

## Effects of Daily Supplementation with High Dose Ascorbic Acid on Blood Lead Levels in Broiler Chicken after Intentional Exposure to a Concentrated Source of Lead

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### ABSTRACT

Lead toxicity is an important global public health issue. The present study was conducted to determine the amount of lead deposited in chicken blood after intentional exposure to a concentrated source of lead for 1 week and to determine the effect of 4 week daily supplementation with high dose ascorbic acid (AA) on the blood lead levels in broiler chicken. Clinically normal mixed-breed adult laying chickens were used in this study. Chickens received lead acetate (200 mg/kg/day) for 1 week. A group of chicken received AA (500 mg/kg/day) for 4 weeks. Another group did not receive any treatment for another 4 weeks. Blood samples were collected and analyzed for blood lead levels using a graphite furnace atomic absorption spectrophotometer. The baseline blood lead level was  $47.5 \pm 38.0 \mu\text{g/L}$  and increased significantly to  $2755 \pm 576 \mu\text{g/L}$  after 1 week of lead acetate treatment ( $P < 0.001$ ). AA treatment reduced blood lead levels significantly ( $P < 0.05$ ). Supplementing lead-contaminated feed with daily high doses of AA might protect from lead exposure when chickens are exposed to environmental pollution.

**Keywords:** Ascorbic acid, lead, pollution, chicken, blood.

### INTRODUCTION

Lead is present ubiquitously in our environment (1). Consequently, lead toxicity is an important global public health issue. Sources of lead contamination are numerous including water, air, and soil (2, 3). Nevertheless, urbanization and industrialization have considerably increased lead levels in these sources (3). Furthermore, lead is present in the plants and grains grown on contaminated soils (4). Not surprisingly, animals and birds grazing on contaminated plants and grains had elevated lead levels (4, 5). Following exposure to contaminated foodstuffs, lead may accumulate in different body tissues and cause deleterious effects on health (4). Hypertension, renal failure, and depression were associated with elevated lead levels in the

organs of affected patients (6-8). Lead was also associated with hematopoietical, hematic, renal, gastrointestinal, mental, cognitive and puberty impairments (9-11). Elevated lead levels were also associated with poor scholastic achievement in children (9-11). The Centers for Disease Control and Prevention (CDC) estimated that there are more than 500,000 children aged between 1 and 5 years in the United States with blood lead levels above  $5 \mu\text{g/dL}$  which is the reference level at which intervention should be initiated (12). In Palestine, our recently published study showed that 19.1% of the breast milk samples analyzed for lead contained higher levels than the WHO's safety reference (13). Similarly, a previous study conducted in Palestine showed that blood lead levels in children were in many cases higher than  $5 \mu\text{g/dL}$  (14).

Chicken meat and eggs are among the major sources of proteins, minerals and vitamins in the diet of different nations (8, 15, 16). Globally, children as well as adults consume large amounts of poultry meat and eggs. According to recent statistics by the Palestinian Central Bureau of Statistics a Palestinian household consumes on average 17.3 kg of poultry meat products and 3.7 kg of chicken eggs per month. Although chicken meat and eggs contain components which are essential for normal body development, growth and tissue repair, accumulating evidence showed that hens exposed to environmental pollution had elevated lead levels (4, 17). Lead can deposit in various chicken tissues. Studies showed that lead can also be transferred from the different tissues of laying hens to eggs (8, 18). Therefore, determination of lead in foodstuffs including chicken is of great importance as exposure to even low levels of lead may cause health related serious deleterious effects, especially in children.

In previous studies, several chelators were used to decrease lead toxicity in the events of exposure to lead. However, none of them was ideally suitable for decreasing the lead burden at chronic exposure level (19). In addition to the fact that these chelators were not able to completely remove lead from body tissues, some of them were potential toxicants (19). Ascorbic acid (AA) was previously used as chelating agent (19, 20). AA was shown to be effective in protecting cells from damaging effects of external stress (20). There is a lack of experimental work to investigate if AA can decrease the concurrent deposition of lead in chicken blood. In the present study, our aim was to investigate if daily dose supplementation with high doses of AA was able to reduce concurrent lead levels in blood of broiler chicken after exposure to a concentrated source of lead.

## MATERIALS AND METHODS

### Experimental design, bird welfare and ethical considerations

Clinically normal mixed-breed adult laying broiler chickens were obtained from raisers in Tulkarem region and used in this study. Each hen weighed 1.7 to 2 kg. Hens were

housed separately in cages in the poultry housing facility of the Faculty of Agriculture and Veterinary Medicine, An-Najah National University (Tulkarem campus).

