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Research Article

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Levels of Heavy Metals in Tissues of Layer Poultry Strains in Maiduguri, Borno State, Nigeria

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Abstract This study is set out to determine heavy metals (As, Cd, Cr, Fe, and Pb) accumulations in tissues of two poultry strains, Black Hacco and Isa brown of odd Chicken Layer *(Gallus spp*). The poultry strains were sampled from two different poultry farms in Maiduguri; Ghumba Consult Poultry Farm, located at Bulumkutu Abuja and Doctor Isa Farm located at Wulari Jerusalem. The samples were collected and treated according to standard procedures. Heavy metals determination was carried out using the inductively coupled plasma – optical emission spectrometry (ICP-OES) technique. Generally, a comparative assessment of the results shows that the odd layer strains of poultry are within safe limits for consumption. The results provided herein will be essential to frame guidelines and standards for heavy metals in poultry meat products in Nigeria.

Keywords Heavy Metals, Tissues, Poultry, Maiduguri

1. Introduction

Poultry meat is nowadays a major component of diet and source of protein. It is also used in the production of several seasoning such as Maggi and Knorr chicken flavor cubes. Therefore, the high demand of poultry meat has also influenced their production and has been enhanced by extensive several factors. Accumulation of toxic substance such as heavy metals in poultry feeds on the other hand are great cause for concern.

The high demand for poultry meat has in recent years influenced their production significantly and alongside is the increased production and extensive modifications of poultry feeds to meet these demands. However, in view of the fact that poultry feeds, whether it is natural or locally sourced or the improved modifications from special manufacturing processes have been reported to be affect the content of heavy metals in poultry feeds [1]. A study was conducted in Nigeria [2] to assess four brands (starter, grower, finisher and layer) of three feeds (Gold medal, Top and Vital) sold commercially. A similar study has also been conducted earlier by Islam *et al* (2007) [1]. All reported that there are various sources of raw materials for poultry feed production.

Contamination with heavy metals is a serious health hazard because of their toxicity, bioaccumulation and bio magnifications in the food chain [3]. The principal route of heavy metals intake into the tissues of poultry is through the feeds [4]. On the other hand, the accumulation of heavy metals varies significantly from one tissue to another within an animal, and varies also between one animal and another [5].

The determination of heavy metals in tissue and organs of poultry animals has therefore received serious attention [6, 7, 8]. For the same reason environmentalist are also concerned about the release of these metals through the poultry droppings [9, 10]. Poultry meat and meat products are important for human diet because they provide a great part of nutrients, including the necessary trace elements [8]. The meat products now include Maggi and Knorr seasoning cubes, due to the fact that poultry meat has high nutrient components. However, the diet of poultry meat constitutes that source of heavy metal accumulations of the poultry meat [8, 11]. Therefore, while the application of poultry droppings to land is an effective disposal of animal waste and applied to solve the problem of animal waste disposal and also improves agricultural productivity [12] issues of the adverse effects have also been reported [7, 9].

2. Materials and Methods

2.1 Sample collection

Samples were collected from two different poultry farms in Maiduguri, Borno State, Nigeria. One of the farms, Ghumba Consult Poultry Farm, is located at Bulumkutu Abuja, and the other farm, Dr (DVM) Isa Farm is located at Wulari Jerusalem. The Black Hacco and Isa Brown poultry species were sampled from the respective farms according to standard sampling protocols as outlined by (Belton, 2006) [13].

2.2 Poultry Species and Tissues

Two strains of odd layer poultry reared under the prevailing conditions and environment of commercial poultry farm were used in this study. These are Black Hacco and Isa brown of odd Chicken Layer *(Gallus spp*). A total of 24 layer chickens of both species, 12 each of Hacco and Isa brown odd layers were, collected in threefold over a period of 8 weeks. Sampling took place between the months of November 2012 and January 2013.

Four tissues (breast meat, gizzard, liver and kidney) from odd layer poultry strain were harvested according to standard veterinary protocols [14]. Dr Isa an expert vet physician identified and performed the procedure. Cutting knives and forceps of sterile stainless steel materials were used. A composite of three each of the tissues were collected from the respective farms for analysis. All samples were collected in sterile polyethylene containers (poly bags) and transported to the laboratory for preparations.

