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Heavy Metals in Selected Tissues of Adult Chicken Layers (Gallus spp)

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ABSTRACT

A study was conducted to determine heavy metals (As, Cd, Cr, Fe and Pb) in tissues (breast meat, gizzard, liver and kidney) of two layers (Hacco and Isa Brown) species of adult chicken layer (Gallus spp). Sampling was carried out in two chicken layer farms in Maiduguri over a three months period (November 2012 – January 2013). Heavy metals were determined by the standard methods of inductively coupled plasma-optical emission spectrometry (ICP-OES). The results of the study reveal that As concentration is in the order: breast meat > kidney > gizzard > liver, Cd: kidney > gizzard > liver >breast meat, Cr: gizzard > breast meat > liver > kidney, Fe concentration in layer poultry tissues studied is: breast meat > gizzard > kidney > liver. Lead was not detected (ND) in all the samples analysed. The result of this study shows that the adult layer chickens are within safe limits for consumption. The information provided herein will be essential to frame guidelines and standards for heavy metals in feedstuff and meat products in Nigeria.

Keywords: *Heavy metals, tissues, Gallus spp, WHO/FAO guideline*

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 in Nigeria. Therefore the high demand of poultry meat has also influenced their production and has been enhanced extensively by several technological inputs. Accumulation of toxic substance such as heavy metals in poultry feeds on the other hand are great cause for concern is it is major source of heavy metal intake.

Due to the fact that 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 affected by the content of heavy metals in poultry feeds (Islam et al., 2007).

Demirezen and Uruc (2006) and Baykov et al., (1996) have indicated that contamination with heavy metals is a severe health hazard since they are toxic, bioaccumulate and biomagnifications in the food chain, which is the principal route of heavy metals intake into the tissues of poultry, is through the feeds.

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 (John and Jean, 1994).

The determination of heavy metals in tissue and organs of poultry animals has therefore received serious attention (Surtipanti et al., 1990; Malak et al., 2007; Iwegbue et al., 2008). According to Cunningham and Saigo (1997), the foremost among toxic metals which accumulate in food chains and have a cumulative effect are lead, cadmium, mercury and arsenic. Direct physiologically toxic effects of heavy metals arising from storage or incorporation in living tissues have been reported by Baykov et al., (1996).

This study aims to provide information on the concentration distribution of heavy metals (As, Cd, Cr, Fe, and Pb) accumulated in tissues of two poultry strains. These are Black Hacco and Isa brown of Adult Chicken Layer (Gallus spp). These chickens are highly grown for commercial purposes in Maiduguri, northeastern Nigeria. The result of this study is anticipated to support monitoring effort and reveal the level of health hazard associated with the continuous consumption of these strains of layer chickens.

2. MATERIALS AND METHODS

2.1 Species and Tissues

Two Adult layer chickens reared under the prevailing conditions and environment of commercial poultry farm were used in this study. These are Black Hacco and Isa brown of Adult 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 Adult layer chickens were harvested according to standard veterinary protocols (OLAW, 2002). 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 and analysis.

2.2 Sample Preparations

Procedure for sample preparations in this study was adopted from the analytical techniques presented in Belton (2006). The harvested tissues and organs were cleaned and washed with demineralized water. They were cut into small sizeable pieces with stainless steel knife and oven dried at 65°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 10mL concentrated HNO_3 at 135°C until the liquor was clear. Thereafter, 10mL of HNO_3, 1mL $HClO_4$ and 2mL H_2O_2 was added and temperature was maintained at 135°C 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 digests was subsequently filtered through Whatman filter No 1 and diluted to 25 mL with 1M HNO_3.

2.3 Heavy Metals Determinations

Standard curves for the metal analytes (As, Cd, Cr, Fe, and Pb) were prepared from stock solutions (standard concentrations of 1000mg/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 and was used. Blank solutions were be also prepared accordingly.

The external standard methods (Boss and Fredeen, 1997; Aglient, 2010) 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 were performed in replicate and integrated computer results of determinations will be recorded.

2.4 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. 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 (Alfassi et al., 2005).

3. RESULTS

Arsenic (As) concentration distribution in tissues of layer poultry analysed in this study is shown on Figure 1. 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.

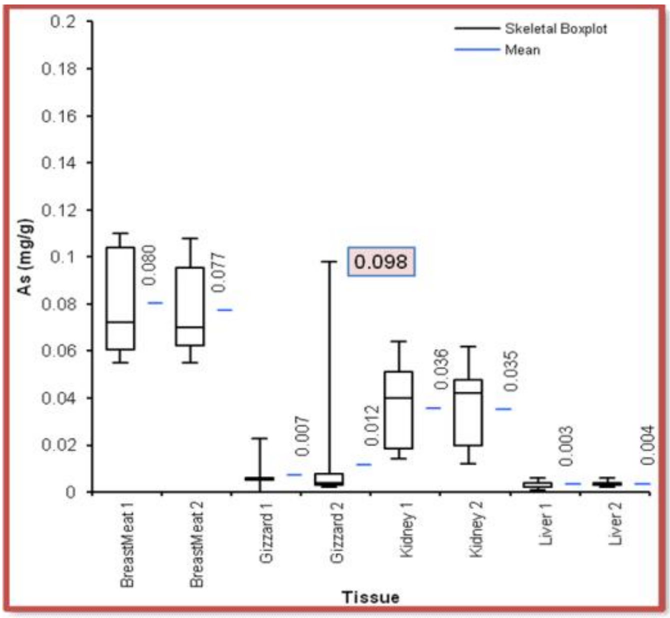

Fig 1: Box-Whisker plot of **Arsenic** concentration (mg/g) in layer **Poultry Tissues**

Cadmium (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 were recorded. The general order of Cd concentration in layer poultry tissues studied is: kidney > gizzard > liver >breast meat.

