Exposure to environmental heavy metal (cadmium) through feed and its effect on biohistomorphological changes in commercial quail

MT Akter, KA Ferdous, T Rahaman, MA Hassan and T Monjur

Abstract
The present study was aimed to investigate the toxic effects of Cd exposure in commercial quail. A total number of 72 quail chicks (12 days of old) were assigned in four dietary treatments with three replicates. Control group T0 received only basal diet and the other groups T1, T2 and T3 received supplemented Cd with feed at a dose level of 0.2, 1 and 5mg/kg feed respectively. The body weight of each bird was weighed at 7 days interval and found decreases in weight gain significantly (P<0.01) among the Cd treated groups. Cd caused elevation of ALT (P<0.01) and decreased serum creatinine attributed to gross and histopathological changes in liver and kidney respectively. Gross pathological changes showed diffuse congestion, haemorrhage, presence of necrotic foci on liver and congestion in kidney and haemorrhage in muscle and lung. Accumulation of gas on intestine, mucosal erosion and discolouration of gizzard were also found in Cd treated birds. Histopathological lesions in liver included infiltration of reactive cells in central vein, hepatic vein and sinusoidal space in group T1 and T2 whereas group T3 showed highly necrosis of hepatocyte, picnotic nucleus and disarrangement of hepatic cord. In kidney, tubules were filled with reactive cells in all Cd treated groups while necrosis and disarrangement of tubules were found in groups fed Cd at higher doses (T3).

Keywords: Quail, cadmium toxicity, body weight, alanine transaminase (ALT), serum creatinine, morphological and histopatology

1. Introduction
The poultry sector is an integral part of farming systems that shares a significant contribution to the employment opportunity, food security and thereby economic growth and poverty reduction in the rural area of Bangladesh. Quail farming may be an alternative to chicken and ducks due to its immense potentiality for meat and egg production. Poultry production largely depends on quality feed. There are various sources of raw materials for poultry feed production and in many ways these sources of feed can be associated with anthropogenic heavy metal pollution. Cadmium, a rare but widely dispersed one of the most hazardous heavy metal and environmental pollutant. Most Cadmium is refined during zinc production. It is released into the environment through mining and smelting, usage of phosphate fertilizers, presence in sewage sludge and various industrial uses such as Ni, Cd batteries, plating, pigments and plastics. In the ground, cadmium moves easily through soil layers and is taken up into the food chain by uptake by plants such as leafy vegetables, crops, cereals and grains [25]. Accumulated Cd in various tissues of poultry remains as non-degradable heavy metal that can be transferred to human through poultry meat and impose health impact. ‘International Agency for Research on Cancer’ has classified cadmium and its compounds as a group I human carcinogen [36]. Although several studies have been done on the toxic effect of Cd in quail physiology, in details study of dose dependent impact of dietary Cd in body weight gain, the biochemical alteration and histo-morphological changes in visceral organs of quail has not been well addressed. Therefore, the present research work was undertaken to evaluate the effect of Cd on the growth performance, morphometric, hematological parameters and observe the histo-pathological changes in liver and kidney sample due to Cd toxicity in quail.

2. Materials and methods
In this study seventy-two 12 days old quail chicks about 87gm body weight were randomly allotted into four groups T0, T1, T2 and T3 (having 3 replications containing 6 birds in each replication). The experiment (rearing of quail, body weight measurement and blood collection)
was carried out in the laboratory of Anatomy and Histology department, Hajee Mohammad Danesh Science and Technology University (HSTU) during the period of 6 months from October 2017 to April 2018. The birds of group T0 were kept as healthy control group received only basal diet from Nourish Feeds Ltd. while birds of group T1 received Cd powder @ 0.2 mg/kg feed, group T2 @ 1 mg/kg feed and group T3 @ 5mg/kg feed. The chicks were supplied with fresh drinking water twice daily as per requirement. The chicks were kept under observation for 3 days with basal diet before starting of treatment with Cd. All birds during the treatment period were examined daily for abnormal physical and behavioral changes as well as mortality (if any) due to Cd toxicity. The effect of Cd toxicity on growth performance in quail was evaluated on the basis of average weekly feed consumption, body weight and feed conversion ratio. For average body weights, initial body weight of individual chick on first day of experiment was recorded. Subsequently body weights were recorded at seven days interval up to 29th days of experiment for each group. At the end of the experiment, 16 birds (4 birds from each replicate) from each treatment group were randomly selected and slaughtered after 12 h of fasting to collect theviscera (liver, kidney, lung, gizzard, intestine) and muscle samples to find the gross changes of those organs. Blood samples were collected from the wing vein at initial day and before slaughtering for blood analysis [Alanine Transaminase (ALT) and Serum Creatinine]. For histopathological study the collected samples were preserved for fixation in the Bouin’s fluid for 24 hours. The tissues were then dehydrated by using ascending grades of alcohol (70%, 80%, 90%, 95%, 100% and 100%) and kept for one hour in each grade of alcohol. The tissues were then transferred to the xylene-1 and xylene-2 each for ninety minutes. Then the tissues were infiltrated in the liquid paraffin at 60 degree centigrade temperature for ninety minutes and repeated again. Finally the tissues were embedded in paraffin and paraffin blocks were made. The paraffin blocks were cut at 6 μm thickness using microtome machine (Mu 509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45°C for stretching. The sections with glass slides were stained with Hematoxylin and Eosin’s (H & E) stain for general histological study. Observations of the slides were then done by using a light microscope and photographs were taken with an automatic photo micrographic system. Data were expressed as mean ± standard error (SE) and analyzed using one way Analysis of Variance followed by Duncan’s test as post-hoc test using IBM SPSS Statistics 20.0 software package and the chart was created by Microsoft Excel 2010 software. Results were considered to be statistically significant when P values are less than 0.01.

