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Research Article
Aflatoxins and Heavy Metals Detection in Poultry Feeds Used in Some Farms in Enugu, Nigeria

Obiageli D. Mbegbu, Ngozi F. Onyemelukwe, Nnamdi C. Orgu and Nkemdilim C. Okechukwu

Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus, Enugu, Nigeria

Abstract

Background and Objective: A major threat to the poultry industry is the contamination of poultry feed with heavy metals and aflatoxins. This study was conducted to detect and determine levels of aflatoxins and heavy metals in poultry feeds. **Materials and Methods:** Two categories of poultry feeds were obtained within Enugu Metropolis, Nigeria. A total of 32 samples of company branded feeds were investigated for heavy metals and aflatoxin levels while 10 samples of locally milled feeds were investigated for aflatoxin levels. Heavy metals content was determined using atomic absorption spectroscopy and expressed in mg kg^{-1}. Aflatoxin level was determined through Elisa kit and were expressed in PPb. **Results:** The heavy metals ranged from manganese (6.65-20.30 mg kg^{-1}), cobalt (12.03-39.11 mg kg^{-1}), zinc (1.05-1.47 mg kg^{-1}), nickel (0.56-0.73 mg kg^{-1}), iron (0.04-0.19 mg kg^{-1}), copper (3.82-5.07 mg kg^{-1}), lead (0.01-0.11 mg kg^{-1}) and cadmium (0.01-0.05 mg kg^{-1}). All the metals detected were found to be low in levels except cobalt. The metals were found to be statistically significant ($p>0.05$) in feeds. The total aflatoxin levels of company branded feeds analysed varied from 0.74-4.12 PPb while that of locally milled poultry feeds ranged from 0.53-4.14 PPb. **Conclusion:** Zinc, iron, copper and manganese were found to be low in this study. Feed manufacturers should increase the content of these micro-nutrients and also protect the feed materials from aflatoxin contamination.

Key words: Aflatoxin, atomic absorption spectroscopy, food safety, heavy metals, poultry feeds

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Corresponding Author: Obiageli D. Mbegbu, Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus, Enugu, Nigeria. Tel: +2348034318181

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry feeds are food materials used in raising birds and are designed to contain all the nutritional materials needed for proper growth, meat and egg production in birds. Poultry feed is derived from grains such as maize, wheat, soybean, bone meal, offal and peanut[1,2]. There are different types of poultry feeds based on their purpose, for example starter, grower, layer, finisher and broiler feed. Poultry feed ingredients are often contaminated with micro-organisms, mostly bacteria, fungi and or insects depending on the composition of the feed stuff materials, it's origin, climatic conditions, processing, storage, transportation, technologies employed and packaging materials[3]. It has been reported that most commercial feeds failed to meet up with the requirements of birds and raw materials needed for the production of these feeds have been associated with heavy metals pollution[4] as well as aflatoxins contamination. Certain mineral elements such as zinc, manganese, iron and copper are considered essential dietary micro nutrients for poultry birds apart from other nutrients. However, a high concentration of any mineral element can adversely affect humans and animals if consumed in excessive amounts[5]. Heavy metal contamination in meat is a major concern for food safety and human health due to their toxic effects even at relatively low concentrations[6]. Heavy metals including cadmium, copper, chromium and lead are potential bio-accumulative toxicants that may cause severe health problems even at low concentration[7,8]. Copper is an essential micronutrient that plays a vital role in human and animal's health. For adults, the World Health Organization recommends 1.3mg/day as a minimum acceptable intake[9]. The maximum tolerance limit for copper in animals feed is 40 ppm[10]. Exposure to high doses of copper can be harmful[11] and long term exposure to copper dust can irritate the mouth, eyes, nose, cause dizziness, nausea, headache and diarrhoea[12]. However Poor growth and anemia are symptoms of copper deficiency in grazing cattle. Aflatoxins are a family of toxic metabolites with low molecular weight produced by strains of *Aspergillus flavus* and other related organisms. Because of their high toxicity, the presence of aflatoxins in poultry feeds is believed to pose a risk to the birds and humans. Fungal and aflatoxin contamination of poultry feeds can occur pre-harvest in the field and post-harvest in the store[2,13]. Poultry feeds require adequate safety regulation. This study was designed to determine heavy metals and aflatoxin concentrations levels in poultry feeds in Enugu Metropolis; to facilitate exposure assessment and regulatory risk management.

MATERIALS AND METHODS

All the plastic and glass wares were washed with detergent and rinsed with water before being immersed in 10% nitric acid solution. Deionized water and Analytical grade reagents were used throughout the analysis.

Collection of poultry feed samples for heavy metals analysis: A total of 3 brands (starter, grower, layer and finisher) of poultry feeds coded as A, B, C, D, E, F, G, H, I, J, K and L available in Enugu metropolis were purchased from different locations within the metropolis.

