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SHORT REPORT

# Microbial Contamination of Seven Major Weaning Foods in Nigeria

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## ABSTRACT

Five million children aged less than five years die annually due to diarrhoea. The aim of the study was to identify some possible contributing factors for persistent diarrhoea. Seven weaning foods, including a locally-made food, were evaluated by estimating the microbial load using the most probable number method and aflatoxin levels ($AFM_1$, $AFG_1$, $AFG_2$, and $AFB_2$) by immunoaffinity column extraction and high-performance liquid chromatography (HPLC) with detection of fluorescence. The results showed that the locally-made weaning food had the highest microbial count (2,000 cfu/g) and faecal streptococcal count (25 cfu/g). Moulds isolated were mainly *Aspergillus niger, A. flavus, A. glaucus, Cladosporium* sp., and *Penicillium* sp. The home-made weaning food recorded the highest fungal count (6,500 cfu/g). $AFM_1$ of the weaning foods was 4.6-530 ng/mL. One weaning food had $AFB_1$ level of 4,806 ng/g. Aflatoxin metabolites, apart from $AFM_1$ and $AFB_1$ present in the weaning foods, were $AFG_1$ and $AFG_2$. There were low microbial counts in commercial weaning foods but had high levels of aflatoxins ($AFM_1$, $AFG_1$, $AFG_2$, $AFB_1$, and $AFB_2$). Growth and development of the infant is rapid, and it is, thus, possible that exposure to aflatoxins in weaning foods might have significant health effects.

**Key words:** Aflatoxins; High-performance liquid chromatography; Microbial contamination; Microbial count; Weaning foods; Nigeria

## INTRODUCTION

Microbial contamination leading to infections and poor nutrient associated with weaning foods may contribute significantly to deaths of 13 million infants and children aged less than five years worldwide each year (1,2). After respiratory infections, diarrhoeal diseases are the commonest illness and have the greatest negative impact on the growth of infants and young children (3,4). The causes of diarrhoeal diseases have traditionally been ascribed to water supply and sanitation (5). To prevent such diseases, governments and non-governmental organizations have focused their efforts on and sometimes limited to improving water supply and sanitation and promoting and protecting breastfeeding with less emphasis on food safety. This issue is increasingly becoming important in national and international debates about agriculture, nutrition, and health. Food safety is not a luxury of the rich but a right of all people (6). Based on literature, weaning foods prepared under unhygienic conditions are frequently heavily contaminated with pathogens and may, thus, be a major factor in causing diarrhoeal diseases and associated malnutrition. In particular, traditional gruels used in The Gambia for supplementing breastmilk were found to be heavily contaminated with potentially pathogenic micro-organisms, and such supplements are important factors in weaning-related diarrhoea (7). Therefore, it appears that current efforts are not sufficient to prevent diarrhoeal diseases; thus, education of mothers in food-safety principles, particularly weaning foods, must also receive high priority (2). Educational programmes based on the hazard-analysis-critical-control-point approach, taking into consideration also sociocultural factors, should be integrated into all national infant-feeding or food and nutrition programmes (8).

Food-borne infections can have dangerous and

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long-term effects, especially on nutritional status. Formula-fed infants usually require formula for their first year but they are introduced to other kinds of foods once they reach six months of age. Most food items used for the composition of weaning foods, such as groundnuts, maize, and other oilseeds, are vulnerable crops to moulds, especially *Aspergillus parasiticus* and *A. flavu*s that produce aflatoxins (AFs) (9-11). These toxigenic fungi grow well. However, it is more serious in tropical countries of the world where humidity is high and the temperature is conducive for the growth and production of AFs. AFs are potent carcinogens, mutagens, teratogens, and immunosuppressants. In addition to being potent carcinogens, AFs may contribute to early growth faltering of the child (12), and strong associations have been reported around the weaning stage in Beninese infants (13,14). A study in Beninese children reported that secretory IgA in saliva may be reduced by dietary levels of AF (13). The immune status of Ghanaian adults has been reportedly affected by exposure to AFs (15). The aim of this study was to ascertain the microbiological and AF status of some weaning foods by screening such foods sold in Nigeria.

