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Estimation of Heavy Metals in Liver, Gizzard, Breast and Thigh Muscles of Broiler Chicken in Different Area of Lahore by ICP-OES

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Abstract
Poultry, considered to be healthy type of meat, is the most important animal source of protein in food web. In this study, the concentration of metals in different parts of broiler chicken was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The highest mean level of nickel (43.89 ±0.87 µg/kg) was observed in gizzard muscles collected from Amin Park while lowest level (2.34 ±0.63 µg/kg) was found in gizzard samples collected from Lohari. Thigh muscle samples collected from Lohari did not show any level of nickel. The highest mean concentration of chromium was found in breast muscle samples collected from Amin Park 171.37±0.92 µg/kg while the samples collected from Outfall Road had the lowest chromium concentrations in gizzard 55.46±0.23 µg/kg. No chromium was detected in thigh muscles collected from Lohari. The maximum mean cadmium concentration calculated in liver and thigh muscles sampled from Shahdara were 52.36 ± 0.07 µg/kg and 51.46 ± 0.21 µg/kg respectively. The liver tissue samples of Amin Park had minimum cadmium concentration 28.46 ± 0.28 µg/kg. Higher level of copper among all samples was observed in thigh muscles 1.81±0.15 µg/kg collected from Outfall road, while lowest level was in breast muscle 0.10 ±0.01 collected from Shahdara. The study showed presence of wide range of heavy metals concentration (Ni, Cr, Cd and Cu) in all parts including (Gizzard, Breast muscle, Thigh muscle and Liver) of broiler chicken. All the results are within the safe limits and safe for human consumption.
Keywords: ICP-OES, Broiler, Lahore, Heavy metals.

INTRODUCTION

Elements found in all living bodies have distinctive roles in organizational, structural, and many other functioning of the animal body. These elements are characterized into essential elements, trace elements and non-essential elements (Rehman *et al*., 2012). Trace elements whose little amount is required included Cu, Fe, Zn and Co along with that some elements are whose presence doesn't affect the body activity but their deficiency as well as excess leads to biological malfunctioning in living (Szyczewski *et al.,* 2009). Heavy metals have the adverse effects on human health as they tends to bio accumulate inside the living tissues even a very trace amount can create disturbance in different inter and intracellular biochemical activities (Garelick *et al*., 2008). An interaction of these metals among other heavy metals including (essential and toxic) and with environment can alter their chemistry of absorption, accumulation and level of toxicity, as these interactions are central to mineral balance inside the living body (Pappas *et al*., 2010).

A balance diet is good for the healthiest life which vigor the productivity of the living body (Maqbool *et al*., 2005). Protein is the major macromolecule of our body and constitute of about 16% of body ratio and have a great importance for maintaining the metabolic health and body functioning capacity (Gorissen *et al.*, 2017). The major sources of protein are animals and plants. Poultry is the most important animal source of protein in food web which is considered to be healthy type of meat. There are wide concern with the contamination of chicken and egg by chemical contaminants Pb , Cr ,Hg, Ni, Cu, As (Hossain *et al.*, 2017). The animal feed is the principle rout for the heavy metal intake into the poultry tissues. In poultry industry these metals are being used in feed formation as a mineral supplement to fulfill the protein requirement but their excessive use creating bioaccumulation of these metals in broiler meat (Rehman *et al*., 2012).

Poultry is highly susceptible to the heavy metal intoxication, especially the cadmium as it occurs by feeding on the plant origin food. Its accumulation in body leads to renal failure, osteoporosis and mutagenic effects beyond the threshold limits established by the WHO. Copper metal is essential element for the maintenance of body homeostasis as well act as cofactor in many enzymatic activities inside the body (Strausak *et al*., 2011). Chromium plays role in catabolism of protein, sugar, and fat but its excess lead to cancer. The aim of the study was to determine the concentrations of following metals Ni, Cr, Cd, and Cu in different organs of broiler chicken and comparison among the concentrations found in different organ collected from different localities of Lahore city.

MATERIALS AND METHODS

This research was conducted at Fish Quality Control Labs, Fisheries Research and Training Institute Manawa, Lahore with the permission of Director Fisheries Research and Training.

