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Original Research Article

Assessment of Heavy Metals and Trace Elements in Eggs and Eggshells of *Gallus gallus domesticus*, *Coturnix coturnix* and *Anas platyrhynchos* from Bangladesh

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Abstract

Background: Despite playing an essential role as micronutrients, when exposed to an excess level, heavy metals can augment the risk of potential health diseases among humans and animals. Due to environmental degradation and food adulteration, many people are becoming the victims of those diseases by ingesting heavy metals from those food sources. Eggs are one of the principal sources of proteinaceous food and eggshells are also a good source of calcium, therefore, it is necessary to estimate the frequency of heavy metals as the poultry feeds, nowadays, are contaminated with heavy metals. ***Materials and Methods:*** Five types of egg samples were collected from the Jahangirnagar University area including indigenous, white and brown layer chicken eggs, duck eggs, and quail eggs. The concentration of Pb, Cd, Cr, Cu, Fe, and Zn was estimated both for egg contents and eggshells (10 samples) by atomic absorption spectrophotometry compared with the standard curve. Estimated daily intake (EDI), Target hazard quotient (THQ), and Hazard Index (HI) were also calculated to observe whether egg intake possesses any health hazards or not. ***Results:*** Among all the trace elements estimated, the highest concentration was 35.36±0.4 (Zn) in white layer chicken eggs, and for eggshells, the highest concentration was 0.69875±0.082 mg/kg (Fe) in Quail eggshell. Except for Fe, Pb was relatively higher compared to other elements in eggs, the amount of Pb was the highest in Quail eggs (0.486±0.09 mg/kg). All the six elements were also within the acceptable range in eggshells where the amount of Pb was the highest in Brown layer eggshells, and Cu and Zn were the highest in indigenous eggshells. Cd and Cr were below the detection limit for all types of eggshells. Estimated daily intake (EDI), Target hazard quotient (THQ) and Hazard Index (HI) values indicated that intake of eggs does not possess any health risk. ***Conclusion:*** Interestingly, the heavy metal concentration of our samples belongs within the PTDI and USEPA limit. Therefore, based on our study, all egg samples were safe considering the average egg ingestion in our country. If the average egg consumption rises, people might be at risk for higher Pb consumption through egg intake.

INTRODUCTION

Eggs are consumed as a popular diet throughout the world and it is a highly important and available food source to meet the nutritional requirements providing 151 kilocalories of Energy, 12.5 grams (g) of protein, 391 mg of cholesterol, 200 mg of phosphorus, and adequate amounts of other vitamins and minerals, including Vitamin B_{12}, Vitamin A, Iodine, and Selenium [1]. Eggs of various bird species are consumed in different countries and their nutritional composition also varies significantly. In one Nigerian [2] study, the guinea fowl eggs were found to be superior in phosphorus, sodium, iron, zinc, and potassium contents while the trace elements were compared with exotic chicken, local chicken, turkey, and quail eggs. In another Greek [3] study, it was established egg yolks serve as the major source of trace elements compared to egg albumin. While the trace elements were assessed, turkey eggs contained the highest Zn, Mn, Cu, and Cr contents, Pigeon eggs contained the highest Se content and duck eggs contained the highest Mo contents in their yolk among chicken, turkey, duck, goose, and pigeon egg yolks. Among those species, Zn, Mn, and Co were the highest in pigeon egg albumin, Se and Cu were the highest in duck egg albumin, and Cr and Ni were the highest in goose egg albumin, respectively. The poultry industry is playing a pivotal role in satisfying not only the demand for protein, vitamins, and minerals but also contributing to economic growth, particularly in developing countries. The prices of poultry meat and eggs are declining with the progression over time due to the high production rate, this is why more people are becoming able to afford these food items, and thus food security is attained [4]. Despite being a widespread source of nutrition, concerns have arisen due to the presence of high heavy metal contents in both poultry products and feed. The level of Pb, Cr, and Se was found to be higher than the permissible human intake level both in eggs and feed, whereas, in backyard chicken eggs those levels were within the permissible limit according to a study in Pakistan [5]. Two studies in India and Egypt also depicted that the concentration of heavy metals in meat and eggs depends on the heavy metal concentration in soil and also on geographical variation. In the Indian study, the amount of Ni, Cu, and Co were quite high near the copper mining area, however, it did not possess the risk of developing any health hazards [6, 7]. Eggshell is still treated as a biowaste obtained from households and restaurants; however, researchers are trying to utilize eggshells for potential beneficial applications. Half of the chicken eggshells can satisfy the daily dietary requirement of calcium in humans. The eggshell powder can also be added to bread, fried chicken, spaghetti, and pizza without altering the texture and flavor of foods. Consuming eggshell powder also plays a pivotal role in enhancing bone density in postmenopausal women while consumed for a year, also prevent osteoporosis in animals without affecting blood calcium levels, and can also be used as a biofertilizer and soil stabilizer [8, 9]. Solid tannery wastes are converted into protein concentrate and are used as poultry and fish feed in Bangladesh which is responsible for the higher amount of Cr in the meat and eggs. Although in previous studies in Bangladesh, most of the samples of eggs do not contain heavy metals in hazardous concentrations, the exception was also present, and in one study a single egg contained more than 23μg of Cr [10, 11]**.** If heavy metal ingestion exceeds the accepted level, fatal consequences can occur among humans, including carcinogenesis by DNA damage, inactivation of regulatory proteins like p53, transcription factors, protein misfolding, aggregation, conformational change and may also lead cell damage by free radical generation [12].

