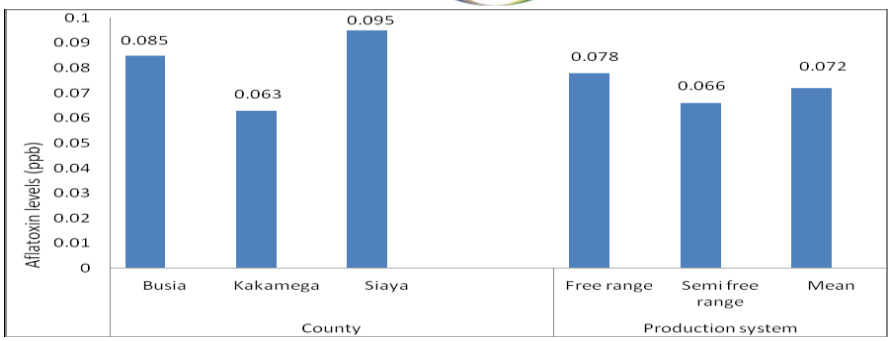

Figure 1: Mean aflatoxin levels (Ppb) in indigenous chicken eggs from Counties and Production system. Safety levels are recommended by FOA/WHO (20ppb) and KEBS (10 ppb).

Eggs contained traces of aflatoxin (Figure 1) which was less than those in tissues (Table 2). Egg samples from Siaya County contained higher levels of aflatoxins compared to those from Busia and Kakamega why? Give a good scientific explanation. Very low aflatoxin levels in egg yolk and albumin could be due to the fact that eggs are made and laid within a very short time, thereby allowing only little amounts of aflatoxin to be deposited.

Chicken tissues from different production systems indicated varied levels of total aflatoxins (Figure 1); this can be due to chicken being exposed to feeds contaminated by mycotoxigenic fungi especially *Aspergillus* species) commonly found in the field and in feeds as they scavenge (free range). Under semi free range, chicken are fed first before being released to scavenge in the field hence minimizing exposure to contaminated feed. These chickens are likely to select what to consume unlike their counterparts, the free rangers.

CONCLUSION AND RECOMMENDATIONS

Presence of aflatoxins in chicken meat is a clear indication of fungal contamination of feeds consumed by chicken in Western Kenya especially those under free range system of production. Eggs had less aflatoxin levels than chicken meat and tissues.

There is need for farmers to be educated on the effects of feeding chicken with feeds contaminated with mould putting more emphasis towards farmer education especially on aflatoxin in feeds, the carry-over to products and effects on consumers as an integrated, multidisciplinary research in the context of a public health. It is therefore important to prevent contamination in products.

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