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Simultaneous Atomic Absorption Spectroscopic Quantization Survey of Toxic Metals in Poultry Feed and Their Translocation in Various Tissues of Chicken in Lahore Region of Pakistan

Jaweria Shamshad, Jawayria Najeeb, Muhammad Naeem, Muhammad Nawaz Chaudhry

Abstract— Addition of trace minerals in appropriate concentrations is an inevitable requirement for achieving the optimal growth and development of poultry birds. However, their excessive intake presents serious carcinogenic and toxic threats to the consumer's health. For keeping this borderline concentration in check, investigation was carried out over feed, eggs, liver, leg and heart tissue samples of chicken and a mineral profile, highlighting the concentration and translocation of trace mineral and electrolytes within the samples, have been built. Quantization of Aluminium, Cadmium, Chromium, Cobalt, Iron, Lead, Nickel, Zinc, Sodium, Potassium, Calcium and Magnesium was done by using techniques of atomic absorption spectrometry and flame photometry; and mean concentrations obtained were compared with allowed Maximum Permissible Limit (MPL) set by EU standards. Data acquired showed an alarming increase of Lead and Antimony levels in samples while other minerals content fell quite low than the set limits. In fact, Iron and Chromium concentrations were so scarce to provide their nutritive value to the consumers. This study not only addresses the pivotal health concerns these toxic trace metals could have over consumers but also shed light over the existing nutritious value of commercially available poultry products.

Index Terms— Atomic absorption spectroscopy, bioaccumulation, carcinogenic effects, chicken, chemical analysis ,metal toxicity, poultry feed.

1 INTRODUCTION

Owing to the inexpensiveness and high accessibility rate of commercially produced broiler chicken in under developed countries, chicken's meat and eggs are considered the respected source for obtaining high quality protein and other essential minerals by layman [1]. In Pakistan, Poultry enterprise has become the second fastest growing sector of decade due to its economical, nutritional and eco friendlier progression approach. Currently, gross revenue of Pakistan's poultry industry is around Rs. 564 billion and nearly 40% of total meat consumption is being acquired from poultry products [2]. This phenomenal growth in poultry sector is however, disengaged with the critical need of doing research regarding food safety and feed safety issues related to poultry and investigations are needed to be carried out to assess the risk it poses to human health.

Poultry feed is crucial factor directly linked with the prevalence of foodborne diseases that is causing tremendous concerns worldwide [3]. Metals, such as Aluminium (Al), Arsenic (As), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Potassium (K), Sodium (Na) and Zinc (Zn) are the essential component of poultry feed because of their contribution in avoiding anemia and premature hatching, for proper extensive feather growth and killing of gastric parasites in Chicken [4].Excessive dietary consumption of these vital metals could pose serious toxic and carcinogenic threats not only to poultry chicken but also among the human beings which are ultimately consuming them. Even small concentrations of non-essential trace elements like Antimony (Sb), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Nickel (Ni) or Lead (Pb) have been reported to induce adverse effects on humans [5]. Moreover, most of the Poultry farms in Pakistan are located around urban areas. Thus, metal adulteration due to industrialization is also needed to be considered while devising dietary feed composition for the chickens, as food consumption has already been recognized to be the cause of ninety percent exposure of mankind to trace metal contamination [6].

Health-risk assessment by developing the trace metal profile has been done by several researchers in different countries, e.g. Bangladesh [7], China [8], Egypt [9], India [10], Nigeria [11], Taiwan [12] and Turkey [13] etc. In Pakistan, an appreciable and inspiring work on heavy metal contamination has been done distinctively on poultry eggs and meat [14, 15].The present research was carried out for simultaneous quantization of selected trace elements (Al, Cd, Co, Cr, Fe, Ni, Pb, Sb and Zn) and electrolytes (Ca, Mg, Na and K) in the chicken's feed, meat, eggs and tissues of heart, liver and kidney with samples collected from five representative poultry farms located in and around the Lahore, Pakistan. To the best of researcher's knowledge, this is the first comprehensive trace metal study conducted exclusively on the poultry feed with the correlation of its translocation in the several tissues of the chicken done in our country. Aside from the quantization of trace elements giving us a special prospect on health-risk as-