## MATERIALS AND METHODS

### Sample collection

Seven weaning foods collected from open markets in Ibadan, Nigeria, and one locally-made weaning food voluntarily donated were examined for the microbial load and AF levels. The major ingredients of the weaning foods were as follows: weaning food A was made of milk and wheat; weaning food B was made of rice and milk; weaning food C was made of maize and soya; weaning food D, E, and F were made of maize and milk; and weaning food G was made of maize, fish, groundnut, and soya.

### Microbiological analysis

The total microbial load was determined using nutrient agar prepared according to the guidelines of the manufacturer. Serial dilution was done using physiological salt solution containing NaCl and NaHPO4 (1.45 g, 10 g, and 6.25 per 2.5 L) as diluents. The aim was to maintain the microorganisms in their physiological state to prevent plasmolysis resulting from osmosis. One hundred mL of the diluent was measured into bottles used for serial dilution containing 11 g of each sample. The mixture was shaken using a horizontal shaker (Model SM, Einrichtungen, Germany) for 30 minutes. Further dilutions were made, and dilution $10^1$ and $10^3$ were plated in duplicates.

Faecal coliform counts were determined using 36.5 g/L of fluorocult media containing durham tubes. The most probable number (MPN) was used, and $10^1$, $10^2$, and $10^3$ dilutions were plated out. Any tube containing gas was an indication of the presence of faecal coliform. The next stage was to confirm faecal coliform by adding 1/2 mL of NaOH to tubes containing gas. Sodium hydroxide will neutralize the acid and enable the media to fluoresce if faecal coliforms are present.

Fungal counts were determined using Dichloran Glycerin (DG) 18. One mL of streptomycin (650 mg/100 mL membrane filtered) was added to the media, and dilution $10^1$ and $10^3$ were plated in duplicates.

The nutrient agar bacteria plates were incubated at 37 °C for 48 hours, and the fungi plates were incubated at room temperature (28 °C) for five days.

### Aflatoxin analysis of weaning foods

Chemicals and solvents used were of HPLC grade or equivalent. All water used was distilled and, for HPLC, passed through a Milli-Q purification system (Millipore, London, UK). Acetonitrile used for mobile phase was of HPLC grade and provided by Merck, Darmstadt, Germany.

### Analysis

Immunoaffinity columns (IACs) (RIDA aflatoxin column, R-BIOPHARM, Darmstadt) were used for cleaning the sample extracts. The IAC was brought to room temperature, plugged unto Luer-attachment of a vacuum pressure facility. Twenty-five g of each weaning food was weighed into a 250-mL Erlenmeyer flask, 125 mL of acetone/water (85:15, v/v) was added and placed on a magnetic stirrer (Telemodule, Labortechnik) at 500 rpm for 45 minutes, and 5 mL of the filtrate was measured into a vessel connected to the IAC. Forty-five mL of Millipore water was added to the barrel attached to the IAC. The sample was allowed to flow through the IAC at a rate of ca. 1 mL per minute. Slight pressure was applied. The reservoir was rinsed twice with 10 mL of phosphate buffer at a flow rate of ca. 2-3 mL per minute. Then the reservoir was removed, and the IAC was dried by applying pressure. $AFM_1$ was collected in glass bottles previously treated with 2N $H_2SO_4$. The solvent used for eluting was 1.25 of mL methanol/acetonitrile (20:30, v/v). The eluate was dried in a water bath at 40 °C and 80 kPa. The dried extract was re-dissolved with 1.25 mL of acetonitrile:water (25:75, v/v). The acetonitrile/water solution was used as the mobile phase for HPLC. $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$ were also determined.

### Determination of aflatoxins by HPLC

The HPLC system consisted of a LDC, Milton Roy, Consta Metric 1 pump, and a Lichrosorb RP-18 (Merck Hibar) column (particle size of 5 µm, length–125 mm, inside diameter–4 mm). The pump pressure was 60 MPa. The injector was an automatic type (Rheotype Gilson Abimed Model 231). The detector had a fluorescence spectrophometer (Shimadzu RF 535, gamma excitation–365 mm and gamma emission–444 nm). The flow rate was 1 mL per minute, and the injection volume was 50 µL. The mobile phase was water/acetonitrile (75:25).