Sample Collection

Samples of broiler chicken and their tissues (Breast muscles, Liver, Gizzard and Thigh muscle) were collected from different markets of Lahore city located at Amin Park, Karim Park, Shahdara, Lohari and Outfall Road. The samples were packed in polyethylene bags after washing with deionized water and tagged with specifications. The samples were transported Fish Quality Control Laboratory Manawa Lahore and kept frozen at -20ºC until further analysis.

Sample preparation

The sample preparation was followed by wet digestion method for the determination of heavy metal (Ni, Cr, Cd and Cu). The method involved the combine use of oxidizing agent conc. HNO_3, and non-oxidizing agent H_2O_2.The key advantage of wet digestion procedure is complete decomposition of sample matrix and to remove the different interference.

The sample preparation was as followed;

- Firstly, the samples were defrosted at room temperature and washed with deionized water.
- 10g of each sample was taken into the 100ml beaker. 10ml of Hydrogen Peroxide and Nitric Acid 1:1 V/V solution was prepared and added. The beakers were covered with watch glass and set aside for an hour.
- The beaker were then placed on the hot plate and allowed gradual rise in temperature up to 160ºC. The beakers with all contents were boiled for 2 hours and the volume is reduced up to 2-2.5ml.
- The contents were allowed to cool down and filtered via a Millipore membrane filter (0.45µ, type HV). Later the contents of beaker were transferred to the volumetric flask of 25ml and diluted up to mark with deionized water and subjected for determination of following heavy metals; nickel, chromium, cadmium and copper by using the Inductively Coupled Plasma (PerkinElmer, model: Optima 7000DV ICP-OES). Results were interpreted in µg/kg (Butt *et al*., 2016).

Standard solutions preparation

Standard solutions of five different concentration 0 µg/kg, 100 µg/kg, 200 µg/kg, 350 µg/kg, 400 µg/kg and 500 µg/kg were prepared from the standard stock solution of value 500 mg/kg by using the formula given as below.

$C1V1=C2V2$

Where

C1 is the value of stock solution

V1 is the volume to be measured

C2 is the value of different concentration that have to be prepared

V2 is the volume given for the dilutions of standard solutions.

Elemental Analysis

For achieving the optimum emission working range further dilutions up to 50 ml were prepared. Metals determination in samples were conducted by using Inductive Coupled Plasma-Optical Emission Spectrometer (ICP-OES Perkin Elmer) under the operating conditions: RF Power 1.5 kW, Outer argon flow 12.0 L/min. Intermediate and Inner argon flow 1.0 L/min and the Nebulizer uptake rate1.0 ml/min. Samples run in replicate and an integrated computer result was recorded, to check the reliability of the instrument, a blank and a standard of known concentration were run after every 5 samples.

RESULTS

The mean level of four metals in different organs of broiler varies between the chicken selling outlets from different area. The values of Ni, Cr, Cd and Cu were determined in different tissue organs collected from different locations. A wide range of variation was found in the results. Table 1 presents the concentration of Ni, Cr, Cd and Cu in different organs of broiler chicken collected from five different localities of Lahore city.

Table 1. Mean concentrations of heavy metals (Ni, Cr, Cd and Cu) in different organ of broiler chicken

