ASSESSMENT OF HEAVY METALS IN CHICKEN FEEDS OBTAINED FROM UYO. AKWA IBOM STATE, NIGERIA.

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ABSTRACT

Assessment of heavy metals was carried out to determine the concentrations of copper, cadmium, nickel and lead in chicken feeds obtained from Uyo metropolis, Akwa Ibom State, Nigeria. Three feed types (starter, layer and finisher) of three commercially available brands (X, Y and Z) were purchased from markets in the city. The samples were prepared for analysis by wet digestion method and heavy metals analysis was carried out using an Atomic Absorption Spectrophotometer. The mean concentration of heavy metals (mg/kg) were in dry weight; the levels of copper varied from 5.69 to 26.79 mg/kg and were found to be present in the sequence; Finisher > Starter > Layer. The observed mean values of Cd ranged between 0.18 to 1.47 mg/kg and the levels of Cd in the different brand of feed increase with the trend, Z > Y > X. The mean concentration of nickel ranged from 0.80 - 4.78 mg/kg and the contamination sequence of nickel for the different feeds were Starter> Layer > Finisher, while the levels of lead in the different brands of feed were in the range of 0.00 - 2.4 mg/kg. The observed levels of copper and lead were low in the feed while amongst all the different brands studied, Cd mean values of 1.02 ± 0.73 for Z was slightly above the recommended limit of 1.0 mg/kg. Furthermore, Nickel concentrations were slightly higher in the feed samples analyzed, when compared with the recommended limits. Statistically, it is observed that the Fcrit > Fcal (3.44 > 0.41) for the types of feed, therefore there is no statistically significant difference between the types of feed evidence at $\alpha = 0.05$, as confirmed by a P value of 0.67. t- test analysis showed that, there was no significant difference in Cd between Y and Z brands as the observed $P_{0.05}$ was for two samples assuming equal variances were 0.41 and 0.82 for one tail and two tail respectively and the observed P value for two samples assuming unequal variances were 0.42 and 0.84 for one tail and two tail respectively. The heavy metals load in the different types of feeds was found to be at non-toxic concentrations, except for cadmium and nickel. These metal contaminations may arise from environmental factors, production line processes, poor handling and storage by the retailers. The deficiency or the elevation of these heavy metals affects the normal physiological activity and biochemical process of the chicken which in turn affect the health of humans.

Keywords: Heavy Metals, Chicken Feeds, Contamination

1. INTRODUCTION

Certain mineral elements such as iron, manganese, copper and zinc are essential dietary nutrients for poultry and livestock. However, all mineral elements, whether considered to be essential or potentially toxic, can have an adverse effect upon the humans and animals if included in the diet at excessively high concentration [1]. A study conducted by [2] shows that all human activities give rise to residual materials (wastes) which are not of immediate use. These wastes are generally made up of organic and inorganic compounds. According to Ubong *et al.*[3], heavy metals like cadmium (Cd), lead (Pb), and nickel (Ni), discharged into the environment by human activities, could be very toxic even at low concentrations. Metal toxicity has proven to be a major threat to humans and there are several health risks associated with them. Trace elements in nutrition depend on total content of elements in soil which have effect on plants, animals and humans. Deficiencies of heavy metals, such as zinc, copper, manganese and selenium in agricultural

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soil are affecting agricultural productivity and human health [4]. The increasing use of metal-based fertilizer in agriculture could result in a rise in metal pollution of fresh water reservoir, due to water run-off [5]. As human population increases, the intensity of anthropogenic threat exerted on the environment increases as a result of industrialization and agricultural activities [6]. Apart from soil environment and aquatic ecosystem, atmospheric inorganic contaminants of natural or anthropogenic sources could lead to serious ecological consequences and pose human health risks [7]. Heavy metals are potentially hazardous to humans and various ecological receptors because of their toxicity, persistence, bio accumulative and nonbiodegradable nature. Therefore, monitoring and evaluation of heavy metal concentrations in soils, groundwater and atmospheric environment is imperative in order to identify hazards to human health, to prevent bioaccumulation in the food chain and further degradation of the ecosystem [7] .

Studies have shown that essential metals can also produce toxic effects when the metal intake is excessively elevated [8]. Intake of toxic metals by animals can largely be attributed to oral ingestion, especially during feeding.

