Aflatoxin B_1 contamination in poultry feeds in Arusha City, Tanzania

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SUMMARY

Aflatoxin B_1 (AFB_1) contamination in food and feeds has significant health problems and economic loss to poultry industry. This study assessed qualitatively and quantitatively AFB_1 in samples of poultry feeds and raw feeds and associated the levels of AFB_1 with certain risk factors in Arusha city, Tanzania using competitive ELISA technique. Samples collected from poultry feed producers, sellers and poultry keepers, AFB_1 was detected in all samples with various concentrations ranging from 1.1 to 80.1 µg/kg. Aflatoxin B_1 concentration above the FAO/WHO tolerable limit of 5 µg/kg were in 65% of starter feed, 72.2% finisher feed, 79% layers mash, 62.5% maize bran and 75% of sunflower seedcake. Overall, 70.8% of all sample tested for aflatoxin B_1 were above FAO/WHO tolerable limit. Aflatoxin B_1 means concentration of poultry raw feeds was significantly higher than that of finished poultry feeds ($p< 0.05$). Questionnaire interview of 38 respondents showed association between appropriate storage facility and AFB_1 contamination above FAO/WHO tolerable limit (OR=0.2, 95% CI:0.03-1.0); while sun drying of poultry finished feeds/ raw feeds had an odd ratio of 0.05 (95% CI: 0.04-0.5). This is the first comprehensive report on prevalence of aflatoxin B_1 in finished poultry feed and poultry raw feeds in Arusha city, northern zone, Tanzania. We recommend control strategies which should based on pre- and post- harvest handling through promoting good farming and production practices.

Key words: Aflatoxin B_1, poultry feeds , risk factors, ELISA.

INTRODUCTION

Mould occurrence in poultry feeds can be one of the major threats to poultry economy and health besides nutritional and organoleptic problems (Shareef, 2010). Aflatoxin (AF) producing fungi particularly *Aspergillus flavus* are common and widespread in nature and most often found when certain grains are grown under stressful conditions such as drought. The moulds occur in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and invade all types of organic substrates when the conditions are favourable for growth, particularly in hot and humid weather (Ominski *et al*., 1994).

There are four major aflatoxins: B_1 (C_{17} H_{12} O_6), B_2 (C_{17} H_{14} O_6), G_1 (C_{17} H_{12} O_7) and G_2 (C_{17} H_{14} O_7) as reported by Morgavi and Riley (2007). There are two additional metabolic products, M_1 and M_2 that are of significance as direct contaminants of human foods and feeds (Kaaya *et al*., 2000). The B designation of aflatoxins B_1 and B_2 resulted from the exhibition of blue fluorescence under ultraviolet light (UV-light), while the G designation refers to the yellow-green fluorescence of the relevant

structures under UV-light (Kaaya *et al*., 2000).

Aflatoxin B_1 (AFB_1) is the most prevalent toxin in cereals used in feeds and presents the greatest toxigenic threat (Leeson *et al*., 1995). Under suitable environment, aflatoxigenic fungi contaminate human foods and animal feeds directly or indirectly. In direct contamination, the product is infected with aflatoxigenic fungi with subsequent toxin production. Indirect contamination occurs when foodstuffs or raw feeds were previously contaminated with aflatoxin producing fungi and although most of the fungi have been removed or killed during processing, some still remain in the final product. Such contamination of cereals and oilseeds is the main source of many mycotoxins in the human and animal dietary systems, particularly in Africa (Smith and Moss, 1985). However, due to genetical and ecological factors, relatively few molds produce mycotoxins (Jemmali, 1979).

The Food and Agriculture Organization of the United Nations (FAO) estimates that mycotoxins contaminate 25% of agricultural crops worldwide (Smith *et al*., 1994). Aflatoxins, a group of mycotoxins mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are of economic and public health importance because of their effects on livestock and human health. *A. flavus* produces only B aflatoxins, while *A. parasiticus* produces both B and G aflatoxins (Pitt, 1993). Feed is the major financial input in poultry production, amounting to 60-70% of the total financial expenditure (Butool *et al.*, 1990). Generally, poultry feed contains 40-60% grains, mainly corn, rice and wheat. Aflatoxins have both carcinogenic and hepatotoxic actions, depending on the duration and level of exposure to the consumer. Chronic dietary exposure to aflatoxin B_1 is a major risk factor for hepatocellular carcinoma, particularly in areas where hepatitis B virus infection is endemic. Ingestion of higher doses of the toxins can result in acute aflatoxicosis, which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Fung and Clark, 2004).

