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Aflatoxin Contamination in Feeds and Eggs From Bafoussam, Cameroon

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Abstract

Safe feed is vital for animal health, and is a precondition for food safety and human health. Dietary exposure of poultry chickens to aflatoxin-contaminated feeds may result in negative health implications, and may be carried-over into their by-products such as eggs. The aim of this study was to determine the contamination levels of aflatoxins in feed and egg samples from layer chickens, as well as egg samples from free-range chickens from Bafoussam in Cameroon. Levels of crude proteins and total aflatoxin were determined in layer chickens' feeds (n=9) from nine poultry farms. Additionally, aflatoxin B1 was quantified in eggs (16 pooled egg samples) from the layer chickens that were fed the studied feeds as well as eggs (10 pooled egg samples) from free-range hens in Bafoussam. All studied feed samples revealed levels of crude proteins (mean: 20.4, range: 17.2-25.6 g/100 g of feed) higher than standard (16 g/100 g feed), and levels of total aflatoxin (mean: 107.6, range: 27-178 µg kg-1) higher than the maximum tolerable limit (20 µg/kg) in feed. Levels of aflatoxin B1 in eggs from layer chickens (mean: 0.0005, range: nd-0.0013 µg/kg; 62.5%) and in eggs from free-range hens (mean: 0.0016, range: 0.0009-0.0024 µg/kg; 100%) did not exceed the maximum tolerable limit (2 µg/kg) of aflatoxin B1 in foods destined for human consumption. The direct proposional relationship between crude proteins and total aflatoxins levels speculate that proteins source for feed production may be a critical control point for aflatoxins contamination in feeds. Although levels of aflatoxins B1 in eggs were generally low, the higher occurrence frequencies may be a concern when considering eggs as a part of diet for all age categories in Cameroon.

Keywords: Aflatoxins, Eggs, Feed, Layer Chicken, Free-Range Hens.

Introduction

The supply of healthy and nutritious food is a key issue in food security and safety (USAID, 2002). Safe feed and feed ingredients are vital for animal health, and is a precondition for food safety and human health. Feed and feed raw materials are often contaminated with fungi secondary metabolites, mycotoxins (Park, 2018; Čolović et al., 2019), and in some cases, at concentration that can affect the health condition of animals. Mycotoxins frequently contaminate agricultural commodities worldwide destined for animal and human consumption (Eskola et al., 2019). Mycotoxins, particularly the Aspergillus toxin, aflatoxins, is known to contaminate feed materials (especially maize) and finished poultry feeds worldwide (Streit et al., 2013; Kovalsky et al., 2016). The occurrence of aflatoxins is exacerbated by factors such as the presence of other microorganisms (plus unpredictable interactions that they may develop), late harvest and moist environment that is favoured by insufficient drying (PACA, 2013). Aflatoxins are well known to exert toxic effects including immunosuppression, and stunting (IARC, 2002, 2012, 2015) in animals and human when aflatoxin-contaminated feeds and foods are consumed, respectively. Exposure to aflatoxins has also been associated with high vulnerability to infectious diseases like malaria, tuberculosis and HIV/AIDS (PACA, 2013; Abia et al., 2013a).

