



Aflatoxin content in cereal-legume blends on the Ghanaian market far exceeds the permissible limit

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Abstract

Cereals and legumes, the main ingredients used in the preparation of complementary foods in Ghana, have been associated with aflatoxin contamination. This study aimed to determine aflatoxin contamination levels in cereal-based complementary foods on the Ghanaian market. A cross-sectional survey design over a two-week period was used to sample 48 commercial complementary food brands on an *as available-basis* from supermarkets or mini-marts in all 10 regions of Ghana. A tablet-assisted aflatoxin mobile Assay (mReader) that uses Reveal Q+ test strips (Neogen Corporation) was used to quantify the level of aflatoxin in the samples. All samples were contaminated with aflatoxin. Concentrations in cereal-legume blends ranged from 1 to 1094 ppb while those in cereal-only samples ranged from 1 to 11.7 ppb. The lowest aflatoxin concentrations were recorded in samples from the Upper East region with a mean of 1.5 ppb (1 to 3.8 ppb) while the highest were in samples from the Central region with a mean concentration of 457 ppb (6.6–1094 ppb). Aflatoxin concentrations in approximately a third of the infant formulations sampled exceeded the acceptable standard of 20 ppb, some by a factor of over 5 (100 ppb), and may contribute to the perennial malnutrition (stunting and iron deficiency) prevalent among children in Ghana.

Keywords Cereal-legume blends · Aflatoxin · Child health

1 Introduction

Poor nutrition resulting from food insecurity and consumption of deficient and contaminated foods seems to be the root cause of poor growth and deaths among infants and young children in Africa (Africa Nutrition Chartbooks 2005; Lombard 2014). In Ghana, between birth and the age of 20 months, the percentage of malnourished children rises rapidly from 7 to 48% (Africa Nutrition Chartbooks 2005). It is very saddening to report that about 40% of deaths of children under-five in Ghana is attributed to malnutrition (Africa Nutrition Chartbooks 2005). Symptoms of malnourished victims include stunting, wasting, underweight, night blindness,

anaemia, rickettsia, kwashiorkor and mental impairment (Aheto et al. 2015; Huffman and Schofield 2011).

The negative effects of malnutrition on children have triggered the identification of strategic approaches to reduce malnutrition, one of which is the recommendation to include protein- and energy-rich foods, such as cereals and legumes, for the feeding of infants and young children (WHO and UNICEF 2008). In view of this, cereals and legumes have been heavily depended on as key ingredients for complementary foods (UNICEF et al. 2012; Soro-Yao et al. 2014), especially in Africa, including Ghana, where cereals and legumes such as maize and groundnuts are staples. As a result, numerous ready-to-use cereal-legume based blends are beginning to flood the Ghanaian market as infant foods.

Cereals and legumes are highly susceptible to aflatoxin contamination, a group of toxins that pose serious health threats to humans and animals (Temba et al. 2016). The toxins are produced by *Aspergillus* species (mainly *A. flavus* and *A. parasiticus*) under the influence of high temperature, high relative humidity/moisture content, mechanical and pest damage, as well as poor storage practices - conditions characteristic of tropical and subtropical areas (Yun Yun Gong et al. 2016). Infants and young children, in particular, are very susceptible to the toxins and their attendant negative effects.

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Aflatoxin, depending on the level of exposure, may cause low birth weight (Lombard 2014), growth impairment such as stunting, underweight and wasting (Wagacha and Muthomi 2008; Yunyun Gong et al. 2004), immune suppression as well as mental retardation by instigating changes in the insulin-like protein growth factor and impeding mineral bioavailability (Shephard 2008).

It is also reported that aflatoxin contributes to infertility in humans (Covic and Hendriks 2016) and thus threatens the reproductive health of children in the future. With cancers claiming many lives, aflatoxin is reported to cause several of them (Wagacha and Muthomi 2008). Children under-five, especially in sub-Saharan Africa, are more vulnerable to aflatoxin exposure (YY Gong et al. 2003) because of the high level of contamination of the main ingredients of weaning foods with aflatoxin, coupled with children's low body weight and the additional nutrient requirement for rapid growth (Egal et al. 2005).