Sample preparation: Dried feed sample weighing 2 g was placed in crucibles. Concentrated nitric acid (1 cm^3) was added as ashing aid and pre-ashed by placing the crucible in a heater until the content is charred. The pre-ashed samples were transferred into a muffle furnace at a temperature of 480°C for 2-3 hrs until a constant weight is obtained. It was then allowed to cool. The cooled samples were dissolved using 5 cm^3 of 30% HCl and then filtered using Whatman filter papers. The filtrates were poured into 50cm^3 volumetric flasks and made up to the mark with deionized water. The sample solution was then kept in a sample bottle for further analysis[5].

Aflatoxin analysis: A total of 10 samples of locally milled poultry feeds and 20 samples of company branded poultry feeds from five brands were purchased from different locations within Enugu metropolis.

Aflatoxin extraction and analysis: The poultry feeds were homogenized using a homogenizer. After this, 2 g of the homogenate sample was placed into the 50ml centrifuge tube. Then 5 mL of 70% methanol was added to it and oscillated for 5 min. The sample was then centrifuged at 4000 r min^{-1} for 10 min at room temperature. Afterward, 0.5 mL of the supernatant was placed into another centrifuge tube and 0.5 mL of deionized water was added and fully mixed.

A total of 50 µL was taken from it for detection and analysis. The level of aflatoxin was determined through Elisa kit and protocol supplied by Elabscience AF (total aflatoxin). After this 50 µL of sample was added into each micro well and 50 µL of HRP conjugate and 50 µL of antibody working solution was also added into each well. The plate was covered with plate sealer and oscillated for 5 sec gently to mix thoroughly. It was incubated for 30 min at 25°C in shading light.

The sealer was uncovered and the liquid was removed. After this, 300 µL of wash buffer was added to each micro well and washed. The washing procedure was done 5 times, 30 sec intervals. The plate was inverted and pat against thick clean absorbent paper. After this 50 µL of substrate reagent A was added to each well and then 50 µL of substrate Reagent B was added. The mixture was oscillated for 5 sec to mix thoroughly and incubated at 25°C for 15 min in shading light. Then 50 µL of stop solution was then added to each well and oscillated gently to mix. The OD value of each well was determined at 450 nm with a microplate reader.

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA), the difference between the feeds was considered significant if the computed P value was less than 0.05.

RESULTS AND DISCUSSION

In poultry feeds, the Standard Organisation of Nigeria (SON) has included some heavy metals, such as copper, zinc, manganese and iron, as micronutrients. Zinc was mentioned as a nutrient ranged from 40-50 mg kg^{-1} for starter, grower and finisher, 30-40 mg kg^{-1} for layer. In the current study, zinc content in the feed ranged from 1.05-1.47 mg kg^{-1} (Table 1). Zinc content in all feeds investigated was below SON requirements. However, the values obtained in this study were lower than (33.95-49.950 mg kg^{-1}) that obtained by Okoye *et al.*[5] in their analysis of poultry feeds. These low levels of zinc in feeds could cause decreased immune system, slow wound healing and skin sore[14].

Iron levels were presented in Table 1. Iron was found in all the feed samples but below the WHO/FAO[15] permissible level of 45-80 mg kg^{-1} when compared to SON standard (90-95 mg kg^{-1}) for starter, grower and finisher while 50-60 mg kg^{-1} for layer.

In the current study, iron levels in the feeds ranged from 0.04-0.19 mg kg^{-1} which was lower than the values obtained by Bukar and Sa'id[14] who recorded 08.79-19.74 mg kg^{-1}.

The copper content of the feeds varied from 3.82-5.69 mg kg^{-1}. Copper was detected in all the feeds and was mentioned as a micro nutrient at 0.0-10 mg kg^{-1} for starter and 9-10 mg kg^{-1} for grower, finisher and layer. All the feeds for starter were within the permissible limit but feeds for layer, grower and finisher were below the limit. Manganese content of the feeds varied from 6.65-20.03 mg kg^{-1}. Manganese values compared with WHO/FAO's[15] maximum acceptance limit of 20-60 mg kg^{-1} were below the limit, all the feeds analysed were below the limit except HM starter feed (20.03 mg kg^{-1}). Nickel and cadmium were detected in all feeds but were below the permissible limit. Cobalt was also detected in all feeds sample and was found to be above the permissible limit (1 mg kg^{-1}) stipulated by WHO/FAO[15]. Lead content levels were recorded in Table1. The value varied from 0.01-0.08 mg kg^{-1}. The European Union regulations set a maximum level of lead in feed materials at 10 PPm[16] and NRC[10] also sets the maximum level of lead in poultry at 10 ppm. The values obtained in this study were below the maximum level.

