Research Article

Concentrations of Heavy Metals and Minerals in Poultry Eggs and Meat Produced in Khyber Pakhtunkhwa, Pakistan

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Abstract | The contamination of the food stuffs by toxic metals is a threat and their exposure for long time has detrimental effects on the health status of animals. Chicken eggs are one of the main sources of protein but if contaminated by toxic heavy metals will cause a harmful effect on human health. The concentration of heavy metals (Pb, Cd, Cr, Fe, Mn and Zn) present in poultry egg and meat were determined in the following three districts; Peshawar, Dir Lower and Malakand of Khyber Pakhtunkhwa by using atomic absorption spectrophotometer. In all the three districts the egg albumen was found to contain significantly higher levels of Pb, Cd and Cr as compared to egg yolk which contains significantly higher levels of essential elements (Fe, Mn and Zn). The mean concentrations of toxic heavy metals; Pb, Cd and Cr in albumen samples collected from Peshawar were 0.13, 0.06 and 0.09 (ppm) respectively. Concentrations of Pb, Cd and Cr in egg albumen of Dir Lower were 0.13, 0.05 and 0.05and in Malakand were 0.12, 0.05 and 0.07 ppm, respectively. The mean concentrations of essential elements Fe, Mn and Zn in egg yolk of Peshawar were 1.27, 0.31 and 2.05 while that of Dir Lower were 1.05, 0.19 and 1.97±0.04 ppm, respectively. The mean levels of Fe, Mn and Zn in egg yolk from Malakand were 1.13, 0.20 and 2.00±0.06 ppm, respectively. The finding of the present study revealed that liver contains significantly higher concentration of lead (Pb), Cadmium (Cd), Chromium (Cr), Iron (Fe), Manganese (Mn) and Zinc (Zn) as compared to thigh and breast muscle. The level of Pb in liver and Cd and Cr in liver, thigh and breast in district I , the level of Pb in liver and level of Cr in liver, thigh and breast in district II and level of Cd and Cd in liver, Cr in liver, thigh and breast in district III were found higher than the permissible limits. The poultry meat produced in Peshawar contribute a slight amount of essential minerals (Fe, Mn and Zn) to the total daily intake while considerable amount of toxic heavy metals (Pb, Cd and Cr) were contributed in the total daily intake of these heavy metals reflecting poor quality of poultry meat. It is concluded from the present study that in all the three districts the daily intake values of Cd, Cr and Zn through egg were found above the permissible values. Findings of the present study are reflecting a threat for public health.

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Introduction

The heavy metals are potentially toxic source and the elements included in this category are Pb, Cd, As and Hg. Those elements which have specific gravity greater than five are called heavy metals. Other mineral elements which are nutritionally important and also fit this category include cobalt, vanadium, iron, copper, manganese, molybdenum, chromium and zinc (Henry and Miles, 2001). Heavy metals resulting from human activities are the sources of pollution and are continuously released into aquatic and terrestrial ecosystems. Heavy metals contamination is a serious threat because of their toxicity, bio-magnification and bioaccumulation in food chain (Demirezen and Uruc, 2006). These contaminants often have direct physiological toxic effects as they are stored or accumulated in tissues, sometimes permanently (Bokori et al., 1996; Mariam et al., 2004). The deficiency of elements leads to impairment of vital biological process but when they are present in excess, they become toxic such as cadmium exposure causes bone and kidney damage. It has also been identified as a potential human carcinogen, causing lung cancer. Pb exposures have developmental and neurobehavioral effects on fetuses, infants and children, and elevate blood pressure in adults.

Most elements enter the body through diet. Poultry meat and egg have always been sources of protein for human and their consumption have increased recently (Magdelaine et al., 2007). In children approximately 40% uptake of lead is from egg. Hui-Fen et al. (1994) found the concentration of Pb 0.59 mg/kg egg in China, while William and David (1973) found Cd 0.07 mg/kg egg and Cu 0.78 mg/kg egg in Australian foods.

During recent years the presence of toxic heavy metals in food of animal's origin has got great importance. Mostly we ingest heavy metals such as Pb, Cd, Cr, Cu, Zn and Co through food in our daily diet, although their levels vary from place to place depending on dietary habits, level of environmental pollution and recycling of poultry food.