Aflatoxins have been reported to be carried-over from aflatoxins-contaminated feeds fed poultry chickens to eggs laid by such chickens (Aly Salwa and Anwer, 2009; Völkel et al., 2011). Eggs provide a well-balanced source of nutrients, and remains valuable in therapeutic diets for adults partly due to its low caloric value and relative ease of being digested (Kuang et al., 2018; Réhault-Godbert et al., 2019). Egg is widely used in several meals for all ages. In Cameroon, reports on aflatoxins in feeds, feedstuffs and /or eggs (Tchana et al., 2010; Simo et al., 2011; Kana et al., 2013; Abia et al., 2013b) revealed aflatoxins levels higher than the maximum tolerable limits fixed by the Food and Agriculture Organizations (FAO, 2003) and the European Commissions (EC, 2006). For example, Kana et al. (2013) revealed that 9 percent of maize used for human and animal needs in Cameroon were tainted with aflatoxins (range: 2-42 µg/kg), meanwhile, aflatoxins were detected in all studied samples of peanut-cake (range: 39-950 µg/kg). Abia et al. (2013b) reported mean aflatoxin concentration of 40 µg/kg in 95 percent of studied poultry feed samples from Cameroon. The need for safe ingredients for safe poultry feed production, cannot be over emphasized when considering health of poultry birds, as well as human health when we consume poultry birds and or their by-products. This necessitates regular monitoring of aflatoxins in layer chicken feeds as well as in eggs, when considering the risk of carry-over of aflatoxins from aflatoxins-contaminated feeds into eggs. The aim of this study was to determine the contamination levels of aflatoxins in feed and egg samples from layer chickens, as well as egg samples from free-range chickens from the Bafoussam I district area in Cameroon.

Material and methods

Study Site and Target Population

This study was carried out in Bafoussam (Figure 1), the headquarter of the West region of Cameroon. Bafoussam is known for breeding of layer chickens as well as eggs production. About 70% of these eggs are conveyed to the country's major cities, Yaoundé and Douala, as well as being exported to the neighbouring countries such as Gabon, Chad, Central African Republic and Congo Republic. Sixty to seventy percent of poultry feed is maize (Abia et al., 2013b), a staple food in Bafoussam. The targeted population for this study were poultry farmers in Bafoussam with at least 1,000-layer chickens or hens (age: ≥25 weeks) in their poultry farms.

Sampling

Poultry feeds for finisher layer chickens (n=9; 200 g each per farm) were collected by probing the feed bag from different sites. For poultry egg samples (n=32 eggs), two eggs each were collected from seven of the nine studied poultry farms on days three (n=14 eggs) and seven (n=14 eggs) following feed administration. Specimen collection was done on day five for the other two farms (n=4 eggs) due to no feed storage on day seven in the two farms.

Figure 1: Map of Cameroon showing the study site, Bafoussam

Additionally, twenty egg samples from free-range hens were purchased from two local community markets in Bafoussam I. It is vital to mention that feed from the free-range hens could not be collected as they feed on uncontrolled items in the environment. Sampling was done in August 2014. Altogether, the collected feed and egg samples were kept in refrigerated containers at 7-8 °C, conveyed to the laboratory of Pharmacology and Toxicology, of the University of Yaounde 1, Cameroon, for analysis of total crude protein, aflatoxin B1 and total aflatoxin contents using an ELISA kit.

Analysis of Samples

Total Crude Protein Content

The crude protein contents in feed samples were assessed by means of the total nitrogen content, done after mineralization according to Kjeldahl and by colorimetric technique as described by the Devani et al. (1989). In brief, organic nitrogen was converted into mineral nitrogen ammonium sulphate and quantified after which it was used to estimate total crude protein level. Specifically, a mixture containing 0.25 g of the sample, 5 mL of concentrated H_2SO_4 (98%, density = 1.83 g/cm^3), and a pinch of mineralization catalyst, was transferred into a mineralization matrix. The set was thereafter heated (temperature > 100 °C) with a mineralization ramp until a clear solution was obtained. After allowing at ambient temperature, the product recovered from the mineralization process was transferred into a volumetric flask and completed with distilled water to 50 mL flask graduation. The same protocol was followed for the blank control (distilled water). Nitrogen quantification was then performed according to Devani et al. (1989) for which color change intensity positively correlates nitrogen content in the tested specimen. The amount of nitrogen in each assay (q) was estimated by reference to the calibration curve of the OD (OD = aQ + b) as plotted in supplementary Figure 1.