### Standard solutions

$AFM_1$, $AFB_1$, $AFB_2$, $AFG_1$, and AFG2 were obtained from Sigma-Aldrich (St. Louis, MO, USA). The commercial stock solution of $AFM_1$ was 1,000 ng/mL. The spike solution was made by diluting the stock solution 1:40 to give approximately 25 ng/mL using HPLC grade acetonitrile/water. Of the diluted stock solution, 140 µL was added to 70 mL of defatted Hipp baby milk. Calibration curve was prepared by diluting 2 µg/L of $AFM_1$ in a 1:500 dilution.

The stock solutions were stored at 4 °C when not in use.

### Validation and repeatability of measurements

Validation and repeatability of measurements were done using the Hipp Baby formula bought from a supermarket in Munich, Germany. Seventy g of powdered milk was weighed into a one-litre beaker, and 450 mL of Millipore water added. The mixture was stirred vigorously with a stirrer. The temperature of milk was raised to 50 °C to ensure proper dispersion of milk fat. Thereafter, the milk temperature was lowered to room temperature. Four centrifuge tubes were half-filled and centrifuged at 3,000 g for 15 minutes. To aid the removal of fat, the milk samples were kept in the cold room (4 °C) for 15 minutes. The solidified fat was scooped off, and the milk samples were filtered using ashless filter circles (MN 640W, diameter–150 mm) (Macherey-Nagel, Germany). Seventy mL of defatted Hipp milk was measured into five 100-mL volumetric flasks. Three of the flasks containing Hipp-defatted milk were spiked with 25 ng/mL $AFM_1$, and two samples served as controls. $AFM_1$ was extracted as previously described above. The average recovery rates were calculated. $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$ were also determined using standard $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$ as reference.

### Statistical analysis

Comparison of statistical analysis between means was evaluated by Student's *t*-test and analysis of variance. A value of $p<0.05$ was considered significant.

## RESULTS

Seven weaning foods were bought from an open market, including a locally-home made weaning food, which was voluntarily donated. These foods were examined for $AFM_1$, $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$ by immunoaffinity column extraction and HPLC with detection of fluorescence. $AFM_1$ was detected in three samples. Two samples were above the 500 ng/g $AFM_1$ approved by the Nigeria National Agency for Food and Drug Administration and Control while $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$ were found in five samples. Three weaning foods had $AFB_1$ ranging from 181.6 to 4,806 ng/g (Table 2). One sample had $AFB_1$ level of 4,806 ng/g, and another baby food had $AFM_1$ of 530 ng/g. Only one sample and the locally-made weaning food had no AFs. AF metabolites present in weaning foods, apart from $AFB_1$ and $AFM_1$, were $AFG_1$ and $AFG_2$. All positive samples had extremely high $AFB_1$, $AFB_2$, $AFG_1$, and AFG2. The main raw ingredients of the weaning foods were maize, cassava, yam, melon, groundnuts, and foods rich in mycotoxins.

**Table 1.** Validation and repeatability of measurement using Hipp baby formula by HPLC

| Sample name | Ret time min | Area Mv min | Height Mv | Amount ppb | % of recovery |
|---|---|---|---|---|---|
| Std 2.0 ppb | 5.48 | 221,323 | 878,118 | 2.00001 | 100 |
| Std 1.0 ppb | 5.49 | 110,008 | 43,988 | 1.0003 | 100 |
| Std 0.5 ppb | 5.47 | 54,896 | 219,905 | 0.4985 | 99.7 |
| Std 0.25 ppb | 5.48 | 27,419 | 111,156 | 0.2504 | 100.16 |
| Spiked milk | | | | | |
| 2.5 ppb (1) | 5.57 | 55,298 | 246,355 | 2.23 | 89 |
| (2) | 5.57 | 56,604 | 256,889 | 2.33 | 93 |
| (3) | 5.57 | 59,533 | 27,180 | 2.58 | 103 |

HPLC=High-performance liquid chromatography; min=Minutes; Mv=Measurement unit; Ret=Retention; Std=Standard; 1, 2, and 3 with 2.5 ppb indicate replicate 1 , 2, and 3