Feeding materials contaminated by aflatoxins to animals, especially monogastric animals, impairs feed intake, decrease efficiency of feed utilization, reduces body weight gain, increases disease incidence (due to immune – suppression) and reduces reproductive capacities, which leads to economic losses (Morgavi *et al*., 2007). The significance of aflatoxins in health and its effects to poultry industry can never be underestimated. Due to the nature and storage conditions of most commercial, local chicken feeds and pet feed as well as mode of feeding it can easily be speculated that AFs contaminations are common.The rank order of toxicity of aflatoxins is $AFB_1 > AFTG_1 > AFTB_2 > AFTG_2$ (Erkmen and Bozoglu, 2008; Set and Erkmen, 2010).

A study by Muriuki and Siboe (1995), reported contamination of maize meal in Nairobi with AFB_1 and AFB_2 of 0.4–20 µg/kg. This result indicates a high exposure to aflatoxins to the public, considering the consumption rate of maize meal of 0.4 kg/person/day, as reported by Lewis *et al.*, (2005) that, contamination of maize during the aflatoxicosis outbreak in Eastern Kenya in 2004 with many samples exceeding 100 ppb. Limited studies have been carried out to establish level of AFB_1 in animal feeds in Tanzania. Kajuna *et al*., (2012) studied the occurrence of AFB_1 in chicken feeds in Morogoro municipality and found that 68% of the feeds were contaminated with AFB_1.

Poultry feeds present in Arusha region Tanzania, are compounded using the ingredients such as maize grains, maize bran, sunflower seed cake and cotton seed cake, which are potentially favourable substrates for fungal growth. In addition, Arusha region shares both formal and informal trade of animal feeds and feed inputs like premix, concentrates and maize. Due to inadequate knowledge and facilities of AFB_1 detection at zoosanitary/ phytosanitary border post, it is possible for Arusha region to have AFB_1 contaminated products from neighboring country.

The optimum condition for *Aspergillus* spp to grow is between 25^0C to 30^0C temperature, at 0.90 - 0.99 humidity (Giorni, 2009). Arusha region climate ranges across the mentioned conditions that support fungal growth and subsequent mycotoxins contamination, particularly if storage, transport and processing environments are not controlled. Therefore, it is important to established the status on mycotoxins accumulation in poultry feeds in Arusha city. This study was to conducted to establish presence and level of AFB_1 in finished poultry feeds and raw feeds as well as associated risk factors in Arusha City.

MATERIALS AND METHODS

Study area

The study was carried out in Arusha city located within Arusha region which is at $3^0$22'S, $36^0$41'E, northern Tanzania. Sample were analyzed at the Toxicology Laboratory, at the Faculty of Veterinary Medicine, Sokoine University of Agriculture (SUA) in Morogoro, approximately 621 km to the south of Arusha city.

Study organization

The study was organized in two parts. The first part dealt with interview on sources and storage of poultry feeds. The interviewees were poultry keepers, poultry feeds sellers, and poultry feed compounders while the second part was analysis of the aflatoxin B_1 in poultry feeds. Feed samples were randomly collected from different areas of Arusha city specifically from animal feed sellers, animal feed compounders and poultry farms. Interviews were conducted to the animal feed sellers, animal feed compounders and poultry keepers during the sample collection.

Sample size calculation

Sample size was calculated by the formula: $n = 1.96^2pq / L^2$ (Martin *et al*., 1987); where by n = sample size, p = Prevalence, q = 1-p and L = desired limit of error of the prevalence was 10%. The similar study that was done in Pakistan by Khan *et al*., (2011) showed a prevalence of 61% of AFB_1 contamination in Poultry feeds and raw feeds. Adopting this prevalence in the above formula, a total of 92 samples were collected.

Interview on sources and storage of chicken feeds

Four poultry feed producers, nine chicken feed sellers and 25 chicken farmers were interviewed. The assessment was conducted using an open ended questionnaire which was designed for collecting information on poultry feeds and raw feeds including date of collection, type of feed/ingredients, sources of feed/ingredients, name of feed mill, farm/shop, storage duration, storage facility, drying of feeds/ingredients through

sun exposure and pest control to feeds/feeds ingredients.

Analysis of aflatoxin B_1 in poultry feeds

Poultry feed samples were collected from different sources for analysis of AFB_1 contamination. Feed samples were categorized into two groups finished feed (starter feeds, finisher feeds, layers feeds) and raw feeds (maize bran and sunflower seed cake) as shown in Table 1.

Table 1. Categories of feeds sampled during a study to analyse for aflatoxin B1 contamination in Arusha city, Tanzania, 2013.

