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Estimation of Iron in Liver, Gizzard, Breast and Thigh Muscles of Broiler Chicken

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Abstract
The aim of this study was to determine the concentration of iron (Fe) in different tissues (e.g. liver, gizzard, breast and thigh muscles) of broiler chicken collected from various areas including Karim Park, Amin Park, Shahdara, Lohari and Outfall Road. The concentration of Fe was determined by the standard method of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The concentration of Fe in thigh muscle, breast muscle, gizzard and liver selected from different localities of Lahore ranged from 10.63±0.08mg/kg to 17.58±0.93mg/kg, 2.67±0.08mg/kg to 21.99±o.25mg/kg, 10.62±0.01mg/kg to 21.66±0.41mg/kg, 56.97±0.48mg/kg to 160.45±0.57mg/kg respectively. The results showed significant differences among various locations ($p<0.05$). The concentration of Fe was below the permissible limit (150mg/kg) except in the sample of liver of Outfall Road. There is further need to investigate the addition of iron in broiler chicken feed.
Keywords: Broiler chicken, inductively coupled plasma optical emission spectrometry, concentration, Iron.

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INTRODUCTION

Heavy metals have been consumed for thousands of years. Exposure to heavy metals continuously increased day by day. Heavy metals are described as the metals which have density more than 5g/cm^3. Heavy metal is a group of harmful elements that demonstrate the metallic characteristics. Metallic elements are present in all living organisms where they play a vital role in the body. Heavy metals incorporate or store in living tissues and have direct physiological effects (Baykov *et al*., 1996; Mariam *et al.,* 2004). Living organisms require different amount of heavy metals. Some metals (e.g. Lead, Cadmium, Mercury, Iron and Arsenic) may accumulate in the body and show cumulative effect (Cunningham and Saigo, 1997). The toxic effects of metals have been deeply studied at level of metal assemblage in tissues. All metals are poisonous at higher concentration (Chronopoulos *et al.,* 1997).

Heavy metal mass varies from one tissue to another within the animal or differ between one animal and another. Heavy metals transfer via food chain and from there they eventually make their way into the tissues (Tchounwou, and Centeno, 2008).

Poultry meat is a major component of diet and source of protein now a day. Chemical composition of meat relies on the kind and degree of feeding animal. Due to the increase demand of poultry meat by people, their production is greatly influenced. To meet the demands of people, improvements in the food production and modifications have been done in the poultry feeds; these are naturally or locally sourced (Thornton, 2010). Modifications have been reported to be affected by the contents of heavy metals in poultry feeds. Ingestion of these contaminants by animals results in deposition of residues in meat. Due to the grazing on contaminated soil, higher levels of metals have been found in meat (Sabir *et al.,* 2003). Too much iron intake can be harmful to health. The most common iron overload disorder is hereditary hemochromatosis. This leads to the buildup of iron in tissues and organs (Kowdley, 2004). Iron deficiency leads to anemia (Hamid *et al.,* 2016).

The risk of heavy metal contamination is of great concern for both food safety and human health, due to the toxic nature of these at relatively minimum concentration (Demirezen and Uruc, 2006).

The specific objective of this study was to determine Iron concentration in different tissues of broiler chicken of different places to check whether the broiler chicken contains the concentration of Iron within the safe limits or not.

MATERIALS AND METHODS

This research project was conducted under the supervision of Govt. Post Graduate Islamia College for Women Cooper Road Lahore. The experimental work had been performed at Fisheries Research and Training Institute, Manawan, Lahore.

Sample Collection

Different parts (e.g. liver, gizzard, breast muscle, and thigh muscle) of broiler chicken were collected from some selected places of Lahore city including Lohari, Amin Park, Karim Park, Malik Park, and Shahdara. The samples were slaughtered; organs were separated and were packed in polyethylene bags and transported to Laboratory at Fisheries Research and Training Institute, Manawan, Lahore and frozen until further analysis.

Sample preparation

The collected samples were decomposed by wet digestion method for the determination of Iron. A great advantage of wet digestion procedure is that it is sufficient to solubilize all of the trace elements and destroy most of the organic matter, which then do not interfere with the determination.