2.3 Sample preparation

Procedure for sample preparations in this study was adopted from the analytical techniques presented in Belton [13]. Water samples were digested by treating 50 ml of water with 10 ml 2 M HNO_3, heat gently for 30 minutes at 80 ^oC. The remaining volume will be made to mark in 100 ml volumetric flask with distilled water. Similar digestion procedure was applied to drug samples, and heating made to produce a clear solution before diluting with distilled water. The harvested tissues and organs was cleaned and washed with demineralized water. They were cut into small sizeable pieces with stainless steel knife and oven dried at 65^{o}C for about 48 hours discontinuously until constant weight is obtained.

The wet digestion procedure was used. Two grams (2.00 g) samples was placed in a digestion tube and predigested in 10 mL concentrated HNO_3 at 135 ^oC until the liquor was clear. Thereafter, 10mL of HNO_3, 1 mL $HClO_4$ and 2 mL H_2O_2 was added and temperature was maintained at 135 ^oC for 1 h until the liquor became colourless. The product of the digestion was allowed to slowly evaporate to near dryness (avoiding prolonged baking). It was cooled and dissolved in 1M HNO_3. The digest was subsequently filtered through Whatman filter No 1 and diluted to 25 mL with 1M HNO_3

2.4 Sample analysis

Standard curves for the metal analytes (As, Cd, Cr, Fe, and Pb) were prepared from stock solutions (standard concentrations of 1000 mg/ml) of metal analytes. To cover the optimum emission working range (0.01 to 5.00 mg/ml) further serial dilutions was prepared. Usually freshly stored standard curves in the system software where available was used. Blank solutions will be also prepared accordingly.

The external standard methods [15, 16] of the inductively coupled plasma – optical emission spectrometry (ICP-OES) were used for the determination of the heavy metals (Cr, Cd, As, Fe, and Pb). The analysis was conducted at the Central Laboratory of Geology Department University of Maiduguri, Borno State, Nigeria.

The Aglient 710 Series ICP-OES (USA), operational with SPS auto-sampler was used for the determination of heavy metals. Samples were analyzed under the instrumental operating conditions: RF Power 1.0 kW, Outer argon flow 12.0 L/min, Intermediate and Inner argon flow 1.0 L/min and the Nebulizer uptake rate (ml/min) 1.0. Samples run was performed in replicate and integrated computer results of determinations will be recorded.

2.5 Quality assurance

Applicable quality assurance procedures and precautions were carried out to ensure reliability of the results especially in the aspect of samples preparations by keeping to procedural guidelines.

2.6 Data analysis

Data analysis was conducted using Analyse-it v.2.26 statistical software for Microsoft Excel. Summary results are presented as mean and standard deviation. Analysis of variance (ANOVA) with Tukey post-hoc test was applied to determine significance difference of variations between multiple variables, e.g. metal concentrations in tissues of poultry species. Pearson's correlation analysis was applied to determine level of association between pairs of variables. Possibilities less than ($P<0.05$) was considered statistically significant [16].

3. Results

3.1 Arsenic (As)

Figure 1 shows As concentration distribution in tissues of layer poultry analysed in this study. It shows that breast meat of Hacco (0.0802±0.021 mg/g) and Isa Brown (0.0772±0.018 mg/g) form the respective Farm1 and Farm2 accumulated the highest in As concentrations, while liver tissues were least Liver1 (0.0033±0.001 mg/g) and Liver2 (0.0037±0.018 mg/g) from Farm1 and Farm2 respectively.

Concentrations variations of As between similar tissues from the two farms show no statistical significance (ANOVA with Bonferroni post hoc, $p<0.05$), but variation between the different tissues does, with exception of gizzard and liver. An observed outlier in gizzard2 (0.098 mg/g) maximum concentration was excluded due to the high uncertainty. The general order of As concentration in layer poultry tissues studied is: breast meat > kidney > gizzard > liver.

3.2 Cadmium (As)

Cd concentration distribution in tissues of layer poultry is shown in Figure 2. It revealed that kidney of Hacco (0.019±0.001 mg/g) and Isa Brown (0.018±0.001 mg/g) form the respective Farm1 and Farm2 accumulated the highest in Cd concentrations, while liver tissues were least Liver1 (0.003±0.001 mg/g) and Liver2 (0.004±0.000 mg/g) from Farm1 and Farm2 respectively, breast meat indicated absence of Cd.

