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Toxicity of Cadmium and Lead in *Gallus gallus domesticus* Assessment of Body Weight and Metal Content in Tissues after Metal Dietary Supplements

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Abstract: The influence of dietary cadmium on the accumulation and effects of dietary lead, examined in chicken. This experiment was conducted to investigate the toxic effects of dietary Cd and Pb on chick's body weight and organ, content of the tissues of these two metals was also detected. One day age chicks of *Gallus gallus domesticus* fed diet supplemented with 25, 50, 100 ppm of Cd, second group exposure to 300, 500, 1000 ppm of Pb in feed daily during 4 weeks. The control groups were fed without supplementation of metals. The concentrations of Cd and Pb resulted in increased of Cd and Pb content in liver, gizzard and muscle. While Cd 100 ppm and Pb 1000 ppm were increased metals content in feather. Body weight of chicks was not influenced by Cd treatment. In contrary Pb treatment was significantly ($p<0.05$) decreased body weight of chicks after dietary treatment. On the other hand, Liver weigh in chicks was significantly ($p<0.05$) decreased after Cd and Pb treatments.

Key words: Cadmium, lead, toxicity, body weight, chicks

INTRODUCTION

Lead is one of the heaviest metals toxic to the environment. It has a toxic effects in the bird practically reduced the growth rate, growth retardation and increased mortality. Lead causes reproductive effects such as reduced egg production in Japanese quail and reproductive failure in cattle egret (*Bubulcus ibis*). It can cause oxidative damage (DNA, lipids, proteins (Hoffman *et al*., 1985; Grue *et al*., 1986; Burger *et al*.,1992; Mateo *et al*., 2003). According to Burger and Gochfeld (1995) humans and other animals exposure to lead affects the development anatomical, physiological, behavioral and intellectual. Up till now it is largely unknown whether the effects occur gradually or are more pronounced if exposure occurs at particular stages. There is ample evidence that lead have negative effects on the resistance of the body of the disease.

Chronic Cd reduced reproductive success in bird by reduce the intake and egg production in mallard, chicken, egret and increased susceptibility to stress, disease and oxidative and histopathological damage (Leach *et al*., 1979; Nicholson *et al*., 1983; Scheuhammer, 1987; Burger *et al*., 1992). It is hardly capable of generating free radicals directly, but may increase the oxidation of lipids in tissues after exposure. Exposure to Cd is known to cause harmful effects of different levels of the trophic chain, because of bioaccumulation (Stoeppler, 1991). Toxic effects of Cd on birds causes reduced egg production, kidney spoil, testicular damage and modified behavioral reaction (Furness, 1996). Bioaccumulation is defined as the accumulation of a metal in a tissue of interest or a whole organism that results from exposure. In other term, bioaccumulation represents the amount of metal adsorbed or absorbed by an organism from the bioaccessible metal encountered by the organism. Bioaccumulation of metals comes from all environmental sources, including air, water, solid phases and feed (McGeer *et al*., 2004). While, metal bioavailability includes metal species that are biologically available and are absorbed or adsorbed by an organism with the potential for the distribution, metabolism, elimination and bioaccumulation (McGeer *et al*., 2004). In this regard, the present study was an investigation of the exposure to Cd and Pb during the developmental period which includes chicks body weight and tissues content of metals. Few studies aiming at enhanced understanding of the impact of heavy metals in birds have been demeanor.

MATERIAL AND METHODS

Animals and experimental design: *Gallus domesticus* fertile eggs were obtained from a commercial (poultry farm, Malaysia). The eggs were cleaned, labeled and weighed

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58.437±8.717 g, rang 52-64 g, then put in incubator. After 21 days, eggs hatchling, one day age chicks were randomly assigned into seven groups (n = 10 each) according to dietary cadmium and lead. (1) control, (2) Cd 25 ppm, (3) Cd 50 ppm, (4) Cd 100 ppm, (5) Pb 300 ppm, (6) 500 ppm and (7) Pb 1000 ppm. The animals were housed for 4 weeks in groups in stainless steel cages home (90×60×50 cm) with warmth provided by OSRAM lamps (100 W). Diet and water was available daily. Drinking water and commercial diets were offered ad libitum.

