

Original Article**Bioaccumulation of Lead in the Tissues of Japanese Quails and Its Effects on Blood Biochemical Factors**Fatemeh Hamidipour^{*1}, Hamid Reza Pourkhabbaz², Mahdi Banaee³, Saeideh Javanmardi²

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ABSTRACT

Background: Lead is the oldest known toxic metal, physiologically and biologically harmful to living creatures. This study aimed to evaluate the lead accumulation in the liver and breast muscles of Japanese quail (*Coturnix japonica*) and to compare concentrations in both sexes and its effect on blood biochemical factors.

Methods: Twenty-four young farm Japanese quails (25 day old) prepared from local breeders in December 2014 and randomly divided into control and treatment group. Treatment group were exposed to 0.4 mg per kg diet of "Lead Acetate" for 21 days. We studied the effects of lead on survival and blood biochemical factors. The lead accumulation in the liver and breast muscles of Japanese quail was determined using atomic absorption.

Results: Exposure to lead caused a significant increase in the activity of enzymes (AST), (ALT), (LDH), glucose, creatinine and uric acid in poultry treated with lead compared with the control group ($P<0.05$). In addition, significant decrease in the activity of ALP, AChE, total protein, albumin, globulin, and triglycerides was found ($P<0.05$). The treated group had no significant change in the activity of CPK and cholesterol. Lead accumulation was more in the liver rather than the breast muscle. There was no significant difference between males and females as for concentration of lead in muscle and liver of quail.

Conclusion: Quail have capabilities to accumulate lead in their tissues. In addition, it can lead to apparent changes in enzymes and blood biochemical factors, which show adverse effects of heavy metals on the immune and physiological system of birds.

Keywords: Bioaccumulation, Biochemical Factors, Japanese Quail, Lead.

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INTRODUCTION

Lead is a dangerous heavy metal with negative effects on birds in natural and laboratory environment especially on the endocrine system [1]. Weight loss and impaired reproduction [2], poisoning, poor performance and death are other consequences of lead poisoning [3]. Birds can maintain high level of metals in their tissues, including liver and kidney [4], then these compounds may find their way through the food chain to human and other creatures causing a range of physiological and biochemical abnormalities in central and peripheral nervous system, red blood cells, cardiovascular system, kidneys and reproductive organs [5, 6].

It can influence natural behaviors of living creatures [7, 8]. Therefore, considering

continues increasing of environmental pollution, the use of biological implement to assess and monitor environmental contamination seems necessary. Japanese quail (*Coturnix japonica*) is suitable as a model for the study of toxicity in animals for being readily available, easy to handle, maintainable under laboratory conditions [9], much lower feed and space requirement than the domestic fowl [10], disease resistance [10, 11], economical viability in farming [11] and small size which accelerates the accumulation of heavy metals in their tissues just in two weeks [9]. Japanese quail recently emerged as one of the important species of poultry in Iran and it gradually found its place in food basket. Quail's meat with its unique capabilities, quick digestion, low in cholesterol, high in protein, is best for children, elderly and hypertensive

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peoples [10, 12]. Japanese quail is very similar physiologically and biologically to the wild breed.

The purpose of this study was to determine the concentration of lead in the liver and breast of the Japanese quail and its biological effects on blood biochemical factors

MATERIALS AND METHODS

This experimental study was carried out in Behbahan Khatam Alanbia University of Technology in December 2014. Twenty-four young Japanese quails (25-day-old) weighted average 152.97 ± 54.44 gr were used in the present study that the university ethics committee approved the study according to National Ethical Framework for Animal Research in Iran [13].

The birds maintained and adapted for 15 days under lab conditions: 16-h lighting, 8 h darkness, at temperature $25 \text{ }^\circ\text{C} \pm 2$ and relative humidity 25% to 50%. After the acclimatization period, the birds were randomly kept into two groups of 12. Treatment group were fed a diet containing 0.4 mg lead acetate per 1 kg diet for 21 d, while the control group were fed with normal diet. The density of lead acetate considered in this experiments according to a tenth of a lethal concentration of Pb (LD50 value was 4 ppm) [3].

Blood Sampling

Blood sample were prepared from 24 adult quail (60 d) of both sexes. They were healthy and were kept in the cage. Before sampling, they were kept in good condition, catching and wrapping them with towels and put them in a position to reduce tension. After chloroform made them unconscious and cutting feathers and cleaning sampling area (Wing Vein), blood was taken by 2 ml heparinized syringe and poured into micro-tubes with identified labels. Micro-tubes were put in a centrifuge for 15 min at 6000 g. After plasma separation, it stored at $-21 \text{ }^\circ\text{C}$ for biochemical tests.