The above lines indicate the sensitivity of hazard being posed to general public health. This problem requires immediate attention of the health regulatory authorities and the researchers as well. There is a serious need of local database or risk assessment studies in local animals and foodstuffs to evaluate the potential risk or threat to humans from heavy metals because the Asian countries have different environmental and topographical conditions under which a large number livestock and poultry are growing. The information regarding diseases, health and management of poultry are usually acquired from the literature of western countries where the nutritional and the environmental conditions are different from the Asian countries. Therefore the present study was designed to evaluate the levels of selected heavy metals (Pb, Cd, Cr, Fe, Mn and Zn) in poultry meat and egg in Khyber Pakhtunkhwa to safeguard the local public health. This study will be useful in determining the potential risks from the toxic effects of heavy metals and to make recommendations for future implementations by the local health regulatory authorities.

Materials and Methods

Collection of Samples

One hundred and fifty (150) samples of poultry/broiler meat & eggs were collected from sales outlets situated in widely spread localities of Khyber Pakhtunkhwa (KP). The KP was divided into three regions i.e. Peshawar (District I), Dir Lower (District II) and Malakand (District III). From these total 150 samples, 90 were from broiler meat (30 livers, 30 breast, 30 thigh muscles) and 60 of eggs (30 albumen, 30 yolks). From 90 meat samples, 30 samples (10 Liver, 10 Thigh, 10 Breast) and from 60 egg samples, 20 samples (10 albumen and 10 yolks) were taken from each district in four weeks intervals. The egg samples were split into egg yolk (Y) and albumen (A).

The samples were collected in polyethylene bags and stored in refrigerator and were analyzed as soon as possible. The samples were transported to the laboratories of Centre of Animal Nutrition, Livestock Research & Development Khyber Pkhtunkhwa Peshawar and Soil and Environmental Sciences Department of the University of Agriculture, Peshawar Khyber Pakhtunkhwa, for analysis.

Sample Preparation

The collected poultry samples were subjected we digestion/acid digestion with a mixture of nitric acid and perchloric acid by the method as reported by Richards (1968) for the determination of heavy metals. Each egg was first broken into egg yolk and albumen and each part was then examined separately. One gram of each sample (liver, breast, thigh, egg white and egg yolk) was taken into 100 ml digestion flask and then 10 ml of concentrated nitric acid (65%) was added.

The flask was heated for 20 minutes. The sample was cooled at room temperature then 5 ml perchloric acid was added. The sample heated vigorously until a clear solution was obtained and the sample volume reduced to 2-3 ml. The content of the flask were filtered into a 50 ml volumetric flask and was made up to the mark with distilled water (Akan et al., 2010).

Elemental Analysis of the Samples

The determination of heavy metals Pb, Cd, Cr, Fe, Mn and Zn in liver, thigh, breast and egg of chicken samples were made directly on each of the final solutions using Perkin-Elmer Analyst 300 Atomic Absorption Spectroscopy (AAS).

Statistical Analysis

The data was analyzed by using completely randomized design (CRD) using SAS. Comparisons among organs were made by using LSD.

Results and Discussion

Heavy metals can easily enter the food chain (Llobet et al., 2003) and have been reported to have detrimental effects on human body (Hooda et al., 1997). Therefore, attention of the researchers has been focused on its bio-hazardous potential. Once heavy metals are absorbed, they are stored in the body even throughout the life (Bernard, 2008). Some heavy metals are so toxic that a low concentration can adversely effect on a number of metabolic processes in the body (Bernard, 2004; Nordberg et al., 2007).

Heavy Metals in Poultry Meat

The finding of the present study revealed that liver contains significantly higher concentration of lead (Pb) as compared to thigh and breast muscle. This is in agreement with most reports which show that liver accumulates lead more than other tissues (Miranda et al., 2005; Korenekova et al., 2002). The inter district differences in the residual concentration of Pb in liver, thigh and breast indicate that liver, thigh and breast in district I contains higher concentration (0.28±0.01, 0.24 ± 0.01 and 0.23 ±0.01 ppm respectively) followed by district II (0.25±0.01, 0.23±0.01 and 0.21±0.01 ppm respectively) and district III (0.25 ± 0.01, 0.22 ± 0.01 and 0.21±0.01 ppm respectively) (Table 1). Excess Pb can lead to reduce cognitive development and intellectual performance in children and high blood pressure and cardiovascular disease incidence in adults (Commission of the European Communities, 2001). Level of Pb in liver is above and level in thigh and breast is within the range of FAO/WHO permissible level of 0.2 ppm.