- *Jaweria Shamshad is currently pursuing Ph.D degree program in University, of Punjab, Pakistan, PH-+923368780450. E-mail: chem_guru@yahoo.com*
- *Jawaryia Najeeb is currently pursuing Master degree program in University, of Punjab, Pakistan,E-mail: jiyanajeeb73@gmail.com*
- *Mohammad Naeem is currently working as Senior Scientific officer in Pakistan Council for Scientific and Industrial Research, Lahore, Pakistan, Email: chmnaeem32@yahoo.com.*
- *Muhammad Nawaz Chaudhry is currentlyProfessor of Emeritus in University,of Punjab,Lahore, Pakistan,.E-mail: muhammadnawazchaudhry@yahoo.com*

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sessment in poultry field, the information acquired could also be valuable for improving the existing nutritive conditions in Pakistan.

2 Experimental Section

2.1 Materials and Instruments

Reagents used for analysis were of analytical-grade standards. Hydrochloric acid (HCl), Hydrogen peroxide (H2O2), Nitric acid (HNO3), Per-chloric acid (HClO4), Sulfuric acid (H2SO4) and salts/oxides/bulk form of metals (used for the preparation of standard solution) were purchased from Sigma-Aldrich (Germany). Perkin-Elmer® AAnalyst 100 Atomic absorption spectrometer and Flame photometer AE-SFP 40 A&E Lab® (UK) were used during experimentation. Glass wares used were of Pyrex brand. For solution preparation and rinsing, Ultra purified deionized (grade 1) water was used. All measurements were taken at room temperature.

2.2 Sampling and sample indexing

Eleven samples of several kinds of commercially available poultry brands (Grower, Breeder layer, Pre-breeder, Breeder starter, Broiler starter, Breeder layer, Broiler grower, Broiler finisher, Layer, Layer chick and Breeder grower) from nationally distributing feed company i.e. Big Bird were acquired and named as sample SF-1 to SF-11 respectively. Plastic bags were used for sample conservation. Three samples of chickens egg specified as sample SE-12 to SE-14 from various markets (Iqbal Town, Model Town and Muhammad Nagar) in Lahore city along with four set of meat's samples each containing muscle, liver and heart tissues of chicken respectively from Model Town (SM-15m to SM-15h), Barket Market (SB-16m to SB-16h), Johar Town (SJ-17m to SJ-17h) and Wapda Town (SW-18m to SW-18h) were collected and analyzed. Iceboxes were used for sample storage and preservation.

2.3 Sample treatment

Oven-drying of 3g of feed samples at 1040C was done for ensuring the removal of water. Samples were milled for uniformity in size and afterwards were digested in aqua regia (HNO3 and HCl in 1:3 respectively). Obtained clear solution was separated by filtration and stored. For egg samples, yolk was removed and white was homogenized by mixing. 10g weighed sample was treated with aqua regia and 30% v/v H2O2 solution was added during digestion for nitrogen dioxide removal. Digested clear solution was preserved in 50ml volumetric flask. Decomposition of tissue's sample was done by applying wet digestion in open system technique [16]. Washed organs (liver, heart and leg muscles) were sliced by stainless steel knife and 3g of sample was introduced in long neck digestion flask. At the same time, 30mL mixture of HNO3, HClO4 and H2SO4 in ratio 4:1:1 was introduced and assembly was heated up to 800C for 2-3 hours. After that extract was cooled up to room temperature, 20mL double distillated and demineralized water was added and was again heated for four hours up to 1500C. Clear solution was separated and stored. Volume of all the samples was brought to 50mL with Ultra-pure water.