Sample preparation steps are as follows:

1: First of all, samples were removed from the deep freezer and allowed time for them to defrost before opening the plastic tubes.

2: Each tube was opened and a known quantity of almost 10g was introduced into a clean 100-ml beaker.

3: 10 ml of freshly prepared 1:1 v/v hydrogen peroxide/nitric acid, prepared in a small measuring cylinder as requisite, were added and beakers were covered with a watch glass.

4: The beakers were set aside for an hour until the first reaction settled.

5: Then the beakers were placed on the hot-plate and carefully permitted the temperature to rise about 160°C.

6: Solutions were boiled gently for about 2 hours to reduce the volume between 2-5 ml. To cease any reaction, the samples were not permitted to go dry. If it happened then it must be discarded.

7: The solutions were allowed to cool and the solutions were filtered by using the Whatman filter paper. Later the solutions were transferred to the volumetric flask of 25 ml and diluted to the mark with distilled water (i.e. the test solution will be about 4-10% v/v nitric acid and not more) (FAO, 1983).

Control Sample Preparation

Blank and known amounts (10 ml of freshly prepared 1; 1 v/v hydrogen peroxide/nitric acid) of the working standard solutions taken through this wet ashing procedure to test for methodic and operative errors (FAO, 1983). In these solutions samples were not present.

Standard Solution Preparation and Elemental Analysis

These solutions were prepared according to their permissible limits. The permissible limit of iron was 30-150mg/kg (Demirezen and Uruc, 2006).

Standard curves for iron were prepared from the stock solutions. The stock solution value of iron was 10,000mg/kg. Five different concentrations of standard solutions were made according to the permissible limits include 0 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm but the solution of 0 ppm was blank.

A formula was used for making these concentrations 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 concentrations that have to be prepared and

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

For the optimum emission working range further serial dilutions were prepared up to 50 ml in volumetric flask. An instrument called Inductive Coupled Plasma- Optical Emission Spectrometer (Perkin Elmer) was used for the determination of heavy metal iron. Samples were analyzed under the instrumental operating conditions: RF Power 1.5kW, 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 an integrated computer result of determinations was recorded. In order to determine the reliability of instruments, a blank and known standard were run after every 6 samples.

Statistical analysis

Collected data was presented as mean and standard deviation and were subjected to one way analysis of variance (ANOVA) ($p<0.05$) to assess whether Fe concentration varied significantly between samples of different places. All statistical calculations were performed with SPSS 9.0.

RESULTS

Concentration of iron in liver, gizzard, thigh and breast muscles collected from different localities of Lahore city was determined in present study.

The concentration of Iron in different tissues of broiler chicken collected from Karim Park was highest in liver (86.69±1.48mg/kg), followed by breast muscle

(21.99±0.25mg/kg), thigh muscle (10.63±0.08mg/kg) and the lowest (10.62±0.01m/kg) in Gizzard (Table 1).

The concentration of Iron in different tissues of broiler chicken collected from Amin Park was highest in liver (56.97±0.48mg/kg), followed by Gizzard (16.93±0.62mg/kg), thigh muscle (12.02±0.34mg/kg) and the lowest (9.28±0.05mg/kg) in breast muscle (Table 1).

The concentration of Iron in different tissues of broiler chicken collected from Shahdara was highest in liver (61.24±1.01mg/kg), followed by Gizzard (19.25±0.63mg/kg), thigh muscle (17.58±0.93mg/kg) and the lowest (2.67±0.08mg/kg) in breast muscle (Table 1).

The concentration of Iron in different tissues of broiler chicken collected from Lohari was highest in liver (76.79±2.34mg/kg), followed by Gizzard (21.66±0.41mg/kg), breast muscle (10.42±0.07mg/kg) and the lowest (7.59±0.07mg/kg) in thigh muscle (Table 1).

The concentration of Iron in different tissues of broiler chicken collected from Outfall Road was highest in liver (160.45±0.57mg/kg), followed by Gizzard (17.46±0.41mg/kg), thigh muscle (8.13±1.01mg/kg) and the lowest (6.48±0.13mg/kg) in breast muscle (Table 1).

Table 1. Concentration of Iron in different organs of broiler chicken collected from different localities of Lahore.

