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

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## Abstract

The study was conducted to determine 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 of Rawalpindi and Islamabad. Mercury, Lead, Cadmium, Chromium and Iron were analysed 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 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. Liver contains significantly higher concentration 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.

**Keywords**: Trace Minerals, Bioaccumulation, Lead, Poultry, Liver, Food Chain, Rawalpindi, Islamabad.



## 1. INTRODUCTION

Poultry is a tempting and inexpensive source of nutrients. With 38% 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]. 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 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 Non-essential trace elements (Aluminium (Al). Lead (Pb), Mercury (Hg), Boron (B), Silver (Ag), bismuth (Bi) etc.). Some trace minerals are also referred as Heavy Metals on the basis of their particular density greater than 5 $g/cm^3$ [5, 1]. 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].

Poultry industry in Pakistan, contributes to 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 of 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 the capacity ranging from 5,000 to 500,000 broiler chicken, more than 15000 fowl farms are located within the rural areas throughout the country from Karachi to Peshawar. Forty-five (45) % of the total meat intake is drawn from fowl products. Pakistan poultry industries produce 17,500 million eggs and

1,322 million kilo grams bird meat yearly. Consumption of meat is just 6.61 kilo grams and 88 eggs per capita each year in the country. Contrary to this, intake in evolved international locations is 40 kilo grams meat and over three hundred eggs according to 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 Kasur district of Punjab with Mercury present at 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 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 egg 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 for common population. This is an indication of heavy metal contamination in the environment

Safety of poultry feed is vital for poultry health and productivity and health of consumers. Poultry chicken and poultry products are important part of human diet. Accumulation of heavy metals in regularly consumed food items is of grave concern. 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 the feed which, if polluted with heavy metal, might be poisonous for the health of chicken 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.

## 2. MATERIALS AND METHODS

### SAMPLE COLLECTION

Six samples of layer breed chicken were collected from each of six different poultry farm houses of Rawalpindi and Islamabad along-with their solid and liquid feed and eggs. All chicken were slaughtered with 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 same poultry farms. Egg Albumen and egg yolk was analysed separately. Total number of samples analysed for presence of five heavy metals under consideration were 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 hot air oven (Memmert) until constant weight was obtained [2]. Dried body tissues were then grinded to a powder form using a grinder machine [15] and passed through sieve of 0.25 mm mesh size [16]. Composite sampling was done for same tissue from individual farm e.g., all sieved liver from farm A were combined and then homogenized using a homogenizer stirrer (Daihan Scientific HS-30E). Thus total number of analysed 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 separately labelled glass petri dish [9] and dried at 105 °C in hot air oven (Memmert) until constant weight was obtained [2].

#### Sample Digestion

Analytical grade chemicals were used in sample digestion. Each sample, prepared on dry basis, was taken in conical flask. 15 ml of concentrated Nitric acid and Perchloric acid in 4:1 was added. Digestion was carried out using condenser and stirring was done at 80°C for 2 hours until the solution turned colourless. 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].

## 3. 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 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 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 was not detected in egg samples. In egg albumen, Chromium, Iron and Lead showed average concentration 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 standard are shown in Table 1.

