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Occurrence of Aflatoxins in Feedstuffs Used For Feeding Indigenous Chicken in Baringo and Kisumu Counties, Kenya.

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Abstract: *Aflatoxins (AF) were analyzed in 16 feed ingredient samples fed to Indigenous Chicken collected from Baringo and Kisumu Counties, Kenya. The concentrations of Total Aflatoxins in the samples were determined using direct competitive Enzyme-Linked Immunosorbent Assay (ELISA) and overall incidence of Total Aflatoxins recorded. According to the results, most of the samples were found contaminated with Total Aflatoxins. The highest mean level of Total Aflatoxins were found in growers mash fed to indigenous chicken, that is 19.4ppb in Kisumu County and 19.7 ppb in Baringo County. Similarly, Maize grains was the highest contaminated feed ingredient used for supplementing indigenous chicken in Baringo County with Total Aflatoxins of 18.6ppb, while millet grain was the least contaminated with Total Aflatoxins (2.9-3.2parts per billion) in both Baringo and Kisumu Counties of Kenya. The results showed that the levels of the Total Aflatoxins in the feed ingredients are above regulatory limits of 10 parts per billion and the feedstuffs not generally safe. It also indicates the need to establish a continuous monitoring program to prevent and manage the occurrence of the Total Aflatoxins in feed ingredients of indigenous Chicken in order to improve the health status of the chicken and consumers of indigenous chicken and products.*

Keywords: *Aflatoxins, Chicken feed ingredients, Indigenous Chicken.*

I. Introduction

Aflatoxins (AF) are mycotoxins that are produced by various Aspergillus species including A. flavus, *A.parasiticus* and *A. nominus*. As secondary metabolites of these fungi, AF may contaminate a variety of food and feedstuffs, especially corn, peanuts and cottonseed. Chemically, AF are difuranocoumarin compounds and include aflatoxin B1, B2, G1, G2, M1 and M2 depending on their structures. Toxigenic Aspergilus flavus isolates generally produces aflatoxins B1 and B2, whereas *A. parasiticus* produces aflatoxins B1, B2, G1 and G2. The major hosts of *A. flavus* among food and feed commodities are cereal grains, rice germ, cotton seed, peanut and protein sources such as rapeseed meal, soyabeen meal , cotton seed meal, sunflower meal, corn gluten meal, copra meal, and palm kernel meal (Anjum et al., 2012). Aflatoxin producing fungi utilize the nutrients present in the ingredients for their metabolism and propagation, and thereby reduce the nutritional quality of the feed ingredients (Akande et al., 2006). Indigenous chicken feed ingredients such as rice, maize, sorghum cultivation is practiced in sub-tropical environment which are characteristically warm and humid. They are generally dried after harvesting, but under inappropriate storage conditions, they can be ideal substrate for mycotoxins producing fungi therefore they can be contaminated with fungi during cultivation and subsequent handling if conditions are favorable. According to the survey that was done in Baringo and Kisumu County in April 2014 by the author, most farmers supplemented their chicken with mouldy and broken grains that are not utilized by humans that may contain mycotoxins. Mycotoxins are often found as natural contaminants in raw ingredients of poultry feeds (Khan et al., 2011). Aflatoxins sub types B1, B2, G1 and G2 present significant danger to humans if the concentration is at a high level. Aflatoxin B1 is the most toxic and has been implicated in human health disorders such as hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis (Helica Total Assay Kit, 2011). The International Agency for Research on Cancer (IARC, 1993) has designated AFBI as carcinogenic to humans. To ensure food safety in the European Union the maximum aflatoxins level have been set within the commission Regulation No. 1881/2006. The limit for total aflatoxins in grains intended for direct consumption is 10 parts per billion (ppb) (European Commission, 2006) while the U.S. Food and Drug Administration (FDA) has established guidelines for the maximum toxin level that can be safely fed to immature poultry in Corn & peanut products as 0.02 mg/kg or 20 ppb. Mycotoxins of importance causing contaminations found world-wide, generally occurs in the tropical and sub-tropical regions in the world. They are often found as natural contaminants in raw ingredients of poultry feed (Khan et. al., 2011). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins (TA) and ochratoxins (OTA) (Anjum et al., 2012). In chicken, Total Aflatoxins impairs most of the important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance (Hussain et al., 2010). As a common rule, poultry should not get more than 10ppb Total Aflatoxins in the feed. Aflatoxin contamination in feed may cause reduction of immune response in