Location	Organs	Concentration of Iron (mg/kg)		
		Replicate 1	Replicate 2	Mean±SD
Karim Park	Thigh muscle	10.565	10.685	10.63±0.08
	Breast muscle	22.17	21.81	21.99±0.25
	Gizzard	10.625	10.61	10.62±0.01
	Liver	87.735	85.64	86.69±1.48
Amin Park	Thigh muscle	11.78	12.255	12.02±0.34
	Breast muscle	9.238	9.315	9.28±0.05
	Gizzard	17.37	16.495	16.93±0.62
	Liver	57.305	56.625	56.97±0.48
Shahdara	Thigh muscle	18.23	16.92	17.58±0.93
	Breast muscle	2.616	2.725	2.67±0.08
	Gizzard	19.69	18.8	19.25±0.63
	Liver	61.955	60.52	61.24±1.01
Lohari	Thigh muscle	7.5345	7.637	7.59±0.07
	Breast muscle	10.47	10.37	10.42±0.07
	Gizzard	21.955	21.37	21.66±0.41
	Liver	75.14	78.445	76.79±2.34
Outfall Road	Thigh muscle	7.4105	8.8405	8.13±1.01
	Breast muscle	6.5745	6.387	6.48±0.13
	Gizzard	17.165	17.745	17.46±0.41
	Liver	160.85	160.05	160.45±0.57

Mean concentration of Iron in thigh muscle of broiler chicken collected from different localities of Lahore city was highest at Shahdara followed by Amin Park, Karim Park, Outfall Road and lowest at Lohari (Table 1). The results of one-way ANOVA ($p<0.05$) indicated that there were significant differences in Iron concentration in thigh muscle of broiler chicken collected from various places (Figure 1).

The results showed that breast muscle of broiler chicken collected from Karim Park of Lahore city contained the highest content of Iron as followed by Lohari, Amin Park, Outfall Road and Shahdara respectively (Table 1). The P value ($p<0.05$) indicated that there were significant differences in Iron concentration in breast muscle of broiler chicken collected from various places (Figure 2).

Mean concentration of Iron in gizzard was highest in broiler chicken collected from Lohari followed by Shahdara, Outfall Road, Amin Park and Karim Park respectively (Table 1). Results of one way ANOVA ($p<0.05$) indicated that there were significant differences in Iron concentration in gizzard of broiler chicken collected from various places (Figure 3).

The results showed that liver of broiler chicken collected from Outfall Road of Lahore city contained the highest content of Iron as followed by Karim Park, Lohari, Shahdara and Amin Park respectively (Table 1). Results of one way ANOVA ($p<0.05$) indicated that there were significant differences in Iron concentration in liver of broiler chicken collected from various places (Figure 4).

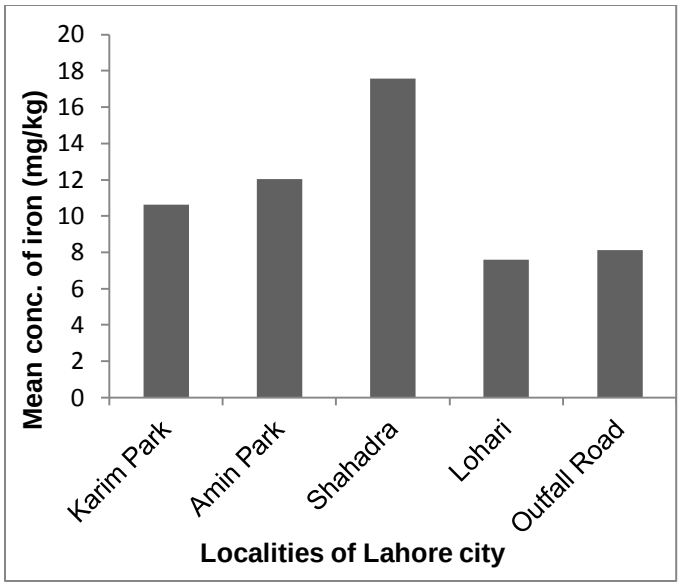

Fig. 1. Mean concentration of Iron in Thigh muscle of broiler chicken (p<0.05) collected from different localities of Lahore city.