2.4 Opted analytical method

Mineral concentration within each sample was identified by using Atomic absorption spectroscopy (AAS) and Flame emission photometry (FEP). Standard calibration curve and stock preparation was done by following the approved method of American Association for Clinical Chemistry (AACC) [17]. 99.99% pure Aluminium metal in HCl, granules of Sb metal in HCl along with small spiking of HNO3 in mixture, Cadmium Sulfate in HNO3, Calcium Carbonate in HNO3, Cobalt metal strip in HNO3, Potassium dichromate in HCl with moderate heating, 99.99% pure iron metal wire in HCl, Pb metal in HNO3, Magnesium Carbonate in HNO3, Ni metal strip in HNO3 and Zn metal granules in HCl were used as standard materials for Al, Sb, Cd, Ca, Co, K, Fe, Pb, Mg, Ni and Zn respectively. Materials were used as received for experimentation. All these standard materials were then diluted with ultra-pure water to make 1000ppm stock solutions which were further diluted into various concentrations for attaining calibration curve for these metals for performing AAS. Sodium Chloride and Potassium Chloride dissolved in distilled water were diluted up to one liter to get stock solution of Na and K for its further usage in FEP. Functioning parameters for both AAS and FEP used are given in Table 01 and 02 correspondingly.

Table 01 Atomic Absorption Spectrophotometer functioning parameters

Mineral	Lamp Current	Fuel used	Oxidant applied	Wavelength specified	Flame Nature	Spectral band width
Aluminium	10mA	Acetylene	Nitrous oxide	309.3nm	Reducing	0.7nm
Antimony	EDL	Acetylene	Air	217.6nm	Oxidizing	0.2nm
Cadmium	4mA	Acetylene	Air	228.8nm	Oxidizing	0.5nm
Calcium	10mA	Acetylene	Nitrous oxide	422.7nm	Reducing	0.5nm
Chromium	7mA	Acetylene	Air	357.9nm	Reducing	0.7nm
Cobalt	7mA	Acetylene	Air	240.7nm	Oxidizing	0.2nm
Iron	5mA	Acetylene	Air	248.3nm	Oxidizing	0.2nm
Lead	5mA	Acetylene	Air	283.3nm	Oxidizing	0.7nm
Magnesium	4mA	Acetylene	Air	285.2nm	Oxidizing	0.5nm
Nickel	4mA	Acetylene	Air	232.0nm	Oxidizing	0.2nm
Zinc	5mA	Acetylene	Air	213.9nm	Oxidizing	0.7nm

Table 02 Flame Emission Spectrophotometer functioning parameters

Electrolyte	Wavelength	Fuel used	Oxidant applied	Spectral band width
Sodium	589.0nm	Acetylene	Air	0.1nm
Potassium	766.5nm	Acetylene	Air	0.1nm

Statistics

One Way ANNOVA was applied over the obtained data for performing multiple comparisons Turkey's test by using Graph Pad Prism (Version 7.00) software. Significant difference among the mean values of elements was calculated and analyzed as done by several researchers[9], [15].

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3.0. Results and discussion

Minerals and electrolyte quantization studied for Aluminium, Antimony, Cadmium, Calcium, Cobalt, Chromium, Iron, Lead, Magnesium, Nickel, Potassium, Sodium and Zinc in various samples have been summarized in graphical form (as shown in Fig.1 and .2) while elements are individually discussed below.

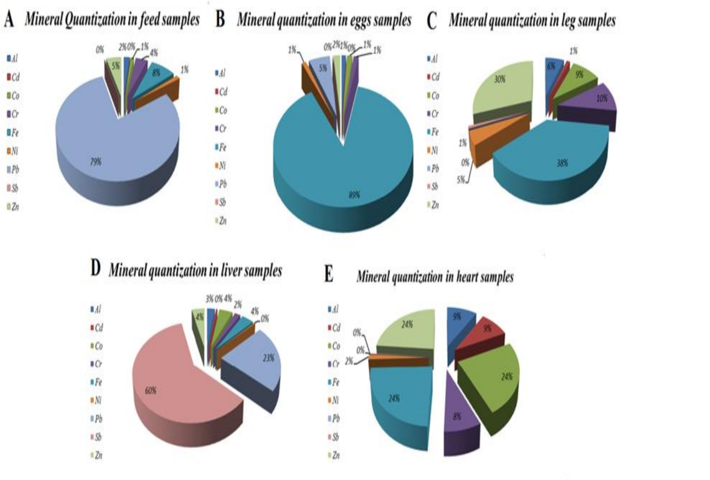

Fig.1 Mineral quantization in samples of A) feed B) eggs C) leg D) liver E) heart of chicken

