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OCCURRENCE AND LEVELS OF AFLATOXIN CONTAMINATION IN POULTRY FEED INGREDIENTS AND LAYER MASH IN FARMS AND FEED MILLS IN GHANA

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ABSTRACT: The study assessed the incidence and contamination levels of total aflatoxins (TAF) and aflatoxin B_1 (AFB_1) in feed ingredients and compound layer mash from six regions in Ghana. Thirty-five facilities comprising commercial poultry farms and feed mills were used in the study. There was 100% incidence of TAF and AFB_1 in the samples of layer mash and feed ingredients (maize, soybean meal and wheat bran). The TAF of layer mash, maize and soybean meal (55.2, 54.0 and 47.6 µgkg^{-1}, respectively) were significantly higher ($P < 0.05$) than TAF of wheat bran (28.6 µgkg^{-1}). Most of the layer mash, soybean meal and wheat bran samples had TAF concentrations exceeding the US Food and Drug Administration (USFDA) maximum limit of 20 µgkg^{-1}. Mean TAF concentrations in layer mash and maize samples were strongly and positively correlated ($r = 0.50$; $P < 0.018$). Layer mash, maize and soybean meal had significantly higher ($P < 0.05$) AFB_1 concentrations (33.0, 35.1, 26.5 µgkg^{-1}, respectively) when compared to wheat bran (13.8 µgkg^{-1}). Most layer mash and maize samples exceeded the European Commission's maximum limits of 20 and 50 µgkg^{-1} respectively for AFB_1. Mean AFB_1 concentrations in layer mash and maize samples were strongly and positively correlated ($r = 0.54$; $P = 0.01$). High aflatoxins contamination of poultry feed is a persistent problem in Ghana. The use of toxin-binders, education of poultry farmers and feed millers on the implication of aflatoxins contamination in poultry feeds and the enforcement of regulation by Ghana's food and drugs authority is recommended.

Keywords: Aflatoxin B_1, Compound feed, Maize, Soybean meal, Total aflatoxin, Wheat bran.

INTRODUCTION

Poultry production plays a major role in poverty reduction and improving food security in developing countries including Ghana. Feed is a major input in livestock production, accounting for approximately 70% of total production costs (Donohue and Cunningham, 2009; Etuah et al., 2021) directly impacting animal health, productivity and public health. Consequently, the need to ensure quality and safety of feeds in animal production cannot be overemphasized.

The feed industry in Ghana has gradually shifted to production of layer feed due largely to bulk importation of chicken meat and the attendant decline in domestic broiler bird production (Ashitey, 2017). As a result, layer feed constitutes approximately 80% of feed manufactured by commercial feed mills and farms (Ashitey, 2017). Poultry feed formulated mostly from feed ingredients including maize, soybean meal, wheat bran, cotton-seed cake, palm kernel cake, copra cake and fish meal (Ashitey, 2017). The above ingredients are however susceptible to contamination by mycotoxin-producing fungi, including *Aspergillus flavus* and *Aspergillus parasiticus* (Nemati et al., 2014) which produce secondary fungal metabolites a major one being aflatoxin. The presence of aflatoxins in feed could reduce nutritive value of feeds and adversely affect animal health and performance as well as compromise the resultant animal products (meat, milk, eggs) and public health (Mokubedi et al., 2019; Ochieng et al., 2021; Thakur et al., 2022). For example, chronic exposure of layer birds to dietary aflatoxins reduced egg production, decreased weight gain and increased liver fat levels (Rosmaninho et al., 2001; Aly Salwa and Anwer, 2009), with attendant economic implications. Aflatoxins contamination of feed can occur at any point along the feed value chain, especially during cultivation and production of feed ingredients as well as production, handling and storage of compound feed (Obonyo and Salano, 2018; Omari et al., 2020). Reducing aflatoxin contamination of feed will help improve human and animal, and productivity.