The result of present study reveal that the levels of Cd in the district I, II and III were significantly higher in the liver (0.68±0.02, 0.54 ± 0.01 and 0.55 ± 0.01 ppm respectively) than thigh (0.63±0.02, 0.51 ± 0.01 and 0.51 ± 0.01 ppm respectively) and breast (0.63±0.02, 0.51 ± 0.01 and 0.51 ± 0.01 ppm respectively) (Table 1). Statistically, no significant difference was observed in thigh and breast muscles. The finding of our research study was in agreement with the finding of Doganoc (1996) and Mariam et al. (2004) who also found higher concentration in liver than muscles. Similarly, Skalicka et al. (2002) reported higher concentration in liver (0.0985 ppm) and the lower concentration were found in breast muscles (0.0187 ppm) and thigh muscles (0.0210 ppm) while dealing with poultry birds from polluted areas in Eastern Slovakia. It is also evident from the results of this study, that the concentration of cadmium in the liver, thigh and breast samples collected from district I and liver of district II and III are higher while the concentration in thigh and breast muscles of II and III are within the range of 0.5 ppm permissible limit set by (FAO/WHO, 2000).

Table 1: *Concentration (mean± SE; ppm) of heavy metals in liver, thigh and breast muscle of chicken*

Part	Pb	Cd	Cr	Fe	Mn	Zn
	District I					
Liver	0.28±0.01[a]	0.68±0.02[a]	0.11±0.01[a]	54.0±7.02[a]	0.45±0.02[a]	110.2±8.11[a]
Thigh	0.24±0.01[b]	0.63±0.02[b]	0.06±0.01[b]	43.3±4.72[a]	0.43±0.01[a]	107.4±7.60[a]
Breast	0.23±0.01[b]	0.63±0.02[b]	0.06±0.00[b]	41.39±5.10[a]	0.43±0.01[a]	107.82±7.66[a]
P-value	0.00	0.03	0.00	0.26	0.17	0.96
	District II					
Liver	0.25±0.01[a]	0.54±0.01[a]	0.08±0.01[a]	54.93±6.86[a]	0.47±0.02[a]	109.76±8.63[a]
Thigh	0.23±0.01[b]	0.51±0.01[b]	0.06±0.00[ab]	43.91±4.51[a]	0.44±0.01[a]	106.6±7.37[a]
Breast	0.21±0.00[b]	0.51±0.01[b]	0.06±0.00[b]	43.12±4.84[a]	0.43±0.01[a]	107.4±7.49[a]
P-value	0.00	0.89	0.07	0.25	0.31	0.96
	District III					
Liver	0.25±0.01[a]	0.55±0.01[a]	0.10±0.01[a]	53.53±6.76[a]	0.46±0.02[a]	110.33±7.24[a]
Thigh	0.22±0.01[b]	0.51±0.01[b]	0.06±0.00[b]	43.01±4.48[a]	0.43±0.01[a]	106.78±7.48[a]
Breast	0.21±0.00[b]	0.51±0.01[b]	0.06±0.00[b]	42.98±4.42[a]	0.43±0.01[a]	107.95±7.73[a]
p-value	0.00	0.00	0.00	0.29	0.34	0.94

Chromium (Cr) is an essential element which helps the body to use sugar, protein and fat at the same time it possess carcinogenic properties as well (Institute of Medicine, 2002). Excess amount of Cr may cause adverse health effects (ATSDR, 2004). According to our findings, highest concentration of Cr was found in the liver from district I (0.11±0.01 ppm) followed by III (0.10 ± 0.01 ppm) and the lowest level was observed in the liver from II (0.08 ± 0.01 ppm). The values of Cr in thigh and breast from all the three districts were same (0.06 ± 0.01 ppm) (Table 1). The findings of our study are comparable to the work done by Akan et al. (2010) who found higher concentration in liver (0.65 ppm) than muscle (0.29 ppm). The results of our study are different to that of Mahmud et al. (2011) who found highest concentration of Cr in thigh (1.26 ppm) followed by breast (0.734 ppm) and liver (0.415 ppm). The concentration of Cr in liver is higher while that of thigh and breast is slightly above the WHO permissible level of 0.05 ppm.

In district I, II and III, liver of chicken contains the highest concentration of Fe (54.0 ±7.02, 54.93±6.86 and 53.53±6.76 ppm respectively) followed by thigh (43.3 ± 4.72, 43.91±4.51 and 43.01±4.48 ppm respectively) while breast contains the lowest (41.39±5.10, 43.12±4.84 and 42.98±4.42 ppm respectively) (Table 1). The findings of our study are comparable to the findings of Akan et al. (2010) who found that liver of chicken has the high levels of Fe (4.65±0.30 ppm) than chicken meat (1.92 ppm).

