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Lead Levels in Tissues of Local Scavenger Chickens in Maiduguri, Nigeria

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ABSTRACT

Mineral mining activities with environmental pollution in Nigeria resulted in lead intoxication in humans and livestock. The likelihood of lead contamination of the environment from non-mining sources may exist in Maiduguri, Borno state, Nigeria. The study was carried out to evaluate the bioaccumulation of lead in extensively reared local scavenger chickens-LSC (*Gallus gallus domesticus*) in Maiduguri. Two hundred samples each of the liver, kidney, intestine, muscle and blood were tested for lead using standard methods in toxicologic evaluation. There were significant ($p<0.05$) variations in lead levels in the kidney, liver, muscle, intestine and blood of local scavenger chickens. The highest mean lead level (17.0±12.0µg/kg) was observed in the liver, and the lowest lead level (4.0±7.0µg/kg) was in the blood. No significant ($p>0.05$) sex differences existed in the frequency of lead detection. Mean lead level in the liver of males was significantly ($p<0.05$) higher than that of females with the highest and lowest mean lead levels occurring in the liver and kidney of male chickens, respectively. The highest lead level detected was 414 µg/kg; in the kidney of a female LSC. It was therefore concluded that environmental pollution with lead may be detected in LSC that bioaccumulate lead; with the liver having the highest affinity.

Keywords: Local scavenger chicken, Lead, Maiduguri, Nigeria

INTRODUCTION

Lead is an ubiquitous environmental contaminant that is poisonous to animals and humans throughout the world (Khan *et al.,* 2008). The increased occurrence of lead pollution in the environment has been associated with human activities (Hernberg, 2000; Tong *et al.,* 2000) such as agricultural and industrial practices that promote its use. These include mining, smelting, use of leaded petrol (gasoline); production of lead-acid batteries and paints, jewellery making, soldering, ceramics and leaded glass manufacture in informal and cottage industries; electronic waste and use in water pipes and solder (UNEP, 2008).

In developing countries like Nigeria, local chickens (*Gallus gallus domesticus*) are raised in subsistence households and serve as important source of animal protein; making up about 30% or more of the total protein consumed (Gueye, 1998; Egahi *et al.,* 2010). Chicken population in Nigeria has been estimated at about 137 million with the local scavenger chicken (LSC) being about 90% of this estimate (Bourn *et al.,* 1994; Egena *et al.,* 2012). Most of the chickens are kept on free-range systems where they scavenge for food. As such they are likely to be more exposed to environmental contaminants like lead; which may concentrate in their tissues and can be passed up the food chain. Local scavenger chickens are known to feed freely in the environment and drink water from ponds, streams, rivers and other sources that may be contaminated making them potential sentinels for lead. Animals and poultry especially chickens graze along the roads which might be contaminated with exhaust from vehicles that contain lead and this could serve as a source of intoxication (Nwude *et al.,* 2010). They also scavenge on refuse dumps with materials containing lead such as batteries, paints, glazed ceramics and electrical wires. These metals bio-accumulate in the organs and tissues of animals causing toxicity. This was demonstrated in several studies in which there were varying concentrations of lead in tissues and blood of exposed animals including poultry (Iwegbue *et al.,* 2008; Iwegbue, 2008; Akan *et al.,* 2010; Okoye *et al.,* 2015; Tyokumbur, 2016).

Toxicity, clinical manifestation, levels in tissues and pathology of lead in various animal species has been studied (Erdogan *et al.,* 2005; Nwude *et al.,* 2010; Abduljaleel and Shuhaimi-Othman, 2013). Furthermore, lead level in chickens has been reported in other parts of the country (Iwegbue *et al.,* 2008; Oforka *et al.,* 2012; Okeke and Okeke, 2015; Tyokumbur, 2016). Most of the work done in the country were conducted in areas where there were outbreaks of lead poisoning due to mining and smelting activities or from abattoir surveys (Okoye and Ugwu, 2010; Okoye *et al.,* 2011; Ihedioha and Okoye, 2012; Bala *et al.,* 2013; Orisakwe *et al.,* 2017). The possibility of long-term low level environmental contamination from other sources (used batteries, waste water irrigation, use of pesticides and

herbicides, leaded paints) and consequent transfer of this ubiquitous metal to the LSC; a highly favoured source of animal protein has not been explored in most parts of Nigeria including Maiduguri. Thus, there is need to check for the presence of lead in tissues of the LSC.

