Effects of dietary supplementation of lead (Pb) on biochemical, gross and histo-morphological changes in different organs of broiler chicken

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Abstract

Background

Nowadays poultry industry, an important sector is becoming a serious threat to public health due to the heavy metal exposure & accumulation in poultry tissues. Therefore, our recent study was aimed to investigate the toxic effects of lead (Pb) exposure in broiler chicken.

Methods

A total number of 72 broiler chicks (Cobb-500, 12th day old) were assigned to four dietary treatments with three replicates. Control group received only basal diet without any supplementation. The other groups T1, T2 and T3 received feed with supplemented Pb @ 10, 30 and 50mg/kg feed, respectively. The body weight of each bird was weighed at 3 days interval.

Results

Lead caused elevation of SGPT/ALT (P<0.01) and decreased serum creatinine attributed to pathological lesions including enlarged, pale & friable liver, swollen kidneys and splenomegaly in experimental groups. On histopathological examination, liver shows cirrhosis and necrosis in all treated groups. In the kidney, glomerulus was filled with reactive cells in group T1 while fibrosis and necrosis were found in groups T2 & T3.

Conclusions

Lead toxicity in broiler had a dose-dependent effect on body weight gain, blood parameters, gross and histological changes.

Key words: Lead toxicity (Pb), body weight, SGPT/ALT, serum creatinine, histopathology

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Introduction
Over the last several years, poultry has become very popular and promising sector in Bangladesh. Poultry industry contributes 1% to the country’s GDP while millions of people are involved in the sector for their livelihood. It met up 106.21 gram/day/head of total protein while the demand is 120 gram/day/head in Bangladesh (DLS, 2016). Such blessing of poultry sector has resulted in an increase in the number of commercial poultry feed producers. The raw materials for the production of poultry feeds are of various origins. The exposure of these sources to various anthropogenic pollutants, especially heavy metals like lead (Pb) may affect poultry food chain through the feed. Heavy metals found in all living organisms where they play different roles. They may be components of control mechanisms (e.g. in muscles and nerves) and enzyme activator or redox systems. Rice bran and maize the two major components of poultry feed can be the source of Pb by absorbing the excess Pb from contaminated soil. Nearly 50% poisonous metal (Pb, Hg and Cd) comes via the contaminated food of plant origin (fruit, vegetables and cereals). In poultry industry, use of lead paint in different equipment’s (drinkers and feeders) as well as litter material from woods (North and Bell, 1990); Fumes from gasoline (Genevieve & Greg, 1994); contaminated bone and blood meal is the sources of lead poisoning. Pb toxicity results in biochemical alterations including changes in certain enzyme levels (ALT/SGPT, serum creatinine) in extracellular fluids that may affect the growth and productivity of broilers. Ingested Pb is accumulated in liver and transfer to the kidney, from which small amount passes through urination and the rest conjugated in most of the important body organs and hamper their ability specially the kidney as a target site for Pb toxicity (Jarrar et. al., 2000; Jarrar et. al., 2001, Taib et. al., 2004 and Wynee et. al., 2007). Supplementation of heavy metals with a large safety margin in broilers has resulted into higher mineral excretion and ends up in the environment (Demirezen and Uruç, 2006; Abdul et. al, 2012). However, accumulated Pb in various tissues of poultry remain as non-degradable heavy metal that can be transferred to human through poultry meat and impose health impact. Therefore our present study was conducted to evaluate the dose dependent impact of dietary Pb in body weight gain, the biochemical changes including gross & microscopic changes of many visceral organs of broiler.

Materials and Methods

Study area and duration
The experiment was conducted from May 2016-October, 2017 at Hajee Mohammad Danesh Science and Technology University (HSTU), Basherhat, Dinajpur, and histopathology were performed at laboratory of the Anatomy and Histology, HSTU.