As eggs obtained from various birds are a growingly popular food source in Bangladesh, we aimed to represent the amount of six trace elements in five widely available egg species in Bangladesh.

METHOD AND MATERIALS

Sampling

The study was carried out in the Jahangirnagar University area (23°52'56.6"N 90°16'01.6"E). Egg and eggshell samples (10 samples) were taken from Indigenous chicken, White layer chicken, Brown layer chicken (*Gallus gallus domesticus*), Duck (*Anas platyrhynchos*), and Quail (*Coturnix coturnix japonica*). Egg samples were taken from the local market and washed thoroughly with clean water, then labeled, stored in a sterile plastic bag, and then separated the eggshells from those eggs. The eggs content and eggshell were dried separately in sunlight and, after drying, grounded with agitating mortar to a fine powder.

Method

This experiment has similarities with a slight modification of Anwar *et al*., [13]. We have taken 10 samples (five kinds of eggs and their eggshells). At first, we weighted 1 gram of each sample powder and transferred it into a 100ml glass beaker. Added 25 ml of 65% HNO_3 to each beaker, gently shaken, and kept aside for a few minutes. Then the beaker was placed on the hot plate and cooled down. After that, 10 ml of perchloric acid was added to each beaker; which turned the solution to turn into orange color.

Then continued, the digestion process on the hot plate at a temperature between (100-300°C). When the color of the sample solution in the beaker changed from orange to a yellowish color by giving away the orange color fume. Then the digestion process was completed. Remove the beaker from the hot plate and cool down. After cooling, added 2ml of HCl and deionized water up to 60 ml. This time the color of the sample solution was turned into the color of sample solution was turned into light green color. Filtered the solution in 250 ml of the conical flask through Whatman filter paper and added 40ml of deionized water to make the total volume of the sample 100ml. Finally, the content of the flask was transferred into a plastic bottle for assessment by Atomic Absorption Spectrophotometer (Shimdzu AA7000).

Absorption of standard and sample solution by Atomic Absorption Spectrophotometry

A standard solution was prepared for all six trace elements and heavy metals that were tested at different concentrations. The concentration of those in the samples was calculated by plotting the absorbance on the standard curve constructed by AAS software.

Estimated daily intake (EDI) of heavy metals through consumption of egg

The EDI of heavy metals and trace elements is measured by the following equation:

$EDI = C_{metal} \times W_{food}$ / Body weight [C_{metal}=concentration of heavy metal, W_{food}=Weight of food]

Followed by Giri *et al.*, [6] According to the Bangladesh Bureau of Statistics, the average egg consumption was 13.58 grams/day in 2016, and the average chicken and duck egg consumption was 17.33 grams/day [14] C_{metal} was calculated by measuring the mean of three types of chicken eggs for estimating the EDI of chicken eggs, and for estimating the EDI of chicken and duck eggs, the mean of duck and three types of chicken eggs were considered. Quail eggs were not considered because they are rarely taken among the samples considered here and no data on quail egg consumption is available yet in Bangladesh.