Quantitation of aflatoxins in feed and egg samples

Extraction of total aflatoxins from feed samples

The samples were treated as recommended by the protocol provided with the ELISA kit (RENEKABIO, California, USA, lot No: 080414). In brief, 200 mL of extraction solvent (acetonitrile: distilled water, 80:20%, v/v) was added into 2 g of each feed sample (1:100 dilutions (w/v), stirred vigorously for 10 mins, and then centrifuged at 3500 rpm for 5 min. Then, 0.5 mL of the supernatant was mixed with 4.5 mL of a washing buffer (PBS) (1 L of distilled water into 4.5 mL of the original solution), and vortexed thoroughly to serve for the assay. Standards did not require pre-dilution. The extraction products were kept frozen in lidded glass vials until analysis.

Extraction of aflatoxins in egg samples

This was performed as described by Micco, adapted from that of AOAC (Micco et al., 1994). In brief, into 100 g of the egg sample, 42 mL of saturated 40% NaCl was added, and shaken vigorously for 10 min. Thereafter, 300 mL of acetone was added, stirred for 5 min and filtered (Whatman N° 1). The filtrate was deproteinized in 20 mL of 20% lead acetate, stirred for 3 min then, mixed with 150 mL of distilled water, and further stirred for 10 min and thereafter allowed to stand for 5 min. Thereafter, 10 g of diatomaceous earth were added, once again stirred for 10 min and allowed to stand for 5 min prior to filtration with a filter paper (Whatman N° 1). Lipid was removed from the filtrate twice using 100 mL of hexane and thereafter, the extraction process was repeated twice with 50 mL of pure chloroform. The resulting aqueous phase was subjected to acid hydrolysis as described by Gregory and Manley (1981). This phase was further diluted with an equal volume of 0.4 N HCl solution and heated in a water bath at 90 °C for 2 h; then extracted with 20 mL of chloroform and 20 mL of chloroform-acetone consecutively (1:1 v/v).

The various chloroform extracts were combined and evaporated under vacuum. Thereafter, the residues of extraction were retaken up in 5 mL of chloroform and transferred into test tubes, then evaporated at 50 °C under a hood in a water bath and away from UV light. The final product was used for the quantification of aflatoxin B1 using an ELISA kit. It is worth noting that eggs from layer chickens collected on the same day were pooled together to make a single 100 g samples (thus, 32 eggs were then pooled to 16 eggs samples). In the same way, eggs of local free-range hens were also pooled together (thus, 20 eggs were then pooled to 10 eggs samples of 100 g each). Therefore, a total of 26 pooled eggs samples of 100 g each were analysed.

ELISA Assay for aflatoxins quantification in feed and egg samples

Total aflatoxins levels in poultry feeds were analysed as described in the protocol of the Total Aflatoxin LOW MATRIX ELISA Kit (Lot No: 080414) for animal feeds. Aflatoxin B1 concentrations in eggs were determined as described in the Aflatoxin B1 Low Matrix ELISA Kit (Lot No: 080414) for chicken eggs. The limit of detection (LOD) for these kits was 1-20 µg/kg.

For each feed sample, the necessary steps (mixture and incubation, washing, revealing) were followed accordingly to the ELISA Kit protocol. Each standard or sample was tested in duplication. The optical densities (OD) were determined with an APPAR-V230 ELISA plate reader at 450 nm wavelength.

For each egg sample, extraction of residues was done using 500 µL of 80% methanol (washing buffer and methanol: 100 µL/900 µL). The resulting mixture was assayed as described in the protocol of the ELISA kit.

Aflatoxin B1 content in egg samples as well as total aflatoxins in feed samples were determined as indicated by the ELISA Kit protocol with reference to optical density in the feeds and eggs submitted to the assay; then interpolated as indicated by the standard binding curve (supplementary Figure 2).

Statistical Analyzes

Data was analyzed with the use of a one-way analysis of variance (ANOVA) of the Statistical Package for the Social Sciences (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA). Furthermore, the non-parametric tests were used to compare paired and independent samples. Thus, the Mann-Whitney U test and the simple regression linear model were applied.