**Table 1: Heavy metals Concentration in Samples and WHO standard**

| S. # | Sample Type | Sample ID | Heavy Metals Concentration (ppm) | | | | | Permissible Level (ppm) (WHO) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cadmium (Cd) | Chromium (Cr) | Iron (Fe) | Lead (Pb) | Mercury (Hg) | | |
| 1. | Solid Feed | AF | ND | 0.65 | 8.70 | 2.32 | ND | Cd | - |
| 2. | | BF | ND | 0.61 | 30.98 | 1.22 | ND | Cr | 0.1 |
| 3. | | CF | ND | 0.84 | 15.96 | 1.08 | ND | Fe | - |
| 4. | | DF | ND | 0.61 | 12.50 | 0.93 | ND | Pb | 0.05 |
| 5. | | EF | ND | 10.20 | 38.36 | 8.87 | ND | Hg | - |
| 6. | | 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 |
| 8. | | BL | 0.19 | 0.30 | 676.84 | 0.54 | ND | Cr | 0.5 |
| 9. | | CL | 0.09 | 0.41 | 461.16 | 1.65 | ND | Fe | - |
| 10. | | DL | 0.12 | 1.10 | 266.25 | 4.11 | ND | Pb | 0.1 |
| 11. | | EL | 0.11 | 0.95 | 528.49 | 8.42 | ND | Hg | - |
| 12. | | FL | 0.10 | 0.43 | 181.51 | 0.94 | ND | | |
| 13. | Breast Muscle | AB | ND | 0.32 | 22.16 | 0.89 | ND | Cd | - |
| 14. | | BB | ND | 0.43 | 29.29 | 3.04 | ND | Cr | 1.0 |
| 15. | | CB | ND | 0.64 | 24.10 | 0.41 | ND | Fe | - |
| 16. | | DB | ND | 1.28 | 29.14 | 2.96 | ND | Pb | 0.1 |
| 17. | | EB | ND | 0.32 | 33.96 | 1.68 | ND | Hg | - |
| 18. | | FB | ND | 1.02 | 22.72 | 6.95 | ND | | |
| 19. | Thigh Muscle | AT | ND | 0.66 | 51.62 | ND | ND | Cd | - |
| 20. | | BT | ND | 0.35 | 40.87 | 0.49 | ND | Cr | - |
| 21. | | CT | ND | 0.20 | 39.50 | 0.88 | ND | Fe | - |
| 22. | | DT | ND | 0.25 | 37.80 | 2.58 | ND | Pb | - |
| 23. | | ET | ND | 0.25 | 55.46 | 5.98 | ND | Hg | - |
| 24. | | FT | ND | 0.21 | 41.22 | ND | ND | | |
| 25. | Egg Albumen | AA | ND | ND | 5.48 | 0.81 | ND | Cd | - |
| 26. | | BA | ND | 0.25 | 3.73 | 0.46 | ND | Cr | - |
| 27. | | CA | ND | 0.94 | 6.52 | 1.70 | ND | Fe | - |
| 28. | | EA | ND | 0.26 | 3.95 | 1.24 | ND | Pb | - |
| 29. | | FA | ND | 0.46 | 15.52 | 7.40 | ND | Hg | - |
| 30. | Egg Yolk | AY | ND | 0.96 | 75.18 | 7.49 | ND | Cd | - |

| S. # | Sample Type | Sample ID | Heavy Metals Concentration (ppm) | | | | | Permissible Level (ppm) (WHO) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cadmium (Cd) | Chromium (Cr) | Iron (Fe) | Lead (Pb) | Mercury (Hg) | | |
| 31. | | BY | ND | 0.57 | 58.44 | 8.21 | ND | Cr | - |
| 32. | | CY | ND | 0.74 | 53.12 | 4.64 | ND | Fe | - |
| 33. | | EY | ND | 0.95 | 57.43 | 5.22 | ND | Pb | - |
| 34. | | FY | ND | 4.63 | 67.91 | 7.61 | ND | Hg | - |
| | | | | | | | | Ref: (14) | |
| 35. | Whole Egg | AE | ND | 0.96 | 80.66 | 8.3 | ND | Cd | 0.06-0.07 |
| 36. | | BE | ND | 0.82 | 62.17 | 8.67 | ND | Cr | 0.05 |
| 37. | | CE | ND | 1.68 | 59.64 | 6,34 | ND | Fe | 44.0 |
| 38. | | EE | ND | 1.21 | 61.38 | 6.46 | ND | Pb | 0.43 |
| 39. | | FE | ND | 5.09 | 83.43 | 15.01 | ND | Hg | - |

The standard deviation between 6 farm house for each of Chromium, Lead and Iron is shown in Table 2, 3 and 4, respectively.

**Table 2: Standard Deviation between different Farm Houses for Chromium**

| | CHROMIUM | | | | | | |
|---|---|---|---|---|---|---|---|
| POULTRY ITEM | FEED | LIVER | BM* | TM** | EA*** | EY**** | WE***** |
| FARM A | 0.65 | 0.46 | 0.32 | 0.66 | 0 | 0.96 | 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 DEV | 3.8474 | 0.3306 | 0.3995 | 0.1748 | 0.3520 | 1.7182 | 1.7844 |

BM* Breast Muscle
TM** Thigh Muscles
EA*** Egg Albumen
EY**** Egg Yolk
WE***** Whole Egg

**Table 3: Standard Deviation between different Farm Houses For Lead**

| | LEAD | | | | | | |
|---|---|---|---|---|---|---|---|
| POULTRY ITEM | FEED | LIVER | BM | 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 DEV | 3.1089 | 2.9711 | 2.3577 | 2.3236 | 2.8764 | 1.5925 | 3.5438 |