Measuring the Blood Biochemical Parameters

Biochemical parameters were measured using a UV/ VIS spectrophotometer (model UNICO 2100) and standard biochemical reagents (Pars Azmoon Company, Tehran, Iran).

Total plasma protein was determined by measuring the absorbance at 540 nm by the Biuret reaction. Albumin level was measured by the immediate Bromocresol Green reaction at 630 nm.

The plasma globulin was measured based on the ratio of albumin to total protein [14]. Plasma glucose was determined by the glucose-oxidase method at 500 nm [15], plasma cholesterol levels by the CHOD-PAP method at 510 nm, triglyceride levels by GPO-PAP method at 546 nm [16] and creatinine by the JAFFE method at 510 nm [17]. Furthermore, uric acid was determined by kinetic colorimetric assay by TOOS method at 546 nm [18]. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined by NADPH consumption and its conversion to NAD^+ at 340 nm. Lactate dehydrogenase (LDH) in plasma was determined based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) by converting nitro phenol phosphate into nitrophenol and phosphate at 405 nm, and creatinine phosphokinase (CK) based on the conversion of creatinine phosphate into creatinine at 340 nm [19]. Plasma acetylcholinesterase (AChE) activity was determined by adding an adequate volume of sample into a cuvette containing 0.1 M phosphate, pH 8.0, and acetylcholine iodide (0.015 M) and dithiobis nitrobenzoic acid (0.01 M) as substrates. AChE activity was recorded during 180 s at 405 nm [20] and based on optical density (OD) absorption and the formula provided in the kit's manual. All biochemical parameters were measured according to the instructions provided in the manual.

The Preparation of Tissue Samples

In atomic absorption method, all the material and tools should be soaked in 5% nitric acid for about 24 h then washed twice with distilled water. This is done to minimize possible error. The birds were weighted after labeling and morphometry (including length of body and wingspan). Weighting precision was 0.001g. Sex determination was accomplished with reproductive organs located on top of the kidneys and breast feathers appearance. Finally liver tissue and breast muscle were separated and placed in a contamination free zip bag and after

passing the freezing time (at -20°C) were dried in the oven (at 60°C for 48 h).

Dried samples (liver and muscle) were powdered by pestle and mortar [21]. For chemical digestion of the samples, the amount of one gram of tissue powder poured into 50 ml Erlenmeyer flasks and a ratio of 1 to 2 nitric acid and perchloric Acid was added [22]. For this purpose first, 10 ml of 65% nitric acid were added to samples in each Erlenmeyer flask, and fully closed with Parafilm. All samples were kept overnight at room temperature (20°C) to digest gradually. On the second day, 5 ml perchloric acid 72% were added to each sample and were put on a hot plate at 150°C for 6 h until the solution was clear and the samples fully digested. After digestion, samples were placed in the ambient to cool. The obtained solutions were passed through 42 microns Whatman filter and reached the volume to 25 ml using deionized water. Besides digested samples, in each set of 15 hot plates, 3 witness samples were prepared along with other analyzed samples for verification of atomic absorption spectrometry and the purity of the acid used. Finally, the samples were injected to "ICP atomic absorption" (*Jobin iyvan* 138 models) to determine concentration.

Lead levels were measured in triplicates and measurements were repeated three times. Ultimate lead concentration calculated using relation 1.

$$M = CV/W \quad (\text{relation 1})$$

C: concentration obtained from the device (ng/ml)

V: ultimate sample volume (ml) (in this study was 25ml)

W: dry matter intake for digestion (g) (in this study was one gram)

M: ultimate concentration of sample was measured by ppb or ng/g dry weight.

Statistical Analysis

Statistical analysis were performed using SPSS (IBM, 22) and excel (2013). All the data were tested for normality by "Shapiro-Wilk" test. The statistically analysis for compare between two groups was done with the "Students *t*-test" (at 95% assurance). Relationship between gender and lead concentrations in the tissues of

quail was determined by Pearson correlation coefficient. Data are presented as mean \pm SD.

RESULTS

Quail bioassay results are presented in Table 1.

