



#### **Research Article**

# **Evaluation of Heavy Metals Concentration in Poultry Feed and Poultry Products**

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## Abstract

The study was conducted to determine the absorption of essential and non-essential trace minerals from poultry feed to poultry products. Poultry feed, liver, muscles, and egg samples were collected from six poultry farms in Rawalpindi and Islamabad. Mercury, Lead, Cadmium, Chromium, and Iron were analyzed in the samples using Inductively Coupled Plasma Optical Emission Spectrophotometer. Iron, Lead, and Chromium exceeded the permissible limits set by World Health Organization and National Research Council in Poultry feed. Lead was high in the liver, breast muscles, thigh muscles, egg albumen, and egg yolk. Chromium was found in feed, egg yolk, egg albumen, and two (02) of the liver and breast muscle samples. Mercury was not detected in any of the samples. The liver contains significantly higher concentrations of detected heavy metals as compared to thigh and breast muscles and egg yolk contained significantly high concentrations of Iron, Cadmium, and Lead as compared to egg albumen. Standards requirements for feed manufacturers and poultry farmers should be maintained to monitor and mitigate routes of entry of contaminants in the food chain.

#### More Information

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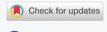
Submitted: May 02, 2023 Approved: July 10, 2023 Published: July 11, 2023

How to cite this article: Iqbal H, Shafique MA, Khan MJ. Evaluation of Heavy Metals Concentration in Poultry Feed and Poultry Products. J Sports Med Ther. 2023; 8: 030-035.

DOI: 10.29328/journal.jsmt.1001069

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Keywords: Trace minerals; Bioaccumulation; Lead; Poultry; Liver; Food chain; Rawalpindi; Islamabad



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#### Introduction

Poultry is a tempting and inexpensive source of nutrients. With 38% of global production, poultry meat is widely produced meat after beef meat. It accounts for about 30% of meat production globally [1]. Meat, liver, eggs, and offal are generally used poultry products acting as primary sources of vitamins, minerals, protein, and energy [2]. The dynamically growing poultry industry in South Asian countries has also increased the demand for feed and raw materials [3].

Micro-minerals are an important need of the human body in amounts less than 100 mg/day. They are subdivided into three categories i.e., Essential trace elements (Iron (Fe), Copper (Cu), Iodine (I), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Cobalt (Co), Fluoride (F), Selenium (Se) And Chromium (Cr)); possible essential trace elements (Nickel (Ni), Vanadium (V), Cadmium (Cd) and Barium (Ba)) and Nonessential trace elements (Aluminium (Al). Lead (Pb), Mercury (Hg), Boron (B), Silver (Ag), bismuth (Bi), etc.) [4]. Some trace minerals are also referred to as Heavy Metals on the basis of their particular density greater than 5 g/cm<sup>3</sup> [1,5]. These metals have the potential for bioaccumulation and bio-magnification [6,7]. They are stored more rapidly than excreted [8] and are constantly found in the environment [9].

The poultry industry in Pakistan contributes to the agriculture sector, total meat production, and overall GDP in the proportion of 5.76%, 26.8% and 1.26%, respectively. Pakistan has produced 834,000 tonnes of poultry against the target of 758,000 tonnes in the year 2011–2012. Currently, more than one hundred forty (140) feed generators are running with the potential of around 4 million tons of compound feed. Current funding in fowl enterprise is around 200.00 billion rupees [10]. With a capacity ranging from 5,000 to 500,000 broiler chickens, more than 15000 fowl farms are located within the rural areas throughout the country from Karachi to Peshawar. Forty-forty-five (45)% of the total meat intake is drawn from fowl products. Pakistan's poultry industries produce 17,500 million eggs and 1,322 million kilograms of bird meat yearly. Consumption of meat is just 6.61 kilograms and 88 eggs per capita each year in the country. Contrary to this, intake in evolved international locations is 40 kilograms of meat and over three hundred eggs per capita every 12 months.