**Table 4: Standard Deviation between different Farm Houses for Lead**

| | IRON | | | | | | |
|---|---|---|---|---|---|---|---|
| | FEED | LIVER | BM | TM | EA | EY | WE |
| FARM A | 8.7 | 597.62 | 22.16 | 51.62 | 5.48 | 75.18 | 80.66 |
| FARM B | 30.98 | 676.84 | 29.29 | 40.87 | 3.73 | 58.44 | 62.17 |
| FARM C | 15.96 | 461.16 | 24.1 | 39.5 | 6.52 | 53.12 | 59.64 |
| FARM D | 12.5 | 266.25 | 29.14 | 37.8 | | | |
| FARM E | 38.36 | 528.49 | 33.96 | 55.46 | 3.95 | 57.43 | 61.38 |
| FARM F | 15.36 | 181.51 | 22.72 | 41.22 | 15.52 | 67.91 | 83.43 |
| MEAN | 20.31 | 451.9783 | 26.895 | 44.4116 | 7.04 | 62.416 | 69.456 |
| STD DEV | 10.6369 | 175.7653 | 4.2495 | 6.6407 | 4.3616 | 8.0023 | 10.3485 |

Graphical representation of results with permissible levels of Chromium, Iron, Lead and Cadmium is shown in Figure 1, 2, 3 and 4, respectively.