MATERIALS AND METHODS

Study Area

The study area (Maiduguri) is located in the semi-arid Sahel zone of Northeastern Nigeria (latitudes 11º 5" N and longitudes 13º 09"E) at about 350m above sea level. It occupies an area of 50,778 square kilometres (Ijere and Daura 2000).

Study Population

A total of 200 adult local scavenger chickens were purchased from Maiduguri live chicken market and local households. Sexes of the chickens were noted based on morphology and vent characteristics (Maguelonne, 2009).

Tissue Sample Collection and Preservation

Birds were slaughtered by cutting through the neck with a sharp knife at the base of the lower beak to sever the trachea, esophagus and blood vessels. Five milliliters (5 ml) of blood was collected into heparinised sample bottles and refrigerated. Tissue samples included the liver, kidney, muscles and intestine were collected after opening up the birds. The tissues were packed in sterile polythene bags, properly labelled and frozen prior to digestion for determination of lead concentration.

Processing of Samples

Dry Digestion of Tissue samples

The tissue samples were first dried in an oven at 45 ^{0}C. After drying, individual samples were pulverised using motar and pestle. One gram (1g) of the fine powdered sample was weighed into a porcelain crucible. The sample and crucible were then ignited in a muffle furnace at 550^{0}C for an hour. The samples were then removed from the furnace and allowed to cool in desiccators and weighed again. The difference between the weight of the crucible and ash and the weight of the crucible alone was used to calculate the percentage ash content of the sample. Three millitres of trioxonitrate (v) acid (HNO_3) solution was added to the left-over ash and evaporated to dryness on a hot plate. It was returned to the furnace and heated again at 400^{0}C for 15-20 minutes until perfect greyish-white ash was obtained. The samples were then allowed to cool in desiccators. Nine millilitres (9 ml) of hydrochloric acid (HCL) was then added to the ash to dissolve it and the solution was filtered into 100 cm^3 volumetric flask. The volume was made up to 50 ml with deionized water.

Digestion of Blood Sample

The blood sample was prepared for analysis in the following steps. Two and half millilitres (2.5 ml) of blood were put in a beaker and 10 ml of concentrated Nitric acid was added to it. The mixture was heated to dryness and allowed to cool at room temperature. Then 1.5 ml of 30% hydrogen peroxide was added. This was again heated to dryness and allowed to cool at room temperature. Thereafter 15 ml of 0.5% Nitric acid in de-ionized water was added and the solution filtered with Whatman filter paper size one.

Quantitative Metal Analysis

Lead concentration in the digested tissues was measured by atomic absorption spectrophotometry using a Varian, AA240FS Spectrophotometer. The actual concentration of lead in the tissues was determined as described by Olaifa *et al.* (2003) thus:

Actual concentration of lead in sample = Result × Dilution factor

Where Result = AAS reading

Dilution Factor = $\frac{\text{Volume of digest used}}{\text{Weight of sample digested}}$

Statistical Analysis

Data from the study were summarized as Mean ± Standard Deviation and analyzed using Kruskal-Wallis test (Non-parameteric ANOVA) followed by Dunn's post-test for comparison of means. Chi-square test for independence and 2x2 chi-square was used to assess level of association between tissues and frequency of lead detection in tissues. Student's T-test was used to assess the difference between lead levels in tissues of male and female chickens. Values were considered significant at $p<0.05$. Statistical analysis was done using computer software GraphPad Instat Version 3.10©

RESULTS AND DISCUSSION

Frequencies of detection and mean lead levels in tissues of local scavenger chickens

Table 1 summarizes the frequencies of lead detection and the mean lead levels in the liver, kidney, muscle, intestine and blood of the local scavenger chickens in Maiduguri. Significant ($p<0.05$) variation in the frequencies of lead detection occurred among the different types of tissues. However, there was no significant ($p<0.05$) difference in the frequencies of lead detection in the liver and blood on one hand and the muscle and intestine on the other. The kidney had the lowest ($p<0.05$) detection frequency among all the tissues while the highest ($p<0.05$) was recorded in the blood. This study has established the presence of lead in tissues of the LSC in Maiduguri. Lead levels in Maiduguri are much lower than those recorded by Tyokumbur (2016) who reported much higher values of 2.94, 3.66, 3.40 and 3.98mg/kg in the liver, kidney, muscle and intestine respectively of domestic chickens in Ibadan. In addition, assessment of lead levels in the internal organs of local chickens in Awka, Anambra State of Nigeria also showed higher values ranging from 0.046 to 0.478mg/kg (Okoye *et al.,* 2015) than what was obtained in this study. Another study in southern Nigeria by Iwegbue *et al.,* (2008) also showed mean lead level in chicken meat to be 0.77±0.01mg/kg which is much higher than what was obtained in the current study. Other studies from different parts of the world reported higher values of lead concentration in offals and muscle of chickens and other poultry species. Reem *et al.* (2012) reported that in chicken samples in the local markets of Basrah city, Iraq, lead contents were in the range of 0.171 to 3.269 mg/kg while the