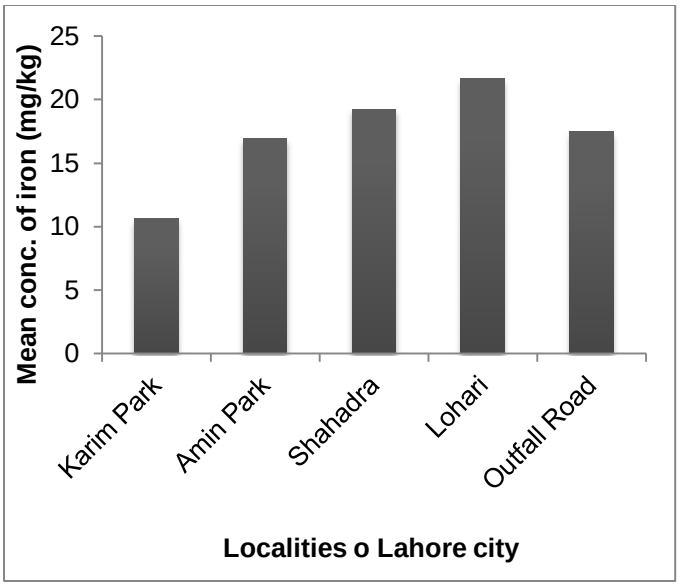

Fig. 3. Mean concentration of Iron in Gizzard of broiler chicken (p<0.05) collected from different localities of Lahore city.

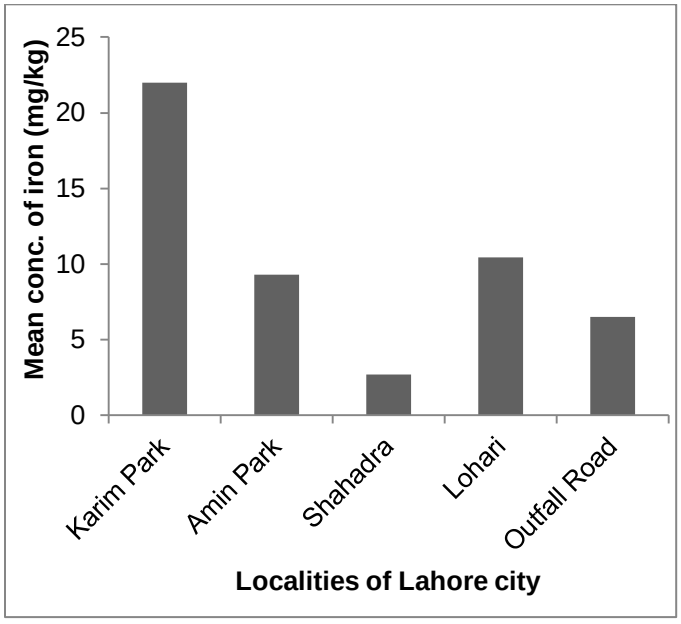

Fig. 2. Mean concentration of Iron in Breast muscle of broiler chicken (p<0.05) collected from different localities of Lahore city.

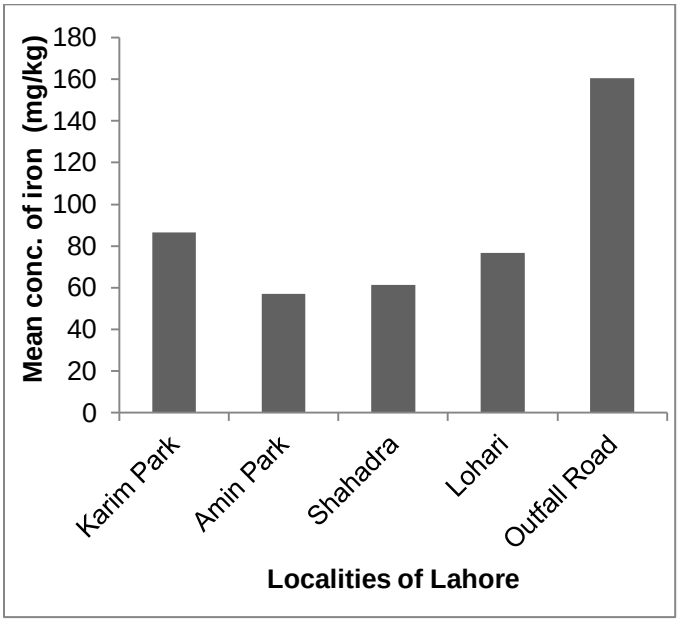

Fig. 4. Mean concentration of Iron in Liver of broiler chicken (p<0.05) collected from different localities of Lahore.