Results

Crude protein and aflatoxin levels in poultry feeds and eggs from layer chickens

Table 1 below provides amounts of crude proteins (mean±standard deviation: 20.38±2.82 g/100 g; range: 17.19-25.63 g/100 g of feed) in the studied feed samples. All studied feed samples were positive (100%) for total aflatoxin (range: 27-178 µg/kg). Meanwhile, aflatoxin B1 was detected in six (85.7%; mean: 0.000917 µg/kg) and four (57.1%; mean: 0.00095 µg/kg) egg samples collected on days three and seven following administration of the studied feeds, respectively.

Comparison of aflatoxin B1 amounts in eggs from layer chickens and free-range hens

Table 2 provide information on the levels of aflatoxin B_1 in studied egg samples from layer chickens and free-range hens from the same locality, Bafoussam in Cameroon. Aflatoxins B1 levels in egg samples from layer chickens and free-range hens were in the ranges of nd-0.0013 µg/kg and 0.0009-0.0024 µg/kg, respectively.

Table 1: Protein and AF contents in the studied feed and egg samples

Farm code	Crude protein content (g/100 g)	Total aflatoxins in feeds (µg/kg)	Aflatoxin B_1 in eggs from layer (µg/kg)	
			⸸Day 3	⸸Day 7
1	19.05	27	0.0006	0.0011
2	18.09	94	0.0013	0.0011
3	20.29	175	0.0006	0.0010
4	18.91	120	nd	nd
5	20.2	97	0.0013	nd
6	25.63	115	0.0006	nd
7	24.44	95	na	na
8	17.19	67	na	na
9	19.63	178	0.0011	0.0006
F (%)	9 (100)	9 (100)	6 (85.7)	4 (57.1)
Mean	20.38	107.6	0.000917	0.00095
Range	17.2-25.6	27-178	nd-0.0013	nd-0.0011
MTL	16^{β}	20*	2**	2**

⸸Day of egg collection from the layer chickens following exposure to the studied feeds. nd: not detected. na: not analysed. β: ISA Brown (2011); *: FAO (2003); **: EC (2006); F (%): Frequency (percentage)

Table 2: Aflatoxin B1 contents in egg samples from layer chickens and free-range hens

Bird type	Number of samples	Aflatoxins B1 positive samples		Aflatoxins B1 levels (µg/kg)		Mean ± SD^{α}
		Number	%	Minimum	Maximum	
Layer	16^{π}	10	62.5	nd	0.0013	0.0005 ± 0.0005
Free-range hens	10^{π}	10	100	0.0009	0.0024	0.0016 ± 0.0006

$^{\pi}$Pooled eggs samples; SD^{α}: Standard Deviation

Discussion

Food safety concerns raised from consumption of animal-based food products is of major interest nowadays that natural food contaminants can easily go through the complex food production and supply chains. Surveying foods at various stages in the supply chain to ensure tolerable levels, or even absence of contaminants where possible, remains vital. Provision of such information could be a vital contribution towards protecting consumers' health. The present investigation was carried out to quantify aflatoxin contents in feed and egg samples from layer chickens, and egg samples from free-range hens from the Bafoussam I geographic area in Cameroon. Nine poultry farms in Bafoussam, known to be famous in Cameroon for layer chicken breeding and eggs production were surveyed and their layer feed and egg samples collected and analyzed, alongside with egg samples from free-range hens in same localities, for presence of aflatoxins. In addition, crude protein levels were quantified in studied feeds only. All the studied feed samples had crude protein and total aflatoxin levels higher than their respective maximum tolerable limits (MTLs). Higest frequency and levels of aflatoxin B1 were detected in egg samples collected on day three relative to day 7. This is indicative of transfer of aflatoxins from aflatoxin-contaminated feeds fed the layer chicken into its eggs. Egg samples from free-range hens had higher occurrence frequencies (100%), and relatively higher levels of aflatoxin B1 when compared with levels in layer chickens.

The crude protein content found in layer chickens' diets were higher than the standard value of 16 g/100 g complete feed (ISA Brown, 2011). This was not strange to the farmers who however did not disclose the proportions in the formulation. The observed high levels of crude proteins in feeds could be attributed to the use of CMAVs (Nitrogen Mineral Concentrates and Vitamins, very high protein compounds) used in animal diets as a vector for growth.