Heavy metal contamination of poultry feed with Cd, As, Cr, Cu, Pb, Mn, Ni, Zn, Fe, and Hg was reported in the Kasur district of Punjab with Mercury present at an alarming level [11]. A similar study done in Lahore, Punjab showed high levels of Arsenic, Lead, and Mercury in the lean and organ meat of beef, mutton, and poultry [12]. A study done in Jamshoro, Sindh showed the presence of Zn, Cd, Pb, and Cu in commercial rooster feeds with Cadmium and Lead in excess [10]. In another work in Hyderabad, Sindh relatively higher concentrations of Lead (Pb) were observed in industrial feed samples [13]. Poultry eggs and meat in three districts of Khyber Pakhtunkhwa were found polluted with heavy metals Pb, Cd, Cr, Fe, Mn, and Zn. Egg albumen contained considerably higher levels of Lead (Pb), Cadmium (Cd), and Chromium (Cr) as compared to egg yolk which contained significantly higher levels of essential elements Iron (Fe), Manganese (Mn) and Zinc (Zn). These results are threatening to the common population [14]. This is an indication of heavy metal contamination in the environment

The safety of poultry feed is vital for poultry health and productivity and health of consumers. Poultry chicken and poultry products are important parts of the human diet. Accumulation of heavy metals in regularly consumed food items is of grave concern. The slow accumulation of different heavy metals in various body compartments may lead to organ failure. Kidney, lungs, muscle, and neurological complications are major effects of heavy metals exposure. Exposure to harmful substances can be minimized by eliminating sources of their entry into biological systems.

As chickens are nourished with feed which, if polluted with heavy metal, might be poisonous to the health of chickens therefore, the study aimed to monitor poultry feed as a source of escalating concentrations of heavy metals in poultry products in the poultry farms of twin cities of Rawalpindi and Islamabad.

### Materials and methods

#### Sample collection

Six samples of layer breed chicken were collected from each of the six different poultry farmhouses of Rawalpindi and Islamabad along with their solid and liquid feed and eggs. All chickens were slaughtered with a sterilized knife and collected in sterilized plastic bags and containers. Liver, breast muscle, and thigh muscles were separated from each chicken and immediately frozen at ambient temperature till analysis.

#### Sample size

From a total of six (6) farms, 6 Solid feeds, 6 liquid feeds (water), 33 liver, 33 breast muscles, 33 thigh muscles, and 25 egg samples were collected. Composite sampling was done for livers, breast muscles, thigh muscles, and eggs from the same poultry farms. Egg Albumen and egg yolk was analyzed separately. The total number of samples analyzed for the presence of five heavy metals under consideration was 40.

#### Sample preparation

Sample preparation of feed samples: Solid Feed Samples were placed in separate glass Petri dishes and dried at 105 °C in a hot air oven (Memmert) until constant weight was obtained [2]. Weight was determined using Electronic Balance (LS-EXD). Water Samples were filtered and kept in sterilized containers.

Sample preparation of body tissues: Each liver and muscle sample was cleaned, weighed, and washed with deionized water. It was then placed in clean and dry glass Petri dishes and cut into small pieces. Next, they were dried at 105 °C in a hot air oven (Memmert) until constant weight was obtained [2]. Dried body tissues were then ground to a powder form using a grinder machine [15] and passed through the sieve of 0.25 mm mesh size [16]. Composite sampling was done for the same tissue from individual farms e.g., all sieved liver from farm A were combined and then homogenized using a homogenizer stirrer (Daihan Scientific HS-30E). Thus the total number of analyzed body tissue samples was 18.

**Sample preparation of egg samples:** Edible parts of egg, albumen, and yolk, were separated by plastic bottle method. Each part was placed in a separately labeled glass petri dish [9] and dried at 105 °C in a hot air oven (Memmert) until constant weight was obtained [2].

#### Sample digestion

Analytical-grade chemicals were used in sample digestion. Each sample, prepared on a dry basis, was taken in a conical flask. 15 ml of concentrated Nitric acid and Perchloric acid in 4:1 was added. Digestion was carried out using a condenser and stirring was done at 80 °C for 2 hours until the solution turned colourless. The digested sample was then allowed to cool and filtered using filter paper. Double distilled water was used to rinse the conical flask and filtrate was transferred into a 25 ml volumetric flask. Volume was made with distilled water. Clear filtrate of each sample was refrigerated to avoid evaporation until analysis [17].

### Sample analysis

Analysis of all digested samples was done using Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) (iCAP 6500 Thermo Scientific, UK) [17].

#### Results

Heavy Metals found in the samples were compared with different tolerable limits proposed by WHO, NRC, EU, and research work.