3. Results and Discussion
3.1 Clinical findings
In the present study, the salient clinical symptoms including depression, reduced feed intake, ruffled feathers, generalized weakness and gastrointestinal signs such as bloody diarrhoea were observed in birds treated with Cd. Intoxication were primarily related to the effects of Cd on the nervous, GI, hematopoietic and renal systems. The above findings were similar to Agency for Toxic Substance and Disease Registry that was observed by ATSDR., Osama SEO, Mohamed AL. etal., [22-2]. Diarrhoea in Cd poisoning that might be due to regurgitation and decreased motility of the upper GI tract (esophagus, proventriculus, and ventriculus) and signs related to hematopoietic impairment including weakness.

3.2 Body weight
During the experiment, supplemental dietary Cd significantly reduced body weight (P<0.01) compared to control (Table1). Decreased body weight was found throughout the experimental period in Cd treated birds and the rate of decrease was proportional to consumption of Cd. At 29th day of treatment, group T3 (Cd 5mg/kg feed) showed lowest body weight (78.7250±1.9gm) whereas group T2 (Cd 1mg/kg feed), group T1 (Cd 0.2mg/kg feed) and control T0 (no Cd) had body weight (91.7500±4gm), (99.5000±4.94gm) and (130.7500±1.49gm) respectively. Such finding is in agreement with previous findings of Anderson O at el, Omid K et al, Sajjad S et al., Osama SEO et al., Elmonem HA et al., Okeke OR et al., Vodella JK et al., Sant’Ana MG et al., [14, 7, 9, 26, 4, 37, 10]. The decreased body weight gain may be associated with several factors such as interruption in absorption and imbalanced metabolism produced by impairing zinc- dependent enzymes which are necessary for many metabolic processes and decreased level of erythropoietin hormone which has anabolic effect that was observed by Padilla MA et al., [14]. Lowered body weights in the heavy metal treated birds could be also due to the reduction in the feed utilization or due to the metabolic disarray resulting in loss of the cellular functions and tissue damage which was shown by Erdogan Z et al.,[28].

3.3 Gross findings
At the end of experiment, postmortem examination grossly showed diffuse congestion (Fig.1), haemorrhage (Fig.2) and presence of necrotic foci (Fig.5) on liver and in kidney congestion (Fig.4). These findings are in accordance with previous work of Jin T et al., Dehn PF et al., Tohamy HG et al., El- Sharak AS et al., [33, 25, 43, 27]. In muscle (Fig.5) and lung (Fig.6) there was also haemorrhage. Cadmium toxic effects induced the detoxification in liver and kidney enzymes which may lead to kidney dysfunction, hepatic injury, lung damage that was similar with the Foulkes EC., Foulkes EC., Webb M., [30, 29, 38]. Accumulation of gas on intestine (Fig.7), mucosal erosion (Fig.8) and discoloration of gizzard (Fig.9) were also found in Cd treated birds Cadmium initiates the generation of free radicals that was reported by Berzina N et al., [23], which may induce metal othinein (MT) production also reported by Markov J et al., [44] and participate in the manifestation of intestinal injury. No gross lesion was found in the quails slaughtered from the control group.