There was no statistical significance in concentrations variations of Cd between similar tissues from the two farms, but variations between kidney and the other tissues was recorded. The general order of Cd concentration in layer poultry tissues studied is: kidney > gizzard > liver >breast meat.

3.3 Chromium (Cr)

Figure 3 shows Cr concentration distribution in tissues of layer poultry analysed in this study. It shows that gizzard of Hacco (0.691±0.042 mg/g) and Isa Brown (0.684±0.036 mg/g) form the respective Farm1 and Farm2 accumulated the highest in Cr concentrations, while liver tissues were least Liver1 (0.104±0.029 mg/g) and Liver2 (0.102±0.029 mg/g) from Farm1 and Farm2 respectively.

Concentrations variations of Cr between similar tissues from the two farms show no statistical significance, but variation between the different tissues does, with exception of kidney and liver. The observed outlier in breast meat 1 & 2 (0.176 & 0.172 mg/g) minimum concentration was excluded due to the high uncertainty. The general order of Cr concentration in layer poultry tissues studied is: gizzard > breast meat > liver > kidney.

3.4 Iron (Fe)

Figure 4 present the concentration distribution of Fe in tissues of layer poultry analysed. It revealed that breast meat of Hacco (1.719±0.11 mg/g) and Isa Brown (1.710±0.09 mg/g) form the respective Farm1 and Farm2 accumulated the highest in Fe concentrations, while liver tissues were least, Liver1 (0.645±0.10 mg/g) and Liver2 (0.642±0.10 mg/g) from Farm1 and Farm2 respectively.

Concentrations variations of Fe between similar tissues from the two farms show no statistical significance, but variation between the different tissues was marked between breast meat and other tissues, with exception of kidney and liver. The general order of Cr concentration in layer poultry tissues studied is: breast meat > gizzard > kidney > liver.

3.5 Lead (Pb)

Lead was not detected (ND) in all the samples analysed.

3.6 Metal Interaction Analysis in Layer Poultry Tissues

The inter-metal correlation analysis (Table 1) indicated a strong positive association levels between Cr – Fe (r = 0.83) and As – Cd (r = 0.78) in the trend of metal accumulation on the tissues of layer poultry. A moderately weak anti-correlation level of association was observed between Cr – As (r = -0.49)

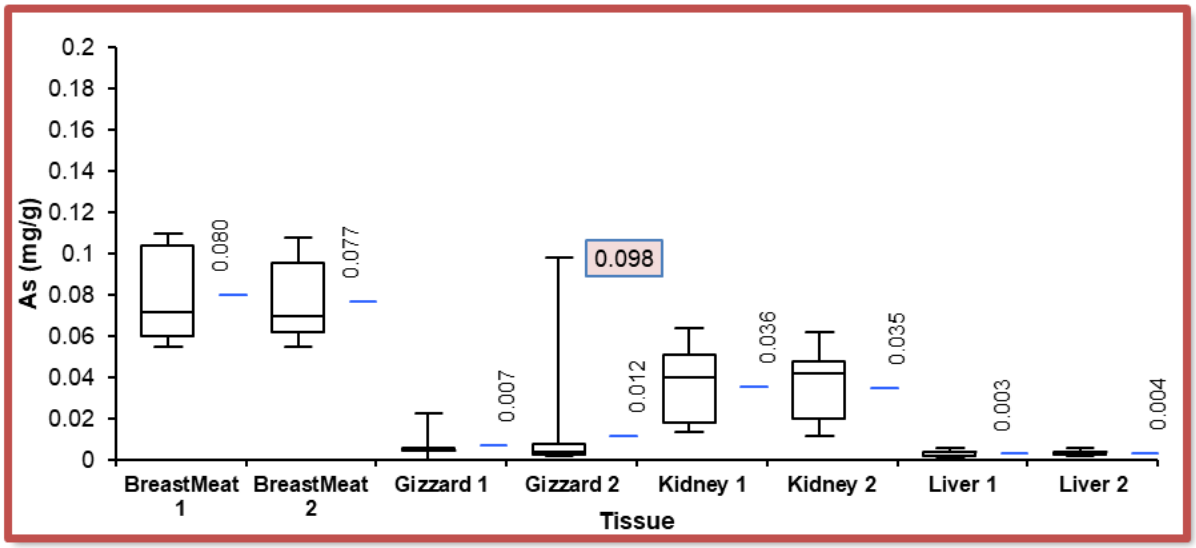

Figure 1: Box-Whisker plot of Arsenic concentration (mg/g) in layer Poultry Tissues