Despite the alarming health effects of aflatoxin and its association with cereals and legumes, little has been done on the cereal-based complementary foods on the Ghanaian market to ascertain their wholesomeness. It is therefore of a great concern and a necessity to evaluate the aflatoxin levels of cereal-only and cereal-legume complementary foods on the Ghanaian market across the nation to ascertain their safety for use as feed for infants and young children.

2 Materials and methods

2.1 Study design and site

A cross-sectional survey design was employed with samples taken from all 10 regional capital towns in Ghana. This was because, across the nation, several locally prepared cereal-only and/or cereal-legume blends are on the Ghanaian market for sale as complementary foods for children but are not registered with the Food and Drugs Authority.

2.2 Sampling

Forty-eight branded cereal-only ($n = 6$) and cereal-legume blends ($n = 42$) were obtained in triplicate on an "as available-basis" from supermarkets or mini-marts in all 10 regions of Ghana. The samples collected were grouped on a company basis into small scale (SS) and medium-to-large scale (ML); and on food type: cereals-only; and cereal-legume blends. The cereal-legume samples were further sub-grouped according to the main legumes added i.e. cereal-soybean (CS) and cereal-soybean-groundnut (CSG). The cereals-only blends had one of the following as the main ingredient: maize, millet or wheat.

2.3 Aflatoxin analysis

Thirty millilitres of a 65% ethanol solution was added to 10 g of each sample. The mixture was shaken for 3 min and filtered through Whatmann No 1 filter paper into a glass bottle. A dilution series of the solutions was prepared by serially diluting 100 μ l samples in 500 μ l of diluent (65% alcohol). A reveal Q+ strip (Neogen Corporation) was put into each dilution for 6 min. The strip was removed after development and tested using the mobile Assay (mReader), an equipment originally designed for AccuScan^R readers. The Reveal Q+ test strip was fully inserted into the cartridge and the cartridge inverted and inserted into the mReader. The test strip was then analyzed and the results for aflatoxin concentrations (ppb) displayed on the mReader. This was done in triplicate for each of the samples. Prior to testing the samples, the reader was calibrated using two spiked groundnut paste standards (2.5 ppb and 12 ppb) to ensure that the reader was functioning properly. The testing was done using two different in-built calibration curves pre-designed in the reader for cereals and legumes.

2.4 Statistical analysis

Data from the study were subjected to analysis of variance using the general linear model procedure in Minitab (Minitab[®] Inc. USA, version 16.2.4) statistical package. The Tukey's student range test was used to determine which of the differences among means were significant at ($p < 0.05$).

3 Results and discussion

3.1 Aflatoxin contamination by companies

All samples analyzed were contaminated with aflatoxin with individual concentrations ranging from 1 ppb to 1094 ppb (Tables 1 and 2). Sixty percent of the samples had aflatoxin contamination above 20 ppb and 35% of the companies had products with aflatoxin contamination above 20 ppb on the Ghanaian market. The range for the ML samples was from 5.83 ppb to 1045 ppb with 2 out of the 7 samples having contamination above 20 ppb (Table 1).

All samples from the ML companies except ML_A_P1 and ML_A_P2 were below the maximum allowable limit of 20 ppb (Table 1). This could partly be due to the small sample size ($n = 7$ ML companies) and possibly better internal monitoring by management as compared to the SS samples ($n = 41$ see Table 2).

The concentrations of aflatoxins in the 41 SS samples varied greatly with an average range of 1.00 ppb to 686.00 ppb and a third of them recording aflatoxins above the acceptable standard limit of 20 ppb (Table 2).