Feed category	Type of feeds	Number of feed samples
Finished feeds	Starter	20
	Broiler Finisher	18
	Layers mash	19
Raw feeds	Maize bran	16
	Sunflower seed cake	16
Total		89

Samples and sampling procedure

Samples of selected raw feeds and finished feeds were randomly collected directly from poultry farms, poultry feed production sites, and feed sale centers. Analysis was done in the Toxicology Laboratory, Sokoine University of agriculture, Morogoro during the period from 15th December 20013 to 1st February 2014. Sampling procedure followed the principles of the Romer® guide on "Sampling and sample preparation for mycotoxin analysis as explained by Richard (2000). Initially, several small samples weighing about 100 g each were collected randomly from a whole lot batch of poultry feeds. There after, the several small samples were mixed together to form one lot of 500 g. This one lot sample of 500 g was ground and a sub-sample of 25 g was taken for actual analytical process.

Reagents

Aflatoxin B_1 ELISA kit No: 20131210 was purchased from Shenzhen Lvshiyuan Biotechnology Co., Ltd China. Other chemicals (methanol, chloroform) were purchased from Merck AG, Germany. The AFB_1 ELISA test kit had the following accuracy specifications: Sensitivity of 0.1 µg/kg and specificity of 100%.

Sample preparation and cleaning up procedure

For AFB_1 clean-up, a total amount of 25 g of a sample was ground (grinding of sample was accomplished using blender Bomino®, Italy). Ten grams (10 g) of milled sample, weighed using a beam balance, was introduced into 50 ml methanol solution (1 part methanol + 1 part water) in 100 ml flask. Struers® flask shaker was used to shake the mixture (methanol solution and sample) for 15 minutes after which the mixture was filtered using Frissennette ® filter paper. One quarter of the filtrate was discarded and the remaining filtrate was collected into flat bottom flask.

Ten millilitres (10 ml) of the filtrate was introduced into a 125 ml separatory funnel, then 20 ml of chloroform ($CHCl_3$) was added into the separatory funnel, thereafter the solutions in the separatory funnel were shaken for 3 minutes and let still for stratification. The lower layer of chloroform was released and the upper layer was filtered through rapid qualitative filter paper which was pre-filled with about 5 g anhydrous sodium sulphate (Na_2SO_4) and pre-wetted with chloroform solution into a 100 ml evaporation dish. The separatory funnel was rinsed by 5 ml

chloroform and the rinsing mixture was similarly filtered into the evaporation dish. The evaporation dish was let to float on 65°C water bath. After drying the evaporation dish was allowed to cool and 10 mls of methanol solution (1 part methanol + 1 part water) was added to dissolve the contents in the evaporation dish in order to obtain sample extract solution.

Laboratory analysis of AFB1 by Competitive ELISA

Competitive ELISA principle

The toxin of interest (AFB_1) has been coated in the micro-titre plate wells. During analysis a sample is added along with primary antibody specific for the AFB_1. If AFB_1 is present in extract, it will compete for antibody and therefore preventing antibody from binding to the AFB_1 attached to the well. The secondary antibody, tagged with peroxidase enzyme, target the primary antibody that is complexed to the toxin coated on the plate wells. The resulting colour intensity after addition of substrate has inverse relationship with the AFB_1 concentration.

ELISA procedure

ELISA kit was brought to the room temperature 20-25^{0}C (as per ELISA kit operation manual) for 30 minutes, and each reagent was shaken evenly before use. The ELISA plate wells were numbered as follows: On well number one (1) was labelled zero, well number 2-7 was for aflatoxin B_1 standards and other 89 wells were for samples. Fifty micro-litres (50 µl) of sample diluent (Reagent A) was introduced into well number 1 and AFB_1 standards (B reagent) (AFB_1 standards. 0, 0.1, 0.25, 0.5,1, 2 ng/ml) into wells number 2-7 accordingly. The sample extracts duplicates were added into the 89 sample wells. Fifty micro-litres (50 µl) of enzyme antigen diluent (Reagent D) was added to well number 1 while in the other wells enzyme antigen solution (Reagent C) was added.