The chicks were fed starter diet to 3 week of age and a grower diet until the end of experiment (Table 1), The toxic concentration of metals (Cd and Pb) was chosen depend on Nutrient Requirement of Poultry by National Research Council (NRC, 1994). The first treatment groups were fed the experimental diets which consisted of the basal diet supplemented with 25, 50 and 100 ppm of Cd as ($CdCl_2$. H_2O) since the toxic concentration of cadmium was 25 and 40 ppm for immature chicken (NRC, 1994). While the second groups were fed the food supplemented with 300, 500 and 1000 ppm Pb as Pb $(NO_3)_2$. Lead was added to the diet at this level, since the toxic dietary concentration of lead was reported at between 200-1000 ppm for chickens (NRC, 1994). Dietary supplements to control group without addition of metals. Body-weight and data were obtained bi-weekly. At the end of the 4 week exposure period, the chicks were weighed and slaughtered and the liver, gizzard, heart and feather were removed and samples of liver, gizzard breast muscle and feather were collected and stored prior to analysis.

Determination of metal concentration in chick tissues: Sample of liver, gizzard and breast muscle were thawed. The samples of feather were washed in de-ionized water which was alternated with acetone to remove loosely adherent external contaminations (Burger and Eichhorst, 2007). All the tissue samples were dried in an oven at 70°C for 24 h or until a constant dry mass was achieved (Van Eeden, 2004). Then the dryer samples ground using a mortar to powder.

The feathers were homogenized using electric blender (Philips-HR-1741) (Rattner *et al.*, 2008). The samples weighted about 0.5 g of each tissue except feather and blood were weighted 0.1 g (Burger and Gochfeld, 2001). The individual tissue samples (including blood) were digested with 70% nitric acid and 30% hydrogen peroxide (2:1), according to standard method of analytic (AOAC, 1984) and left in room temperature overnight. The samples were completely digested in block thermostat (150°C) for 4 h until the solutions became clear. After cooling, the solution was made up to 50 and 25 mL with de-ionized water. Cd and Pb concentration determined using inductively coupled plasma-mass spectrometry (ICP-MS, model Perkin-Elmer Elan 9000 A). Quality assurance procedures included the analysis of reagent blanks and appropriate standard reference material for lobster hepatopancreas (TORT-2, National Research Council Canada). The recovery of Cd was 73.6% and that of Pb was 107.3%. The analytical detection limit for Cd was 0.006 $\mu g\ g^{-1}$ and that for Pb was 0.05 $\mu g\ g^{-1}$.

Statistic analysis: Data were expressed as Means±SD. The values were analyzed by Independent sample t-test, was performance to found significant differences between metals concentration in treatment group and control. Differences at $p<0.05$ were considered statistically significant.

Table 1: Composition of poultry feed (starter and grower)

Specification	Starter	Grower
Crud protein	20%(min)	16%(min)
Crud fat	3%(min)	3%(min)
Crud fiber	6%(max)	6%(max)
Total ach	8%(max)	8%(max)
moisture	13%(max)	13%(max)
phosphorus	0.6%(min)	0.5%(min)
Calcium	0.8-1.2%	0.8-1.2%

RESULTS AND DISCUSSION

Body weight of the chicks in the control group and the groups treated with metals increased in pace from the age of one day to a ripe old age of 28 days. Whereas chicks have a lower average body weight weekly throughout the experimental period in four weeks compared with the control group. According to Table 2, the chicks weight after 2 week of dietary supplement Pb was decreased significantly ($p<0.05$) in group Pb 1000 ppm compare with control. While dietary Cd at concentration 5, 25 and 100 ppm did not affect significantly the final body weight.

After 4 week dietary Pb supplementation in the diet at 300, 500, 1000 ppm were significantly affect the chicks weight gain compared with control. On the other hand, supplementation of either diet with 25, 50 and 100 ppm cadmium had slight effect on chicks weight and growth of the three groups of treated chicks.

Table 2: Mean body weight gains (g), after 2 and 4 week of feed supplement metals concentration

Treatment	After 2 week	After 4 week
Control	306.2±33.79	628.3±79.44
Cd 25 ppm	265.2±46.03	604.3±25.14
Cd 50	270.1±49.41	538.3±83.60
Cd 100	241.2±40.62	581.5±75.66
Pb 300	270.2±35.51	501.5±72.83*
Pb 500	268.3±31.42	493.3±25.16*
Pb 1000	237.1±18.01	490.6±58.32*

*$p<0.05$ ANOVA between metal treatment and control

Metallic lead is one of the main pollutants and it is known to be toxic for birds especially during their early development (Pain, 1995). Present result showed that body weight of chick was significantly decreased than control after diet treated with Pb concentration. Same observation was previously recorded by Abd EL-Galil *et al*. (2006) who demonstrated that chicken fed diets supplemented with 1000 and 1500 ppm of lead as lead chloride was decreasing body weight. In addition, similar results have been reported by Dicheva and Ctanchev (1988) they found that birds fed diets with 1000 mg kg^{-1} of lead the body weight gain was 6% lower than the control. El-Sharrawi *et al*. (1988) found that chickens of lead exposure at doses of 750 and 1500 ppm diet caused a major reduces in body weight gain. In addition, Wittmann *et al*. (1994) found that Pb 2400 and 3600 mg kg^{-1} resulted diminution in body weight compared with controls in chicken. Further, Rahman and Joshi (2009) concluded that poultry chicks treated with lead 250 and 400 ppm were gain lower average weekly body weight when compared with the control group and there are undesirable effects of lead acetate on performance of broilers in these concentrations. In this regard, Erdogan *et al*. (2005) investigated the dietary lead exposure causes body weight gain decrease and inhibitory effect on the growth of broilers.