Table 1 Aflatoxin concentrations in medium-to-large (ML) scale processed complementary foods in Ghana

Company code (ML)	Concentration (ppb)	Blend
ML_A_P1 (<i>n</i> = 3)	348.50 (324–373) ^a	Cereal-soybean
ML_A_P2 (<i>n</i> = 3)	1045.00 (996–1094) ^b	Cereal-soybean
ML_B_P1 (<i>n</i> = 3)	8.17 (7.7–8.5) ^c	Cereal only
ML_B_P2 (<i>n</i> = 3)	9.27 (8.1–10.3) ^c	Cereal only
ML_D (<i>n</i> = 3)	11.30 (11.1–11.7) ^c	Cereal only
ML_E (<i>n</i> = 3)	7.37 (5.4–9.4) ^c	Cereal only
ML_F (<i>n</i> = 3)	5.83 (5.3–6.4) ^c	Cereal only
<i>P</i> value	0.001	

Values are means (range). Values in the same column with different superscripts are statistically significantly different. The letters attached to ML are codes for the companies from which the samples were obtained while the “*n*” in parenthesis represents the number of samples of that brand obtained for the analysis

3.2 Aflatoxin contamination based on food composition

All the food compositions tested positive for the toxin with a range of 1 ppb to 1094 ppb (Table 3) with no significant difference ($P = 0.059$). However, the cereals only samples had the least aflatoxin concentrations ranging from 1 ppb to 11.70 ppb, lower than the cereal-legume blends.

The low aflatoxin contamination level in the cereal-only complementary food compared to the cereal-legume blends suggests use of good quality cereals for their preparation. A closer look at the data, however, showed that the millet-only and wheat-only samples had lower contamination than the maize-based samples. Although millet and wheat are not totally resistant to aflatoxin contamination, a number of studies have shown these two to have a higher level of resistance to aflatoxin producing *Aspergillus* species compared to maize and legumes (Kumar et al. 2017; Bandyopadhyay et al. 2007). In Kenya, for example, where high aflatoxin contamination has previously been reported in maize, a study conducted in 2014 showed that only 10% of millet sampled from rural households in 5 counties had aflatoxin B1 values above 5 ppb, the permissible limit for the Kenyan Bureau of Standards compared to 26% in maize (Sirma et al. 2016). When compared with other Nigerian food commodities including rice, millet tested negative for aflatoxin B1 whilst values in rice ranged from 37.26–113.2 µg/kg. This suggests that if cereals are to be used for complementary food, then millet and wheat would be better options than maize or rice. However, the price of imported wheat to Ghana would anyway make it unaffordable for rural households.

The high aflatoxin contamination in the cereal-soybean mix is somewhat surprising as soybean has some level of resistance to aflatoxin contamination compared to other legumes and cereals (Stossel 1986). The resistance of soybean

to aflatoxin contamination has been attributed to a number of factors including the tough seed coat that ensures low moisture content as well as acting as a primary barrier to *A. flavus* attack (Stossel 1986). In situations where the integrity of the seed coat is compromised, it has been demonstrated that the lipoxygenase pathway is activated leading to the release of aldehydes that target invading fungi (Doehlert et al. 1993). It was thus expected that its inclusion in the blend would to some extent lower the level of aflatoxin contamination due to the dilution effect. The high aflatoxin in this blend thus suggests that the cereals used could have been highly contaminated.

The high aflatoxin contamination of cereal-soybean-groundnut blend, on the other hand, is not very surprising due to the inclusion of groundnuts. Groundnut is known to be one of the most susceptible legumes to aflatoxin contamination, with market samples having much higher values than farmgate samples. In stored samples, concentrations can reach values as high as 3276 µg/kg (Awuah and Kpodo 1996). In a nationwide survey of Ghana, Amissah et al. (2017) reported a maximum value of 337 ppb in market samples. The high values reported for aflatoxin contamination in groundnut is even more disturbing when it is considered that local producers of groundnut-based food and feed tend to use cheap and poor-quality groundnut, presenting a high risk of aflatoxin contamination in their products.