Up to date, An-Najah National University has not established an Institutional Animal Care and Use Committee (IACUC) and the Institutional Review Board (IRB) of An-Najah accepts to evaluate research protocols involving human subjects only. In the United States, chickens are exempt from the Humane Slaughter Act. However, this study was conducted in compliance with the international animal care and use regulations. All surgical and tissue harvesting procedures were performed by a licensed veterinarian. Vital signs of all hens were recorded throughout the different stages of the study.

Blood samples were collected in blood collection tubes (vacutainer) via the wing vein. Blood samples collected were analyzed for blood lead levels separately.

### Acclimatization and baseline lead levels

Hens were given a 1 week acclimatization period during which hens had access to feed and water *ad libitum*. Water and feed provided during this period were sampled and analyzed for potential lead contents. Blood samples during this period were collected to determine the baseline blood lead levels.

### Treatments

**Week 1:** Following the acclimatization period, chickens were divided into 2 groups: G1 and G2. G1 served as control and received normal feed and water *ad libitum*. G2 received lead acetate (200 mg/kg/day) mixed with small portions of feed and water for 1 week. Researchers made sure that the feed portions mixed with lead acetate were completely consumed by each chicken before giving free access to water and feed and before the next day dose. At the end of *Week 1*, blood samples were collected from all chickens.

**Week 2 through Week 5:** To investigate if daily supplementation with high dose AA can reduce elevated lead levels in blood, chickens in G2 were further divided into 2 groups. A group received AA (500

mg/kg/day) mixed with water for 4 weeks. At the end of *Week 5*, blood samples were collected and analyzed for blood lead levels separately. To investigate if the reduction in elevated blood lead levels resulted from AA treatment and not from another endogenous detoxification pathway, the second group did not receive any treatment during the 4 weeks period. At the end of *Week 5*, chickens were sacrificed humanly.

### Analytical procedure

The determination of lead in blood samples was performed using a graphite furnace atomic absorption spectrophotometer (iCE™ 3500 Atomic Absorption Spectrometer, Thermo Scientific, UK). Glass ware and crucibles were incubated overnight in 10% HNO<sub>3</sub> to prevent adsorption of lead on the surfaces of the containers. Lead levels were determined against calibration curves in similar matrix (blood). Blood samples were analyzed on wet-weight basis. All chemicals used were obtained from the central chemicals store of An-Najah National University and were of analytical grade.

Aliquots of 100 µL from the blood were mixed with 0.5 mL deionized water and 0.4 mL 0.2% Triton X-100. Aliquots of 1g of blood were mixed with 1 mL of concentrated HNO<sub>3</sub> into crucible and ashed for 1h at 540 °C. Ash aliquots were soaked with 15 mL 1:3 mixture (1M HNO<sub>3</sub> and 1M HClO<sub>4</sub>) for 1h. Aliquots were then filtered and supernatants were analyzed for lead contents.

The analytical method used had a limit of detection (LOD) and limit of quantification (LOQ) of 1 µg/L and 2 µg/L, respectively as previously described in (21, 22).

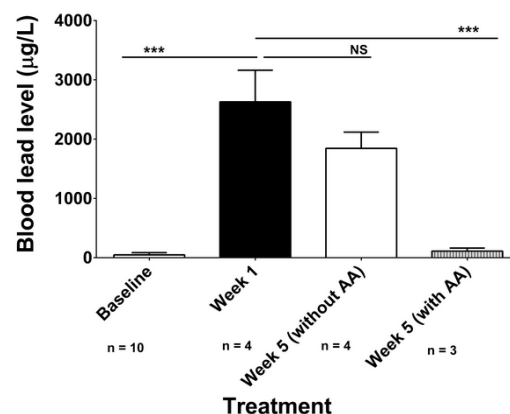
### Statistical analysis

Baseline blood lead levels, decline in blood lead levels by the end of *Week 5* with and without daily supplementation with AA from same chickens were compared using ANOVA with multiple comparisons. Statistical analysis was performed using Graphpad Prism v.6.0 (GraphPad Software Inc., San Diego, CA, USA). Results were considered statistically significant when the P value was less than 0.05.

## RESULTS

Feed and water supplemented before the commencement of the study were analyzed for potential lead contents. Feed and water were found to contain 151 µg/kg and 30 µg/L, respectively.

After acclimatization period, the baseline blood lead level was determined to be 47.5 (SD ± 38.0). At the end of *Week 1*, the mean blood lead level increased significantly ( $P < 0.001$ ) to  $2755 \pm 576$  µg/L, as shown in Figure 1. Blood lead levels declined following daily supplementation with high dose AA. Interestingly, by the end of *Week 5* blood lead levels were significantly reduced by almost 24.7 folds ( $P < 0.001$ ). However, blood lead levels did not decrease significantly in the group that did not receive lead acetate (Figure 1).