In the same time, present result in contrary with Jeng *et al*. (1997) who found body weights of laying ducks were not influenced by Pb 10 or 20 mg kg^{-1} b.wt. treatment, he attributed that domestic birds may be able to tolerate higher levels of Pb as well, the difference between Pb tolerance domestic birds and wild birds can often be attributed to environment, species and sex. Similar observation was recorded by Custer *et al*. (1984) he conclude that weights of American kestrel did not differ among treatment groups after feed treated with Pb (120, 212 and 448 ppm) at 60 day. Body weight of Japanese quail showed significantly decreased rates of weight gain at the level of 500 ppm of lead in the diet (Morgan *et al*., 1975). Lowered body weights in treatment groups birds could be due to reduce in the feed utilization or due to metabolic disarray related with lead, such as inhibition of enzyme involved in the haem synthesis and the oxidase system resulting in loss of cellular functions and tissue damage (Erdogan *et al*., 2005).

Cadmium has less effect on chick weight gain after four week of treatment by 25, 50, 100 ppm compare to control as we detected in current study. Opposite result was achieved by Sant'Ana *et al*. (2005) who found that the exposure to $CdCl_2$ 100 ppm for 28 days significantly reduced the body weight in Japanese quail. While present result is consistent with (Sant'Ana *et al*., 2005) who revealed no significant correlation between cadmium concentration and body mass of willow tits (*Poecile montanus*). Sant'Ana *et al*. (2005) explained the body weight of birds decreased after Cd exposure might be associated with the action of Metallothioneins (MT). The major function of MT in cadmium exposure is associated with increased preservation of Cd in tissues, resulting in a protective mechanism. Sant'Ana *et al*. (2005) found more toxic affects in bird after cadmium exposure such as induced hepatic toxicity while kidney function and cellular immune response were not affected by the Cd exposure.

Table 3, showed that the liver, gizzard and heart weights in chicks treated with Cd concentration and in the control treatment. It noted that the chicks treated Cd 25 ppm, Cd 50 ppm and 100 ppm of Cd was significantly ($p<0.01$) in liver weight decreased compared with control treatment. In contrast the weight of gizzard and heart did not affect significantly compare with control treatment. In addition chicks were treated Pb concentration seems that the gizzard and heart wear does not affect the concentration of Pb while the liver weight was significantly ($p<0.05$) decreased by Pb 300 ppm, 500 and 1000 ppm.

Bioaccumulation of trace metal in chicks tissues after metals feed supplement: The effect of dietary cadmium on the cadmium content of selected tissues in 4 week old broiler chickens was studied in the experiment result summarized in Table 4. All levels of cadmium resulted in significant increases in the cadmium content of liver compare with control Cd content and other tissues of Cd

Table 3: Organ body weight of chicks affected by nutritional supplement Cd and Pb

	Organ weight/g (4 week)		
Treatments	Liver	Gizzard	Heart
Control	22.57±3.314	13.82±1.98	3.42±0.76
Cd 25 ppm	6.205±1.907*	11.47±0.28	2.95±0.91
Cd 50 ppm	8.020±0.492*	9.95±1.615	2.75±0.58
Cd 100 ppm	8.689±0.853*	11.27±0.99	3.38±0.02
Pb 300 ppm	15.22±0.52*	13.68±1.00	2.23±0.36
Pb 500 ppm	11.73±1.34*	12.48±2.55	2.26±0.44
Pb 1000 ppm	13.253±1.43*	15.27±1.16	2.77±0.61

*p<0.05 ANOVA between metal treatment and control

Table 4: Effect of dietary cadmium on the tissue cadmium concentration of liver, gizzard, muscle and feather of young chicks

	Tissue Cd content (4 week) μg g^{-1} D.W.#			
Treatment	Muscle	Liver	Gizzard	Feather
Control	0.0017±0.0002	0.0096±0.0009	0.009±0.0007	0.044±0.013
Cd 25 ppm	0.050±0.017	4.116±1.732*	0.787±0.194*	0.211±0.103
Cd 50 ppm	0.059±0.011*	5.243±0.755*	0.388±0.096*	0.278±0.091
Cd 100 ppm	0.067±0.013*	7.617±0.671*	0.583±0.163*	0.312±0.087*