Figure 1 shows the heavy metals contamination in starter category of company branded poultry feeds. All the heavy metals detected in starter feeds except cobalt, copper and manganese were below the WHO/FAO[15] permissible limit. The cobalt values obtained in the current study was higher than values obtained by Bukar and Sa'id[14].

Figure 2-4 show the heavy metals contamination in grower, finisher and layer category of company branded poultry feeds. All the heavy metals detected in this category of feeds were below the FAO/WHO[15] permissible limit except cobalt. Cobalt values over 1mg/kg in the diet of chickens are considered toxic by FAO/WHO[15], especially if the diet is low in iron. Depressed growth rate is the most important sign of excessive intake of cobalt in poultry birds.

Table 1: Heavy metal contamination in company branded poultry feeds Heavy metals (mg kg^{-1})

Heavy metals	HM starter	TM starter	VM starter	HM grower	TM grower	VM grower	HM finisher	TM finisher	VM finisher	HM layers	TM layers	VM layers
Mg	20.30	10.75	12.83	16.05	14.77	6.65	9.80	15.41	9.77	8.62	10.23	18.740
Pb	0.06	0.03	0.11	0.08	0.04	0.05	0.01	0.10	0.07	0.06	0.03	0.080
Cu	4.43	4.65	4.69	4.26	4.83	4.88	4.32	5.05	5.07	3.82	4.82	4.630
Cd	0.01	0.03	0.03	0.01	0.01	0.01	0.05	0.01	0.01	0.04	0.01	0.010
Ni	0.66	0.58	0.73	0.71	0.65	0.67	0.61	0.72	0.56	0.59	0.59	0.610
Fe	0.06	0.06	0.07	1.10	0.14	0.11	0.05	0.07	0.19	0.05	0.16	0.040
Zn	1.13	1.33	1.44	1.46	1.17	1.47	1.31	1.35	1.05	1.20	1.12	1.390
Co	34.52	19.02	35.04	24.01	39.11	37.04	12.83	32.02	35.02	32.00	26.05	12.030

HM: Hybrid meal, TM: Top meal and VM: Vital meal

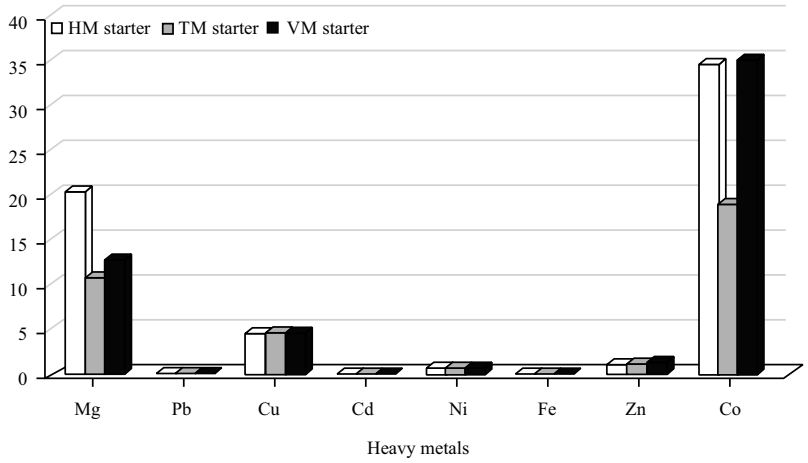

Fig. 1: Heavy metal contamination in starter feeds of company branded poultry feeds

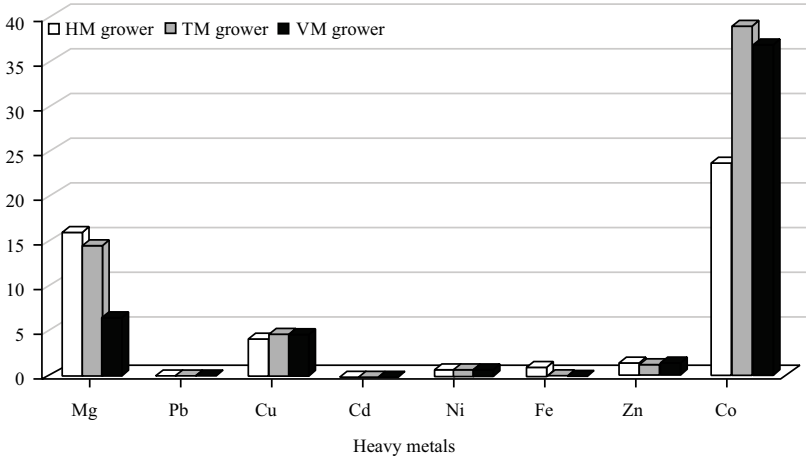

Fig. 2: Heavy metal contamination in grower category of company branded poultry feeds