For the even reaction in each well the reaction plate was shaken slightly for 10 minutes and then incubated at 37 ^{0}C for 30 minutes after which it was washed by adding 250 µl of washing buffer four times, 2 minutes each time and flapped to dry on absorbent paper. For coloration two substrates 50 µl each were added to each well, A solution (Reagent F), followed by substrate B (reagent G) solution. Solutions in the wells were mixed gently by shaking the plate manually followed by incubation at 30 ^{0}C for 15 minutes in a dark area. Fifty micro-litres (50 µl) of the stop solution (reagent H) was added into each well, then the solution was mixed by shaking. The plate was read at the wavelength of 450 nm using Multiskan RC ® microplate reader and optical density (OD) was determined for each well. The optical density (OD) value was then compared to the standard curve and aflatoxin B_1 concentrations were subsequently obtained.

Data recording and analysis

Aflatoxin B_1 concentration in each sample was recorded in Microsoft Excel® worksheet. Data were analysed to obtain means ± standard deviations (SD) for AFB_1 concentration. Frequencies and Logistic regression were calculated for the questionnaire and other categorical data. The analysis was performed in Epi Info™ version 7 program.

RESULTS

General results

A total of 34 respondents were effectively interviewed, Fifty seven (57) finished poultry feed and 32 poultry feed ingredient samples were collected from different areas in Arusha city, Tanzania, and tested for AFB_1 contamination levels.

Table 2. Levels of aflatoxin B_1 (AFB_1) in the feed samples analysed and categorized by feed type

Maize bran		Sun flower seed cake		Starter		Finisher		Layers Mash	
Sample number	AFB_1 Level µg/kg	Sample number	AFB1 Levelµg/kg	Sample number	AFB_1 Level µg/kg	Sample number	AFB_1 Level µg/kg	Sample number	AFB_1 Level µg/kg
1	80.08	17	28.30	33	4.92	53	3.72	71	3.74
2	79.27	18	15.42	34	24.04	54	3.74	72	10.90
3	2.63	19	23.43	35	4.92	55	6.07	73	2.19
4	68.72	20	4.33	36	22.50	56	6.16	74	7.18
5	58.37	21	73.43	37	3.57	57	3.36	75	24.53
6	2.01	22	74.18	38	51.12	58	2.63	76	8.41
7	3.78	23	62.69	39	3.81	59	2.90	77	9.75
8	20.42	24	44.55	40	1.77	60	11.07	78	8.41
9	30.24	25	3.87	41	35.96	61	9.03	79	19.30
10	1.09	26	2.60	42	6.93	62	10.47	80	4.70
11	23.67	27	64.97	43	3.74	63	25.42	81	7.83
12	24.28	28	66.99	44	6.62	64	47.12	82	6.68
13	2.84	29	9.80	45	10.00	65	14.51	83	18.91
14	1.90	30	12.51	46	12.01	66	6.19	84	2.87
15	10.47	31	4.85	47	8.89	67	8.41	85	13.86
16	5.48	32	15.58	48	14.29	68	7.18	86	10.52
				49	12.20	69	5.42	87	14.81
				50	8.28	70	6.13	88	9.70
				51	4.82			89	8.03
				52	41.69				

Table 3. Prevalence of AFB_1 contamination in poultry feeds in Arusha city, Tanzania 2013

Types of feed	Number of Sample analysed	Contaminated Samples ≥ 5µg/kg	Prevalence of AFB_1 contamination %	Mean ± SD µg/kg
Starter feed	20	13	65	14.1 ± 14
Finisher feed	18	13	72.2	10 ± 10.7
Layers mash	19	15	79	10.1 ± 5.9
Maize bran	16	10	62.5	26 ± 29.1
Sunflower seed cake	16	12	75	37.4 ± 27.8
Groups of feed				
Finished feed	57	41	71.9	11.5 ± 10.8
Raw Feed	32	22	68.8	24.7 ± 27.4
All feed	89	63	70.8	16.2 ± 19.5

Aflatoxin B_1 occurrence in different poultry feeds types.

Table 2 gives an overview of the contamination of raw feeds and finished feeds tested, while (Table 4) gives the information on the total number of each feed tested, the number of contaminations that exceeded the FAO/WHO tolerable level arithmetic mean and median of contaminated samples as well as the maximum level detected in each feed type. For all feed samples analysed, AFB_1 contaminations ranged from 1.1 to 80.1 µg/kg and their arithmetic mean concentration was 16.2 ± 19.5 (Table 3). The frequency of sample with AFB_1 contamination that exceeds the FAO/WHO maximum tolerable limit was 70.8% (Table 3).

Aflatoxin B_1 occurrence in raw feeds.