The finding of the present study reveals that the concentrations of Mn in liver, thigh and breast region do not differ significantly in all three districts (I, II and III). However, numerically the concentration of Mn in liver was higher than thigh and breast. The findings of our result are comparable to the results of Oforka et al. (2012) who also reported higher level of Mn in liver than muscle. Concentration of Mn ranged from 1.1290-0.0922 ppm, 0.6963-0.0380 ppm and 0.8027-0.0353 ppm for chicken liver, breast and thigh muscle respectively. Likely, Rahman et al. (2012) also found very high concentration of Mn in liver (340±6.48 ppm) than muscle (102±6.11 ppm). They also indicated that manganese and iron are accumulated in body tissues of broiler chicken but their accumulation in muscles and skin was depending upon their concentrations in broiler feed. The concentrations of Mn in chicken liver and muscles in our study are below the WHO reference standard of 0.5 ppm.

Zinc (Zn) being an essential element is involved in protein synthesis and also a part of many metalloenzymes. The requirement of Zn for all domestic species is generally 35 to 45 ppm (Underwood, 1977). Homeostatic control of the body is generally adequate to eliminate excess amount of Zn from the body (Miller, 1969). Most animals can tolerate large amount of metal. However, Zn becomes toxic when given in excess quantity (Doyle and Spaulding, 1978). Animals' can also become Zn deficient (Ott et al., 1965). The Zn concentration was observed in the chicken liver, thigh and breast collected from three different districts of Khyber Pakhtunkhwa and it was found that the liver in district I, II and III showed the highest concentration (110.2±8.11, 109.76±8.63 and 110.33±7.24 ppm) followed by breast (107.82±7.66, 107.4±7.49 and 107.95±7.73) and thigh (107.4±7.60, 106.6±7.37 and 106.78±7.48 ppm) respectively (Table 1). Mariam et al. (2004) detected high level of Zn in the liver of poultry (54.53 ppm) than muscle (28.52 ppm). Another scientist Anke et al. (1970) also evaluated that the Zn concentration in chicken liver was 158 ppm and in muscle 125 ppm. The result of our study was also different to the work done by Akan et al. (2010) who found concentration of Zn to be (3.11 ppm) in the liver and (1.1 ppm) in the muscle of chicken.

The result showed that the Zn concentration in the liver, thigh and breast in all districts was lower than the permissible level of 150 ppm (ANZFA, 2001) and were above the permissible limit of 50 ppm by Codex standard. The level of Zn may be due to Zn deficient soils; as a result the water available to poultry is deficient of Zn. This might be one of the reasons for low tissue content of Zn. Rahman et al. (2012) showed that trend of heavy metal accumulation was, mostly, higher in metabolic organs.

From the results of this study it is obvious that in district I the concentration of most heavy metal residues Pb, Cd and Cr have been found higher than district II and III particularly in the liver of broiler chicken as liver is the main metabolic organ. This might be due to continuous pouring of heavy metals rich industrial waste effluents to the sewerage drains from many industries present in this area and ultimately the use of this contaminated water for drinking purpose (Mariam et al., 2004). This subsequently led to rapid intake of heavy metals from the underground water by poultry. In this respects, a local study has also been reported showing water contamination in four industrial estates of Peshawar, Gujranwala, Haripur and at Warsak Canal in Pakistan. This water has been used for

Table 2: *Concentration (means ± SE; ppm) of heavy metals in albumen (A) and yolk (Y) of chicken egg*

Egg Parts	Pb	Cd	Cr	Fe	Mn	Zn
	District I					
A	0.13±0.00[a]	0.06±0.00[a]	0.09±0.01[a]	1.27±0.11[b]	0.31±0.06[b]	2.05±0.04[b]
Y	0.09±0.00[b]	0.04±0.00[b]	0.04±0.01[b]	3.36±0.11[a]	1.37±0.13[a]	40.42±0.58[a]
p-value	0.00	0.00	0.00	0.00	0.00	0.00
	District II					
A	0.13±0.00[a]	0.05±0.01[a]	0.05±0.01[a]	1.05±0.04[b]	0.19±0.02[b]	1.97±0.04[b]
Y	0.07±0.00[b]	0.03±0.00[b]	0.03±0.00[b]	3.19±0.13[a]	1.33±0.08[a]	40.34±0.87[a]
p-value	0.00	0.00	0.01	0.00	0.00	0.00
	District III					
A	0.12±0.00[a]	0.05±0.00[a]	0.07±0.01[a]	1.13±0.08[b]	0.20±0.01[b]	2.00±0.06[b]
Y	0.06±0.01[b]	0.03±0.01[b]	0.03±0.01[b]	3.29±0.09[a]	1.40±0.06[a]	39.85±0.47[a]
p-value	0.00	0.00	0.00	0.00	0.00	0.00

drinking as well as for irrigation resulting in the entry of heavy metals to the food chain (Rahman et al., 2012). The concentration of heavy metals in drinking water of Peshawar and Hayatabad industrial estate were above the maximum allowable concentration fixed by WHO and US-EPA (Tariq et al., 2006).