3.3 Aflatoxin contamination based on regions

Wide variation was observed in samples taken from the various regions with mean aflatoxin values ranging between 1.46 and 457.4 ppb, and this variation was statistically significant ($p = 0.000$). Blends from the Central region had the highest aflatoxin contamination with that from the Upper East region having the least (Fig. 1). The difference in aflatoxin contamination in samples from the different regions is not properly understood but some speculations can be made for some of the regions. The Central region, for example, is known to produce cereals but not much groundnuts or soybean. It is, therefore, reasonable to assume that most of the soybean and groundnuts is sourced from the northern parts of the country where they are produced in larger quantities and may take a couple of days to transport. Farm produce is not transported under the best of conditions in sub-Saharan Africa and consequently may be exposed to conditions that favour attack and growth of *Aspergillus* species and subsequent aflatoxin contamination. Poor storage conditions do contribute to postharvest aflatoxin contamination in cereals and legumes. Retailers do not store cereals and legumes properly, allowing for re-introduction of moisture into them mainly through insect and rodent activities that, when coupled with favourable temperatures, leads to aflatoxin build up in these commodities. This may, however, not be the reason for the high

Table 2 Aflatoxin concentrations in small scale (SS) complementary foods in Ghana

Company code	Concentration (ppb)	Blend	Company code	Concentration (ppb)	Blend
SS_A (<i>n</i> = 6)	6.72 (1.9–22.3) ^a	Cereal-soybean	SS_V (<i>n</i> = 3)	152.00 (128–176) ^{bcd}	Cereal-soybean-groundnut
SS_B (<i>n</i> = 3)	2.27 (1.3–2.8) ^a	Cereal-soybean	SS_W (<i>n</i> = 3)	443.50 (385–502) ^{bc}	Cereal-soybean-groundnut
SS_C (<i>n</i> = 3)	1.70 (1.0–2.5) ^a	Cereal-soybean-groundnut	SS_X (<i>n</i> = 3)	631.50 (516–747) ^d	Cereal-soybean-groundnut
SS_D (<i>n</i> = 3)	9.23 (6.9–10.7) ^a	Cereal-soybean-groundnut	SS_Y (<i>n</i> = 3)	153.50 (153–154) ^{cd}	Cereal-soybean
SS_E (<i>n</i> = 3)	5.10 (4.3–6) ^a	Cereal-soybean-groundnut	SS_Z (<i>n</i> = 3)	454.00 (323–585) ^{bc}	Cereal-soybean-groundnut
SS_F (<i>n</i> = 3)	38.57 (37.3–40.1) ^{ab}	Cereal-soybean-groundnut	SS_AA (<i>n</i> = 3)	1.70 (1.1–2.5) ^{aΔ}	Cereal-soybean
SS_G (<i>n</i> = 3)	7.63 (7.1–8.1) ^a	Cereal-soybean-groundnut	SS_AB (<i>n</i> = 3)	99.23 (84.7–108) ^{abc}	Cereal-soybean
SS_H (<i>n</i> = 3)	3.67 (3.1–4.7) ^a	Cereal-soybean-groundnut	SS_AC (<i>n</i> = 3)	4.23 (1.0–10.2) ^a	Cereal-soybean
SS_I (<i>n</i> = 3)	42.00 (39.1–47) ^{abc}	Cereal-soybean-groundnut	SS_AD (<i>n</i> = 3)	1.73 (1.0–2.9) ^a	Cereal-soybean-groundnut
SS_J (<i>n</i> = 3)	1.77 (1.6–2.1) ^a	Cereal-soybean-groundnut	SS_AE (<i>n</i> = 3)	1.83 (1.3–2.2) ^{aΔ}	Cereal-soybean
SS_K (<i>n</i> = 3)	686.00 (576–796) ^d	Cereal-soybean-groundnut	SS_AG (<i>n</i> = 3)	2.37 (1.8–3.3) ^a	Cereal-soybean-groundnut
SS_L (<i>n</i> = 3)	7.97 (7.3–8.7) ^a	Cereal-soybean-groundnut	SS_AH (<i>n</i> = 3)	1.00 ^{a*}	Cereal only
SS_M (<i>n</i> = 3)	6.77 (5.1–7.9) ^a	Cereal-soybean-groundnut	SS_AI (<i>n</i> = 3)	1.07 (1.0–1.2) ^a	Cereal-soybean-groundnut
SS_N (<i>n</i> = 3)	264.50 (253–276) ^{cd}	Cereal-soybean-groundnut	SS_AM (<i>n</i> = 3)	13.67 (6.6–26.5) ^{ab}	Cereal-soybean-groundnut
SS_O (<i>n</i> = 3)	221.50 (220–223) ^f	Cereal-soybean-groundnut	SS_AN (<i>n</i> = 3)	1.30 (1.0–1.9) ^{ab}	Cereal-soybean
SS_P (<i>n</i> = 3)	10.33 (9.2–11.5) ^a	Cereal-soybean-groundnut	SS_AQ (<i>n</i> = 3)	1.00 ^{a*Δ}	Cereal-soybean
SS_Q (<i>n</i> = 3)	8.40 (4.5–11.1) ^a	Cereal-soybean-groundnut	SS_AX (<i>n</i> = 3)	4.57 (3.6–5.8) ^a	Cereal-soybean-groundnut
SS_R (<i>n</i> = 3)	4.57 (4.3–5.8) ^a	Cereal-soybean-groundnut	SS_AY (<i>n</i> = 3)	360.00 (348–372) ^{bc}	Cereal-soybean-groundnut
SS_S (<i>n</i> = 3)	15.33 (8.4–28.9) ^a	Cereal-soybean-groundnut	SS_BD (<i>n</i> = 3)	11.27 (4.3–22.3) ^a	Cereal-soybean
SS_T (<i>n</i> = 3)	370.00 (320–420) ^{cd}	Cereal-soybean-groundnut	SS_BQ (<i>n</i> = 3)	1.00 ^{a*}	Cereal-soybean-groundnut
SS_U (<i>n</i> = 3)	265.50 (243–288) ^{cd}	Cereal-soybean-groundnut			
P value	0.000			0.000	