Experimental birds
In this study, 72 broiler chicks (Cobb-500 strain) of 12th day old were randomly allotted into four groups (T₀, T₁, T₂, and T₃). Each group consists of 6 birds with 3 replications. Birds were randomly distributed into the cages. The birds of the group T₀ were kept as healthy control received only basal diet broiler grower from CP feed while, birds of group T₁, T₂, and T₃ received lead powder @10, 30, 50 mg/kg feed, respectively. The chicks were supplied with fresh drinking ad libitum. The chicks were kept under observation for 3 days with basal diet before starting of treatment with Pb. Birds were reared up to 42 days. All birds during the treatment period were examined daily for abnormal physical and behavioral changes as well as mortality (if any) due to lead toxicity.

Recording growth performances after lead (Pb) treatment
The effect of lead toxicity on growth performance in broilers was evaluated on the basis of average weekly feed consumption, body weight, and feed conversion ratio. For average body weights, the initial body weight of individual chick on the first day of the experiment was recorded. Subsequently, body weights were recorded at three days interval up to 42 days for each group.
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Collection of sample

Blood and viscera
At the end of the experiment, 12 hens (3 hens from each replicate) from each treatment group were randomly selected and slaughtered after 12 h of fasting. Viscera (liver, kidney, heart, gizzard, spleen) and muscle samples were collected to observe the gross changes and further histopathological study. Blood samples were collected from the wing vein before slaughtering labeled into EDTA bottles from each group of bird for blood analysis [Serum glutamic pyruvic transaminase (SGPT)/ Alanine Transaminase (ALT) and serum creatinine.

Histopathology of sample
Histopathology of liver and kidney samples from each group were performed as described elsewhere (Bancroft et. al., 2002).

Statistical analysis
Data were expressed as the mean ± standard error (SE) and analyzed by using one-way analysis of variance (ANOVA) followed by Duncan’s test as a post-hoc test using IBM SPSS Statistics 20.0 software package. P<0.01 or less were considered as statistically significant in comparison with control group.

Results and Discussion

Clinical findings
In the present study, lead treated birds showed noticeable clinical symptoms (mild depression, reduced feed intake, dullness, rough feathers and greenish diarrhoea that stained feathers around the vent). The above findings were similar to other researchers (Jordan et al., 1996; Puschner and Robert, 2009 and Ritesh et al., 2011) that might be due to regurgitation and decreased motility of the upper GI tract (esophagus, proventriculus, and ventriculus) and signs related to intoxication.

Effects on body weight
During the experiment, supplemental dietary lead significantly (P<0.01) reduced body weight compared to control (Table1). Decreased body weight was found in Pb treated birds and the rate of decrease was proportional to consumption of lead. At 42th day of treatment, group T₃ showed lowest body weight (1825.6±0.89 gm) whereas group T₂, T₁ and control T₀ had body weight (2227.2±1.29 gm), (2231.1±1.59 gm) and (2447.5±1.23 gm), respectively which is in agreement with previous findings by (Morgan et al., 1975; Erdogan et al., 2005 and Ibitoye et al., 2011). Different factors may responsible for body weight reduction such as interruption in absorption and abnormalities in metabolism (Marchlewicz et al., 2007; Sakata et al., 2007; Richardson et al., 2006; Abd et al., 2006; Rahman and Joshi, 2009; Salwa et al., 2013; Haouas et al., 2014).

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Table 1. Effects of different levels of lead on growth performance (gm) of broilers from 15 days to 42 days old