Heavy Metals in Poultry Egg

Results of the present study reveal that egg yolk contained significantly higher concentrations of Fe, Mn and Zn whereas significantly higher concentration of Cd, Pb and Cr were observed in egg albumen (Table 2). The findings of the present study were in agreement with the findings of (Flores and Martins, 1996). It might be due to the fact that migration of Pb and Cd across the membrane form albumen to yolk has been inhibited due to larger ionic radii of Pb^{+2} and Cd^{+2}.

The daily intake of heavy metals through commercial chicken eggs was calculated on the basis of consumption of two eggs per person per day and the results are reported in Table 3 along with the recommended and tolerance levels of WHO. The average concentration of each metal in the whole egg (albumen and yolk) was used for such calculations. The estimated daily intake of Zn in district I, II and III (84.94, 84.62 and 83.7 mg respectively), Cd (0.2, 0.16 and 0.16 mg respectively) and Cr (0.26, 0.16 and 0.2 mg respectively) through commercial chicken eggs was found higher than their corresponding minimum recommended values (77, 0.06-0.07 and 0.05 mg respectively) while the estimated daily intake of Fe, Mn and Pb were within range of WHO recommended value. Zmudzki and Szkoda (1996) also found higher Cd in egg. The recent review by Kan and Meijer (2007) gives a general insight on transfer of toxic metals from feed to eggs. According to Zmudzki and Szkoda (1996), it has been noted that farm's egg samples have higher amount of cadmium than permissible limit (0.05 ppm).

Table 3: *Estimated daily intakes of heavy metals through farm eggs mg/person) (adopted from Khalid et al. 2007 and Expert Group on Vitamins and Minerals (EVM; 2003)*

Metal	Estimated daily intake			Requirement / Tolerance level
	District I	District II	District III	
Pb	0.44	0.4	0.36	0.43
Cd	0.2	0.16	0.16	0.06 - 0.07
Cr	0.26	0.16	0.2	0.05
Fe	9.26	8.48	8.84	44.00
Mn	3.36	3.04	3.2	0.5 - 5.0
Zn	84.94	84.62	83.7	77.00

Conclusion

Liver contains significantly higher level of toxic metals (Pb, Cd and Cr) whereas no significant difference was observed between thigh and breast muscles. The level of Pb in liver and Cd and Cr in liver, thigh and breast in district I, the level of Pb in liver and level of Cr in liver, thigh and breast in district II and level of Cd and Cd in liver, Cr in liver, thigh and breast in district III were found higher than the permissible limits. The poultry meat produced in Peshawar contribute a slight amount of essential minerals (Fe, Mn and Zn) to the total daily intake while considerable amount of toxic heavy metals (Pb, Cd and Cr) were contributed in the total daily intake of these heavy metals reflecting poor quality of poultry meat. Significantly higher levels of Fe, Mn and Zn were found in egg yolk whereas the levels of Pb, Cr and Cd were higher in egg albumen. The estimated daily intake values of Cd, Cr and Zn through egg were found above the recommended permissible levels while the estimated daily intake of Fe, Mn and Pb were within range of WHO recommended value. This indicates that the chicken eggs are not adequate source of essential element Fe and Mn.

Recommendations

To provide quality food products for human consumption, appropriate legislation are required for monitoring of quality of poultry meat and meat products. Necessary measures should be taken detoxicate the waste effluents from industries which are to be used as drinking water for poultry birds. All those poultry products which exceed the maximum permissible limits may be declared as unhygienic for human consumption. Further studies are needed to find out the exact sources of minerals and heavy metals in poultry meat and eggs. Both the public and private sectors must cooperate in tackling the problem.

Authors' Contribution

Zafar Khan Main researcher awarded M. Sc (Hons) degree on the basis of successful completion of this research project. Asad Sultan Major supervisor of this research project. Rajwali Khan Major member of this research project and also corresponding author of the paper. Sarzamin Khan Member of supervisory committee and also checked draft of the manuscript. Imran Ullah helped in field work. Kamran Farid helped in laboratory and analytical work.

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