Table 1. Quail bioassay results.

	means \pm S.D	max	min
Total length of body (cm)	21 \pm 0.52	22	20
Wing length (cm)	16 \pm 0.7	17	15
Wing width (cm)	6.5 \pm 0.44	7	6
Wing span (cm)	37 \pm 1.6	38/5	35
Weight (gr)	221.75 \pm 40.16	270	173

Blood Biochemical Parameters

The results of the measurement of biochemical parameters are presented in Table 2. The activity of the enzyme AST, ALT, LDH), glucose, creatinine and uric acid in treated quail with Pb showed a significant increase compared to the control group ($P < 0.05$). In addition, it showed a significant decrease in the activity of ALP, acetylcholinestrace (AChE), total protein, albumin, globulin, and triglycerides, compared to the control group ($P < 0.05$). The treated quail with lead did not show a significant change in the activity of creatine phosphokinase (CPK) and cholesterol (Table 2).

The Concentration of Lead in the Tissues of Quails

The average level of lead in the liver and breast of quail is shown in Table 3. The average concentration of lead in the liver of male and female was respectively, 0.182 ± 0.004 and 0.184 ± 0.002 $\mu\text{g/g}$ DW, and in breast tissue in males and females were respectively, 0.16 ± 0.002 and 0.159 ± 0.007 $\mu\text{g/g}$ DW. The highest concentration of lead in the liver was 0.187 and the lowest in the breast tissue was 0.150. According to the *t*-test there is a significant difference in terms of accumulation between liver and muscle of the chest ($P < 0.05$) (Table 3).

Table 2. Changes in the blood biochemical parameters of Japanese quail after feeding 0.4 mg lead acetate per 1 kg food for 21 days.

Parameters	Control	Experimental
AChE (U/L)	2430.48 ± 162.70a	1950.98 ± 114.79b
AST (U/L)	115.50 ± 7.25b	133.80 ± 7.62a
ALT (U/L)	35.80 ± 3.47b	43.67 ± 6.37a
ALP (U/L)	986.19 ± 117.35a	378.93 ± 36.69b
LDH (U/L)	176.92 ± 41.70b	260.04 ± 40.95a
CPK (U/L)	385.34 ± 52.16a	327.71 ± 140.90a
Glucose (mg/dL)	188.51 ± 6.32b	203.40 ± 1.70a
Total Protein (g/dL)	5.81 ± 0.23a	4.65 ± 0.39b
Albumin (g/dL)	3.28 ± 0.15a	2.93 ± 0.19b
Globulin (g/dL)	2.55 ± 0.43a	1.72 ± 0.55b
Cholesterol (mg/dL)	148.61 ± 16.05a	134.60 ± 25.82a
Triglyceride (mg/dL)	186.92 ± 29.71a	127.66 ± 9.30b
Creatinine (mg/dL)	0.28 ± 0.05b	0.48 ± 0.08a
Uric acid (mg/dL)	4.96 ± 0.06b	5.71 ± 0.04a

Significant differences between values when compared with control groups were characterized by alphabet symbol ($P < 0.05$). Values represent mean \pm S.D.

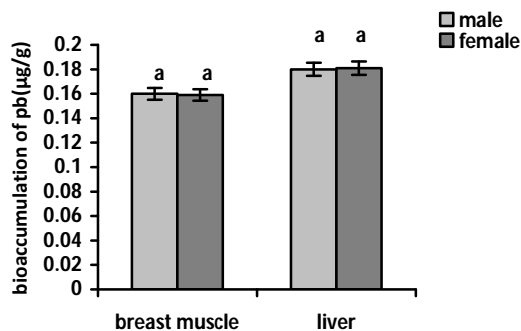
Table 3. Showing levels of lead ($\mu\text{g/g}$ DW) in different tissues after the exposure period (21 days).

metal	Tissue	Experimental	Min	max	Control	P value
Lead	Liver	0.181 (± 0.003)	0.177	0.187	0	0.00*
	Breast muscle	0.159 (± 0.005)	0.150	0.167	0	

*significant differences ($P < 0.05$)

Effect of Gender on Bioaccumulation of Lead in Different Tissues of Quail

Obtained results of *t*-test compare the amount of lead in the male and female sexes showed no significant difference between the sexes in terms of accumulation in various tissues (Figure. 1).

**Figure 1.** Mean concentration of lead ($\mu\text{g/g}$ DW) in the male and female sexes of quail.

Relationship between Gender and Lead Concentration in the Tissues of Quail

Pearson correlation test results shows that the relation between gender and the accumulation of lead in breast is negative and in

liver is positive, though this relationship is not statistically significant and meaningful (Table 4).