Some research on aflatoxins contamination of poultry feeds has been carried in Ghana (Kumi et al., 2019; Aboagye-Nuamah et al., 2021), this notwithstanding, information on source tracking of compound poultry feed contamination in Ghana is not readily available. Source tracking of compound feed contamination is particularly imperative on the premise that major feed ingredients (maize, soybean and wheat bran) used in poultry feed compounding in Ghana are susceptible to aflatoxins contamination due to prevailing high temperatures and humidity, poor agricultural, postharvest handling and

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storage practices (Akowuah et al., 2015; Ashitey, 2017; Nakavuma et al., 2020) during feed production that serve as favorable conditions for aflatoxins contamination. The main objective of this study was therefore to determine incidence and concentrations of total aflatoxins (TAF) and aflatoxin B_1 (AFB_1) in feed ingredients in locally manufactured compounded feed (layer mash) from commercial poultry farms and feed mills in the country.

MATERIALS AND METHODS

Location of study and data collection

Thirty-five facilities comprising commercial poultry farms and feed mills were selected randomly from six regions (Bono, Ashanti, Eastern, Western, Greater Accra and Northern) in Ghana for the study (Figure 1). Ghana is located on longitude 3° 11´W and 1° 11´E and latitude 4° 44´N and 11° 11´N (MOFA, 2011). The country is divided into 16 administrative regions, across five main agroecological zones namely, Rainforest, Deciduous forest, Transitional, Northern Savannah (Guinea and Sudan Savannah) and Coastal Savannah (MOFA, 2011). The climate of Ghana is generally warm and humid with mean annual rainfall of approximately 1187 mm and mean annual temperature ranging between 26.1°C at the coasts to 28.9°C at the extreme north. The Northern region, a relatively dry savannah agroecological zone, has annual rainfall of 1100 mm. The Bono region falls within the moist deciduous ecological zone which has annual rainfall of 1500 mm. The Ashanti and Eastern regions are in the moist semi-deciduous forest agroecological zone and have an annual rainfall of 1500 mm, while the Western Region is located in the rainforest agroecological zone and is the wettest part of Ghana.

The region has annual rainfall of 2200 mm. At each of the facilities, four samples comprising three feed ingredients (maize, soybean meal and wheat bran) and one compound layer mash were collected. In total, eighty-eight samples (n=88) were collected; consisting of feed ingredients (n = 66) and compound layer mash (n = 22). A breakdown of the samples collected per region is shown in Table 1.

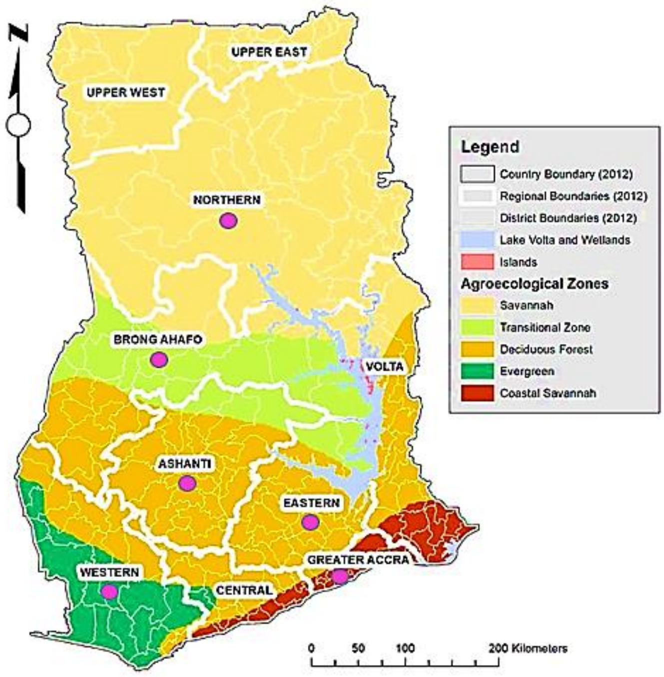

Figure 1 - Regional map of Ghana with agro-ecological zones (Abbam et al., 2018). Sampling points indicated with dots.