**Figure (1):** Baseline blood lead level, following exposure to lead acetate (200 mg/kg/day) for 1 week, and 4 weeks with supplementation with high daily dose AA (500 mg/kg/day).

## DISCUSSION

To our knowledge, this study is the first report on the effects of daily supplementation with high dose AA on blood lead levels in hens intentionally exposed to a concentrated source of lead. A group of mixed race broiler chicken was used in this study. This group was identical to most family-owned hens domesticated for eggs in the vicinities of their homes (23).

Our findings showed that ingestion of a concentrated source of lead significantly increased blood lead levels in chickens. The mean blood lead level after 1 week of lead

acetate ingestion was significantly higher than the baseline blood lead level before ingestion of lead acetate (Figure 1). These findings suggest that hens absorbed significant amounts of lead. Our findings were consistent with those reported by Trampel et al in which broiler chicken blood lead level significantly increased after ingestion of paint chips containing lead (4). Similarly, blood lead levels were significantly increased after dosing laying ducks with lead nitrates orally (24). In case chicken blood lead levels reflected levels in other edible tissues, this could be of special interest as contaminated tissues might be consumed repeatedly by humans and present a significant source of lead exposure which may cause negative consequences on their health.

Prior studies showed that lead can distribute in various body tissues including bones and can also be sequestered in eggs. Chicken eggs in India and Nigeria were shown to contain detectable lead levels (19, 25). The eggshell is composed of calcium carbonate (94%), calcium phosphate (1%), magnesium carbonate (1%) and organic substances (4%) (26). It is possible that a considerable quantity of lead is stored in the eggshell forming lead carbonate.

Blood samples collected during the acclimatization period contained detectable lead levels. This might suggest that hens might have been previously exposed to lead from the environment before the study. In their analysis, Fakayode et al showed that commercial chicken feed contained variable concentrations of contaminant lead (25). Provisional tolerable weekly intake of lead was previously established as 50 µg/kg for adults and 25 µg/kg of body weight for children (5).

Interestingly, our findings showed that daily supplementation with high dose of AA brought the blood lead levels after exposure to a concentrated source of lead back close to the baseline level. Dawson et al showed that a daily supplementation with 1000 mg of AA resulted in a significant decrease in blood lead levels in smokers (9). A previous study in albino rabbits showed that co-administration of lead acetate with AA corrected the alterations in blood indices to control values (15). When AA was supplemented

to lead exposed rats, it significantly reduced lead levels in the blood, liver, and kidneys. AA is thought to provide greater protection against lead acetate due to its ability to chelate lead and increase urinary elimination of lead (15). AA is also thought to be able to scavenge free radicals and form complexes with lead, decrease its intestinal absorption and increase renal clearance of lead (15, 18).

Our results can be explained considering the following limitations. First, blood lead levels were not correlated with lead levels in other tissues. Second, in this investigation we used a graphite furnace atomic absorption spectrophotometer, using coupled plasma mass spectrometry could have yielded lower detection limits. However, in this study lead levels were detectable and quantifiable in the samples analyzed. Finally, the number of hens used was comparatively small. A larger number of hens would have allowed a more rigorous study design.

## CONCLUSIONS

In conclusion, contamination of food products with potential toxic metals like lead presents an important public health challenge. In this study, daily supplementation with high dose AA could reduce lead levels in the blood of broiler chicken. Supplementing feed and water ingested by chicken with AA might help in detoxifying lead from chicken tissues and might protect consumers from exposure to lead. More research is needed to investigate if supplementing feed with food residues containing AA like citrus fruits can reduce environmental lead exposure in chicken.

**Footnote:** Baseline blood levels, decline in blood lead levels by the end of *Week 5* were compared using ANOVA with multiple comparisons. Statistical significance was considered \*\*\* when the  $P < 0.001$ . NS: Not significant.

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## CONFLICT OF INTERESTS

Authors declare no conflicts of interest.