The raw materials for the production of poultry feed are of various origin. The exposure of these sources to various anthropogenic pollutants especially the heavy metals, may affect our food chain through the feeds. Poultry is one of the main sources of protein for humans. Over the years, poultry chicken has been genetically selected for improve feed conversion and rapid growth or production. This has led to two different major types of chickens namely broiler and layer type chickens as a consequence of this selection for economically important production traits, with their corresponding kind of feed. These chickens differ in body weight gain, duration of life and immune system caused by genetic differences. Therefore, this study is largely focused on the levels of heavy metals in chicken feed; highlighting the fact that humans are subsequently exposed to these heavy metals by consuming the affected poultry. Furthermore, this study is significant because there is insufficient data on the possible metal contamination of commercially available feeds consumed by chicken in the country. Although, some studies have been carried out on the levels of heavy metals in poultry feed [14,15,16], there is currently limited study on this subject in Uyo, Akwa Ibom State, Nigeria; therefore, this research aims to fill that knowledge gap.

2.1 SAMPLE COLLECTION

Three feeds (broiler starter, broiler finisher and layer mash) of three commercial feeds (X, Y and Z) were purchased from different commercial areas within Uyo metropolis, Nigeria and was transported to the Chemistry laboratory of Akwa Ibom State University for analysis.

SAMPLE PREPARATION

2.2 Sample digestion

Each brand of the feed sample obtained was mixed, homogenized thoroughly and reduced to smaller sizes (through conning and quartering). The samples were oven dried at the temperature of 50 – 60 °C until constant weight was obtained. The dried samples were pulverized using mortar and pestle. The powdered samples were sieved and 1.0 g of each sample was weighed. Reagent grade chemicals were used in all

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cases. Sample digestions were carried out by adopting method 3050B sample digestion protocol (US EPA 3050B) [9]. 10 ml nitric acid (HNO_3) was added to beakers containing 1g feed sample, then covered with watch glass and heated for 15 minutes without boiling. Samples were cooled, 5 ml HNO_3 was added and heated for 30 minutes (brown fumes was given off). More 5 ml HNO_3 was added and no brown fumes was given off. Solution was allowed to evaporate to < 5 ml and allowed to cool. 2 ml water and 3 ml 30% hydrogen peroxide (H_2O_2) was added and heated for 2 hours until effervesces ceased. Solution was reduced to 5ml via evaporation. 10 ml hydrochloric acid (HCl) was added and heated for 15 minutes without boiling. After cooling, the digested samples were filtered using a Whatman filter paper (grade 41, pore size 20 µm) into 100 ml volumetric flask. The filtrate was diluted to the mark with ultrapure water of resistivity 18.2 MΩ-cm at 250C and ready for analysis using Flame atomic Absorption Spectrophotometer (FAAS). Each sample was digested in triplicates for the purpose of reproducibility. The blank determination was also carried out in similar manner. The presence of heavy metals was determined using Atomic Absorption Spectrophotometer in the Soil science laboratory of University of Uyo, Nigeria [10].

3.0 RESULTS AND DISCUSSIONS

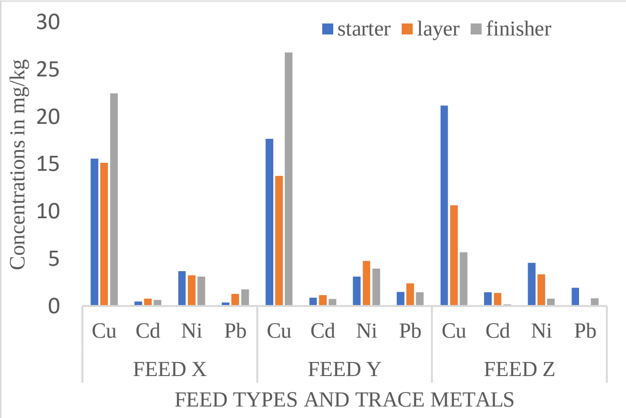

Figure 1.0: Comparative data of the trace metals in the different types of feed under study

3.1 COPPER

Figure 1.0 shows that copper from feed Y had the most prominent peak when compare to levels of trace metals in the different types of feeds. Considering Table 3.1, the mean concentrations of copper varied from 5.69 to 26.79 mg/kg. The highest mean concentration was observed in finisher (18.31±11.14 mg/kg), while layer had the lowest concentration of 13.18±2.30 mg/kg.