| Days | Various treatment groups showing mean ± SE values | Level of Significance |
|------|---------------------------------------------------|----------------------|
|      | T₀       | T₁       | T₂       | T₃       |                      |
| D₁₅ | 480.83±2.07<sup>a</sup> | 476.67±1.85<sup>a</sup> | 475.83±1.53<sup>a</sup> | 475.28±1.37<sup>a</sup> | NS                   |
| D₁₈ | 663.06±1.00<sup>a</sup> | 537.22±1.16<sup>d</sup> | 579.11±1.26<sup>b</sup> | 562.22±1.68<sup>c</sup> | **                   |
| D₂₁ | 855.56±1.06<sup>c</sup> | 726.11±1.79<sup>d</sup> | 735.00±0.1<sup>c</sup>  | 740.56±1.56<sup>b</sup> | **                   |
| D₂₄ | 1069.4±1.75<sup>a</sup> | 952.22±1.29<sup>c</sup> | 961.11±1.59<sup>b</sup> | 955.00±0.1<sup>c</sup>  | **                   |
| D₂₇ | 1291.1±1.59<sup>a</sup> | 1229.4±1.20<sup>b</sup> | 1227.8±1.29<sup>bc</sup> | 1224.4±1.20<sup>c</sup> | **                   |
| D₃₀ | 1546.1±1.49<sup>a</sup> | 1441.9±1.46<sup>d</sup> | 1431.9±1.52<sup>c</sup> | 1320.0±0.1<sup>d</sup> | **                   |
| D₃₃ | 1798.9±1.59<sup>a</sup> | 1642.2±1.29<sup>b</sup> | 1636.1±0.95<sup>c</sup> | 1431.9±1.46<sup>d</sup> | **                   |
| D₃₆ | 2070.6±1.66<sup>a</sup> | 1847.8±1.29<sup>b</sup> | 1838.9±1.59<sup>b</sup> | 1500.0±1.4<sup>d</sup>  | **                   |
| D₃₉ | 2178.9±1.59<sup>a</sup> | 2150.0±0.1<sup>b</sup>  | 2131.1±1.59<sup>c</sup> | 1678.9±1.59<sup>d</sup> | **                   |
| D₄₂ | 2447.5±1.23<sup>a</sup> | 2231.1±1.59<sup>b</sup> | 2227.2±1.29<sup>c</sup> | 1825.6±0.9<sup>d</sup>  | **                   |

Means on the same row with different superscripts are significantly different (P < 0.01). SE: Standard Error, NS: Non-significant, ** Means: Significant at 1% level
Changes in blood parameters
According to the current study, lead treatment influenced the serum creatinine level (Table 2). In group T_3, T_2 and T_1 had lowered creatinine 0.2±.002, 0.2±.0032 and 0.3±.0019 mg/dl, respectively compared to the control T_0 (0.4±.004). Significant (P<0.01) decrease in creatinine was reported in lead acetate treated rats (Rumana et. al., 2002) which is similar to this study. Decrease serum creatinine could be associated with different dose, route of administration, species and other environmental or dietary factors.

On the other hand, SGPT was significantly (P<0.01) increased in group T_3, T_2 and T_1 compared to control group T_0 (Table 2). Increased transaminases in serum levels which are normally remain in cytosol of hepatocytes, an indication of primary liver dysfunction in treated birds. This finding is similar to (Moussa and Bashandy, 2008; Mehana et. al., 2010; Monira et. al., 2012; Sayed et. al., 2015 and Yaseen et. al., 2015). SGPT is present in highest concentration in liver and the smaller amount in intestines, heart, kidney, skeletal muscles and RBC and its elevated level is a cardinal sign of hepatocellular damage. Increased blood SGPT level is also important sign of cellular damage, impaired metabolism due to Pb toxicity and impermeability of plasma membrane (Upasani and Balaraman, 2001). Such biochemical changes in kidney and liver associated with structural alteration in those organs are more supported by the histological study.

Table 2. Serum biochemical parameters of broilers at 42th days fed varying doses of Lead

| Parameters             | Various treatment groups showing mean ± SE values | Level of Significance |
|------------------------|--------------------------------------------------|-----------------------|
|                        | T_0         | T_1         | T_2         | T_3         |                      |
| Serum creatinine (mg/dl)| 0.4±.004^a  | 0.3±.0019^b | 0.2±.0032^c | 0.2±.0024^c | **                   |
| SGPT (U/L)              | 6.32±.017^d | 10.02±.039^c| 11.17±.038^b| 35.83±.019^a| **                   |

Means on the same row with different superscripts are significantly different (P < 0.01). SGPT: Serum Glutamic Pyruvic Transaminase
SE: Standard Error, ** means significant at 1% level

Gross findings
On gross observation, birds of different treatment group showed pale color liver and hepatomegaly (Fig. 1), hemorrhage in thigh muscle (Fig. 2), enlarged kidney (Fig. 3), and splenomegaly (Fig. 4). These findings are in accordance with previous work of (Suradkar et. al., 2009; Yaseen et. al., 2015; Shah et. al., 2016). Similar changes were also observed elsewhere (Chauhan et. al., 1995 and Radostits et. al., 2006) they showed gastroenteritis, diffuses congestion of lung and degeneration of liver and kidney. The possible reasons for the occurrence of gross changes in liver and kidney are their involvement in lead metabolism and excretion as they are the primary target organs for lead fate. Chauhan et. al., (1995) reported that splenomegaly may occur secondarily due to increased removal of the lead damaged erythrocyte. Splenomegaly also reported by Riggs et. al., 2002. Gross examination of the control group (group T_0) revealed normal liver, heart, lung, kidney, spleen and muscles.