Average body weight was considered for the Asian race (57.7 kg) as the study was conducted in Bangladesh according to Walpole *et al.*, [15].

The risk from the intake of metals through ingestion Target hazard quotient (THQ) and Hazard Index (HI)

Noncarcinogenic health risks from the consumption of chicken and egg by the local inhabitants were assessed based on the THQ. THQ values were obtained by dividing the EDI divided by the reference dose of the metal (RfD) mg/kg.

THQ= EDI/RfD

If the ratio is higher than 1, it is unlikely that there would be any obvious adverse effects on the exposed population. The method of estimating risk using THQ and Provisional Tolerable Daily Intake (PTDI) is provided by the U.S. Environmental Protection Agency (EPA) [16, 17].

The overall Hazard Index (HI) is calculated by adding all estimated THQ values of six metals for each type of egg.

Statistical analysis

Statistical analyses were done using Microsoft Excel 2019 by Microsoft Windows 10.

RESULT

Concentration of heavy metals and trace elements in the egg samples

Here in this table, the Quail's Egg has the highest amount of lead (Pb) and, cadmium (Cd), Chromium (Cr) among all egg samples. Brown colored Egg, Indigenous chicken's egg, and Duck's Egg has a Cd and Cu level of BDL. Cr and Pb are found in all types of samples. Duck's egg has the highest level of iron, and white-colored chicken has the highest level of zinc.

Table-1: Metals concentration (mg/kg) in the egg's samples

Sample(egg)	Pb	Cd	Cr	Cu	Fe	Zn
Brown Layer Chicken's egg	0.044±0.002	BDL	0.00208±0.0007	BDL	8.9±0.9	21.38±1.6
Indigenous Chicken's egg	0.0421±0.07	BDL	0.001±0.0005	BDL	11.8±1.05	22.1±1.5
Duck's egg	0.022±0.005	BDL	0.00168±0.0004	BDL	23.822±1.1	30.84±0.2
Quail's egg	0.486±0.09	0.024±0.00325	0.002475±0.0006	BDL	18.549±1.08	33.14±0.3
White layer Chicken's egg	0.0001±0.00001	0.0131±0.00106	0.00194±0.0004	0.018±0.0035	18.486±1.07	35.36±0.4

Concentration of heavy metals and trace elements in the eggshells

Here in eggshells, brown-colored eggshell has the amount of lead. Quail eggshell has the highest level of iron, and Indigenous chicken eggshell has the highest level of zinc. All eggshells have below the detection limit of Cd and Cr. Quail eggshell has the highest level of iron, and Indigenous chicken eggshell has the highest level of zinc.

Table-2: Metals concentration (mg/kg) in the eggshell samples

Sample (Eggshell)	Pb	Cd	Cr	Cu	Fe	Zn
Brown Layer Chicken's eggshell	0.4609±0.064	BDL	BDL	0.029±0.007	0.41±0.061	0.04595±0.006
Indigenous Chicken's eggshell	0.3496±0.0283	BDL	BDL	0.0375±0.003	0.4195±0.072	0.0635±0.003
Duck's eggshell	0.3607±0.065	BDL	BDL	0.0127±0.002	0.3115±0.053	0.035±0.005
Quail's eggshell	0.18855±0.01	BDL	BDL	0.0265±0.0035	0.69875±0.082	0.03845±0.004
White layer Chicken's eggshell	0.22185±0.057	BDL	BDL	0.0	0.2575±0.021	0.05375±0.003

Estimated daily intake (EDI) of heavy metals through consumption of eggs

Among all the six heavy metals and trace elements considered, each of them was under the tolerable level. Except for Pb, all the heavy metals were well below the PTDI. However, for consuming both chicken and duck eggs, people should be cautious as the level is quite similar to PTDI. If egg consumption increases above the current level, then people might ingest a higher amount of Pb.