lead level in chicken meat and offals in El-jaber Alakhder region of Libya ranged from 0.093 to 2.391ppm (Abdolgader *et al.,* 2013). In the Tenerife Island of Spain, the mean lead level in chicken meat was reported to be 3.16mg/kg (Gonzalez-Weller *et al.,* 2006). Such levels of lead in chicken meat and offal samples in the study area and from different parts of the world could indicate a certain level of environmental pollution probably aggravated by human activities that promote such contamination. These high values recorded in other studies within Nigeria and other countries could be as a result of high industrial activities in these locations as compared to Maiduguri which is principally an agrarian community. As such the level of contamination of the environment is probably much less.

Table 1: Frequencies of lead detection and mean lead levels in tissues of local scavenger chickens in Maiduguri

Tissue	Number Sampled	Number (Frequency,%) of Lead Detection	Tissue Lead Level (µg/kg) (Min. to Max.)	FAO/WHO Maximum Level (µg/kg)*
Liver	200	189 (94.5)[a]	17.0±12.0[a] (0.0-129.0)	500
Kidney	200	107 (53.5)[b]	5.0±31.0[b] (0.0-414.0)	500
Muscle	200	169 (84.5)[c]	6.0±8.0[c] (0.0-71.0)	100
Intestine	200	176 (88.0)[c]	6.0±18.0[c] (0.0-244.0)	500
Blood	200	198 (99.0)[a]	4.0±7.0[d] (0.0-66.0)	500

[a,b,c,d]Values with different superscripts are significantly different ($p<0.05$) for Means ± standard deviations (ANOVA) and frequency of detection (Chi-square).
*CAC (2017)

There were significant ($p<0.05$) variations in the mean lead levels of the different tissue types tested. The mean lead level in the liver was significantly ($p<0.05$) higher than values in the kidney, muscle, intestine and blood. The level in the kidney was lower ($p<0.05$) than those of the muscle and intestine but higher ($p<0.05$) than that of the blood. There was no significant ($p>0.05$) difference in the lead levels of the muscle and intestine. The blood had the lowest mean lead level among all the tissues. The highest mean lead level recorded in the liver when compared to other tissues sampled in this study is similar to what was reported by Akan *et al.* (2010) in an earlier study in chickens sourced from Kasuwan Shanu in Maiduguri metropolis. Similarly, an investigation into the lead levels of chicken giblets in Ismailia city of Egypt showed the liver as having the highest level of lead (Ismail and Abolghait, 2013). The high levels of lead observed in the liver could be due to the detoxification function of the liver since lead is a toxic metal. Also, the liver has been reported to be the largest storage site of lead in the soft tissues (Mudipalli, 2007). However, it could also be due to the direct access of the liver through the portal circulation. The blood had the lowest mean lead level among all the tissues probably because lead is actively removed from circulation and deposited in tissues like the liver (Rabinowitz, 1991; Markowitz, 2000). Maximum detectable lead level was in the kidney (414µg/kg). All tissues had mean lead levels lower than FAO permissible levels as shown in Table 1. The kidney with the maximum detectable lead level of 414µg/kg was still below 500µg/kg that was reported as permissible level by the FAO/WHO (2017) codex alimentarius committee on food additives and contaminants. All other tissues also had lead levels lower than the permissible levels. These low levels are probably due to low level of industrialization in the study area which seems to be a major risk factor elsewhere.

Effect of sex on frequencies of lead detection and mean lead levels in tissues of the of local scavenger chicken in Maiduguri, Nigeria.