Table 1 - Number of feed ingredients and compound feed collected

Region	Maize	Soybean	Wheat bran	Compound feed (layer mash)
Bono	5	5	5	5
Ashanti	4	4	4	4
Eastern	2	2	2	2
Western	2	2	2	2
Greater Accra	7	7	7	7
Northern	2	2	2	2
Total	22	22	22	22

Sample preparation

The samples collected were finely ground in a Retsch® ultra-centrifugal mill (ZM 200) with a sieve size of 0.5 mm. The ground sample was thoroughly mixed and spread out on a flat surface and then 500 g sub-sample was obtained by taking samples from different sections of the spread-out sample. The 500 g sub-sample was mixed thoroughly and then 10g was taken from it for analysis.

Aflatoxin extraction

Aflatoxins extraction was performed according to the kit manufacturer's procedure of the R-Biopharm® laboratories (R-Biopharm, 2008). All feed samples and reagents were brought to room temperature (25°C) and the extraction carried out at room temperature. Aflatoxins were extracted from the analytical sample by mixing the 10 g analytical sample with 50 ml methanol (70%). The mixture was shaken manually for 15 minutes at room temperature and filtered through a Whatmann No. 1 filter paper. The filtrate was kept in covered bottles and refrigerated (5°C) prior to analysis.

Determination of aflatoxin concentrations

The TAF and AFB_1 concentrations in feed ingredients (maize, soybean meal and wheat bran), and the compound layer mash were analysed by direct competitive Enzyme-Linked Immunosorbent Assay (ELISA) using the Ridascreen® total aflatoxin and the Ridascreen® aflatoxin B130/15 procedures respectively by R-Biopharm® laboratories. Analysis was performed according to the manufacturer's procedure (R-Biopharm, 2008).

Statistical analysis

Means and standard deviations of results obtained were computed and subjected to Analysis of Variance using SPSS version 26 (2019). Test of significance between mean concentrations of TAF and AFB_1 was determined using Least Significant Difference (LSD) at 5% level of probability. The Pearson's correlation coefficient was computed between concentrations of TAF and feed ingredients, and also between AFB_1 and feed ingredients.

RESULTS AND DISCUSSION

Incidence and mean concentrations of aflatoxins in layer mash and feed ingredients

There was 100% incidence of TAF and AFB_1 in the samples of layer mash, maize, soybean meal and wheat bran. The mean concentrations of TAF in layer mash and the feed ingredients (maize, soybean meal and wheat bran) are indicated in Table 2. Layer mash, maize and soybean meal had higher ($P < 0.05$) TAF concentrations than wheat bran. Most of the layer mash, soybean meal and wheat bran samples had TAF concentrations exceeding the US Food and Drug Administration (USFDA) or the Ghana Standards Authority (GSA) maximum limit of 20 μgkg^{-1}. This is likely due to non-adherence to Good Manufacturing Practices (GMPs) and poor storage practices at majority of the farms sampled. Mean TAF concentrations in layer mash and maize samples were positively correlated ($r = 0.50$; $P < 0.018$). The observed total aflatoxins concentration of layer mash 55.2 μgkg^{-1} (Table 2) was similar to concentrations of 57.2 μgkg^{-1} reported by Aboagye-Nuamah et al. (2021) for TAF in compounded poultry feed sampled from three regions in Ghana (Bono, Ashanti and Eastern), but higher than the mean of 2.85 μgkg^{-1} and upper limit of 22 μgkg^{-1} reported by Kumi et al. (2019), who also observed 100% incidence of TAF in compounded poultry feed from the Greater Accra, Bono, Ashanti and Western regions of Ghana. Furthermore, Nsiah et al. (2023) investigating the incidences of aflatoxin contaminations in ingredients, feed and products of poultry from the Greater Accra and Eastern regions of Ghana recorded 100% incidence of TAF in poultry feed and maize. High aflatoxins contamination of poultry feed is therefore a persistent problem in Ghana. Similar high incidence of TAF have also been reported in broiler and layer mash from South Africa (92%; Mokubedi et al., 2019), Cameroon (83%; Kana et al., 2013) and Kenya (93%; Kemboi et al., 2020).