Table-3: Estimated dietary intakes (EDI) of heavy metals of locally reared eggs (mg/kg)

Sample	Pb	Cd	Cr	Cu	Fe	Zn
Chicken Egg	0.0020	0.0003	$3.93x10^{-5}$	0.00042	0.0307	0.00619
Chicken and Duck Egg	0.0032	Undetectable	$5.03x10^{-5}$	Undetectable	0.0473	0.0082
PTDI	0.0035	0.003	0.5	0.003	0.8	1.0

The risk from the intake of metals through ingestion

Target hazard quotient and Hazard Index (HI)

As no THQ value was higher than 1.0, hypothetically it can be said that no egg samples possess the risk of developing potential health hazards, however, both chicken and duck eggs have a THQ of 0.9142 for Pb, quite close to 1. Therefore, a further increase of chicken and duck egg increase might possess a risk of Pb toxicity.

Table-4: Target Hazard Quotients (THQ) of heavy metals of locally reared egg and eggshell

Sample	Pb	Cd	Cr	Cu	Fe	Zn	HI
Chicken Egg	0.5714	0.0001	$9.825x10^{-7}$	0.00014	$4.391x10^{-5}$	$2.063x10^{-5}$	0.0605
Chicken and Duck Egg	0.9142	Undetectable	$1.257x10^{-8}$	Undetectable	$6.757x10^{-5}$	$2.733x10^{-5}$	0.00109
RfD	0.0035	0.003	0.04	0.003	0.7	0.3	

DISCUSSION

Our study aimed to provide information on the composition of heavy Metal in eggs and eggshells that had been previously lacking in the Savar region. All samples analyzed provided the information that all types of eggs and eggshells are free of risk of health hazards, and their concentration is tolerable according to the limit suggested by USEPA.

The increased content of heavy metals is contributed to the high intake of these elements by birds from feed and water. An increase in cadmium content of egg has a positive correlation with increased cadmium content of the feed [18, 19]. Compared to one study in Nigeria, the concentration of Zn was slightly higher in chicken egg samples, however, the Pb content of Brown and Indigenous chicken eggs was markedly higher than the Pb content in the eggs of Nigeria [20]. Only a small amount of Cd was found in our study ranging from 0.01- 0.02 mg/kg in the White layer chicken and quail eggs, which was even lower in Belgian egg samples. However, the concentration of Pb, Cu, and Zn was remarkably higher on those samples compared to our study [19]. Aendo *et al.,* illustrated in a comparative study on duck eggs that eggs obtained from small scale farms contain higher concentrations of Fe (27.57 mg/kg) and Zn (53.10 mg/kg), although the Fe content was quite similar (23.822 mg/kg) in our sample, the zinc content in that study was 1.77 times higher [21]. The mean level of Fe and Zn was also higher in the chicken eggs sampled from Tummalapalle uranium mine area, India. Another Indian study conducted in the Singhbhum copper belt, India depicted a lower level of Pb concentration among four of the 14 sample areas they considered, Fe content was higher than our samples in most of the samples, however, the zinc content was mostly lower in Indian samples. The EDI and THQ values were within the safe range for all of our egg samples, however, they crossed the ideal THQ for Cr in most of the samples, and in some samples, Pb content was also higher [6, 22]. Burger J in 1994 estimated the heavy metal in Avian eggshells, quite distinct from chicken and duck eggs, and Se, Mn, Cr, and Pb were comparatively higher than our samples and Cd was extremely low, whereas Cd and Cr were not detected in any of the egg samples [23]. Chicken eggshell powder is also familiar as a supplemental source of calcium and strontium. Schaafsma *et al.*, estimated mineral contents of various Dutch and Slovenian egg samples, where Fe and Zn were higher in our samples, however, Cd was significantly high in two of the dutch egg samples (0.018-0.024 mg/kg) and it was undetectable in all five egg samples that we tested [24].

Conclusion and Future Aspects

Health risks of metal exposure from egg consumption based on the EDI of metals and the THQ suggest that the local consumers are not at risk. However, increased consumption may lead to higher consumption of Pb in the future. Fe and Zn were comparatively present in higher concentrations in eggs, and the Fe content was also high among the tested trace elements in eggshells. Other heavy metals or trace elements can be assessed in eggs and eggshells like Co, Ni, Mn, Se, Sr, Ca, and As.

LIMITATIONS

We could assess only six constituents because our limitation of instruments; the HI level does not represent the true value here, because other heavy metals can also be present which we could not detect due to our limitations.

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