Location	Organs	Concentrations of Metals (µg/kg)			
		Ni Mean±SD	Cr Mean±SD	Cd Mean±SD	Cu Mean±SD
Karim Park	Gizzard	37.35 ± 0.78	117.45 ±0.11	28.73 ±0.49	0.58 ±0.01
	Breast	15.33 ±0.06	109.80 ±0.25	42.14 ±0.42	1.41 ±0.02
	Thigh	43.65 ±0.48	111.40 ±1.025	41.35 ±0.07	0.19 ±0.03
	liver	18.52 ±0.74	161.52 ±0.07	44.93 ±0.04	1.47 ±0.01
Amin Park	Gizzard	43.89 ±0.87	109.28 ±0.69	41.50 ±0.14	1.59 ±0.01
	Breast	23.78 ±0.35	171.37 ±0.92	46.56 ±0.28	0.36 ±0.01
	Thigh	3.91 ±0.17	119.57 ±1.13	37.81 ±0.35	0.57 ±0.01
	liver	15.6 ±0.45	151.595 ±0.25	28.46 ±0.28	1.6 ±0.14
Lohari	Gizzard	2.34 ±0.63	62.36 ±0.55	37.31 ±0.21	0.65 ±0.07
	Breast	3.66 ±0.19	91.12 ±0.35	41.79 ±0.15	0.11 ±0.01
	Thigh	N.D	N.D	33.80 ±0.21	0.25 ±0.01
	liver	13.42 ±0.23	151.62 ±0.21	50.31 ±0.07	1.39 ±0.02
Outfall Road	Gizzard	8.50 ±0.44	55.46 ±0.23	37.01 ±0.14	0.48 ±0.01
	Breast	5.02 ±0.11	77.10 ±0.67	47.87 ±0.07	0.27 ±0.01
	Thigh	2.52 ±0.16	69.82 ±0.64	39.24 ±0.07	1.81 ±0.15
	liver	8.48 ±0.16	60.52 ±0.17	39.40 ±0.14	1.62 ±0.01
Shahdara	Gizzard	3.92 ±0.17	55.93 ±0.60	35.37 ±0.28	0.65 ±0.07
	Breast	17.5 ±0.76	98.55 ±2.09	45.89 ±0.14	0.10 ±0.01
	Thigh	12.84 ±0.03	99.34±0.64	51.46 ±0.21	0.25 ±0.01
	liver	10.19 ±1.18	146.67 ±0.21	52.36 ±0.07	1.39 ±0.01

ND: Not detected

The mean concentration of nickel in different organs (gizzard, breast muscle, thigh muscles and liver tissues) ranged from 2.34 ±0.63 µg/kg to 43.89 ±0.87 µg/kg. Amongst all the analyzed samples, the highest mean level of nickel was in gizzard 43.89 ± 0.87 µg/kg and thigh muscle 43.65 ± 0.48 µg/kg samples collected from Amin Park and Karim Park, respectively. The samples collected from Lohari showed the lowest value of nickel concentration in gizzard 2.34 ± 0.63 µg/kg, breast 3.66 ± 0.19 µg/kg and no Nickel was detected in thigh muscle (Table 1).

The mean concentration of chromium in different organs (gizzard, breast muscle, thigh muscles and liver tissues) ranged from 55.46 ±0.23 µg/kg to 171.37 ±0.92 µg/kg. The highest mean concentration of chromium was found in breast muscle samples collected from Amin Park 171.37±0.92 µg/kg while the samples collected from Outfall Road had the lowest chromium concentrations in gizzard

55.46±0.23 µg/kg, followed by Shahdara 55.93±0.60 µg/kg in gizzard. No chromium was detected in thigh muscles collected from Lohari (Table 1).

The mean concentration of cadmium in different organs (gizzard, breast muscle, thigh muscles and liver tissues) ranged from 28.46 ±0.28 µg/kg to 52.36 ±0.07 µg/kg. The maximum cadmium concentration calculated in liver and thigh muscles sampled from Shahdara were 52.36 ± 0.07 µg/kg and 51.46 ± 0.21 µg/kg respectively. The liver tissue samples of Amin Park had minimum cadmium concentration 28.46 ± 0.28 µg/kg.

The mean concentration of copper in different organs (gizzard, breast muscle, thigh muscles and liver tissues) ranged from 0.10 ±0.01 µg/kg to 1.81 ±0.15 µg/kg. Higher level of copper among all samples was observed in thigh muscles 1.81±0.15 µg/kg collected from Outfall road, while lowest level was in breast muscle 0.10 ±0.01 collected from Shahdara.