Table 3.1 Mean concentrations (mg/kg) of copper in chicken feeds obtained from Uyo metropolis

FEED TYPE	FEED X	FEED Y	FEED Z	Mean ±std dev
Starter	15.57± 0.11	17.69 ±0,18	21.18±0.14	18.15±2.83
Layer	15.13 ±0.09	13.76±0.04	10.64±0.10	13.18±2.30
Finisher	22.46±1.64	26.79±0.08	5.69±0.13	18.31±11.14
Mean ±std dev.	17.72±4.11	19.41±6.68	12.50±7.91	

However, the Y feed brand had the highest mean value of 19.41±6.68 mg/kg while the Z had the lowest. These values were lower than the maximum acceptable concentration of 100 mg/kg for copper in feed as recommended by European Union [11]. Copper was observed to be present in the following

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sequence Y > X > Z Figure 1. However, t test statistical analysis showed P value of 0.17 and 0.33 for one tail and two tail respectively for t test assuming unequal variances and P value of 0.11 and 0.22 for one tail and two tail respectively for t test assuming equal variances, These indicated that there was no significant difference between the Y and Z brands of the feed. Similarly, the same observation was made for X and Y brands.

According to [12], copper is an essential trace mineral required for many biological processes, particularly enzyme functions, and they have a positive influence on the livestock growth and reproduction. Due to the low copper content in some home-grown feeds, compared with recommendations and varying bioavailability, supplementation of these metals is necessary for most livestock species; supplements are commonly added to dairy rations as mineral supplement [11]. The value gotten from this study were higher than 6.52 - 14.20 mg/kg obtained by [12] but lower than 12.3 - 65.8 mg/kg obtained by [13]. Copper was found to be present in the feed in the sequence of Finisher > Starter > Layer (Table 3.1). It is necessary to note that copper is regarded as a micro-nutrient in humans, as it function as a cofactor for many enzymes [14]; however, exposure to higher doses can be harmful [15].

3.2 CADMIUM

The comparative concentrations of cadmium at all the study sites are presented in Figure 1.0, the lowest peak of cadmium was observed at the Z brand of feed. The mean result of the different brands of feed stock analysis for cadmium is presented in Table 3.2. The observed mean values of Cd ranged between 0.18 to 1.47 mg/kg. The mean value of the layer feeds (1.12 ± 0.31 mg/kg) was comparatively higher than other feeds, while the mean values of 0.53 ± 0.31 mg/kg of the finisher feed was the lowest. The concentration of Cd in the different brand of feed showed the trend, Z > Y > X.

Table 3.2 Mean concentration (mg/kg) of cadmium in chicken feeds obtained from Uyo metropolis

FEED TYPE	FEED X	FEED Y	FEED Z	Mean ±std dev
Starter	0.49±0.05	0.89±0.05	1.47±0.07	0.95±0.49
Layer	0.80±0.02	1.16±0.10	1.41±0.14	1.12±0.31
Finisher	0.65±0.09	0.77±0.08	0.18±0.06	0.53±0.31
Mean ±std dev.	0.65±0.16	0.94±0.2	1.02±0.73	

The mean value of the layer feed was slightly higher than the maximum recommended limit of 1.0 mg/kg of Cd in feed by European Union [11]. The observed values of the starter and finisher were comparatively lower than the limit. Amongst all the different brands studied, Cd mean value of 1.02 ± 0.73 for Z was slightly above the recommended limit of 1.0 mg/kg. However, the values obtained in this study were higher than 0.033 - 0.463 mg/kg obtained by [12], and comparatively lower than 3.8 – 33.6 mg/kg obtained by [13]. Similarly in a study by [16], cadmium levels were found in all analyzed samples to exceed the permissible limits of FAO/WHO, with the exception of feed C grower (0.532 mg/kg) which falls below limit.

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In this study, cadmium contamination follows the order Layer > Starter > Finisher brands. Statistically, t-test analysis showed that, there was no significant difference between Y and Z as the observed P value for two samples assuming equal variances were 0.41 and 0.82 for one tail and two tail respectively and the observed P value for two samples assuming unequal variances were 0.42 and 0.84 for one tail and two tail respectively. Cadmium was indicated as carcinogen that has harmful effects to human lungs, bones and kidney. Cadmium toxicity has been related to decrease in bird body health [17]. Several studies have shown that cadmium accumulate in liver and kidney in most chicken [18]. Cadmium changes the activities of antioxidant enzymes of erythrocytes and produce oxidative stress by disturbing the oxidative and anti-oxidative balance of the adult poultry birds [19; 20]. Cadmium suppresses the viability of the chicken spleeny lymphocyte and induces the oxidative stress and subsequently DNA damage and apoptosis [20].