DISCUSSION

In order to improve poultry production, different heavy metals are being added in poultry feed to enhance the growth rate of broiler. Excessive use of these heavy metals may cause accumulation in different body parts of the broiler. Concentration of Iron was determined in gizzard, thigh muscle, liver and breast muscle of broiler chicken at different localities of Lahore city.

The concentration of Iron varied significantly in the thigh muscles of broiler chicken collected from different localities. The highest amount of Iron was found in thigh muscle samples of broiler chicken collected from Shahdara (17.58±0.93mg/kg) which was higher than previously reported value 4.96±1.83mg/kg of Iron in thigh muscle by Petrovic et al. (2010). Thigh muscle samples collected from Lohari contained the lowest level of Iron concentration (7.59±0.07mg/kg) higher than previously reported results. This difference in concentration might be due to variation in feed content.

The concentration of Iron varied significantly in the breast muscles of broiler chicken collected from different localities. Breast muscle sample of broiler chicken collected from Karim Park contained highest amount of Iron (21.99±0.25mg/kg), which was higher than the reported value (4.15±1.83mg/kg) by Petrovic et al. (2010). This high amount of Iron might be due to the addition of this heavy metal in feed in large amount. The lowest amount of Iron in breast muscle was found in broiler chicken collected from Shahdara (2.67±0.08mg/kg) which was lower than the previously reported results by Petrovic et al. (2010).

The concentration of Iron varied significantly in the gizzard of broiler chicken collected from different localities. Gizzard collected from Lohari contained highest concentration (21.66±0.41mg/kg) of iron which was less than previously reported value (33.64±2.04mg/kg) of iron in gizzard by Iwebue et al. (2008). The lowest amount of Iron was found in gizzard sample (10.62±0.01mg/kg) of broiler chicken collected from Karim Park, which was lower than previously reported results.

The concentration of Iron varied significantly in the liver of broiler chicken collected from different localities. Liver of broiler chicken collected from Outfall Road contained the highest content of Iron (160.45±0.57mg/kg), which was greater than the previously reported value (75.36±0.11mg/kg) by EI-Salam et al. (2013), and much greater than previous result (4.65±0.34mg/kg) of Iron in liver by Akan et al. (2010). This might be due to change in nutrition. Liver collected from Amin Park contained the lowest content of Iron (56.97±0.48mg/kg). The reported value (75.36±0.11mg/kg) by EI-Salam et al. (2013) was greater than our results and the results of Akan et al. (2010) were lower than our findings. The reported value (2827±20.0mg/kg) of liver by Rehman et al. (2012) was too much higher than our determined value, which might be due to the addition of this heavy metal in feed in large amount. Al-Zuhairi et al. (2015) determined the levels of Iron ranging from 2.1099 to 7.2834 ppm in the heart, kidney and meat of beef, mutton and chicken. Khanum *et al.* (2016) demonstrated that lead toxicity caused hepatotoxicity in broiler chicks.

Our findings indicated that in all the selected tissues of broiler chicken taken from different localities of Lahore city contained iron concentration below the permissible limit, that is 150mg/kg by ((Demirezen & Uruc, 2006), except the liver sample collected from Outfall Road contained 160.45±0.57mg/kg iron. Other organs were deficient in iron as compared to recommended limits. There is further need to investigate the addition of iron in broiler chicken feed.

CONCLUSION

It can be concluded that the levels of the Iron in liver samples collected from Outfall Road were high and above the statutory safe limit. While liver samples collected from other localities and other tissues collected from all localities of Lahore contained safe amount of Iron. Therefore, the samples containing higher amount of Iron can be considered unsafe for human consumption.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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