Values are means (range). Values in the same column with different superscripts are statistically significantly different

* Values without ranges. The letters attached to SS are codes for the companies from which the samples were obtained while the “*n*” in parentheses represents the number of samples for that brand obtained for the analysis

contamination in samples from the Northern region. Unpublished data from two separate nationwide surveys have shown groundnuts in the Northern region to have the highest aflatoxin contamination. Since samples from the Northern region all contained groundnuts, it could be speculated that the high aflatoxin contamination in blends could be due to contamination from the groundnuts used.

The low aflatoxin contamination in blends from the Volta, Western, Upper West, Eastern and Upper East regions can only be attributed to the use of good quality inputs, and the

use of millet and/or sorghum in the Upper East blends in place of maize.

This is not the first report showing high aflatoxin contamination in infant foods in Ghana. A study carried out in the Ejura Sekyedumase district of the Ashanti region showed contamination of complementary foods prepared from groundnuts, maize and beans ranged from 7.9 to 500 ppb (Kumi et al. 2015). The daily demand for infant foods in Ghana will keep rising as the human population is growing rapidly (Godfray et al. 2010). This, coupled with the desire and efforts to earn a living, might lead to the production of diverse kinds of foods including complementary foods whose quality may be compromised. This is a worrying situation since there is a close link between aflatoxin contamination and poor child growth.

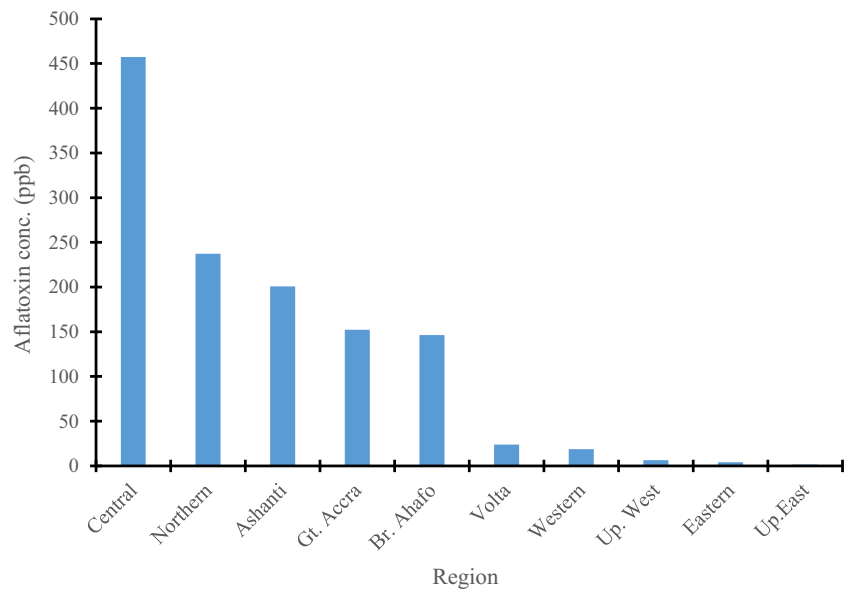
A study conducted to quantify aflatoxin exposure in young children in low-income urban areas of Nairobi, Kenya, and its association with child growth reported that 5% of the affected children were wasted, 41% were stunted and 17% were underweight (Kiarie et al. 2016). Gong et al. (2002), working in Togo and Benin also observed, in a study to determine the effect of aflatoxin exposure on growth of children under five years, that 33% of the children (*n* = 480) were stunted, 29%