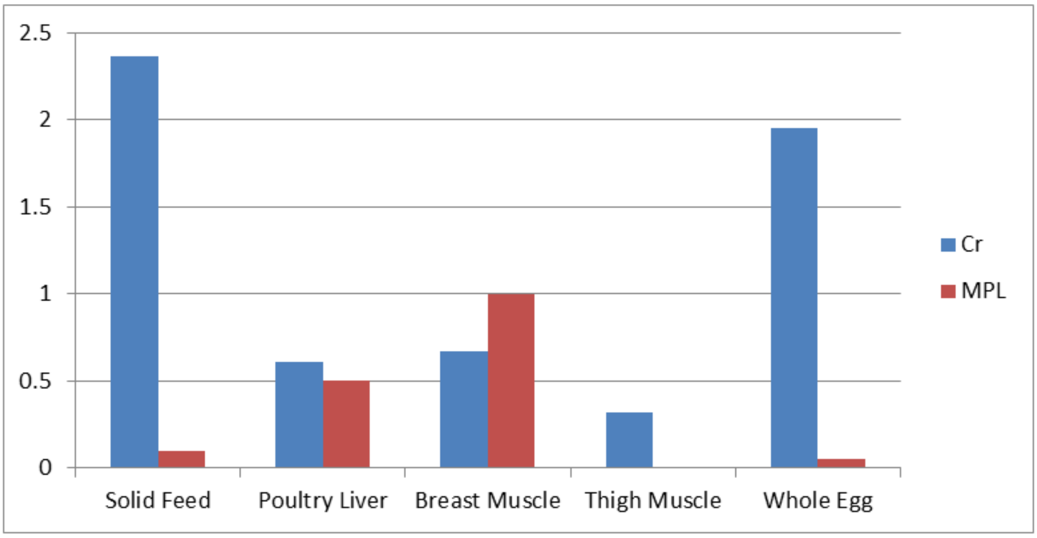


**Figure 1: Graphical Comparison between Chromium Levels and Permissible Level**

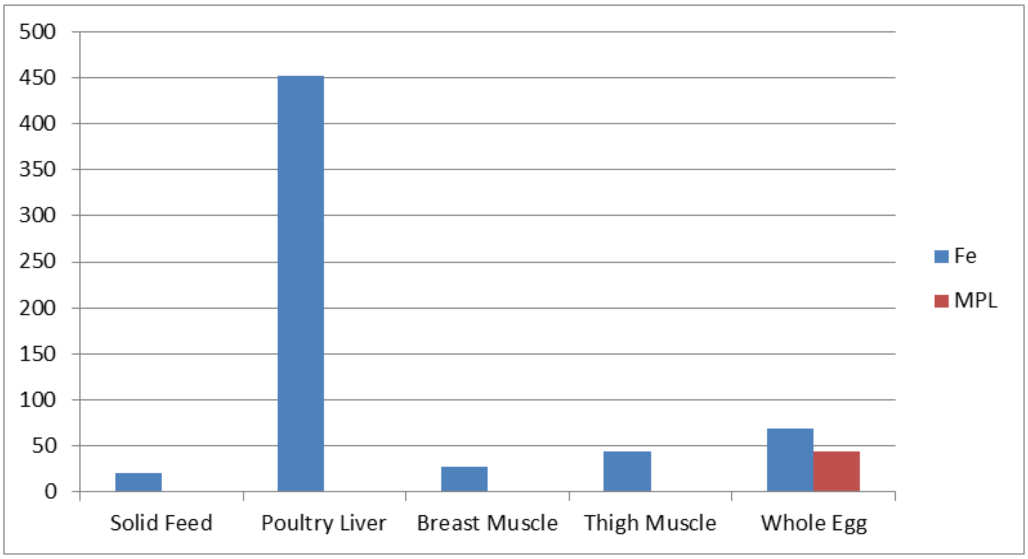


**Figure 2: Graphical Comparison between Iron Levels and Permissible Level**

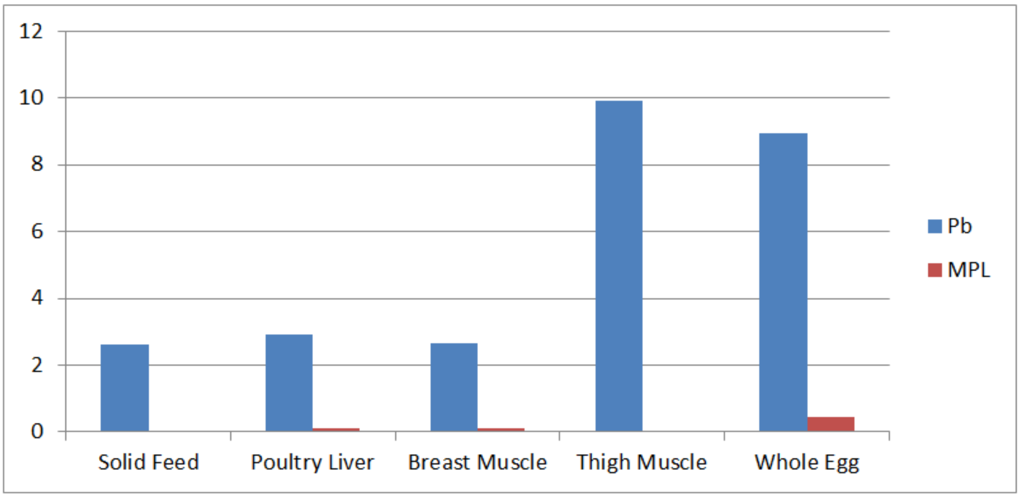


**Figure 3: Graphical Comparison between Lead Levels and Permissible Level**

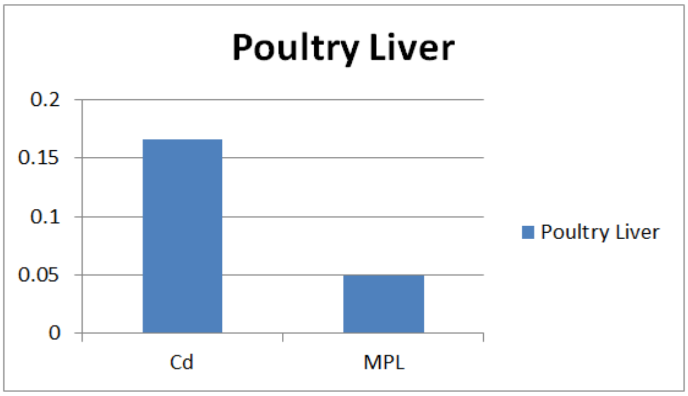


**Figure 4: Graphical Comparison between Cadmium Levels and Permissible Level**

## 4. DISCUSSION

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

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 Liver for detoxification to less toxic metabolites and excretion therefore, concentration of Lead is high in 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 for consumers. Chromium consumption in such high-level leads to liver, kidneys, neural tissues and circulatory system disorders.

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 Liver is due to environmental factors including soil, smoke and food. Also that 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, cardiovascular and auditory structures disorder.

Iron is an essential HM in limited concentration for normal growth and development of human. Presence of this heavy metal within permissible limits shows 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 anaemia.

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: 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 | 0.60 | | 0.06 | 0.32 | 0.23 | 9.93 | 43 | 44.41 |
| 4. | Liver | 0.62 | 0.16 | 0.10 | 0.60 | 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 |

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 practised by poultry farmers to ensure security of final product for safe consumption. Moreover, regular monitoring by food and health authorities is urged along with formulation of strategies to remove these elements from environment. Monitoring of food chain at each step is usually ignored in developing countries. Food chain safety mechanism, if incorporated, may reduce accumulation of Heavy Metals in food network. Feed companies should carry out heavy metal assessment of their feed products periodically in order to keep them at a safe level.

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

The limitations of the study include non-availability of reference limits of the elements for each type of sample from major standardizing bodies like World Health Organization. Also that no such study was done in order to compare and support 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 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 egg comprising of individually analysed egg albumen and egg yolk. This indicates that the chicken eggs are an adequate source of essential element i.e., Iron but liver, breast muscles and eggs are contaminated with lead, chromium and liver with cadmium.

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