Table 2 - Concentration of total aflatoxins (TAF) in layer mash and feed ingredients (Mean ± SE)

Feed/ Ingredient	Mean concentration (μgkg^{-1})	Range (μgkg^{-1})	Percentage of samples exceeding USFDA maximum limit (%)
Layer Mash	55.2 ± 6.08^a	23.2 – 114	100
Maize	54.0 ± 7.29^a	17.0 – 125	13.6
Soybean	47.6 ± 1.43^a	38.0 – 61.0	100
Wheat bran	28.6 ± 2.83^b	15.2 – 79.5	72.7
P-value	$P < 0.001$	-	-

Means within a column with different superscripts (a, b) are significantly (P<0.05) different; SE= Standard Error; USFDA= United States Food and Drugs Authority. USFDA maximum limit for total aflatoxins in layer mash, soybean meal and wheat bran is 20 μgkg^{-1} and for maize is 100 μgkg^{-1}

The concentrations of AFB_1 in layer mash, maize, soybean meal and wheat bran are shown in Table 3. Layer mash, maize and soybean meal had higher ($P < 0.05$) AFB_1 concentrations than wheat bran. Most layer mash and maize samples exceeded the European Commission (EC) maximum limits of 20 μgkg^{-1} and 50 μgkg^{-1} respectively for AFB_1. The mean AFB_1 concentrations in layer mash and maize samples were strongly and positively correlated, ($r = 0.54$; $P = 0.01$). Ezekiel et al. (2012) reported higher AFB_1 levels of up to 1067 μgkg^{-1} in layer and broiler mash in Nigeria. The reported concentrations were over tenfold the upper limit observed for AFB_1 in the current study (74 μgkg^{-1}, Table 3). Kirinyet et al.

(2023) have also reported of high AFBI in broiler feed samples from selected farms in Nairobi City County in Kenya. High AFB_1 contamination of feed is of particular concern as it is the most potent genotoxic and carcinogenic strain of the aflatoxins (Patel et al., 2015), because of its highly reactive metabolite, AFB_1-8, 9-epoxide.

Table 3 - Concentrations of aflatoxin B_1 (AFB_1) in layer mash and feed ingredients (Mean ± SE)

Feed/ Ingredient	Mean concentration (µgkg^{-1})	Range (µgkg^{-1})	Percentage of samples exceeding EC maximum limit (%)
Layer mash	33.0 ± 4.58^{a}	8.50 – 74.0	63.6
Maize	35.1 ± 6.68^{a}	5.00 – 104	54.6
Soybean	26.5 ± 0.91^{a}	19.5 – 35.0	0.00
Wheat bran	13.8 ± 2.39^{b}	5.25 – 60.0	4.55
P-value	0.003	-	-

Means within a column with different superscripts (a, b) are significantly ($P<0.05$) different; SE= Standard Error; EC= European Commission. The EC maximum limit for AFB_1 aflatoxins in layer mash and maize is 20 µgkg^{-1}, and for soybean meal and wheat bran is, 50 µgkg^{-1}.