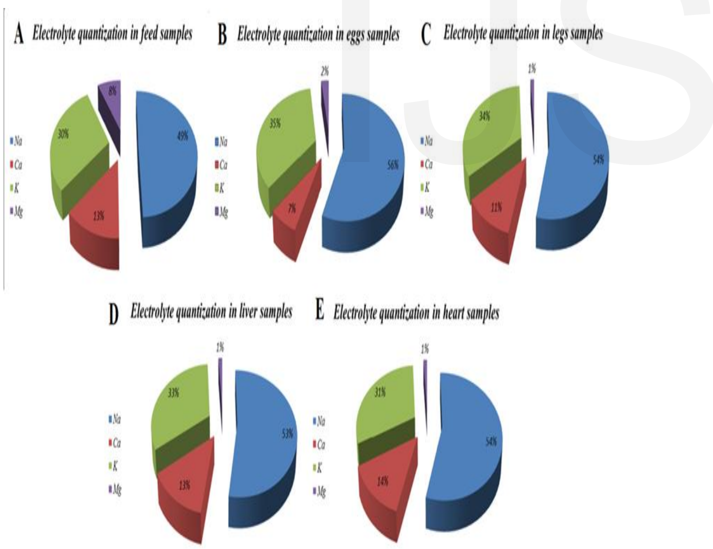

Fig.2 Electrolyte quantization in samples of A) feed B) eggs C) leg D) liver E) heart of chicken

3.1. Trace metals quantization

Aluminium has been termed as a non-essential dietary element associated with various pathological illnesses in humans like Parkinson's disease, Alzheimer's disease and kidney failure etc. [18],[19].

Aluminium concentration normally reaches up to 0.1mg/kg in poultry as reported by Leblanc et al. whereas maximum permissible limit (MPL) for Al is 1mg/kg [20]. Data acquired indicates Al content to be much lower as compared to MPL in all samples.Aluminium quantity was found to be 0.0237±0.02mg/kg,0.0065±0.00mg/kg,0.017±0.02mg/kg,0.055± 0.10mg/kg and 0.04±0.06mg/kg in feed, eggs, leg muscle, liver and heart muscles respectively. Maximum amount of Aluminium i.e. 0.209mg/kg was observed in liver sample. This low concentration of Aluminium is not only in accordance with the fact that exceeding Al amount in feed causes generalized growth repression and progressive decline in bone strength in chicken but also comparable with the results reported by Uluozlu et al. [21],[22].

As a non-essential global contaminant inducing carcinogenetic and genotoxic effects in humans, Antimony and its compounds are principally addressed in the US Environmental Protection Agency (EPA) as hazardous material [23],[24]. Maximum permitted amount of Sb in poultry specified products is 1mg/kg [25]. Antimony was not detected in any of collected feed and eggs samples while leg, liver and heart tissues were found to be contaminated with 0.002±0.00mg/kg, 1.315±1.60mg/kg and 0.001±0.00mg/kg respectively. This is an alarming situation as Sb in liver gravely exceeds the MPL. Further investigation of water and soil in the farms are required for the specification of the Sb source as several researchers have indicated ground water utilized for poultry production to be playing a fundamental hand in Antimony contamination such as Coetzee et al. [26].

Nutritive value of Cadmium for animals and humans is quite negligible. Demineralization of bones, anemia and kidney failures are the few diseases that are generally associated with its excessive usage [27]. MPL of Cd in poultry product has been reported to be 0.05mg/kg [28]. Evaluation of poultry feed and eggs indicated the absence of Cadmium in all collected samples. However, concentration of 0.003±0.00mg/kg, 0.009±0.00mg/kg and 0.038±0.05mg/kg of Cd were found to be deposited in leg, liver and heart muscles correspondingly. Fortunately, the obtained results were far below the MPL and reinforce the suitability of poultry feed and chicken for human use. Skalicaka et al. also acquired the same results in this regard [29].