All the studied feed samples were tainted with aflatoxins (mean: 107.6, range: 27-178 µg/kg) at levels that exceeded the maximum tolerable level (MTL) of 20 µg/kg of total aflatoxins in feeds for layer chickens fixed by the Codex Alimentarius (FAO, 2003). About 90% of feed samples contained total aflatoxin levels three-times higher than the MTL. This may be in part, due to the use of feed ingredients that were moldy, exposed to weevils, and or stored in poor feed storage environment. These findings were consistent with those reported in different agro-ecological zones in Cameroon (Tchana et al., 2010; Simo et al., 2011; Kana et al., 2013; Abia et al., 2013b).

Presence of aflatoxins, especially aflatoxin B1, in various foods items represent a major threat to the health of exposed human populations. The studied egg samples from layer chickens (62.5%) and free-range hens (100%), which are destined for human consumption were contaminated with aflatoxin B_1. Eggs from free-range hens had relatively higher occurrence frequency and aflatoxin B_1 levels ($p<0.05$) partly due to their uncontrolled feeding nature e.g., no restriction to diets, and the commonplace practices of throwing visibly spoiled grains around the house for free-range animals to feed on. The aflatoxin B_1 levels reported here are in line with the report of Pourelmi et al. (2013) that eggs from free-range hens are more tainted with aflatoxin B1 although they did not have any significant difference as it is the case in this present study. Although chicken eggs are generally processed prior to eating, in most cases, if not all, the processing conditions e.g., cooking temperature, is not enough to destroy aflatoxin B_1, thus remaining as a potential health threat to consumer populations. For example, aflatoxin B_1 are known to exert toxic health effects including stunting in children, immunosuppression and cancer in humans (IARC, 2002, 2012, 2015) and or serve as risk factors to common human disorders such as malnutrition (Tchana et al., 2010) and malaria, and HIV/AIDS (PACA, 2013; Abia et al., 2013a). Findings from this present study corroborates with those previously reported in eggs from Cameroon (Tchana et al., 2010) and elsewhere (Pourelmi et al., 2013; Herzallah, 2013).

When compare aflatoxin levels in the studied feed samples and which was fed to the layer chickens whose egg samples were studied, it is obvious that aflatoxins were carried over from the aflatoxins-contaminated feeds into the eggs. This is in line with the report of Pourelmi et al. (2013). Notwithstanding, this study did not capture information such as hens' exposure time to the studied feeds, metabolite synthesis and egg transfer rate which may partly influence egg and egg derivative contamination patterns. Aside from consumers' health protection, egg contamination may influence chick performance at hatch and also lead to a drop in egg production, egg weight and hepatic steatosis in layer chickens exposed to relatively high doses, such as 1000 µg aflatoxin B1/kg of feed for 28 days (Magnin et al., 2016). Furthermore, presence of aflatoxins in eggs may even be responsible for the low shelf-life of eggs complaints from some farmers and consumers in Cameroon, thus, jeopardizing the economy and frustrating consumers' satisfaction. Likewise, little is known on the possible role of aflatoxins in eggs vis-à-vis the assumed rich nutrient contents consumers expect to derive from such aflatoxin-contaminated eggs, suggesting further studies in that direction.

Also, aflatoxin B_1 contamination levels in eggs from layer chickens collected three days after exposure to the studied diets were significant higher ($p<0.05$) relative to aflatoxin B_1 contamination levels in eggs collected after seven days. This may partly be due to conjugation of aflatoxin B_1 in the liver of the studied layer chickens into aflatoxin M1 and other products of metabolism such as aflatoxicol. With a half-life of approximately 67 hours, carry-over rate of ingested aflatoxin B_1 into eggs is about 5000:1 (Lateef et al., 2013). Although aflatoxins are generally excreted by the bile and the intestine, aflatoxin B_1 and aflatoxicol have been detected in ovum and eggs (Lateef et al., 2013).