*p<0.05 ANOVA between metal treatment and control

Table 5: Effect of dietary lead on the tissue lead concentration of liver, gizzard, muscle and feather of young chicks

	Tissue Pb content (4 week) $\mu g\ g^{-1}$ D.W.#			
Treatment	Muscle	Liver	Gizzard	Feather
Control	0.039±0.002	0.048±0.003	0.050±0.002	0.925±0.306
Pb 300 ppm	0.153±0.043	0.460±0.135*	0.394±0.042*	1.626±0.686
Pb 500 ppm	0.194±0.004*	0.501±0.136*	0.397±0.018*	2.738±0.992
Pb 1000 ppm	0.511±0.132*	0.630±0.131*	0.906±0.366*	3.276±0.447*

*$p<0.05$ ANOVA between metal treatment and control

content. Muscle showed a seriously reduced ability to accumulate cadmium although the two levels of Cd in Cd 50 and Cd 100 ppm did result in significant increase compare with control treatment. Gizzard occupy the second rank after liver in Cd accumulation and it content from Cd in three Cd groups were significantly ($p<0.05$) higher than its level at control. Followed by feather which accumulates high level of Cd at Cd 100 ppm compare to control. Compare with other study, these data are in agreement with Leach *et al.* (1979) who revealed that liver and kidney in treated chicken with cadmium in diet were accumulated higher concentration of cadmium while muscle appeared reduced ability to accumulate cadmium. In contrary, Mamabolo *et al.*(2009) found no significant different in metal (Pb) accumulation in tissues of chicken (liver, muscle) after metal ingestion. While, Sharma *et al.* (1979) revealed that no accumulation of cadmium occurred in egg and bone of chicken after treated by Cd (0.3, 1.9 and 13.1 ppm) in their diet for up to 6 months. A slight increase of Cd level in muscle. While liver and kidney had the highest of Cd levels.

Table 5 showed the effect of dietary lead on the tissue lead concentration of liver, gizzard, muscle and feather of young chicks. High level of Pb content accumulated in feather than other tissues. pb content in feather at group Pb 1000 was higher relatively ($p<0.05$) compare with control. In contrary, less Pb accumulated in pectoral muscle, though, the pb levels in Pb 500 and 1000 ppm group were significantly high than control. Different levels of supplemental Pb (300, 500, 1000 ppm) were effect in Pb content in liver and gizzard of chicks and Pb accumulated in this tissue in higher level compare to control. Present study demonstrated that tissues Pb concentration was significantly increased after egg injection (Pb 100, 200 ppm as Pb $(NO_3)_2$ and food treatment (pb 500 and Pb 1000 ppm). Pb levels were higher in feather followed by gizzard and liver while muscle was accumulated less concentration of lead in Pb same observation was record in present study from chicken collected from field which accumulated higher levels of lead in feather followed by gizzard and liver and less levels found in muscle. In this regard, Zraly *et al.* (2008) found significantly increased lead concentrations in liver, kidneys, bones of chicken after food treated with lead acetate. Same observation was detected by Jeng *et al.* (1997) revealed that tissues of laying Tsaiya ducks was accumulate high levels lead after food supplied with 20 mg kg^{-1} Pb. Similar finding recorded by Custer *et al.* (1984) that American kestrels treated by Pb (120, 212, 448 ppm) with diet did not significantly affect concentration of lead in the muscle but there were significant differences among treatment for kidney, liver, femur, brain and blood. Another study was showed the liver of quail was accumulated high level of lead after 21 days of treatment while less concentration in gonads (Mehrotra *et al.*, 2008). In addition, Scheuhammer (1987) found that tissues level of Cd and Pb increased in direct proportion to dietary levels over a particular dose range.

Briefly, the data indicate that dietary Cd did not affect body weight and organ (gizzard and heart) of chick. At the same time Cd was accumulated in high concentrations in young chicken tissues at the end of the experiment. While dietary Pb was decreased chick body weight and liver and accumulated in the tissues of chicken significantly.

CONCLUSION

It was observed from the current study that supplementation of cadmium in chicken diets at 25, 50 and 100 ppm and led at 300, 500 and 1000 ppm, was effect body and liver weight of chicks after 4 week of treatment. Bioavailability of Cd and Pb measured in terms of tissue concentration (liver, muscle, gizzard and feather) was directly related to their levels of supplementation in feed.

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