chicken, thus they become vulnerable to several diseases (Dhanasekaran et al., 2009). Fungi producing aflatoxin utilize the nutrients present in the ingredients for their metabolism and propagation, and thereby reduce the nutritional quality of the feed ingredients (Akande et al., 2006). The major objective of the study was to investigate the occurrence and contamination level of aflatoxins in feed ingredients, for indigenous chicken from Baringo and Kisumu counties in Kenya. The findings presented here provide further data evaluating the occurrence of Aflatoxins in feedstuffs for indigenous chicken and will be valuable in creating awareness among indigenous chicken local farmers and food factories about the risks and health hazards associated with aflatoxins.

II. Materials and methods

Sampling

A total of 16 samples were brought from IC farmers during the survey from Baringo and Kisumu counties. To avoid the sampling error due to highly heterogeneous nature of fungal distribution, each 2Kg composite sample collected from Indigenous Chicken farmers from both Counties and was a composite of all samples (200grammes of each sample). Each 2Kg of each sample was transported to the Biochemistry laboratory in one batch within 24hr of collection and stored at 4 degrees Celsius in the refrigerator and stored until analysis. All samples were ground to a homogeneous particle size and sub-samples of 500grams each were analyzed for aflatoxins. The concentration of total TA in the feed samples was determined by a direct competitive Enzyme-Linked Immunosorbent Assay (ELISA), using HelicaTotal Aflatoxin Assay Kit (CAT. No. 941AFL01M-96 V. 05-April 2011).

Samples extraction procedure

The 16 samples that had been collected according to established sampling techniques were used. The extraction solution (70% methanol) was prepared by adding 30ml of distilled water to 70ml of methanol (reagent grade) for each sample to be tested. The representative sample was grounded to the particle size of a fine instant coffee to pass through a 20 mesh screen. 20g ground portion of the sample was weighed and 100ml of the extraction solvent added at a ratio of 1:5 of sample to extraction solvent (w/v).It was mixed by shaking in a sealed blender for about 2 minutes. The particulate matter was allowed to settle then 10ml of the extract filtered through a whatman # 1 filter paper and then the filtrate collected to be tested for the concentration of aflatoxins.

Total aflatoxin assay procedure

All the reagents were brought to room temperature before use. One dilution well was placed in a microwell holder for each standard plus each of the 16 samples to be tested. An equal number of antibody coated microtiter wells were placed in another microwell holder.200ml of the conjugate was dispensed into each dilution well. A new pipette tip was used for each. 100ml of each standard and sample was added to appropriate dilution well containing conjugate. It was mixed by priming pipette at least three times, recording the location of each standard and sample throughout the test. A new pipette tip for each was used to transfer 100ml of contents from each dilution well to a corresponding antibody coated microtiter well. It was then incubated at room temperature for 15minutes.The contents from the microwells were decanted into a discard basin and then microwells washed by filling each with distilled water then decanting the water into a discard basin, this was repeated for a total of 5 washes. The microwells were tapped (face down) on a layer of adsorbent towel to remove residual water. The required volume of substrate reagent (1ml/strip) was measured and placed in a separate container. 100ml was added to each microwell and incubated at room temperature for 5 minutes and covered to avoid direct light. The required volume of stop solution (1ml/strip) was measured and placed in a separate container. 100ml of stop solution was added in the same sequence and at the same pace as the substrate. The optical density (OD) of each microwell was read with a microtiter place reader using a 450nm filter, and each record done for each microwell. A dose response curve was constructed using the optical density (OD) values expressed as a percentage of the OD of the zero (0.0) standard against the aflatoxins content of the standard. The unknowns are measured by interpolation from the standard curve. The sample dilution resulted in a standard curve from 1ppb to 20ppb.