The much lower TAF and AFB_1 concentrations in wheat bran compared to maize, soybean meal and layer mash (Tables 2 and 3) may be attributed to the fact that all wheat on the Ghanaian market is imported from temperate countries where conditions are unfavorable for aflatoxins contamination coupled with its proper drying postharvest prior to storage and export. Also, the cleaning and sorting procedures carried out during industrial processing of wheat into flour and bran in Ghana may have contributed to reducing aflatoxins contamination. The high TAF and AFB_1 concentrations in layer mash in this current study corroborates reports of high prevalence and contamination of feeds in Ghana and other tropical countries in Africa owing to conducive climatic conditions (warm and humid) which encourage fungal growth and aflatoxins production in feed ingredients (Hell et al., 2000; Ochieng et al. 2021).

Amongst the feed ingredients, high TAF and AFB_1 were recorded in maize, 54.0 µgkg^{-1} (Table 2) and 35.1 µgkg^{-1} (Table 3) respectively. Similar reports of higher aflatoxins concentrations have been reported for maize from Ghana. For example Kortei et al. (2021) found 80% incidence of TAF and AFB_1 in maize samples from various markets in Ghana, at levels ranging from 0.78 µgkg^{-1} to 445 µgkg^{-1} and 0.78 µgkg^{-1} to 339 µgkg^{-1} respectively. Also, Dadzie et al. (2019) found TAF and AFB_1 concentrations of up to 945 µgkg^{-1} and 821 respectively in maize. Furthermore, in a recent study, Nsiah et al. (2023) reported 100% incidence in samples from the Greater Accra and Eastern Regions of Ghana with TAF and AFB_1 ranging from 2.53 to 54.26, and 1.62 to 45.70 µgkg^{-1} respectively. Considering the high aflatoxins contamination of maize and the common practice of channeling bad grains to animal feed, poultry in Ghana may be continually exposed to high concentrations of dietary aflatoxins. Mwalwayo and Thole (2016) and Worku et al. (2019) also reported 100% incidence of TAF in maize from Ethiopia and Malawi respectively. Ayalew (2010) also found 88% incidence of total aflatoxins in maize samples in Uganda similar to the high incidence of total aflatoxins observed in this study.

Concentrations of TAF (28.6 µgkg^{-1}; Table 2) and AFB_1 (13.8 µgkg^{-1}; Table 3) in wheat bran were relatively low. Similarly, Rodrigues et al. (2011) determined concentrations of mycotoxins in maize, wheat bran and soybean meal from seven African countries (including Ghana, Nigeria, and Kenya) and reported lower total aflatoxins contamination in wheat bran (1 µgkg^{-1}) and soybean meal (4 µgkg^{-1}) compared to maize (28 µgkg^{-1}). High aflatoxins concentrations were also found in soybean meal in the current study. Poor storage conditions at majority of the facilities may have led to such high contamination levels.

The high concentrations of aflatoxins observed in the maize ingredient in the current study might be due to infestation of fungi before and at harvest due to poor agricultural and cultural practices (Williams et al., 2004; Chandra and Bishnoi, 2015). Maize cultivation in Ghana and majority of African countries, for instance is predominantly carried out by smallholder resource-poor farmers who often use poor harvesting, drying and storage methods (Kortei et al., 2021). This creates avenues for fungal contamination and aflatoxins production prior to processing into feed. High total TAF and AFB_1 concentrations observed in the layer mash may reflect the high aflatoxins concentrations in maize samples used in formulating this compound feed. This is suggested by the significant positive correlation between the two variables. Similarly, Streit et al. (2013) reported that high aflatoxins concentration in maize used in feed preparation, directly influenced concentration of aflatoxins in the compounded feed. This may be due to the high proportion of maize in compound feed, i.e. approximately 60% of entire formulation (IFPRI, 2017), as well as high susceptibility of maize grains to mycotoxigenic fungal contamination and aflatoxins production (Streit et al., 2013). In the current study however, aflatoxins contamination levels in soybean meal were also found to significantly influence the contamination levels in layer mash.