3.4 Microscopic findings
The microscopic view of liver in current study revealed that varying degree of degenerative changes as well as vascular changes like infiltration of reactive cells in central vein, hepatic vein and sinusoidal space in Group T1 (Fig.11) and T2 (Fig.12) whereas group T3 (Fig.13) showed highly necrosis of hepatocyte, picnotic nucleus, disarrangement of hepatic cord of liver. Appearance of inflammatory cells in the hepatic tissue might be due to the interaction between proteins and enzymes of the hepatic interstitial tissue which interfered with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation which in turn may imitate an inflammatory response that was compliance with Shah TM et al., Anderson O., Omid K et al., [11, 3, 6]. Microstructure of liver of control group T0(Fig.10) seems no
changes. Microscopic view of kidney of group T1 (Fig.15) and T2 (Fig.16) showed reactive cells infiltration in the tubular and peritubular space, kidney tubules (proximal and distal convoluted tubules & Henley’s loop). Necrosis in kidney tubules and deformation of normal structure of kidney tubules found in the group T3 (Fig.17). These findings are in agreement with previous study of Hesaraki S et al., Bharavai K et al., Binkowski L3 et al., Uyanik F et al., Shukla GS et al., Ibraheem AS et al., [13, 15, 16, 19, 26, 27] had reported that cadmium may induce oxidative damage in a variety of tissues by enhancing peroxidation of membrane lipids due to inhibition of antioxidant enzymes. By intake of Cadmium stimulates the formation of beta 2-microglobulin in urine which induces renal tubular dysfunction reported by Suganya T et al., [18] Microstructure of kidney of T0 (Fig.14) was normal.

3.5 Hematological parameter

Birds exposed to Cd showed a significant decrease in serum creatinine (P<0.01) at 29th days of treatment as compared with the control group (table 2). Decreased serum creatinine was found in group T3 (0.12±0.04787), T2 (0.33±0.04082) and T1 (0.40±0.0482) with doses of 5mg/kg feed, 1mg/kg feed and 0.2mg/kg feed respectively compared to control T0 (0.60±0.0482) received normal feed. On the other hand, birds exposed to Cd showed a significant increase in SGPT/ALT (P<0.01) concentrations at 29th day of experiment as compared with the control group (table 2). Highest SGPT was found in group T3 (25±4.78715) followed by group T2 (18.3±3.81452) and group T1 (9±0.23805) compared to control T0 (9.0003±0.04082). The increase in concentration of AST and ALT in blood plasma indicates disease in treated birds. This finding is similar to some findings Omid K et al., Saeed AA et al., Elmonem HA et al., Sajjad S et al., [41, 42, 26, 5] study. The increase in concentration of ALT enzymes in blood may also be used as a stress indicator. The increase in concentration of AST and ALT in blood plasma indicates impairment of liver.

<table>
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<th>Days</th>
<th>T0</th>
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<th>T3</th>
<th>p value</th>
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<td>22nd day</td>
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<td>29th day</td>
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<td>99.5000±4.94132b</td>
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Means on the same row with different superscripts are significantly different (P<0.01). SE: Standard Error, **means highly significant, NS means Non-Significant

<table>
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Means on the same row with different superscripts are significantly different (P<0.01). SE: Standard Error, **means highly significant, NS means Non-Significant

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Means on the same row with different superscripts are significantly different (P<0.01). SE: Standard Error, *means significant at the level of 1%, NS means Non Significant
**Fig 1:** Congestion of liver

**Fig 2:** Haemorrhage on liver

**Fig 3:** Presence of necrotic foci on liver

**Fig 4:** Congestion of kidney

**Fig 5:** Haemorrhage on muscle

**Fig 6:** Haemorrhage on lung

**Fig 7:** Accumulation of gas on intestine

**Fig 8:** Mucosal erosion of gizzard

**Fig 9:** Discoloration of gizzard

**Fig 10:** Microscopic view of liver in T₀ group (H and E; 10x)
4. Conclusions
A large portion of Bangladeshi people consume poultry meat and egg to fulfill their protein demand. The exposure of the heavy metals like Cd may affect the physiology of poultry which in turn may be the issue of public health through feed chain. It was observed from the current study that supplementation of Cd in quail diets at 0.2, 1 and 5 mg/kg feed produced various deleterious effects on growth performance; gross and microscopic study of different organs as well as biochemical parameters. Decreased body weight in Cd treated birds was due to malabsorption and altered
metabolism. Severe gross and histo-morphological changes were recorded in liver and kidney.

5. Acknowledgements
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6. References

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