The frequencies of tissue detection and tissue levels of lead in males and females are compared in Table 2. There were no significant ($p>0.05$) differences between male and female in the frequencies of lead detection in all the tissues between male and female chickens except for the liver where the frequency of lead detection in the male chickens was significantly ($p<0.05$) higher than in female chickens. The highest and lowest mean levels of lead were in the liver and kidney of male chickens respectively. No significant differences were observed in the mean lead levels between the tissues of the male and female chickens except for the liver where lead levels were higher in males than in females. This could be because the scavenging habit of the local chicken is the same regardless of the sex of the bird. However, the mean maximum detectable lead level was 76% higher in females than in males which may be because as females mobilize calcium from the bones for eggshell formation, the intestinal absorption of calcium (and concurrently lead) increases resulting in higher lead levels in tissues of the females than males. Similar findings were reported by Kerr *et al.* (2011) in northern bobwhite quails in which blood lead levels were significantly higher in females than in males.

A comparison of the maximum detectable levels of lead in all tissues tested (liver, kidney, muscle, intestine and blood) of male and female local scavenger chickens in Maiduguri is presented in Figure 1. The mean maximum lead level in females (183.2±148.4) was not significantly ($p>0.05$) different from the level in males (44.4±17.7). However, the mean maximum level was 76.0% higher in females than in males, but the dispersion of estimates was too high to support acceptable level of significance.

Table 2: Effect of sex on frequencies of lead detection and mean lead levels in tissues of local scavenger chickens in Maiduguri

Tissue	Frequency of Detection (%)		Tissue Level (µg/kg) (Min. to Max.)	
	Male (n=78)	Female (n=122)	Male (n=78)	Female (n=122)
Liver	96.2[a]	85.3[b]	16.0±7.0[a] (0-33)	7.0±15.0[b] (0-129)
Kidney	50.0[a]	53.3[a]	2.0±7.0[a] (0-53)	7.0±40.0[a] (0-414)
Muscle	85.9[a]	83.6[a]	6.0±8.0[a] (0-71)	6.0±7.0[a] (0-63)
Intestine	84.6[a]	88.5[a]	4.0±4.0[a] (0-27)	7.0±23.0[a] (0-244)
Blood	100.0[a]	99.2[a]	4.0±4.0[a] (0-38)	4.0±9.0[a] (0-66)

[a,b]Values with different superscripts are significantly different (P<0.05) for Means ± standard deviations (ANOVA) and frequency of detection (Chi-square) along rows.

In the female LSC, 3 out of 5 organs had maximum level of >100µg/kg but no tissue in males had up to this estimated level; and the maximum level (414µg/kg) in a tissue was in the female. In contrast, the male LSC had the lowest detectable level of 27µg/kg in a tissue (intestine) which was 9 folds lower than the maximum detectable level in a comparable tissue in the female.

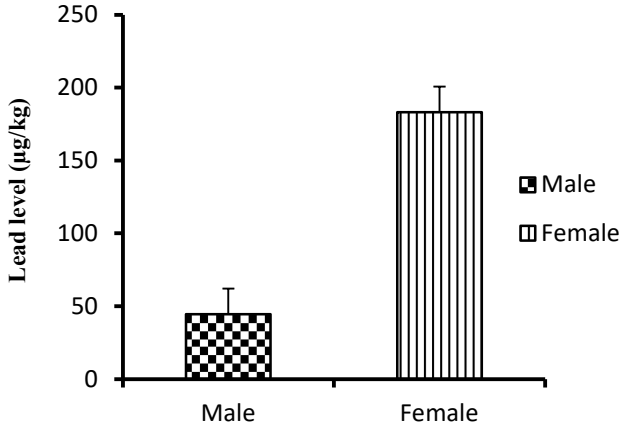

Figure 1: Sex comparison of mean maximum detectable levels of lead in all the tissues sampled (liver, kidney, muscle, intestine and blood) of the local scavenger chicken in Maiduguri

Conclusion

It was concluded that lead is present in the tissues of local scavenger chickens in Maiduguri although mean lead levels are within the permissible limits. The findings of the study have also demonstrated that the local scavenger chicken has the potential to accumulate lead in tissues; with lead having more affinity for the liver as a soft tissue as compared to other organs in chickens.

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Conflict of Interest

The authors have no conflict of interest to declare

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Author's Contribution

SUH, IOI and YAG contributed to the design of the work, supervision and interpretation of data. HIG carried out the laboratory work and prepared the draft manuscript. All authors read and approved the final manuscript.

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