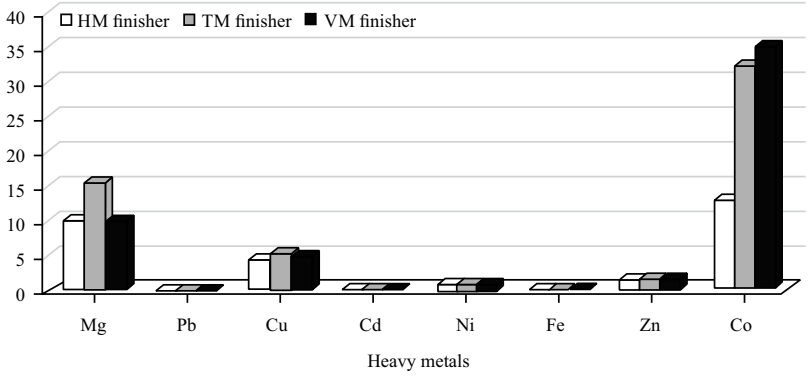

Fig. 3: Heavy metal contamination in finisher category of company branded poultry feeds

The Total aflatoxin levels in 20 company branded poultry feeds varied from 0.74-4.12 PPb (Table 2 and 3). The total aflatoxin levels in 10 local milled poultry feed samples ranged from 0.53-4.14 PPb (Table 4). Total aflatoxin levels in twenty mechanized company compounded poultry feed samples varied from 0.74-4.12 PPb (Table 3). In Ghana, Kumi *et al.*[17]

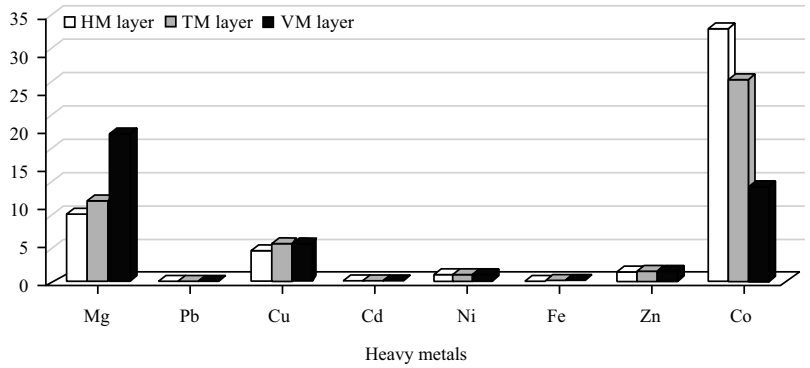

Fig. 4: Heavy metal contamination in layer category of company branded poultry feeds

Table 2: Total aflatoxin levels (PPb) in company branded poultry feeds category

HM starter	HM finisher	HM grower	HM layers	TM starter	TM finisher	TM grower	TM layers	VM starter	VM finisher	VM grower	VM layers
3.96	3.53	4.12	3.92	4.12	4.10	3.64	4.10	1.4	1.2	2.3	3.3

HM: Hybrid meal, TM: Top meal and VM: Vital meal

Table 3: Total aflatoxin levels (PPb) in company branded poultry feeds

SM starter	SM finisher	SM Grower	SM layers	CM starter	CM finisher	NM starter	NM finisher	KPC
4.11	3.95	3.90	4.0	3.84	3.23	3.47	3.20	0.74

Table 4: Total aflatoxin level in locally milled poultry feeds

Blood meal	Rice brown	Bambara nut	Fish meal	Corn flour	Soya fat	Bone meal	Groundnut cake	Palm kernel cake	Spent grain
1.2	0.9	3.8	4.0	2.42	3.17	0.53	4.14	4.11	2.68

reported values between 0.02 and 22 PPb in their study, which is significantly lower than the present study. This low level of aflatoxin could be due to the fact that many feed manufacturers in Nigeria had their feeds mixed with anti-fungal binders to prevent fungal contamination.

CONCLUSION

The micro nutrient levels (zinc, iron, manganese and copper) were found to be low in the feeds investigated. Copper level for starter feeds seems to fall within the permissible limit. One of the heavy metals (cobalt) was found to be above the maximum acceptance limit whereas majority fell below the limit. The metals in all the feeds investigated were found to be statistically significant ($p>0.05$). The total aflatoxin levels in all the feeds investigated were below the permissible limit (20 PPb). Therefore, a definite standard for heavy metals and aflatoxins should be provided as a contaminant so as to keep the food chain safe from heavy metals and aflatoxin and subsequent consequences. Poultry feed manufacturers should be encouraged to put extra care during production in order to eliminate heavy metals and aflatoxin contamination. There is also need for the feed manufacturers to increase the micro nutrient contents of zinc, iron, copper and manganese as they were below the permissible limit stipulated by SON.

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