Figure 3 shows Chromium (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.

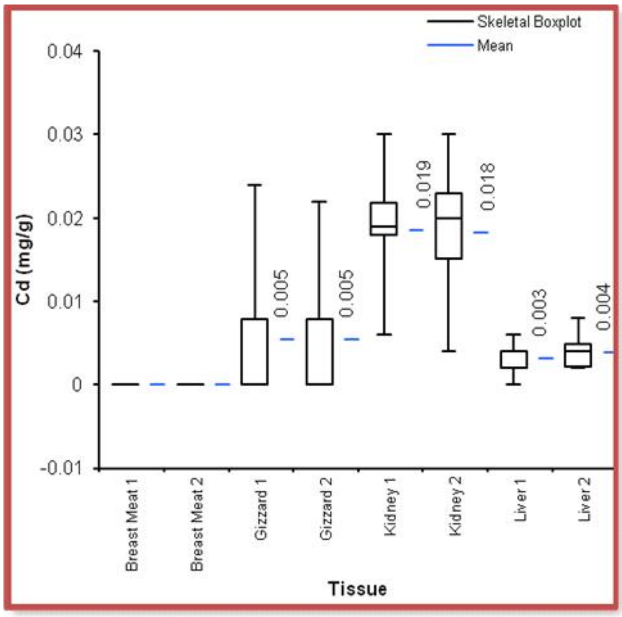

Fig 2: Box-Whisker plot of **Cadmium** concentration (mg/g) in layer **Poultry Tissues**

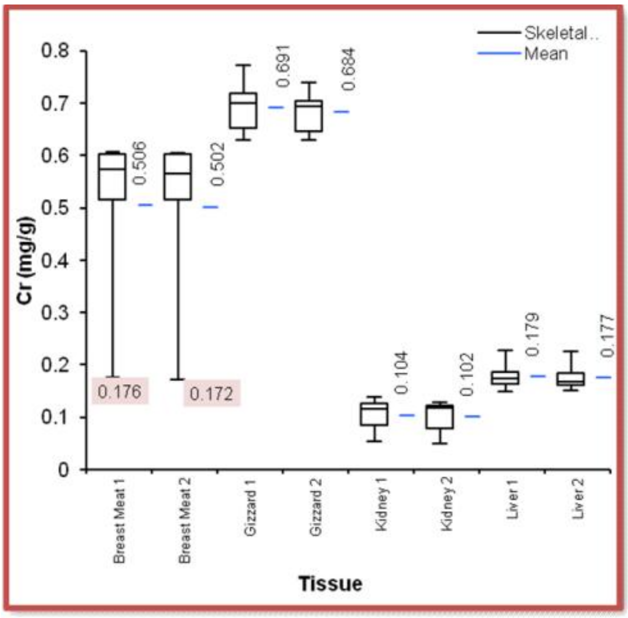

Fig 3: Box-Whisker plot of **Chromium** concentration (mg/g) in layer **Poultry Tissues**

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 Fe concentration in layer poultry tissues studied is: breast meat > gizzard > kidney > liver.

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

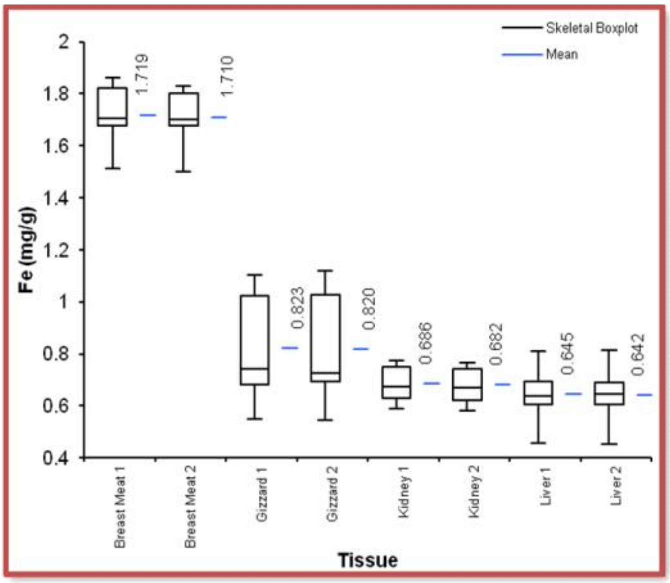

Fig 4: Box-Whisker plot of **Iron** concentration (mg/g) in layer **Poultry Tissues**

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).

Table 1: Inter-metal correlation coefficient matrix of heavy metals 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

In this study, 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 (ANZFA, 2001). However the finding of this study corresponds with Mariam et al. (2004) but less than As found in the liver of local chicken determined in the work of Akan et al (2010).

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 (Codex 1996, FAO/WHO, 2000). The findings of this study are similar to that reported in Skalická, et al., (2002) and Akan et al., (2010).

The order of Cr concentration in tissues studied is gizzard > breast meat > liver > kidney. Iwegbue et al., (2008) 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.

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).

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. Pb occurs in tap water and ground water to a degree, but existing data suggest that Pb concentration is rather low with a maximum permissible contaminant level of 0.01 mg/L. (WHO, 2011). In other studies, Pb was detected in poultry feedstuff (Okoye et al., 2011) and tissue samples including poultry meat (Iwegbue et al., 2008). 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 (Needleman et al, 1979; Goyer, 1993).

5. CONCLUSION

This study set out to elucidate the concentration distribution of heavy metals (As, Cd, Cr, Fe, and Pb) accumulations in tissues of two poultry strains. A generally comparative assessment of the result 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 feedstuff and meat products in Nigeria.

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