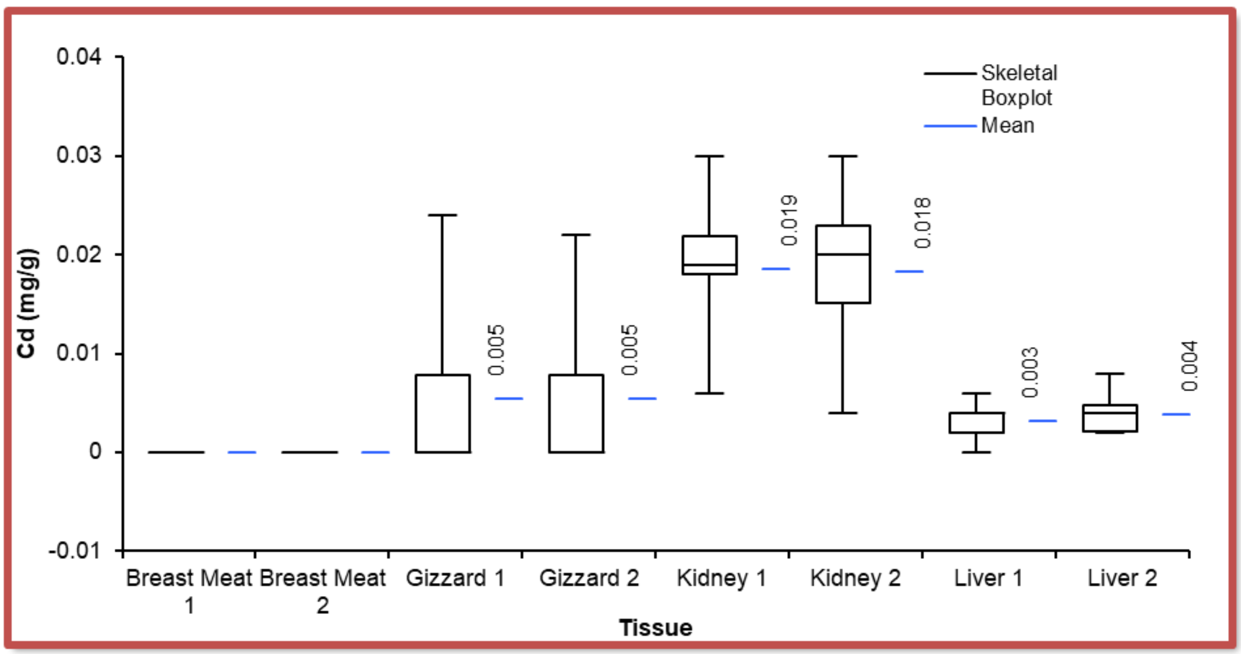

Figure 2: Box-Whisker plot of Cadmium concentration (mg/g) in layer Poultry Tissues