Table 3 Aflatoxin concentrations in complementary foods based on food types

Food type	Concentration (ppb)
Cereal only (<i>n</i> = 18)	7.16 (1.00–11.70)
Cereal-soybean (<i>n</i> = 36)	139.30 (1–1094)
Cereal-soybean-groundnut (<i>n</i> = 90)	139.50 (1–796)
P value	0.059

Values are mean (range). The “*n*” in parenthesis represents different brands (in triplicate) for that food type

Fig. 1 Aflatoxin contamination in cereal-legume blends from different regions of Ghana



were underweight while 6% suffered wasting according to World Health Organization criteria. Similar results, 34% stunted, 30% underweight and 6% wasted, have been reported (Okoth and Ohingo 2004) for Kenyan children under five years of age ($n = 242$). Another study showed that children who were stunted and underweight had 30–40% greater aflatoxin exposure than normal children (Yunyun Gong et al. 2004). Other researchers, in similar studies, have shown that children who tested positive for aflatoxin suffered greater impaired growth as compared to those who tested negative (Castelino et al. 2015).

Nutrient deficiency in humans has also been partly associated with aflatoxin exposure following a proposal that aflatoxin exposure facilitates intestinal damage resulting in a decline in nutrient absorption (Y. Y. Gong et al. 2008). Watson et al. (2015) observed a significant positive link between aflatoxin prevalence and zinc and vitamin A deficiencies. A study in Ghana, on adults, reported that subjects with high exposure to the toxin were more likely to suffer deficiencies of vitamins A and E (Obuseh et al. 2010). A strong association between anaemia and aflatoxin has been reported in Ghana (Shuaib et al. 2010) showing that aflatoxin exposure may contribute partly to the high iron deficiency prevalent in children in developing countries including Ghana. Further, aflatoxins cause immune suppression and mental disorders in children due to their ability to impede nutrient bioavailability (Temba et al. 2016). Other effects of aflatoxin include several forms of cancer in humans, including children (Wagacha and Muthomi 2008) and it is reported to contribute to infertility in humans (Covic and Hendriks 2016) With a suppressed immune system, affected children become pitifully prone to many diseases causing irreversible physical and mental damage and, in the long run, harming the economic prospects of the country.

4 Conclusion and recommendation

To the best of our knowledge, this is the first report of a Nation-wide survey to assess aflatoxin levels in complementary foods intended for infants and children in Ghana. The complementary foods sampled in this study showed high aflatoxin contamination. These complementary foods have, over the years, been promoted to help curb infant and child malnutrition. Unfortunately, their high aflatoxin contamination might rather worsen the health of these infants and children, causing stunting, underweight, wasting, mental retardation and even death. Measures, therefore, have to be put in place to ensure that the ingredients for infant foods as well the infant foods themselves are tested for aflatoxin to ascertain their safety for consumption. The use of roots and tubers as alternative ingredients for complementary foods can be explored since they are less susceptible to aflatoxin contamination compared to cereals and legumes. Most importantly, aflatoxin awareness creation and education should be greatly intensified and regulatory bodies strengthened. These may help to reduce exposure to aflatoxins and their attendant health effects, thus contributing to holistic healthy growth and development in children.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Human or animal studies This article does not contain any studies with human or animal subjects.

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