Microscopic findings
In the microscopic study, liver of treated birds (group T_1 and T_2) showed cirrhosis and necrosis (Fig.5) whereas group T_3 showed highly necrosis and cirrhosis (Fig. 6). Shah et. al., (2016) reported that appearance of inflammatory cells in the hepatic tissue might be due to the interaction proteins and enzymes of the hepatic interstitial tissue which interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation which in turn may imitate an inflammatory response. Similar observation was also found by (Péter et. al., 2003; Taib et. al., 2004; Wynee et. al., 2007; Sayed et. al., 2015 and Yaseen et. al., 2015).

In the present study, the experimental groups showed disorganized hepatic architecture with the
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marked affection of the hepatocytes due to fibrosis which was similar to the study of Sayed et. al., 2015. Liver fibrosis is the ultimate fate of nearly all chronic liver diseases where extra cellular matrix (ECM) is filled with excessive connective tissue. Hepatic stellate cells (HSCs) are indirectly activated by ROS (Reactive oxygen species) which are responsible for liver fibrosis (Bartosz, 2008). HSCs remain in the space between sinusoidal endothelial cells and the hepatocytes known as Disse’s space. HSCs consist of non-parenchymal cell and normal liver cell. In normal liver, HSCs remain inactive to store vitamin A in the body (Pande, 2002). Pathogen or hepatic toxin may cause liver dysfunction and HSCs become activate subsequently proliferation and fibrogenesis of hepatocyte resulting hepatic fibrosis. Active HSCs play a key role in ECM accumulation (Del Monte, 2005). Researcher showed that liver fibrosis is responsible for decreased in storage of retinol (vitamin A) in liver. Decreased vitamin A in liver changes the HSCs to fibroblasts and leading to fibrogenesis (Shinozoka et. al., 1996).

Microscopic view of kidneys belongs to group T1 showed hyper cellularity of glomeruli (Fig. 7) while lining cells are necrosed and fibrous tissue accumulation found in the kidneys of group T2& T3(Fig. 8). These findings are in agreement with the previous study of Sujatha et. al., 2011 and Shah et. al., 2016. These histopathological lesions found in kidneys might be due to accumulation of lead-protein complex which causes discernible changes in proximal tubular linings of cells. Lead deposited predominantly in the proximal tubule may also be considered the main reason for its deleterious effects on the cortex of the kidney.
Fig. 5. Microscopic view of liver: showing cirrhosis (black arrow) and necrosis (blue arrow) in group T₁ & T₂ (H and E; 10x)

Fig. 6. Microscopic view of liver: showing comparatively highly cirrhosis (black arrow) and necrosis (blue arrow) in group T₃ (H and E; 10x)

Fig. 7. Microscopic view of kidney: group T₁ showed no remarkable changes in the kidney tubules (proximal and distal convoluted tubules & Henle's loop). Glomerulus (black arrow) seems to be populated with phagocytic/reactive cells (H and E; 10x)

Fig. 8. Microscopic view of kidney: Lining cells are necrosed (blue arrow) and thereafter fibrous tissue accumulation (black arrow) in group T₂ and T₃ (H and E; 10x)
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Conclusions
Exposure of the heavy metals like Pb 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 Pb in chicken diets at 10, 30 and 50 mg/kg feed produces various deleterious effects on growth performance, gross and microscopic study of different organs as well as biochemical parameters. Therefore, it is recommended for further study to determine the affinity of Pb in different organs. Moreover, the economic losses in the farming sector due to Pb exposure & its preventive strategies should also be undertaken.

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Competing interest
The authors declare that they have no competing interests.

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