3.3 NICKEL

Figure 1.0 gives the comparative levels of nickel in all the study sites; the most prominent peak of Ni value of 4.58 ± 0.12mg/kg was observed at starter of the Z brand of feed.

Table 3.3: Mean concentration (mg/kg) of nickel in chicken feeds obtained from Uyo metropolis

FEED TYPE	FEED X	FEED Y	FEED Z	Mean ±std dev
Starter	3.70±0.07	3.13±0.16	4.58±0.12	3.80±0.73
Layer	3.24±0.03	4.78±0.10	3.35±0.24	3.79±0.86
Finisher	3.11±0.12	3.96±9.31	0.80±0.06	2.62±1.64
Mean ±std dev.	3.35±0.31	3.96±0.83	2.91±1.93	

Table 3.3 gives the mean result of Ni in the different types and brands of feed under study. The mean concentration of nickel ranged from 0.80 - 4.78 mg/kg. The starter feed had the highest mean value of 3.80 ± 0.73 mg/kg while the finisher feed value was 2.62±1.64 mg/kg. The contamination sequence for the different brands were Starter > Layer > Finisher. The different brands of feed revealed the highest Ni concentration of 3.96 ± 0.83 mg/kg in Y, when compared to 2.91 ±1.93 mg/kg and 3.35±0.31 mg/kg for Z and X respectively. These observed values were above the value of 0.05 mg/kg recommended by the European Union [11]. Statistical, t test analysis using two samples assuming equal variances showed that there was no significant difference between Y and Z brands as they had P value of 0.11 and 0.23 for a one tail and two tail analysis, respectively, whereas two samples assuming unequal variances had P value of 0.17 and 0.34 for a one tail and two tail analysis, respectively. Similar observation was made for X and Y brands. The observed values were lower than the values of 2.250 - 4.875 mg/kg obtained by [12]. Comparing with the studies conducted by [21] indicated that all

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samples were above the maximum acceptable limit. According to [21], nickel influences Fe absorption and metabolism, and may be an essential component of the haemopoietic process in humans. However, when in excess, it can cause respiratory disease [16]. There are numerous researches on the adverse effect of heavy metals on animals and humans. In a study conducted by [3], it was stated that minimal amount of Nickel is needed by human to produce red blood cell; however, excessive amount of nickel can become toxic. Short term overexposure is not known to cause any health problem but long-term exposure can decrease body weight, liver damage and skin irritation. In another study conducted by [6], Nickel occur in the environment at very low levels but it can be dangerous when the maximum tolerable amounts are exceeded. Although it is not normally added to chicken diets, but nickel has been detected in liver, kidney and muscle of broilers [2]. Evidence of nickel deficiencies in chicks has been reported [23] and dietary nickel levels of 0.1-0.3 parts/106 dry weight are considered adequate in poultry diets [24].

3.4 LEAD

Lead comparative concentrations in all the study sites are presented in Figure 1.0; the highest lead peak was observed at the starter of Z brand of feed. Table 3.4 gives the mean concentrations of lead. The concentration of lead in the different brands of feed was in the range of 0.00 - 2.40 mg/kg.

Table 3.4: Mean concentration (mg/kg) of lead in chicken feeds obtained from Uyo metropolis

	FEED X	**FEED Y**	**FEED Z**	**Mean ±std dev**
Starter	0.40±0.1	1.50±0.1	1.93±0.2	1.28±0.79
Layer	1.30±0.2	2.40±0.2	0	1.23±1.20
Finisher	1.77±0.2	1.47±0.2	0.83±0.9	1.36±0.48
Mean ±std dev.	1.16±0.7	1.79±0.53	0.92±0.97	

The mean value of 1.23 ± 1.20 mg/kg was observed at the layer feed while the highest level of 1.36 ± 0.48 mg/kg was seen at the finisher. The mean value of 1.79±0.53 mg/kg for Y brand was higher than other types of feed, whereas Z brand showed the lowest lead value of 0.92 ± 0.97 mg/kg. These observed values for the different types and brands of feeds were lower than the recommended value of 5.0 mg/kg by European Union [11]. In t test statistical analysis, the observed $P_{0.05}$ value of 0.07 and 0.13 for one tail and two tails respectively indicated that, there was no significance difference between Y and Z brands of the feed. According to [12], eight of the feed samples analyzed exceeded the lead recommended limit. Furthermore, the values obtained in this study were lower than 23.20 – 32.60 mg/kg obtained by[13] in the analysis of poultry feed,; it can be stated that the feed samples in this study had a minimal lead contamination, in