When comparing raw protein and total aflatoxin contents (Table 1) statistically, there exist a correlation coefficient closer to 17% and a R^2 ≈0.027 as the corresponding ratio, indicating a link between the two variables. The related parametric equation was designed as follows: y = 2.818x + 50.12. The simple linear regression model test showed that the aflatoxin content increase proportionately with the crude protein content. This may partly be associated with the use of soya bean and groundnuts (sometimes spoiled grains) as protein source in poultry feeds and which are known to be vulnerable to aflatoxin contamination (Abia et al., 2013c). On average, at an increase of one unit of the crude protein level corresponding to an increase of three units of the total aflatoxin rate (rate ≈ 1 for 3). Additionally, in view of aflatoxin levels in studied feeds vis-à-vis levels in eggs (Table 1), a positively correlation (≈20%, R^2 = 0.036) was observed. The derived parametric equation could be designed as: y = 2E-06x. The simple linear regression model test disclosed that aflatoxin B_1 contents in layer chickens' eggs appeared to increase with that of total aflatoxins in feed samples (1/1). On the average, an increase of one unit in the total aflatoxin level corresponds to an increase of about 5.10-7 units of aflatoxin B_1 level in laying hens. Although the observed aflatoxin B_1 levels in eggs were low, long-term exposure to such low or moderate concentrations through ingestion of aflatoxin B1-contaminated eggs, especially in children, may results in health implications such as liver cancer (IARC, 2002, 2012, 2015). Again, according to the Scientific Committee on Food, no human exposure levels of aflatoxin B_1 or aflatoxin M_1 is regarded as safe (SCF, 2002, 2003).

Conclusions

Exposures to dietary aflatoxins are well-known to exert toxic effects in animals and man. Aflatoxins are known to be carried-over from aflatoxin-contaminated feeds into eggs when layer chickens are fed with aflatoxin-contaminated feeds. After examination of finisher feed and egg samples from layer chicken from nine poultry farms in Bafoussam, Cameroon, all studied feed samples had crude protein contents higher than the recommended level (16 g/100 g complete feed) (ISA Brown, 2011). Likewise, all studied feed samples were tainted with aflatoxins and at levels higher than the Codex Alimentarius MTL of 20 µg/kg (FAO, 2003). Increase in aflatoxins levels was directly proportional to levels of crude protein partly indicative of aflatoxins contamination via feed protein source especially soya beans and groundnuts in Cameroon (Abia et al., 2013b). Additionally, aflatoxins B_1 were detected in eggs from both layer chickens (62.5%, mean AFB1: 0.0005, range: 0-0.0013 µg/kg) and free-range hens (100%, mean AFB_1: 0.0016, range: 0.0009-0.0024 µg/kg) at high frequencies but at low concentrations relative to the MTL of 2 µg/kg in foods destined for human consumption (EC, 2006). This is partly due to the commonplace practices of throwing visibly spoiled grains around the house for free-range chickens to feed on. Notwithstanding, aflatoxins B_1 levels in eggs were all below MTL (2 µg/kg) of aflatoxins B_1 in human foods (FAO, 2003). Altogether, there is need for educational intervention strategies (such as elimination of visible spoiled grains via landfills) and enforcement by all stakeholders along the feed-animal-food supply chain towards reducing aflatoxins levels in feeds and eventual chicken products for human consumption in

Cameroon as a One-Health approach. Nevertheless, attention is needed to reduce levels of aflatoxins in feeds and eggs produced thereof considering the high assimilability of eggs as well as the fact that no human exposure levels of aflatoxins B_1 or aflatoxins M_1 is regarded as safe (SCF, 2002, 2003).

Author Contributions

AMK, BFF designed the study. AMK and WAA did field work, laboratory analysis, statistical analysis, and drafting of the paper. AMK, WAA, PRKT, SNF, TA, PFM and BFF reviewed the work.

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