The frequency of AFB_1 contamination that exceeds FAO/WHO maximum tolerable limit in raw feed samples was 68.8% whereas mean concentration of AFB_1 contamination was 24.7 ± 27.4 (Tables 3 and 4). In the maize bran, ten (10) of sixteen (16) samples analyzed for AFB_1 had levels exceeding the maximum acceptable level set by the FAO/WHO (5 ppb) and mean concentration of AFB_1 was 26 ± 29.1 (ppb). The frequency of contamination was therefore 62.1%. Twelve (12) out of 16 (75%) sunflower seed cake sample analysed were contaminated with AFB_1 and had levels above the maximum allowed by FAO/WHO. The mean of AFB_1 concentration of sunflower seedcake was 37.4 ± 27.8 µg/kg. (Tables 3 and 4).

Aflatoxin B_1 occurrence in finished feeds

The results indicates that 41 out of the 57 (71.9%) of finished feed samples had AFB_1 levels that exceeds FAO/WHO tolerable limit with mean concentration 11.5 ± 10.8 µg/kg. (Table 3). The result showed that a higher prevalence of AFB_1 contamination in the finished feed samples than in the raw feeds.

However, mean concentration of AFB_1 contamination was also less in the finished feed samples compared to raw feed sample ($p = 0.0053$). Intergroup analysis done on mean difference in concentration of AFB_1 between finished feeds and raw feeds reveal a statistical significance ($p = 0.013$). Among the finished feeds, starter feed had the highest mean of AFB_1 contamination of 14.1 ± 14 µg/kg compaired to finisher feed (10 ± 10.7 µg/kg) and layers mash (10.1 ± 5.9 µg/kg). The difference in mean of AFB_1 contamination among finished feeds was not statistically significant ($p > 0.05$).

Information on sources, storage facility and Sun drying of Poultry Feeds

Table 5 shows relationship between risk factor and aflatoxin B_1 contamination in poultry feed samples that were higher than FAO/WHO maximum acceptable limit. The information obtained from respondents showed that 47.4% (18/38) of them use storage facility to keep their poultry feeds while 13.2% (5/38) had the tendency of drying the poultry feeds before packing/storage. There were three categories of storage durations and these are as shown in Table 6.

Table 4. Descriptive statistics of AFB_1 level in the feeds type analysed

	Maize bran	Sun flower seed cake	Starter	Finisher	Layers	Feed ingredient	Finisher feed	All feeds
Mean	25.95	37.42	14.10	9.97	10.12	24.69	11.47	16.22
SD	29.11	27.77	13.96	10.73	5.91	27.41	10.77	19.47
Median	15.44	19.51	8.59	6.18	8.41	11.49	8.28	8.41
Maximum	80.08	74.18	51.12	47.12	24.53	80.08	51.12	80.08
Minimum	1.09	2.6	1.77	2.63	2.19	1.09	1.77	1.09

SD – Standard deviation

Table 5. Association between storage facilities, sources of feed and aflatoxin B_1 in poultry feed

Risk factors			Aflatoxin B_1 level ≥ 5 µg/kg		OR	Frequency of RF %
	Yes	No	Positive	Negative		
Storage Facilities	18	20	11	7	0.2	47.4
Sun Exposure	5	33	2	3	0.05	13.2
Sources of feed						
Arusha city	21	17	16	5	1	55.3
Out of Arusha city	33	5	28	5	1.6	86.9

RF – Risk factor, Out side Arusha city = Kilimanjaro, Shinyanga, Singida regions, and Mbulu, Kiteto, Babati districts.

Table 6. Different storage duration frequencies of poultry feed and raw feeds

Feed Owner	One week %	Two weeks %	>Two weeks %
Poultry feed seller	33.3	33.3	33.3
Poultry feed producer	25	25	50
Poultry keeper	48	32	20

The poultry keepers showed that, all of them sourced finished feed/ feeds ingredients (ingredients mainly cereals) from areas within Arusha city. Eleven percent (11%) of feeds sellers purchase feeds/ feeds ingredients within Arusha city while for poultry feed producers none of them sourced poultry feeds ingredients within Arusha city. The majority of finished feeds were sourced from a poultry feed producers (Kibo Poultry Feed, Harsho Company) in Kilimanjaro region whereas sources of feeds ingredients were as follows; maize and maize bran obtained from Babati, Kiteto and Mbulu districts and sunflower seedcake was sourced from Singida and Shinyanga regions.

DISCUSSION

Generally samples collected from different sources of Arusha city revealed that all tested feed samples had AFB_1 contamination. Similar results were obtained by Cespedes and Diaz (1997) on analysis of aflatoxin in poultry and pig feeds and feedstuffs in Colombia. Aflatoxin B_1 were found in all commodities analysed except soya beans. Also similar results were obtained in the Middle East and Africa, reported by Rodrigues *et al.* (2011) which included

numerous samples from Western and Central Africa including Nigeria, Sudan, Egypt, Algeria, Kenya, Ghana, South Africa, Israel, Jordan, Lebanon, Syria and Yemen, the authors found that 98% of the ingredients used in animal feed formulation were positive for AFB_1.