Chromium metal exists at a borderline of essentiality and non-essentiality as Cr(III) is an essential dietary element required for carbohydrate-lipid metabolism while Cr(IV) is a known carcinogen [30]. Hence, the delicate balance regarding its concentration should be carefully kept in check. Maximum allowed amount for chromium specifically in poultry product is 1mg/kg [25]. Mean concentrations of 0.047±0.11mg/kg, 0.007±0.00mg/kg, 0.030±0.01mg/kg, 0.041±0.013mg/kg and 0.034±0.01mg/kg were recorded in feed, eggs, leg, liver, and heart muscles separately. Literature on Chromium quantization also supports the study's findings that obtained value was far less than MPL even to provide the nutritional prospect to consumers [20],[31].

The only vital functionality known of Cobalt for humans is being a critical component of Cobalamin also known as Vitamin B-12 [32]. Necrosis relating to stomach and esophageal mucosa along with brain edema was reported owing to Co excessiveness [33]. Highest permissible limit of Cobalt in feeding stuff was found to be 2mg/kg [34]. Amount of Co-

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balt ranged from 0.008-0.13mg/kg in collected samples with feed, eggs, liver, leg, and heart tissues recording a concentration of 0.012±0.00mg/kg,0.008±0.00mg/kg,0.096±0.03mg/kg, 0.027±0.07mg/kg and 0.099±0.02mg/kg respectively. Maximum accumulation of Cobalt was shown in liver and heart tissues of chicken i.e. 0.1mg/kg which also supported by Uluozlu et al. [22].

Iron is considered among one of the most abundant essentially important trace mineral having very high tolerance limit in dietary feeds and livestock owing to its characteristic limited adsorption and bioavailability in food chain. Mean concentration of Fe in feed samples was detected to be 0.099±0.11mg/kg which is quite less as compared to the content detected by Combs [35]. Egg's sample contained 0.604±0.00mg/kg while tissues of leg, liver and heart muscles had 0.111±0.04mg/kg, 0.087±0.04mg/kg and 0.099±0.04mg/kg respectively which are quite lower than its specified Maximum Hygienic value i.e. 500mg/kg [34].

Accumulation of excessive lead in food chain owing to Pb deposition from atmosphere into dietary accessories is one of the fundamental causes of Lead poisoning occurring at an alarming level among children worldwide. Apart from that its agglomeration within human body also adversely affects the cardiovascular tissues and kidney cells [36]. MPL of Pb in feeding stuff as per decided by the EC Regulation law 629/2008 was 0.1mg/kg [34]. Evaluation confirms the absence of Pb in the samples of chicken's leg and heart muscles. However; chicken's feed, eggs and liver tissue samples possessed the mean concentration of 0.846±0.34mg/kg, 0.033±0.05mg/kg and 0.495±0.18mg/kg respectively. The lead content in feed and liver samples gravely exceeds the MPL and is needed to be monitored.

Critical evaluation of Nickel essentiality and toxicity establishes it to be rather non-essential element regarding dietary usefulness and a carcinogenic toxic material in respect of epidemiological issues [37]. Maximum permissible limit for Nickel is proposed to be 1mg/kg [25]. Research conducted indicated the presence of mean concentrations of 0.016±0.02mg/kg,0.007±0.00mg/kg,0.013±0.05mg/kg,0.006± 0.00mg/kg and 0.006±0.02mg/kg in feed, eggs, leg, liver, and heart samples respectively. All the samples evaluated showed lower concentration of Ni than the maximum permissible limit which is also in correspondence with the findings of Miller et al. [31].

Zinc is known for its essentiality as a biochemical catalytic substance involved in several transcription cycles as enzyme's component and also involved in sustaining the mechanical integrity of DNA. Its deficiency causes severe brain impairment among humans[38]. The Maximum allowed limit for Zn in feeding materials is established to be 20mg/kg [15].