Concentration of total aflatoxin (TAF) and aflatoxin Bl (AFB_1) in layer mash and feed ingredients from commercial poultry farms and feed mills

Although the mean TAF, and AFBI concentrations from the commercial poultry farms were higher than those from the commercial feed mills, they were however not significantly (P>0.05) different (Table 4). Generally, aflatoxins contamination was higher in feed ingredients from commercial poultry farms than the commercial feed mills (Table 5). This may be due to non-adherence to good farm management practices (eg. poor facility design, ingredients quality assurance, equipment cleaning, pest management) as well as the poor storage conditions at majority of the farms observed during the study. Similar high concentrations of aflatoxins were reported in layer mashes from smallholder farms (98.4 $\mu g kg^{-1}$) than samples from commercial feed mills (40.4 $\mu g kg^{-1}$) in Uganda (Nakavuma et al., 2020).

Table 4 - Concentrations of total aflatoxins and AFB_1 in layer mash from commercial poultry farms and feed mills (Mean ± SE)

Facility	Total aflatoxins Conc. ($\mu g kg^{-1}$)	AFB_1 Conc. ($\mu g kg^{-1}$)
CFMs	42.6 ± 6.62	24.4 ± 5.73
CPFs	64.0 ± 8.58	39.0 ± 6.29
P-value	< 0.084	< 0.119

Means within a column with same superscripts are not significantly (P>0.05) different; SE= Standard Error; CPFs = commercial poultry farms; CFMs = commercial feed mills

Table 5 - Concentration of total aflatoxins and aflatoxin B_1 in feed ingredients across commercial poultry farms and feed mills (Mean ± SE)

Type of facility	Total Aflatoxins ($\mu g kg^{-1}$)			AFB_1 ($\mu g kg^{-1}$)		
	Maize	Soybean	Wheat bran	Maize	Soybean	Wheat bran
CFMs	38.2 ± 10.90	45.6 ± 2.55	21.6 ± 2.59	22.5 ± 9.68	23.9 ± 0.96^b	9.50 ± 1.61
CPFs	64.9 ± 8.85	49.0 ± 1.62	33.4 ± 3.98	43.8 ± 8.56	28.3 ± 1.16^a	16.7 ± 3.74
P-value	< 0.071	< 0.239	< 0.380	< 0.118	< 0.014	< 0.142

Means in the same column with different superscripts (a, b) are significantly different (P < 0.05). SE= Standard Error; AFB_1= Aflatoxin B_1. CFMs – Commercial feed mills, CPFs – Commercial poultry farms.

CONCLUSION AND RECOMMENDATIONS

All samples (100%) of layer mash, maize, soybean meal and wheat bran were contaminated with total aflatoxins (TAF) and aflatoxin B_1 (AFB_1). Higher concentrations of TAF and AFB_1 were found in layer mash, maize and soybean meal than in wheat bran. Most of the samples of layer mash had TAF concentrations above the Ghana Standards Authority (GSA) and US Food and Drug Administration (USFDA) maximum limit, while AFB1 concentrations were above the EC maximum limit.

Feed millers and poultry farmers should be educated on the implications of aflatoxins contamination in poultry feeds and its impact on animal health, production and public health. The need to include toxin binders in compound poultry feed is being recommended. In addition to continuous surveillance and monitoring of aflatoxin levels in feed and feed ingredients, the Food and Drug Administration (FDA) in Ghana should intensify regulation of commercial feed mills and on-farm feed mills in the country to ensure strict compliance to Good Manufacturing Practices (GMPs) to ensure safety of locally manufactured poultry feed.

DECLARATIONS

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Authors' contribution

Bella Nkansah participated in the proposal design, performed the laboratory analysis and statistical analysis and prepared the manuscript in writing. Leonard Adjorlolo, Regina Appiah-Oppong and Frederick Obese all participated in the reviewing and editing the proposal and write-up. All authors read and approved the final manuscript before submitting for publication.

Ethical consideration

The authors complied with the ARRIVE guidelines and the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

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Consent to publish

All the authors agree to publish this manuscript in this journal.

Competing interests

The authors have no competing interests.

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