The overall prevalence of AFB_1 contamination that exceeded the WHO/FAO maximum tolerable limit was 70.8%. These results are comparable to those by Khan *et al.,* (2011) who reported the incidence of AFB_1 of 69 % in 127 poultry feeds from western part of Pakistan, which was relatively higher than other areas. Our results were above those reported by Kajuna *et al*. (2012), who found that on average 68% of all feed samples were contaminated with AFB_1. Kangethe and Lang'a (2009) in Kenya found that 67% of sampled animal feeds had AFB_1 contamination above FAO/WHO acceptable limit.

The prevalence of AFB_1 contamination that exceeded the WHO/FAO maximum limit was higher in finished feeds than raw feeds, suggesting that mixing different ingredient to make complete feed increases the likelihood of AFB_1 in finished feeds. It is also possible that the longer time taken to transport raw feeds from different sources to feed producer predisposes the feeds to higher risks of AFB_1 contamination. Consistent with the results in the present study, Kajuna *et al.,* (2012) reported that frequency of aflatoxin contamination in compounded feeds was higher than in non compounded feeds in Morogoro Municipality. Cespedes and Diaz (1997) reported that incidence of AFB_1 contamination in Colombia was 41.3% in complete poultry feeds while for raw feeds it was 27.3%.

On the other hand, the mean concentration of AFB_1 contamination in raw feeds was higher than in finished feeds; the reason for this could be due to long storage of maize bran and sun flower seed cake. Normally farmers compound only few bags for their convenience while the feeds ingredients are purchased in huge amounts by most of feed producers and farmers during harvest season which last from May to July of each year. This is because they can get them at cheaper price.

There was relationship between presence of storage facilities and AFB_1 contamination above the FAO/WHO tolerable limit, which shows that respondents who had appropriate storage facilities were 0.83 (based on OR 0.17) less likely to have AFB_1 above 5 µg/kg than those without (Table 5). A similar observation was noted by Richard, (2000) who found that, the increased production of AFB_1 in feedstuffs may be expected if the storage was for a longer period under unsatisfactory ventilation and storage conditions. *Aspergillus flavus* is not normally present at harvest and prevention of the formation of aflatoxins therefore relies mainly on avoidance of contamination after harvest, using rapid drying and good storage practice (Ito *et al*., 2001). It was also observed in Cameroon by Kana *et al*. (2013) that; inappropriate storage condition could be implicated in the fungal growth and aflatoxin production in feedstuffs and poultry feeds. Pitt and Hocking (1997) reported that, the constituted feeds stored under appropriate conditions were subject to lesser direct influence of temperature and humidity.

In view of huge economic losses to the poultry sector due to AFB_1, prevention and control of aflatoxicosis is of great significance. The best control of aflatoxicosis is prevention. Proper

sanitation and prevention of fungal development during harvesting, storage and feeding of feed stuffs is vital. Since fungi need relatively high moisture to grow well, grain and poultry feed should be stored below 13 percent moisture (Cavalheiro, 1981).

The relationship between sun drying practice of poultry feeds before packing into the bags or compounding and occurrence of AFB_1 level beyond the FAO/WHO allowable maximum limit which shows that, the sun dried poultry feeds were 0.95 (based on OR 0.0446) less likely to have AFB_1 contamination above the FAO/WHO allowable maximum limit than those without sun dried (Table 5). Gowda *et al.* (2007) observed that, hot air oven drying of the animal feed resulted in an average reduction of 57.6% in aflatoxin contamination, whereas sun drying reduces the aflatoxin contamination by 83.7%.

Animal feeds are produced from grains; the level of AFB_1 contamination reported in this study suggests that, contaminated grains may have found their way into animal feeds. Tanzania Food and Drug Authority (TFDA) (2012), reported that in Morogoro region (Eastern zone) 43% of maize samples had AFB_1 levels above 5 µg/kg, and in the Shinyanga region (Western zone), 40 percent of the samples were above 5 µg/kg, with average contamination of 50 µg/kg and 28 µg/kg respectively. However contamination was much lower in other zones; in Manyara region (Northern zone) 9% of the samples were above 5 µg/kg, in the Southern Highland regions, Iringa, Mbeya, and Rukwa, only 4% were above 5 µg/kg, and in the Ruvuma region (Southern zone), none of the samples were above 5 µg/kg.