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comparison to other studies reviewed [13] found the amount of lead in most analyzed chicken feeds samples was greater than maximum tolerable level which could be harmful for the chicken and human that consumes the chicken. [25] stated that lead induce oxidative stress suppresses growth performance and feed efficiency in broiler chickens. Lead supplement to chicken diets decreased feed intake, body weight gain and feed efficiency. A study done by [26] in the assessment of the levels of potentially toxic metals in selected poultry feeds sold in Makurdi Metropolis indicated that Copper, Fe, Mn, and Zn were the most abundant toxic metals found in the poultry feed samples. Lead was generally below the allowable limit. Chromium in ABS and ABF was higher than the permissible level. A research done by [27] in the assessment of heavy metals in chicken feeds. Four brands (starter, grower, finisher and layer) of three commercial feeds (X, Y and Z) commonly used by poultry farmers in Delta state were analyzed for cadmium (Cd), lead (Pb), and chromium (Cr). The results of the determinations showed that feed brand and the type studied were contaminated with Cd, Pb, and Cr with amounts within WHO minimum permissible limits.

Another study done by [28] was conducted in order to determine the heavy metals contamination in poultry feed. Results showed the presence of heavy metals in all the analyzed samples. However, none of the metals, except for mercury, were present at alarming levels. Mercury exceeded the tolerable limits set by both European Commission and National Research Council in all the samples [29] determined the level of toxic minerals (Pb, Cd, Ni, Fe, Cu, Mn, Zn, Cr) in poultry feeds using Atomic Absorption Spectrophotometer, the result showed that, the concentration of Mn, Zn and Fe were the highest in all the feed samples with Cd and Pb being the lowest when compared with other researchers and those acceptable standard values for food nutrition as stipulated by European Commission (EU) and World Health Organization (WHO).

Analysis of Variance (ANOVA)

The mean values of the heavy metal contents in the three (3) different brands of the chicken feeds were subjected to Analysis of Variance (ANOVA) to verify if there is a statistically significant difference in the mean.

Table 3.5 Analysis of Variance (ANOVA)

Source of Variation	SS	df	MS	F	P-value	F crit
Feed types	9.408494	2	4.704247	0.409944	0.668653	3.443357
Heavy Metals and feed brands	1571.134	11	142.8303	12.44671	4.83E-07	2.258518
Error	252.4577	22	11.47535			
Total	1833	35				

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From the result of ANOVA in Table 3.5 above, the different heavy metals and brands formed the column and the types of feed formed the rows, it is observed that the Fcrit > Fcal (3.44 > 0.41) for the types of feed, therefore there is no statistically significant difference between the types of feed evidence at $\alpha = 0.05$, a P value of 0.67 confirms it. Analysis of the different heavy metals and brands shows that Fcrit < Fcal (2.26 < 12.45) to show that there is a significant difference in the mean of the heavy metal contents among the 3 different brands of feeds, the P value of 4.83E-07 concurs it.

CONCLUSION

This study examined the levels of heavy metals in commercially available poultry feeds in Uyo, Nigeria. The heavy metals, Pb, Ni, Cd and Cu were analyzed in feed brands X, Y and Z. It was observed that the concentration of copper in Y brand of feed was found to be the highest while Z recorded the lowest. These observed values of copper were below the recommended limit by European Union. The mean levels of lead were lower than the recommended limit by European Union in all samples analyzed. The concentration of cadmium in the different types of feed increase in the following sequence Y > X > Z. Cadmium concentration in the layer feed was slightly higher than the maximum recommended limit of 1.0 mg/kg of Cd in feed. Nickel values were above recommended limit and its contamination sequence for the feeds were Starter > Layer > Finisher. The statistical analysis result has shown that there is no significant difference in the mean of the heavy metal contents among the 3 different brands of feeds. Generally, the heavy metal load in the different types of feeds was found to be at non-toxic concentrations, except for cadmium and nickel. These metal contaminations may arise from environment factors, production line processes, poor handling and storage by the retailers.

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