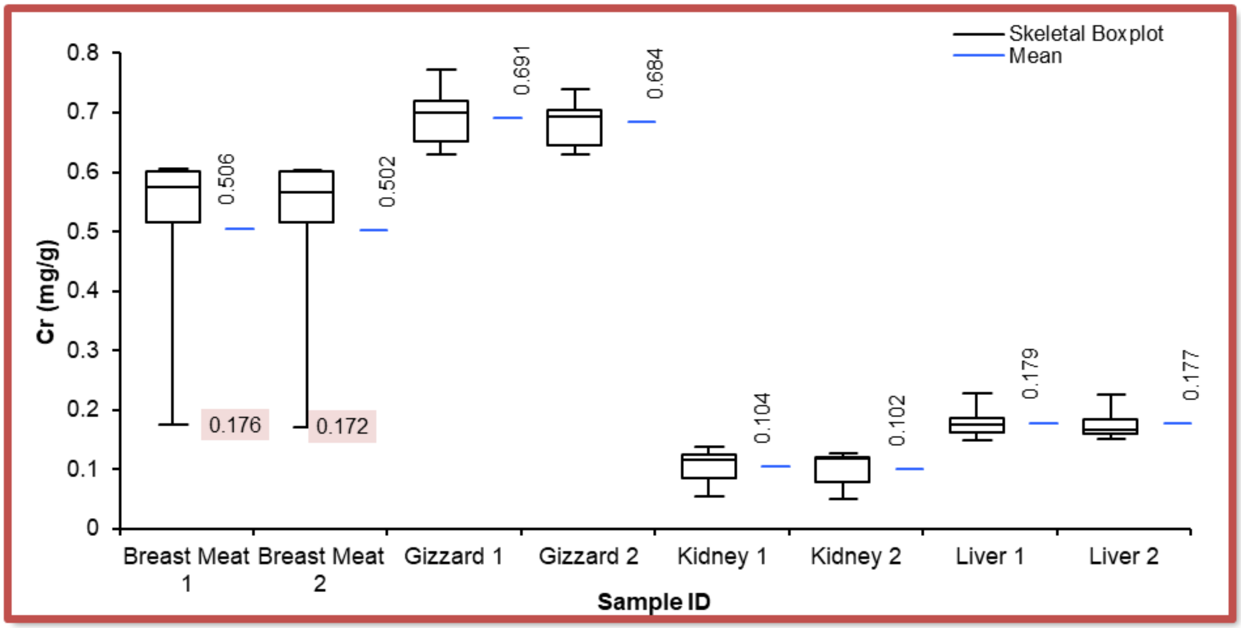

Figure 3: Box-Whisker plot of Chromium concentration (mg/g) in layer Poultry Tissues