DISCUSSION

The presence of metals in broiler meat is due to the excessive usage of metal as food supplements in poultry industry to fulfill the optimum metal requirement. The present study reveals the concentration of nickel varied in the different organs of the broiler chicken. The gizzard samples collected from Amin Park had higher nickel concentrations as 43.89 ±0.87 µg/kg while the lowest concentration found in samples collected from Lohari as 2.34±0.63 µg/kg which was much less than the values (450 µg/kg to 540 µg/kg) reported by (Rehman *et al*., 2013) in domestic cock gizzard. The concentration of Nickel found in breast muscles was from 3.66 ±0.19 µg/kg to 23.78±0.35 µg/kg, thigh muscles 2.52 ±0.16 µg/kg to 43.65 ±0.48 µg/kg. It was not detected in thigh samples collected from Lohari. The lowest level nickel found in liver vary from 8.48 ±0.16 µg/kg to 18.52 ±0.74 µg/kg which was less than the reported values of 24 to 93 µg/kg by (Reddy *et al.*, 2017). Over all nickel concentration found was less than the permissible limits defined by WHO standard which are 500 µg/kg.

The chromium concentration in different analyzed organs were in the order, liver >breast >thigh >gizzard. Our results showed similar trend as reported by (Akan *et al*., 2010) in which the liver has maximum chromium concentration in local chicken. The trend of results in our study was different to the previous research (Iwegbue *et al.,* 2008) where they found highest concentration of chromium in the chicken meat 483 µg/kg followed by gizzard 343µg/kg and turkey meat 233µg/kg respectively. These values are much greater than the present results. On the whole all the observed values were within the hygienic permissible limit 500µg/kg defined by FDA.

The level of cadmium in the Karim Park, Lohari, Shahdara except Amin Park and Outfall Road were slightly higher in the liver 44.93 ±0.04µg/kg, 50.31 ±0.07 µg/kg, 52.36 ±0.07 µg/kg respectively than the concentration of thigh muscle and the breast samples. However, the difference in results observed was not significant. The gizzard samples have lower values as compared to the other organs. The order of the level of cadmium analyzed in the present study is liver> thigh>breast>gizzard. The observed results were within the permissible limit 200µg/kg defined by the FDA. The findings of (Mary Emmanuel *et al.,* 2017) support the results trend of our study. They reported high level of cadmium in liver 110 µg/kg and 30 µg/kg in muscles of local chicken. Khan et al. (2016) also showed the similarities in level of cadmium trend (680±0.02 µg/kg) in liver as compared to the breast and thigh muscles which was 630±20 µg/kg each. Cadmium values of present study were less than the cadmium values 270±10 µg/kg in liver reported by Akan et al. (2010).

The copper concentration varied significantly in different organs of the broiler in present study. The maximum value observed in liver tissues range from 0.10 ± 0.01 to 1.62 ± 0.01 µg/kg was less than the muscle values 2029 to 3536 µg/kg in liver reported by the Thirulogachzandar et al. (2014). In present study, the concentration of copper in breast and thigh muscles ranged from 0.10±0.01 to 1.81±0.15 µg/kg which is far less than the previous reported values 10 µg/kg to 5150 µg/kg in different locations of southern Nigeria. In gizzard, the value of copper vary from 0.48 µg/kg to 1.59 µg/kg is much less than the value 460 µg/kg to 2550 µg/kg reported by Iwegbue et al. (2008) in different locations of southern Nigeria. Our data regarding copper concentration in different organs of broiler chicken were below the permissible limit 200 µg/kg recommended by the WHO/FDA. A previous study reported significant differences of iron level among various locations ($p<0.05$) in Lahore. The concentration of Fe was below the permissible limit (150mg/kg) except in the sample of liver of Outfall Road (Butt *et al.,* 2016). Another study revealed that lead toxicity caused hepatotoxicity in broiler chicks (Khanam *et al.,* 2016).

CONCLUSION

The study showed presence of wide range of heavy metals concentration (Ni, Cr, Cd and Cu) in all parts including Gizzard, Breast muscle, Thigh muscle and Liver of broiler chicken collected from different localities of Lahore city. The samples analyzed for the determination of Ni, Cr, Cd and Cu clearly indicate that the concentrations of relevant metals were found to be under the safe limits and the chicken safe for the human consumption.

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CONFLICT OF INTEREST

The authors declare that no there is no conflict of interest for this study.

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