Table 4. Correlation between gender and Lead concentration in the tissues of quail.

N=24	Sex	Breast muscle	Liver
Sex	1	-0.154	0.337
Breast muscle		1	0.008
Liver			1

*correlation is significant at the 0.01 level

*correlation is significant at the 0.05 level

DISCUSSION

The results of the rate of lead accumulation in the liver and muscle tissues of the breast confirm that lead accumulation in the liver is far greater than the breast muscle and the lowest accumulation is in breast tissue, which corresponds with previous studies [23, 24]. It may be due to liver detoxification mechanism and being the target organ. Due to the accumulation of fat tissue more than pectoral, it receives more lead. In addition, breast muscle tissue may have certain tissue structure and biological activity.

The amount of lead in liver tissue was more than the breast muscle, but there was no significant difference as for the lead levels in males and females. Previous studies on the coot, Siberian gulls and green head ducks (Mallard), showed that there were no significant differences between the sexes in terms of the accumulation of heavy metals [1, 2, 25]. As can be seen in Table 5, the average concentration of lead in this study was 0.181 $\mu\text{g/g}$ DW, which is higher than Uluozlu et al. study on chicken (0.12) [26]. However, in other studies on chicken and quail, the lead level was lower than 0.181 [27, 28]. Based on the table, the average lead concentration is lower than the standard amounts provided by the WHO (2 $\mu\text{g/g}$) for human consumption [29].

Table 5. Comparison of lead concentration ($\mu\text{g/g}$) in tissues of different bird in the present study with previous studies and the permissible limits set by WHO.

	Lead concentration
Present study	0.181
Abduljaleel et al, (2012)	0.35
Akan et al, (2010)	0.22
Uluozlu et al, (2009)	0.12
WHO, (1998)	2

Existing cells in tissues contain enzymes that work in conjunction with the specific function of cells and actually perform catalysis role in biochemical reactions within cells. In disorganized cells, enzymes enter into interstitial fluid, blood serum and cerebrospinal fluid (CSF). So measuring the activity of enzymes in biological fluids helps us to understand how the tissues and organs work [8]. Serum enzymes affected by physiological and environmental factors such as diets, ambient temperature, environmental pollutants [30].

In this study, a significant increase in the activity of AST, ALT and LDH were found in poultry exposed to lead acetate compared with the control group. ASP enzyme is found mainly in liver and heart cells. Therefore, any minor damage, inflammation and necrosis of liver cells release the enzyme then increase its level in plasma.

The ALT is transaminase enzyme mainly found in the cytoplasm of liver cells and to lesser extent in kidney, heart, skeletal muscle, plasma,

and pancreas [31]. Increasing activity of this enzyme plays an important role in the use of amino acids in the oxidation process or glycogenesis [32]. It can be considered a useful clinical index to detect damages to liver [33]. In addition, heart failure, muscular dystrophy, bile duct obstruction, hemolytic and anemia increases level of this enzyme in plasma [31]. Therefore, increasing activity of this enzyme in plasma of quail treated with lead may be an evidence of histopathological damage to various tissues, especially the liver. Concerning the fact that in heavy metal poisoning is one of the major organs involved is usually the liver [28, 34, 35], the enzyme values are likely to increase, especially in acute lead poisoning. Increased level of AST and ALT in plasma of Japanese quail and common carp are in contact with cadmium and Pb, indicating severe damage to liver tissues [8, 36].

The LDH enzyme is also one of the most important liver enzymes. It is a glycolytic enzyme found in the heart and other tissues [37]. Hepatic failures, as well as cardiac failures, renal disorders, glomerulonephritis, acute tubular necrosis, muscle dystrophy, hemolytic anemia and anemia caused by folic acid deficiency can increase plasma levels of this enzyme [31]. Increased activity level of lactate dehydrogenase enzyme indicates damage to various tissues of treated quails.

This study did not show any significant change in the activity of CPK in the quail treated with lead. However, a significant decrease in the level of activity of ALP was observed. Since the ALP plays an important role in the metabolism of glycogen and can disable the phosphorylase enzyme, so it can stimulate glycogen synthesis in the liver. Therefore, the inhibition of enzyme activity in liver associated with glycogen breakdown to provide required energy under stressful conditions reduces the rate of phosphorylation or prevents oxidative phosphorylation in respiratory chain [38]. The decreased activity of ALP has been reported in common carp under the influence of heavy metals [39].