#### Heavy metals in feed

No heavy metals were found in liquid feed (water) samples. Samples were not kept for a long period of time before analysis; therefore the possibility of absorption from watering can is minimal. Mercury and Cadmium were not found in any



of the solid feed samples. However, Chromium, Iron, and Lead were present in the poultry feeds. Average concentrations of Chromium, Iron, and Lead were 2.36 ppm, 20.31 ppm and 2.605 ppm, respectively.

#### Heavy metals in liver and body tissues

In the liver, Mercury was not detected but Cadmium, Chromium, Iron, and Lead were found in average concentrations of 0.1666 ppm, 0.6083 ppm, 451.9783 ppm, and 2.9083 ppm, respectively.

In muscle tissues, Mercury and Cadmium were not detected but Chromium, Iron, and Lead were present. In breast muscles, average concentrations of Chromium, Iron, and Lead were 13.2512 ppm, 493.9212 ppm, and 12.7412 ppm, respectively whereas in thigh muscles, average concentrations of Chromium, Iron, and Lead were found to be 0.24 ppm, 33.3087 ppm, and 1.655 ppm, respectively.

#### Heavy metals in eggs

Mercury and Cadmium were not detected in egg samples. In egg albumen, Chromium, Iron, and Lead showed average concentrations of 0.4775 ppm, 7.04 ppm, and 2.322 ppm, respectively. Concentrations of Chromium, Iron, and Lead in egg yolk were 1.57 ppm, 62.416 ppm, and 6.634 ppm, respectively.

Results of heavy metals in all the samples, except liquid feed, in comparison with WHO standards are shown in Table  $1\,.$ 

The standard deviation between 6 farmhouses for each of Chromium, Lead, and Iron is shown in Tables 2-4, respectively.

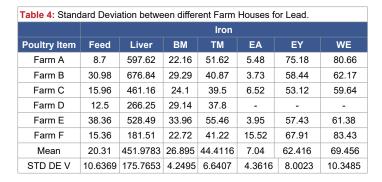
Graphical representation of results with permissible levels of Chromium, Iron, Lead, and Cadmium is shown in Figures 1-4, respectively.

	Sample Type		P	Permissible					
5. #		Sample ID	Cadmium (Cd)	Chromium (Cr)	Iron (Fe)	Lead (Pb)	Mercury (Hg)		(ppm) (WHO
1.		AF	ND	0.65	8.70	2.32	ND	Cd	-
2.	Solid Feed	BF	ND	0.61	30.98	1.22	ND	Cr	0.1
3.		CF	ND	0.84	15.96	1.08	ND	Fe	-
1.		DF	ND	0.61	12.50	0.93	ND	Pb	0.05
5.		EF	ND	10.20	38.36	8.87	ND	Hg	-
S.		FF	ND	1.27	15.36	1.21	ND		
7.	Poultry Liver	AL	0.39	0.46	597.62	1.79	ND	Cd	0.05
B		BL	0.19	0.30	676.84	0.54	ND	Cr	0.5
).		CL	0.09	0.41	461.16	1.65	ND	Fe	-
0.		DL	0.12	1.10	266.25	4.11	ND	Pb	0.1
1.		EL	0.11	0.95	528.49	8.42	ND	Hg	-
2.		FL	0.10	0.43	181.51	0.94	ND		
3.		AB	ND	0.32	22.16	0.89	ND	Cd	-
4.		BB	ND	0.43	29.29	3.04	ND	Cr	1.0
5.		СВ	ND	0.64	24.10	0.41	ND	Fe	-
ŝ.	Breast Muscle	DB	ND	1.28	29.14	2.96	ND	Pb	0.1
7.		EB	ND	0.32	33.96	1.68	ND	Hg	-
3.		FB	ND	1.02	22.72	6.95	ND		
9.		AT	ND	0.66	51.62	ND	ND	Cd	-
).		ВТ	ND	0.35	40.87	0.49	ND	Cr	-
1.		СТ	ND	0.20	39.50	0.88	ND	Fe	-
2.	Thigh Muscle	DT	ND	0.25	37.80	2.58	ND	Pb	-
3.		ET	ND	0.25	55.46	5.98	ND	Hg	-
4.		FT	ND	0.21	41.22	ND	ND		
5.		AA	ND	ND	5.48	0.81	ND	Cd	-
ŝ.	Egg Albumen	BA	ND	0.25	3.73	0.46	ND	Cr	-
7.		CA	ND	0.94	6.52	1.70	ND	Fe	-
3.		EA	ND	0.26	3.95	1.24	ND	Pb	-
9.		FA	ND	0.46	15.52	7.40	ND	Hg	-
0.		AY	ND	0.96	75.18	7.49	ND	Cd	-
1.		BY	ND	0.57	58.44	8.21	ND	Cr	-
2.	Egg Yolk	CY	ND	0.74	53.12	4.64	ND	Fe	-
3.	33	EY	ND	0.95	57.43	5.22	ND	Pb	-
1.		FY	ND	4.63	67.91	7.61	ND	Hg	-
		1							Ref: (14
5.	Whole Egg	AE	ND	0.96	80.66	8.3	ND	Cd	0.06-0.07
6		BE	ND	0.82	62.17	8.67	ND	Cr	0.05
7		CE	ND	1.68	59.64	6.34	ND	Fe	44.0
8		EE	ND	1.21	61.38	6.46	ND	Pb	0.43
19		FE	ND	5.09	83.43	15.01	ND	Hg	-