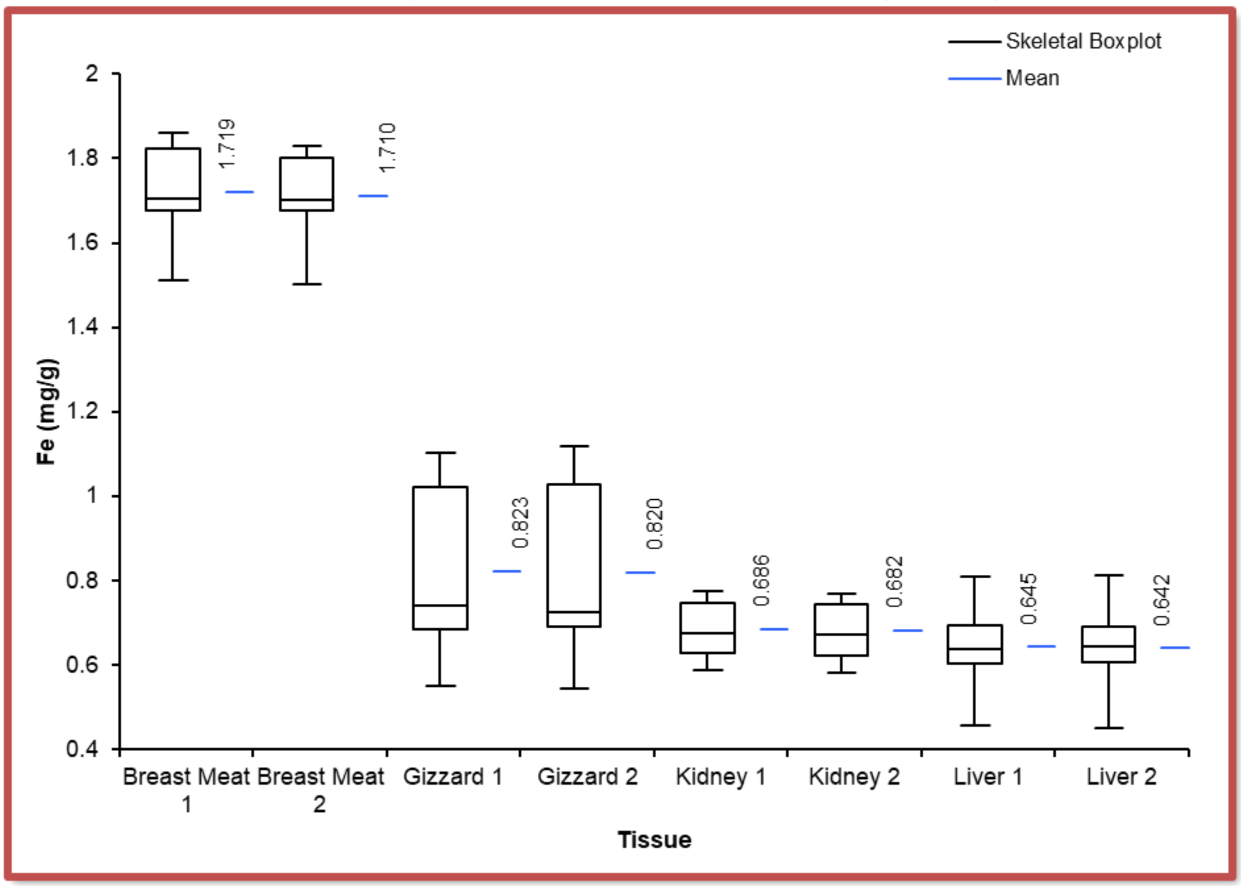

Figure 4 : Box-Whisker plot of Iron concentration (mg/g) in layer Poultry Tissues

Table 1: Inter-metal correlation coefficient matrix in *Poultry Tissues*

Metal	As	Cr	Fe	Cd
As	**1**			
Cr	**-0.49**	**1**		
Fe	-0.21	**0.83**	**1**	
Cd	**0.78**	-0.05	0.15	**1**

*Values in **bold** are significance level alpha=0.05*

4. Discussion

4.1 Arsenic (As)

As in tissues analysed is in the order: breast meat > kidney > gizzard > liver. The range of accumulation is of concern in the breast meat, an essentially edible part of poultry. Even though it was less than the permissible limit set for As (2.0 ppm) in tissue of poultry [18]. However, the finding of this study corresponds with Mariam et al. (2004) [19] but less than As found in the liver of local chicken determined in the work of Akan *et al* (2010) [20].

4.2 Cadmium (Cd)

Cd in tissues samples of this study presented the order: kidney > gizzard > liver >breast meat. On the basis of presented results of Cd analysed it follows that its levels in poultry meat are below the highest permissible hygienic limits for Cd (muscle 0.1mg/kg; internal organs 0.5 mg/kg [21, 22]. The findings of this study are similar to that reported in Skalická, *et al.*, (2002) [23] and Akan *et al*., (2010) [20].

4.3 Chromium (Cr)

Cr (VI) is the toxic form of concern; however, a daily requirement for adults is estimated to be 0.5–2 µg of absorbable chromium (III) has been recommended for humans and some other animals [24].

The order of Cr concentration in tissues studied is gizzard > breast meat > liver > kidney. Iwegbue *et al.*, (2008) [8] reported similar trend in which gizzard, in most cases, was higher in Cr. It was also established in this study that feedstuff accounts for the bulk of Cr intake by the poultry, buttressing most reports of food as being the major source of Cr. On the whole, levels of Cr in this study met the estimated daily requirement for this trace element provided that daily meat intake was no more than 100 g.

4.4 Iron (Fe)

Fe accounts for about 5% of the earth's crust, making it the second most abundant metal [25, 26] therefore the reason it is found in most environmental medium. A general median value of 0.7 mg/L Fe concentration has been reported in rivers, while in anaerobic groundwater where iron is in the form of Fe (II), concentrations usually ranges between 0.5 and 10 mg/L, but concentrations up to 50 mg/L have been recorded. The results of this study is higher, on average, than the usual Fe concentrations levels (0.3 mg/L) in drinking-water [27] and the results of Kolo *et al*. (2009) [28], but lower than the highest limits in ground water.

However, Fe is an essential trace element in living organisms [29].

Therefore, Fe content in all tissue samples were generally high, with breast meat recording the highest value corresponding with the findings of Iwegbue *et al*., (2008) [8].

4.5 Lead (Pb)

Pb was not detected in any of the samples analysed, which may be due to the set limits of detection and quantitation in this study.

It is a favourable condition that Pb was not detected in all the samples because Pb a potentially toxic substance with no known physiological functions. Pb toxicity affects the haematologic, renal and neurologic systems and there is no evidence for a threshold below which Pb has no adverse effects, especially in children health [30,31].

5. Conclusion

Generally, a comparative assessment of the results shows that the odd layer strains of poultry are within safe limits for consumption. The information provided herein will be essential to frame guidelines and standards for heavy metals in poultry meat products in Nigeria.

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