No death was observed in quails treated with lead. Abnormal behavior, restlessness, pecking and feather plucking was most important symptoms of poisoning by this toxin, exacerbated by the concentration and time.

Behavioral changes in birds exposed to lead can be attributed to neurotoxic effects of these pollutants. Neurological disorders in birds is often the dysfunction of ion channels of neurons, particularly sodium ions, and key enzymes involved in nerve function such as $\text{Na}^+ / \text{K}^+ \text{ATPase}$, monoamine oxidase and acetylcholinesterase activity after exposure to pollutants [40]. In fact, prevention of activity of the enzyme acetylcholinesterase (AChE), which is responsible for the breakdown of acetylcholine in the synaptic space may cause overstimulation of cholinergic nerves, which can cause behavioral changes and instability (gait imbalance) [41]. Same as these behaviors was observed on the quail exposed to pesticides (butachlor and atrazine) [42, 43].

In the present study, the concentration of glucose in poultry treated with lead significantly increased. Glucose density adjusts by complex mechanisms hormones such as glucagon, insulin and other hormones, such as corticosteroids, epinephrine and thyroxine. However, environmental stress and tension could result in a significant increase in plasma glucose levels [44, 45]. The increase in glucose may be partly influenced by changes in the endocrine glands and an increase in cortisol, which consequently increases the metabolism of glucose glycolysis [46-48]. Increase blood glucose in treated poultry may have various reasons, such as lead poisoning, impaired carbohydrate metabolism, increase energy demand of cell, cellular ATP decrease or even decrease level of the acetylcholinesterase. The increase in blood glucose level or hyperglycemia indicates disturbances in the metabolism of carbohydrates, caused by an increase in liver glycogen breakdown [49].

In this study, creatinine and uric acid levels in the treated quail significantly increased compared to control group. Creatinine concentration in blood plasma is nearly constant in natural conditions and excessive amounts are excreted through glomerular filtration. Its sharp increase in the plasma is sign of glomerular filtration and kidney malfunction [50]. This combination is an important biomarker for measuring of toxins in the blood [51]. The increase in serum creatinine may be due to the adverse impact of lead on kidney function and glomerular filtration. According to the earlier

research [36, 52], a significant change in the amount of creatinine in the face of cadmium in Japanese quail was not found which is in conflict with this study. Uric acid rise may be due to the adverse effect of lead on kidney function. Because birds are uricotelic measurement of uric acid is more valuable than creatinine and urea to assess kidney function [53].

Based on the results of this study, there was no significant change in cholesterol. However, triglyceride concentration was significantly dropped compared with the control group. Triglyceride drop in the treated birds may result from damage to the small intestine villi and impaired absorption of fatty acids. Moreover, damage to the liver may reduce the synthesis of triglyceride.

In this study, the level of albumin, globulins and total protein in the plasma of the treated quail significantly dropped. Reduced plasma protein level may be related to increased transaminase. Increase the activity of these enzymes may lead to failure to reproduce protein. Destruction of quail muscles under stress may be one of the main reasons for the decrease in tissue protein. In fact, the reduction in stress proteins can be affected by the inability to absorb food, malnutrition, hunger, disorders of eating behaviors by reducing the activity of the enzyme acetylcholinesterase [54, 55], higher energy costs to create homeostasis, tissue repair and detoxification [56]. Reduced total plasma protein levels in hypophthalmichthys molitrix to cadmium [57] and coturnix face to pesticide atrazine [42] have been reported.

Albumin and globulins are a major part of total plasma protein and any changes in albumin and globulins and total proteins in the plasma can be used as a clinical symptom for monitoring the health of the immune system, liver and kidneys of animals [58, 59]. Globulin level drop in blood plasma in lead treated birds may indicate a weakened immune system. In general, reduction of protein synthesis in the liver affects directly on the level of globulins in the plasma and repair of damaged liver tissue, which can increase the level of protein synthesis in the liver and plasma Globulin [59].

CONCLUSION

Quail have capabilities to accumulate lead in their tissues. However, the average lead

concentration is lower than the standard amounts provided by the WHO (2 µg/g) for human consumption. In addition, it can lead to apparent changes in enzymes and blood biochemical factors, which show adverse effects of heavy metals on the immune and physiological system of birds.

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