Table 2: Standard Deviation between different Farm Houses for Chromium. Chromium Poultry Item WE Feed Liver вм ТМ ΕY 0.32 0.66 0.96 Farm A 0.65 0.46 0.96 Farm B 0.61 0.3 0.43 0.35 0.25 0.57 0.82 Farm C 0.84 0.41 0.64 0.2 0.94 0.74 1.68 Farm D 0.61 1.1 1.28 0.25 Farm E 10.2 0.95 0.32 0.25 0.26 0.95 1.21 Farm F 1.27 0.43 1.02 0.21 0.46 4.63 5.09 Mean 2.3633 0.6083 0.6683 0.32 0.382 1.57 1.952 STD DE V 3.8474 0.3306 0.3995 0.1748 0.352 1.7182 1.7844

Table 3: Standard Deviation between different Farm Houses For Lead.										
Lead										
Poultry Item	Feed	Liver	ВМ	TM	EA	EY	WE			
Farm A	2.32	1.79	0.89	0	0.81	7.49	8.3			
Farm B	1.22	0.54	3.04	0.49	0.46	8.21	8.67			
Farm C	1.08	1.65	0.41	0.88	1.7	4.64	6.34			
Farm D	0.93	4.11	2.96	2.58	-	-	-			
Farm E	8.87	8.42	1.68	5.98	1 .24	5.22	6.46			
Farm F	1.21	0.94	6.95	0	7.4	7.61	15.01			
Mean	2.605	2.9083	2.655	1.655	2.322	6.634	8.956			
STD DE V	3.1089	2.9711	2.3577	2.3236	2.8764	1.5925	3.5438			



#### Discussion

Although Mercury was not detected in any of the sample types under study, it is important to highlight its presence in the environment and the risk of exposure.

The prevalence of Lead, a non-essential element, was observed in all of the samples from solid feed to egg yolk. Contamination of the samples with Lead beyond allowed limits is alarming. All chemicals and HMs reach first to the Liver for detoxification to less toxic metabolites and excretion therefore, the concentration of Lead is high in the Liver. But Lead presence in feed, as well as liver, muscles, and eggs, marks its bioaccumulation. Consumption of Lead accumulated meat or eggs may lead to cancer, blood, and nervous disorders and is specifically detrimental for children.

Chromium presence in two of the Liver and breast muscles indicates different patterns of absorption which may be due to mixing of flocks from different sources and at different times. High levels of Chromium in egg albumen and egg white are detrimental to consumers. Chromium consumption at such high levels leads to liver, kidneys, neural tissues, and circulatory system disorders.

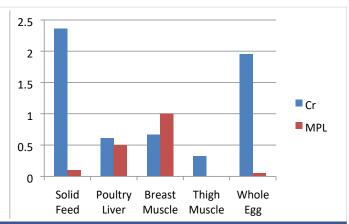


Figure 1: Graphical comparison between chromium levels and permissible level.

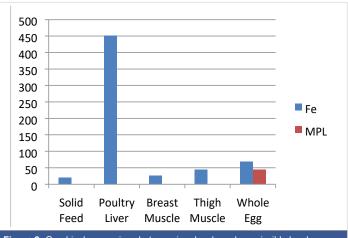


Figure 2: Graphical comparison between iron levels and permissible level.