Mean concentrations of 0.060±0.01mg/kg, 0.010±0.00mg/kg, 0.086±0.02mg/kg, 0.092±0.01mg/kg and 0.099±0.00mg/kg were detected in feed, eggs, leg, liver, and heart samples respectively which are way below than required value thus not even fulfilling the purpose of providing its nutritive value.

3.2. Electrolyte quantization

Electrolytes of Sodium, Calcium, Potassium and Magnesium were studied in the collected samples. Since these are essential components for both humans and chicken, they are required in very higher concentrations than trace minerals. Also, no maximum permissible has been reported in literature for these metals [14].

Sodium-Potassium pumps are involved in the formation of impulses travelling back and forth along the neuron between receptors and effectors with brain and spinal cord acting as its processing unit. Potassium and Sodium are also responsible for formation of trans-membrane potential and buffering of body fluids. Aside for being a fundamental cation involved in muscle activity, Calcium is deposited in bone and teeth, thus providing structural support and mechanical integrity to both. Magnesium also normalize mineral balancing and ATP production within the body [39].

Mean concentrations of electrolytes (13.944±1.48mg/kg, 2.47±0.40mg/kg,6.727±1.54mg/kg,8.827±1.61mg/kg and 7.902 ±0.67mg/kg for Na; 3.657±0.47mg/kg,0.290±0.053mg/kg,1.416 ±1.08mg/kg, 2.275±0.426mg/kg and 1.982±0.45mg/kg for Ca; 8.426±0.89mg/kg,1.565±0.30mg/kg,4.243±1.31mg/kg,5.476±1. 09mg/kg and 4.47±1.10mg/kg for K; 2.136±1.01mg/kg, 0.105±0.00mg/kg, 0.146±0.071mg/kg, 0.204±0.08mg/kg and 0.169±0.043mg/kg for Mg) were calculated in feed, eggs, leg, liver, and heart tissue samples respectively.

3.3. Statistical analysis

Statistical data acquired showed that there exists significant difference among the entire different mineral's content evaluated in the different samples. Following specified symbols were used for each trace mineral in each sample for indicating its significant difference with other metal: Al=β, Cd=α, Co=σ, Cr=Δ, Fe=ρ, Ni=η, Pb=Λ and Sb=ω whereas in case of electrolytes these terms were used: Na=σ, Ca=η and K=ρ. Extent of significant difference was indicated by the number of the symbols as shown in Fig. 3.

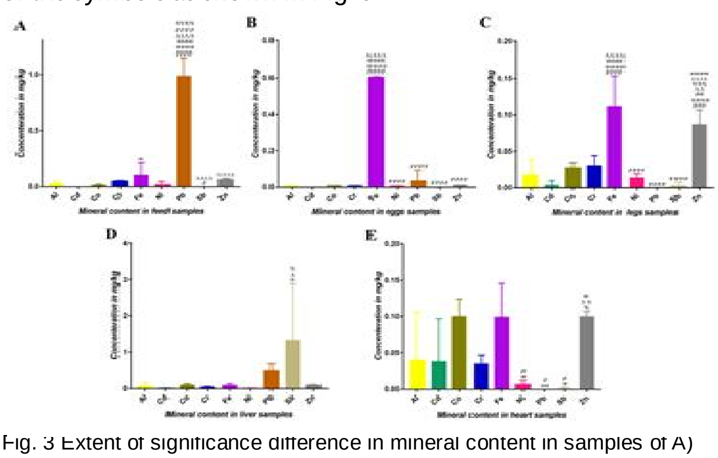

Fig. 3 Extent of significance difference in mineral content in samples of A) feed B) eggs C) leg D) liver E) heart of chicken

In feed samples; Al, Cd, Co, Cr, Fe and Ni showed significant difference of ($p<0.0001$) when compared with Pb concentration and in turn Pb also showed significant difference of ($p<0.0001$) against Sb and Zn. When descriptive statistics was applied over the eggs samples, significant difference of