## REFERENCES

- 1) Clayton BE. Lead: the relation of environment and experimental work. *Br Med Bull.* 1975; 31(3): 236-40.
- 2) Lepow ML, Bruckman L, Gillette M, Markowitz S, Robino R, Kapish J. Investigations into sources of lead in the environment of urban children. *Environ Res.* 1975;10(3): 415-26.
- 3) Gulson BL, Mizon KJ, Davis JD, Palmer JM, Vimpani G. Identification of sources of lead in children in a primary zinc-lead smelter environment. *Environ Health Perspect.* 2004; 112(1): 52-60.
- 4) Trampel DW, Imerman PM, Carson TL, Kinker JA, Ensley SM. Lead contamination of chicken eggs and tissues from a small farm flock. *J Vet Diagn Invest.* 2003; 15(5): 418-22.
- 5) Brams E, Anthony W. Cadmium and lead through an agricultural food chain. *Sci Total Environ.* 1983; 28: 295-306.
- 6) Batuman V, Landy E, Maesaka JK, Wedeen RP. Contribution of lead to hypertension with renal impairment. *N Engl J Med.* 1983; 309(1): 17-21.
- 7) Cooper WC, Wong O, Kheifets L. Mortality among employees of lead battery plants and lead-producing plants, 1947-1980. *Scand J Work Environ Health.* 1985; 11(5): 331-45.
- 8) Fakayode SO, Olu-Owolabi IB. Trace metal content and estimated daily human intake from chicken eggs in Ibadan, Nigeria. *Arch Environ Health.* 2003; 58(4): 245-51.
- 9) Correia P, Oliveira E, Oliveira P. Simultaneous determination of Cd and Pb in foodstuffs by electrothermal atomic absorption spectrometry. *Anal Chim Acta.* 2000; 405(1): 205-11.
- 10) Mostafa GA, El-Shahawi HH, Mokhtar A. Blood lead levels in Egyptian children from high and low lead-polluted areas: impact on cognitive function. *Acta Neurol Scand.* 2009; 120(1): 30-7.
- 11) Tomoum HY, Mostafa GA, Ismail NA, Ahmed SM. Lead exposure and its association with pubertal development in school-age Egyptian children: pilot study. *Pediatr Int.* 2010; 52(1): 89-93.
- 12) Center for Disease Control and Prevention. Blood lead levels in children aged 1-5 years-United States, 1999-2010. *MMWR Morbidity and mortality weekly report.* 2013; 62(13): 245.
- 13) Shawahna R, Zyoud A, Dwikat J, El-Helo M, Yacoub B, Hilal H. Breast Milk Lead Levels in 3 Major Regions of the West Bank of Palestine *J Hum Lact.* 2016; 32(3): 455-61.
- 14) Sawalha AF, Wright RO, Bellinger DC, Amarasiriwardene C, Abu-Taha AS, Sweileh WM. Blood lead level among Palestinian schoolchildren: a pilot study. *East Mediterr Health J.* 2013; 19(2): 151-5.
- 15) Dey S, Dwivedi SK. Toxic metals in hens' eggs in India: a preliminary report. *Arch Environ Health.* 2000; 55(5): 365-6.
- 16) Qabajah M, Awwad E, Ashhab Y. Molecular characterisation of *Escherichia coli* from dead broiler chickens with signs of colibacillosis and ready-to-market chicken meat in the West Bank. *Br Poult Sci.* 2014; 55(4): 442-51.
- 17) Dozier WA, 3rd, Davis AJ, Freeman ME, Ward TL. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. *Br Poult Sci.* 2003; 44(5): 726-31.
- 18) Meluzzi A, Simoncini F, Sirri F, Vandi L, Giordani G. Feeding hens diets supplemented with heavy metals (chromium, nickel and lead). *Archiv fuer Gefluegelkunde.* 1996.
- 19) Haridy M, Al-Amgad Z, Sakai H, Mohi-Eldin M. Ameliorating effects of garlic, calcium, and vitamin C on chronic lead toxicity in albino rats. *Comp Clin Path.* 2014; 23(5): 1215-23.
- 20) Rai DK, Rai PK, Rizvi SI, Watal G, Sharma B. Carbofuran-induced toxicity in rats: protective role of vitamin C. *Exp Toxicol Pathol.* 2009; 61(6): 531-5.

- 21) Othman ZA. Lead contamination in selected foods from Riyadh city market and estimation of the daily intake. *Molecules*. 2010; 15(10): 7482-97.
- 22) Obeid PJ, El-Khoury B, Burger J, Aouad S, Younis M, Aoun A, et al. Determination and assessment of total mercury levels in local, frozen and canned fish in Lebanon. *J Environ Sci*. 2011; 23(9): 1564-9.
- 23) Erdoğan Z, Erdoğan S, AKSU T, Baytok E. The effects of dietary lead exposure and ascorbic acid on performance, lipid peroxidation status and biochemical parameters of broilers. *Turk J Vet Anim Sci*. 2005; 29: 1053-9.
- 24) Jeng SL, Lee SJ, Liu YF, Yang SC, Liou PP. Effect of lead ingestion on concentrations of lead in tissues and eggs of laying Tsaiya ducks in Taiwan. *Poult Sci*. 1997; 76(1): 13-6.
- 25) Zhuang P, Lu H, Li Z, Zou B, McBride MB. Multiple exposure and effects assessment of heavy metals in the population near mining area in South China. *PloS one*. 2014; 9(4): e94484.
- 26) Rahman MA, Rahman MM, Reichman SM, Lim RP, Naidu R. Heavy metals in Australian grown and imported rice and vegetables on sale in Australia: health hazard. *Ecotoxicol Environ Saf*. 2014; 100: 53-60.