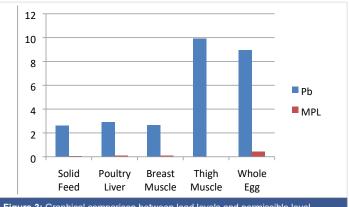


Figure 3: Graphical comparison between lead levels and permissible level.

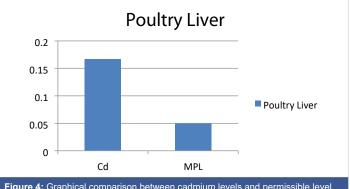


Figure 4: Graphical comparison between cadmium levels and permissible level.



Table 5: Comparison of the Study with Relevant Previous Studies.

S#	Sample Type	Reported Studies	Samples under study	Reported Studies	Samples under study	Reported Studies	Samples under study	Reported Studies	Samples under study
		Cadmium (ppm)		Chromium (ppm)		Lead (ppm)		Iron (ppm)	
1	Solid Feed	0.44-33.6		1.93	2.36	7.9-32. 6	2.605	91.86	20.31
2	Breast muscle	0.57		0.06	0.66	0.21	2.655	42	26.89
3	Thigh Muscle	0.6		0.06	0.32	0.23	9.93	43	44.41
4	Liver	0.62	0.16	0.1	0.6	0.26	2.908	54	451.9
5	Egg Albumen	0.06		0.09	0.47	0.13	2.32		7.04
6	Egg Yolk				1.57		6.63	1.27	62.41

Cadmium found only in Liver samples shows that accumulation of Cadmium is not reported via feed intake as feed was not found contaminated with Cadmium. However, its presence in the Liver is due to environmental factors including soil, smoke, and food. Also, the liver is the primary site for detoxification of contaminants. Consumption of food contaminated with Cadmium leads to carcinogenesis, lung damage, renal disorder, hepatic injury, high blood pressure, mental retardation, and cardiovascular and auditory structures disorder.

Iron is an essential HM in limited concentration for normal growth and development of humans. The presence of this heavy metal within permissible limits shows the normal composition of feed. Excessive presence of Iron in egg yolk was determined which may cause cardiac arrest, breathing failure, and convulsions. Lower levels of Iron in egg albumen were found which may be attributed to relatively low mineral contents in egg albumen. Low levels of albumen may lead to anemia.

In already available data of relevant studies, chromium concentration was found to be comparatively high whereas Lead and Iron were found to be less than the reported concentration in poultry feed. In the liver, breast muscles, thigh muscles, egg yolk, and egg albumen, Chromium, Lead, and Iron were found in comparatively high levels.

However, Cadmium was lower than the reported concentration in the muscle samples Table 5.

Production of quality feed is important for which periodic quality control of feed may be adopted by poultry farm management. Poultry feed manufacturers may take steps to use safe and hygienic raw materials in feed production. Safety and quality control checks may be practiced by poultry farmers to ensure the security of the final product for safe consumption. Moreover, regular monitoring by food and health authorities is urged along with the formulation of strategies to remove these elements from the environment. Monitoring of the food chain at each step is usually ignored in developing countries. Food chain safety mechanisms, if incorporated, may reduce the accumulation of Heavy Metals in the food network. Feed companies should carry out heavy metal assessments of their feed products periodically in order to keep them at a safe level.

This study may be furthered at the national level to evaluate the total impact on the health system. Awareness regarding exposure and the effects of heavy metal toxicity should be given. Along with this, reference limits for feed, eggs, and poultry meat should be prepared at the national level. By taking these steps, exposure to toxic substances may be reduced and the overall population may be saved from harmful impacts of heavy metals.

The limitations of the study include the non-availability of reference limits of the elements for each type of sample from major standardizing bodies like the World Health Organization. Also that no such study was done in order to compare and support the chances of absorption of heavy metals from feed to meat and then to eggs.

#### Conclusion

In solid poultry feed samples, average concentrations of Chromium and Lead were higher than the recommended range. In the liver, Cadmium, Chromium, and Lead were found in higher-than-normal concentrations. In breast muscles, high levels of Chromium and Lead were found. Concentrations of Chromium, Iron, and Lead were found to be high in whole eggs comprising of individually analyzed egg albumen and egg yolk. This indicates that chicken eggs are an adequate source of essential elements i.e., Iron but the liver, breast muscles, and eggs are contaminated with lead, chromium, and liver with cadmium.

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