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($p<0.0001$) was found in Al, Cd, Co and Cr against Fe while Fe also showed the same significant difference against Ni, Pb, Sb and Zn. Multiple comparison done on leg muscle samples also showed significant difference which are comparable with the above results. However, Al vs. Zn was found to be ($p<0.001$), Cd vs. Zn was ($p<0.0001$), Co vs. Zn and Cr vs. Zn established p value of ($p<0.01$), Ni vs. Zn gave ($p<0.001$), Pb and Sb vs. Zn showed a difference of ($p<0.0001$) while Fe vs. Zn concentrations showed no significant difference respectively. Cd, Cr and Ni presented the significant difference of ($p<0.05$) against Sb in liver samples. Co vs. Ni, Co vs. Sb, Fe vs. Ni, Fe vs. Sb, Fe vs. Pb, Ni vs. Zn and Sb vs. Zn all displayed the significant difference of ($p<0.05$) among each other in heart samples. When electrolyte concentrations were compared (as shown in Figure 4), Feed samples exhibited a difference of ($p<0.0001$) in Na against Ca, K and Mg, K vs. Mg and Ca vs. K while ($p<0.01$) was observed when Ca was compared with Mg. Significant increase in the p value was observed in eggs samples during each comparison. Leg muscles also showed significant difference against each other. Ca against K and Na against K were significantly different with ($p<0.01$) while Ca and Mg didn't show any significant difference against each other in liver samples. Heart samples also showed significant difference among the mean concentrations of all electrolytes.

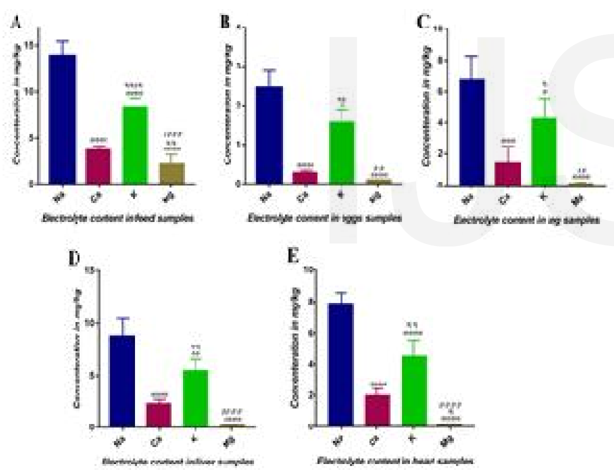

Fig. 4 Extent of significance difference in electrolyte content in samples of A) feed B) eggs C) leg D) liver E) heart of chicken

4.0 Conclusion

Identification and isolation of toxic metals is quite difficult task owing to the presence of these materials in trace concentrations in environment. However, their profiling and quantization is an essential undertaking needed to be addressed as they directly or indirectly affects the human's health by bioaccumulation and agglomeration. Research conducted highlights the mean concentrations of trace minerals and electrolyte not only with in different feeding materials but also among the several tissues of Chicken. Aluminium, Cadmium, Chromium, Cobalt, Iron, Nickel and Zinc showed the concentration quite lower than that of Maximum Permissible limit. However, Lead and Antimony showed higher concentration than MPL. Increase in Pb concentration could be attributed to its swift depositing ability that has dangerously enhanced its ability to bio-accumulate in the food chain. Furthermore, Antimony is a carcinogenic element and it's amassing in liver samples gravely heightens the probability of occurrence of carcinogenic diseases in humans. Electrolyte concentrations were all found to be in accordance with the previously reported concentrations in academic literature.

4.1. Recommendations

Following are the few suggestions that seemed noteworthy during the conduction of this research.

- Maximum Permissible Limit (MPL) of the hazardous trace minerals should be specified on national level by the Federal Government of Pakistan as done by several other countries[7], [34].
- Mineral profiling of water, soil and food should be done to estimate the health risk coefficient within the country.
- Lead accumulation indicated towards increase in pollution in the environment. That problem also requires attention.
- In case of Antimony, further studies should be conducted for identification of its source.

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