III. Results and discussion

The results of incidences and contamination of total aflatoxins B_1 and total aflatoxins (TA) B_2 in feed ingredient samples fed indigenous chicken collected from Baringo and Kisumu Counties are presented in figure 1. The results show that total aflatoxins contamination is more widespread than previously thought, occurring in growers mash used in feeding indigenous chicken from both Baringo and Kisumu Counties of Kenya. It also shows that, the higher the absorbance in feed ingredient, the lower the concentration of total aflatoxins in the feed sample samples collected from the two counties and that most feed ingredients had higher levels of

aflatoxins above the recommended thresh hold level of 10ppb which is allowed in Kenyan grains, as well as the United Nations World Food Programme in maize that is intended for human consumption and chicken feed.

In this study maize was the most commonly used feed ingredient in supplementing chicken diets in Baringo County, in which almost 100% TA incidence was recorded. Maize is more susceptible for TA production throughout the world as compared to canola, soybean and rapeseed (Firdous, 2003). Results indicated that, incidence and average concentration of TA were higher in the finished feed samples (growers mash) as compared to feed ingredients. Contamination levels were higher in the maize and sorghum grains stored by farmers after the harvest. Most sorghum and maize samples from farms in both Baringo and Kisumu Counties, Kenya, all collected between August and December 2014, had aflatoxin amounts greater than 10 ppb. Contamination levels varied from 13.4-17 ppb in sorghum from Baringo and Kisumu Counties and from 18.6 to 19.7 ppb in maize and growers mash from both Counties. Contamination levels vary considerably between farmers and villages, as well as between Counties.

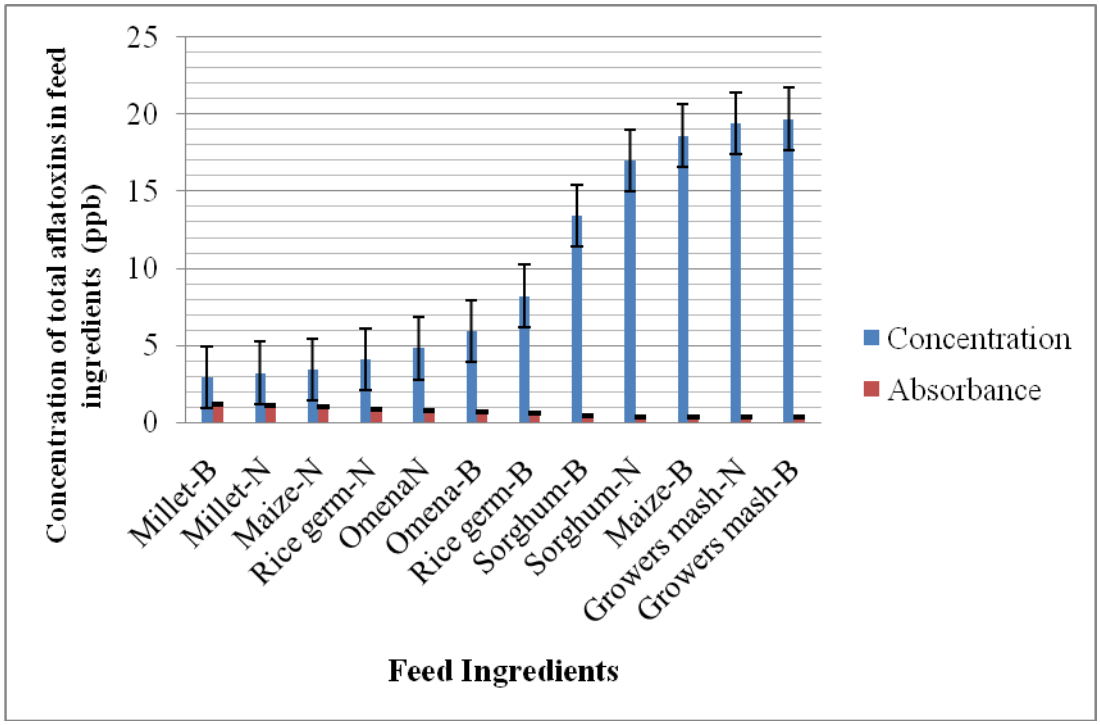

Figure 1: *Aflatoxins levels in feedstuffs used in IC*

Nb: Concentrations are reported as or ppb (parts per billion): 1 mg/kg = 1 ppm = 1000 ppb.;B=Baringo, N=Kisumu