**Table 2.** Aflatoxin levels (ng/mL) of some weaning foods sold in Nigeria

| Sample | $AFM_1$ | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ |
|---|---|---|---|---|---|
| WFA | 127.6 | - | 464.0 | - | 1,699 |
| WFB | 4.6 | - | 8,290 | - | 1,169 |
| WFC | - | 4,806 | - | - | - |
| WFD | - | - | - | - | - |
| WFE | 530 | - | 387 | 144 | - |
| WFF | - | 181.6 | 103 | - | - |
| WFG | - | - | - | - | - |

- Indicates not detected; Af=Aflatoxin; WF=Weaning food

**Table 3.** Microbiological analysis of some weaning foods sold in Nigeria

| Weaning food/major ingredient | Total viable count (cfu/g) | Faecal coliform count (cfu/g) | Fungal count (cfu/g) and species |
|---|---|---|---|
| WFA (wheat+milk) | $2.2x10^6$ | <2 | 10, *Cladosporium* sp. |
| WFB (rice+milk) | No growth after 72 hours | <2 | 10, *Cladosporium* sp. |
| WFC (maize+soya) | No growth after 72 hours | <2 | 15, *Penicillium* sp. |
| WFD (maize+milk) | 20 | <2 | 30, *A. flavus* and *Cladosporium* sp. |
| WFE (maize+milk) | 50 | <2 | 50, *A. flavus* *Cladosporium* sp. |
| WFF (maize+milk) | 500 | <2 | 15, *A. niger*, Mucor |
| WFG (maize, groundnut, fish +soya) | $2x10^3$ | 25 | 6,500, *A glacus*, *A. niger*, and *A. flavus* |

WF=Weaning food

Microbiology of the weaning foods revealed low microbial counts in commercial weaning foods but high AF levels ($AFM_1$, $AFG_1$, $AFG_2$, $AFB_1$, and $AFB_2$). The results showed that the home-made weaning food had the highest microbial count (2,000 cfu/g) and faecal streptococcal count (25 cfu/g) using MPN in a fluorocult medium (Table 3). Moulds isolated were mainly *A. niger, A. flavus, A. glaucus, Cladosporium* sp., and *Penicillium* sp., and the home-made weaning food recorded the highest fungal count of 6,500 cfu/g.

## DISCUSSION

Children are a highly-susceptible population group for exposure to environmental toxicants for various reasons, including lower detoxification capacity, rapid growth, higher intakes of air, food, and water per kg of body-weight (16), and early childhood exposure to bacterial and carcinogenic AFs may, therefore, be the critical determinants of immediate and later health effects.

The bacterial and fungal counts of most commercial weaning foods sold in Nigeria were low probably due to good food-handling and good manufacturing practices. Fungal counts may be low in processed foods but not AF levels since AFs cannot be destroyed by normal cooking temperature (17). Table 2 shows that bacterial and fungal counts were low but heat-processed commercial weaning foods had unacceptable high levels of $AFB_1$, $AFB_2$, $AFG_1$, and AFG2. Exposure in early infancy is occurring at levels that are not safe for the development of the child.

In developing countries, such as Nigeria, growth faltering is often associated with the quantity and/or poor quality of foods, in addition to multiple infectious hazards (18). However, high levels of AF-albumin adducts have been associated with growth faltering in Beninese infants (13,14). Egyptian infants had a high prevalence of stunting and moderate frequency of being underweight, based on the criteria of the World Health Organization (19,20). The exposure of children to AFs may be high in Nigeria. Genotoxic, carcinogenic, immunosuppressive, teratogenic substances, such as AFs, do not have a threshold value for human health below which the risk value is equal to zero. The Joint FAO/WHO Expert Committee on Food Additives does not have tolerable daily intake (TDI) of AF. This simply means that no level of AF is safe from the toxicological point of view but strongly recommends that the AF level should be as low as possible (16).

Therefore, the toxicological significance of the presence of AFs in foods should not be overlooked. To reduce the exposure of infants to AFs, education of mothers is highly recommended. A reduction in AF levels in weaning foods is desirable. Reductions in exposure to AFs can be achieved by several approaches. In Nigeria, the source of contamination is clearly defined, such as poor post-harvest handling and storage of risk foods (13). In addition to controlling post-harvest changes, dietary modulation, e.g. with chlorophyllin (21) or probiotics (22,23), antioxidants, such as selenium and vitamins (24), are effective.

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