Lewis *et al*., (2005) reported that 35% of maize samples collected during the 2004 human aflatoxicosis outbreak in Kenya were contaminated with aflatoxin exceeding 100 ppb and 7% above 1000 ppb. Okoth and Ohingo (2004) reported that 29% of children weaning flour in Kisumu, Kenya contained aflatoxin with levels ranging from 2 to 82 µg/kg. In Tanzania, AFB_1 was reported by Nekander *et al*. (1991) in brewed beverages arising from use of contaminated grain or fruit during their preparation. Rushunju *et al*., (2013) reported on the AFB_1 contamination in commercial locally produced cereals based complementary food from Arusha city, Tanzania; that 3.3% of them had exceeded maximum tolerable level of AFB_1 set by FAO/WHO.

It is thought that the variations in the levels of AFB_1 in poultry feeds and raw feeds could be due to marked fluctuations in the environmental temperature, and humidity conditions during the course of the year in areas where the raw feeds were purchased. Kan'gethe and Lang'a (2009) surveyed four urban centers in Kenya and observed that AFB_1 concentration that exceeded the FAO/WHO limit were 70, 58 and 60% from farmer, manufacturer and feed sellers respectively.

Most of the feed producers reported purchasing raw materials for production of animal feeds from other regions outside Arusha city like Manyara (Kiteto district), Shinyanga and Singida. There was no significant difference in concentrations of AFB_1 contamination between different sources of feeds/raw feeds. This could be due to high prevalence of AFB_1 in cereals in areas where the raw feeds were purchased.

Based on the findings of this study, it is concluded that AFB_1 is present in all poultry feeds in Arusha city. AFB_1 occurrence was higher in finished feeds

than in raw feeds. However many finished feeds and raw feeds had AFB_1 level exceeding the maximum tolerable level established by FAO/WHO as well as TFDA. The level of contamination of AFB_1 in poultry feeds reported in this study should be a 'wake up" signal for appropriate intervention toward control of AFB_1 in the animal feeds and human food. The control strategies should base on control of pre- and post- harvest handling through promoting good farming and manufacturing practices. Also further research in bio - control of mycotoxins and good storage practices of feeds at all levels and regular testing of cereals for AFB_1 should be mandatory in order to asses the scale of problem.

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REFERENCE

Butool AK, Hussin SS, Ahmed MA. Response of three commercial broiler chickens strains to aflatoxins. *Journal Islamic Academy of Science* 3: 27 – 29, 1990.

Cavalheiro ACL. Aflatoxin and aflatoxicosis – A Review. *World's Poultry Sci. Journal* 37: 34-38, 1981.

Cespedes EA, Diaz JG. Analysis of aflatoxin in poultry and pig feedstuffs used in Colombia. *Journal of AOAC international* 80(6): 1215 – 1219, 1997.

Council for Agricultural Science and Technology. *Mycotoxins: Risk in Plants Animals and Humans.* Task Force Report, Iowa, USA, 139pp, 2003.

Erkmen O, Bozoglu, TF. Food Microbiology 1. Microbiology organism in food, Microbial Growth, Food borne disease and detection of microorganism and their Toxin. Iike publishing company, Ankara. 354pp, 2008.

Food and Agricultural Organisation/World Health Organisation. *Standard Programm Codex Alimentarius Commission*, Alinorm. 93pp, 1992.

Fung F, Clark RF. Health effects of mycotoxins: a toxicological overview. *J Toxicol Clin* 42: 217 – 234, 2004.

Giorni P, Magan N. and Battilani P. Environmental factors modify carbon nutritional patterns and niche overlap between Aspergillus flavus and Fusarium verticillioides strains from maize. *Intern J Food Microb* 130: 213–218, 2009.

Gowda NKS, Suganthi RU, Malathi V, Raghavendra A. Efficacy of heat treatment and sun drying of aflatoxin contaminated feed for reducing harmful biological effect in sheep. *Anim Feed Sci Technol* 133: 167-175, 2007.

Ito Y, Peterson SW, Wicklow DT. *Aspegillus Pseudotamarii*, a new aflatoxin producing specie. In: Aspegillus section, Flavi mycology Res. 233- 239pp, 2001.

Jemmali M. Les moisissures et leurs toxines. *Moulds Toxins* 10: 124 – 131, 1979.

Kaaya AN, Warren H, Adipala E. Moulds and aflatoxin contamination of maize and groundnuts in Mayuge and Kumi districts of Uganda. *MUARIK Bulletin* 3: 33 – 41, 2000.