The study also found differences in contamination levels from season to season. In Kisumu and Baringo Counties study sites, most of samples collected from farmers keeping indigenous chicken in August 2014 had aflatoxin levels greater than 10 parts per billion (ppb), which is the maximum level allowed by the Kenyan government, as well as the United Nations World Food Programme, in maize that is intended for livestock and human consumption (Policymakers at International Workshop, 2011). According to findings with Policymakers at International Workshop (2011) acute exposure to high levels of aflatoxins can result in liver failure and rapid death in animals. Chronic exposure, in both humans and animals, exacerbates infectious diseases and can lead to cancer, liver cirrhosis, weakened immune systems, and stunted growth in children (IARC, 1999). Aflatoxin B1 adversely influences egg quality by decreasing shell thickness, egg weight and egg energy deposition. The negative impacts of AF on laying hens can be induced when feed contains 1-2 mg/kg (Azzam and Gabal, 1998; Verma et al, 2007). In addition, TA in laying hen feed can result in an AF residue in the eggs therefore it is very important to control AF concentrations in feeds for laying hens (Oliveira et al, 2000). TA affect laying hens and lead to reduced egg production, poor egg quality and increased mortality of

challenged hens. Following absorption of AF in the upper part of the small intestine (80-90 percent of what is eaten is absorbed), aflatoxins undergoes an extensive transformation into metabolites in the liver. In fact, TA are not toxic per se, but require metabolic conversion by hepatic enzymes (the cytochrome P450 family) to the metabolically active metabolite exo-AFB1-8, 9-epoxyde (AFBO) to exert its toxicity. This metabolically active form of TA can bind with particular cellular compounds (proteins, DNA and RNA) to influence normal cellular activities, and is considered the active form responsible for the carcinogenicity and mutagenicity of TA. Aflatoxins acts as an inhibitor of protein synthesis thereby, dividing cells and tissues with a high protein turnover such as that found in the liver, immune system or gut epithelium, which is most susceptible to the toxic effects of TA. In this respect, exposure to TA has been demonstrated to suppress the immune response in chicken. TA can depress the development of the thymus gland or influence the relative weight of the bursa of Fabricius, which may result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system (Celik et al, 2000). Inhibition of macrophage functions, T- lymphocyte activity or cytokine expression by TA results in vaccine failure or pathogen persistence, as exemplified in many studies by reduced immunoglobulin production (Verma et al, 2004; Yunus et al, 2011).

Recent epidemiological data indicates a high correlation between outbreaks of Newcastle disease and TA contamination of broiler rations (Yunus et al, 2011). In general, the dose of AFB1 needed to affect the immune system is considered less than the dose required to elicit a reduction in bird performance. The threshold dose of AFB1 is reported to be approximately 0.4 and 1 mg/kg for the negative effects on cell mediated and humoral immunity, respectively, in broilers (Yunus et al, 2011). Therefore, chronic consumption of feed contaminated with low AF content may pose a serious risk to animal health, increasing susceptibility to infections or reducing vaccination efficacy. The gastrointestinal tract is the first organ coming into contact with mycotoxins of dietary origin, and should be expected to be affected by aflatoxins B1 with greater potency as compared to other organs. In addition, TA has been shown to reduce energy utilization through a significant increase in the maintenance energy requirement of the hen (Verma et al, 2007). There is a loss of energy availability in the feeds because of the feeding of moldy maize grains containing mycotoxins.

IV. Conclusion

Higher incidence and contamination level of Total Aflatoxins was detected in Indigenous Chicken feedstuffs. This situation demands for immediate necessary control measures. The metabolic pathways of AFB1 in the liver are very complicated, and not all species go through the same reactions. While exact dosages that chicken will receive cannot be predicted, feed ingredient testing strategies in times of higher occurrence can help. Additionally, sequestration/adsorption of AF by feed additives such as probiotics can lessen the severity of these impacts.

V. Recommendation

Use of mycotoxin binders in feeds given to the Indigenous chicken is recommended to adsorb the Total Aflatoxins, strict regulations and surveillance programs for testing feeds and feed ingredients for Total Aflatoxins contamination are highly recommended to improve the health status of both indigenous chicken and the consumers. Adequate post-harvest drying should be done, and proper storage condition should be maintained.

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