Kajuna FF, Temba BA, Mosha RD. Surveillance of Aflatoxin B1 contamination in chicken commercial feeds in Morogoro, Tanzania. *Liv Res Rural Devel* 25 (3): 51, 2013.

Kana JR, Gnonlonfin BGJ, Wainaina J, Wanjuki I, Skilton RA. Assessment of Aflatoxin Contamination of Maize, Peanut Meal and Poultry Feed Mixtures from Different Agroecological Zones in Cameroon. *Toxins*, 5 (5): 884-984, 2013.

Kangethe EK, Langa KA. Aflatoxin B_1 and M_1 contamination of animal feeds and milk from Urban Centres in Kenya. *African Health Sci* 9:4, 2009.

Khan HS, Hasan S, Sardar R, Anjum, AM. Occurrence of Aflatoxin B1 in Poultry Feed and Feed Ingredients in Pakistan. *Intern J Agro Vet Med Sci* 5(1): 30-42, 2011.

Leeson DR, Diaz GJ, Summers JD. Poultry Metabolic Disorders and Mycotoxins. University Books. Guelph, Canada, pp. 352, 1995.

Lewis L, Onsongo M, Njapau H, Schurz RH, Luber G, Kieszak S, Nyamongo J, Backer, L. Kenya Aflatoxicosis Investigation Group Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environ Health Persp* 113(12): 1763 – 1767, 2005.

Martin, SW, Meek, HA, Willeberg P. Veterinary epidemiology: *Principles and Methods*. Iowa State University Press, Ames, Iowa.45pp, 1987.

Morgavi D, Riley RT. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. In: *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. *Anim Feed Sci Technol* 137: 201 – 212 , 2007.

Muriuki GK, Siboe GM. Maize flour contaminated with toxigenic fungi and mycotoxins in Kenya. *African J Health Sci* 2:236–241, 1995.

Nikander P, Seppala T, Kilonzo GP, Hutten P, Saarnen L, Kilima E, Pitkanen T. Ingredients and contamination of traditional alcoholic beverages in Tanzania. *Trans Royal Soc Trop Med Hyg* 85: 133 – 135, 1991.

Okoth SA, Ohingo M. Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya. African *J Health Sci* 11: 43 – 54, 2004.

Ominski KH, Marquardi RR, Sinha RN, Abramson D. *Ecological Aspects of Growth and Mycotoxin Production by Storage Fungi.* In: Mycotoxins in grains: Compounds other than aflatoxins (Miller JD. and Trenholm HL.), Eagan Press, St. Paul Minnesota, USA. pp. 287 – 314, 1994.

Pitt JL. Corrections to species names in physiological studies on *Aspergillus flavus* and *Aspergillus parasiticus*. *J Food Prot* 56: 265 – 269, 1993.

Pitt JL, Hocking AD. Fungi and food spoilage, 2nd Ed. Springer publication, New York, pp. 593, 1997.

Richard J. *Romer(R) labs Incooperation Sampling and Sample Preparation for Mycotoxin Analysis*. To Mycotoxins, Romer Guide Publisher Stylemaster Drive, Union, USA. 32pp, 2000.

Rodrigues I, Handl J, Binder EM. Mycotoxin occurrence in commodities, feeds and feed ingredients sourced in Middle East and Africa. *Food Addit. Contam. B Surveill* 4: 168 – 179, 2011.

Rushunju BG, Laswai HS, Ngowi, HA, Katalambula LK. Aflatoxin contamination of local processed cereal based complementary food in Tanzania. *Tanz Vet J* 28: 82- 95, 2013.

Set, E, Eskmen, O. The aflatoxin contamination of ground red pepper and psistachionuts sold in Turkey. *Food Chem Tox* 48:2532-2537, 2010.

Shareef AM. Molds and mycotoxins in poultry feeds from farms of potential Mycotoxicosis. *Iraqi J Vet Sci* 24(1): 17 – 25, 2010.

Smith JE, Solomons GL, Lewis, CW, Anderson, JG. Mycotoxins in Human Nutrition and Health. *European Commission* 100: 513 – 523, 1994.

Smith JE, Moss MO. Mycotoxins: formation, analysis and significance. *J Basic Microb* 26: 312, 1986.

Tanzania Food and Drug Authority. Aflatoxin Contamination and Potential Solutions for Its Control in Tanzania. In: *A Summary of the Country and Economic Assessment and the Aflatoxin Stakeholder Workshop*. (Abt associates) held in, Dar es Salaam, Tanzania, on 3 – 4 December, 2012.