Bull. Environ. Contam. Toxicol. (1995) 55:833–839

Environmental Contamination and Toxicology

δ-Aminolevulinic Acid Dehydratase: A Sensitive Indicator of Lead Exposure in Broiler Chicks (*Gallus domesticus*)

R. I. Bakalli,[1,2] G. M. Pesti,[1] W. L. Ragland,[2] V. Konjufca,[1] R. Novak[2]

[1]Department of Poultry Science, The University of Georgia, Athens, Georgia 30602, USA
[2]Department of Avian Medicine, The University of Georgia, Athens, Georgia 30602, USA

Received: 31 March 1994/Accepted: 2 June 1994

Delta-aminolevulinic acid dehydratase, EC 4.2.1.24 (ALAD) is one of the enzymes participating in heme synthesis. It catalyzes the condensation of two molecules of delta-aminolevulinic acid, yielding one molecule of porphobilinogen (PBG) and two molecules of H_2O (Finelli *et al.*, 1974).

The inhibition of ALAD activity in blood and various tissues by lead has been documented in humans (Hernberg *et al.*, 1972; Tomokuni, 1974; 1981; Prpic-Majic *et al.*, 1984), rats (Rozhaja *et al.*, 1990), cattle (Rice *et al.*, 1987; Prpic-Majic *et al.*, 1990), Japanese quail (Stone *et al.*, 1977), laying hens (Elezaj *et al.*, 1988, Bakalli *et al.*, 1990), broiler chicks (Bakalli *et al.*, 1995b). Studies by Nakao *et al.* (1968), Hernberg *et al.* (1970) and McIntire *et al.* (1973) have shown a highly significant negative correlation between blood lead concentration and erythrocyte ALAD activity.

Neiburg *et al.* (1974) reported that the activity of blood ALAD was closely correlated with amounts of chelatable lead in the body, and that the degree of inhibition of this enzyme may be a better indicator of lead toxicity than actual lead concentrations for the purposes of identifying individuals with an increased soft tissue lead content.

In a previous study (Bakalli *et al.*, 1995a) lead was fed to broiler chickens at 0, 1, 10 and 100 μg/g for 42 days; blood ALAD and tissue lead levels were measured. After 42 days of feeding Pb, an inverse relationship between blood ALAD and tissue Pb levels was apparent.

The study reported here was designed to determine the activity of erythrocyte ALAD and the relationship between this enzyme and tissue lead levels in chickens, during Pb intake and after withdrawing Pb from the feed.

Correspondence to: G. M. Pesti

MATERIALS AND METHODS

All chemicals and reagents were American Chemical Society Reagent Grade. Deionized water was used for preparing solutions.

Forty-five three-week-old male commercial chickens (Peterson x Arbor Acre strain) were housed in wire floor battery brooders. A 24:0-hr light:dark cycle was maintained throughout the studies.

Table 1. Diet composition.

Ingredients and composition	Amounts
	%
Ground yellow corn	57.34
Soybean meal (dehulled)	33.48
Poultry fat (stabilized)	3.15
Poultry by-product meal	3.00
Iodized sodium chloride	.21
DL-Methionine (98%)	.19
Vitamin premix[1]	.25
Trace mineral premix[1]	.05
Defluorinated phosphate	1.54
Limestone	.79
Estimated Composition	
Protein	23.13
Energy, kcal/g	3.13
Methionine (%)	.57
Cystine (%)	.35

[1]Bakalli *et al.* (1995b).

For 14 days the chickens were fed a commercial-type broiler starter diet (Table 1) *ad libitum*. For the first 7 days only, 50 μg/g Pb as lead sulfate was mixed into the feed.

Approximately 10 ml of blood was taken by heart puncture, and the same birds were killed by cervical dislocation at the start (5 chickens) and 4 chickens at 1, 2, 3, 5 and 7 days from the start and 1, 2, 3, 5 and 7 days after giving feed without supplemental Pb. Blood lead level was determined by wet mineralization (Detection limit = .01 μg/ml; Prpic-Majic, 1985).

Tissue samples were frozen at -20°C and thawed before analysis. Bursa of Fabricius, brain, liver and kidney were dried in a vacuum oven and dry-ashed as described by Blanusha and Breshki (1981). Legs were heated in water to facilitate removal of soft tissue from the tibia before dry ashing. Tissue lead

was measured by the method of flame atomic absorption spectrophotometry (Perkin Elmer 5000).

Blood δ-aminolevulinic acid dehydratase (ALAD, EC.4.2.1.24) was determined by the measurement of porphobilinogen (PBG) synthesized when an aliquot of hemolyzed blood was incubated for 1 hour at 37°C in the presence of excess delta-aminolevulinic acid (Buchet et al., 1988).

Data were analyzed using one-way analyses of variance (SAS, 1985). Means were compared by Duncan's New Multiple Range test. Correlation coefficients were computed using SAS (1985) Proc Corr for the results of this experiment and an earlier experiment described by Bakalli *et al.* (1995a).

RESULTS AND DISCUSSION

The results in Table 2 show that 50 μg lead/g feed reduces enzyme activities to 62% within 24 h, and after 7 days to 31% of normal values. Twenty four hours after the lead was withdrawal from feed, enzyme activity increased 32%, and after 7 days to 90%. Two days after the Pb was withdrawn from the feed, the activity of ALAD was significantly increased. After seven days, the activity of ALAD was near normal (not significantly different from the start).

Lead levels in blood, bursa, brain, kidney and tibia were significantly higher 24 h after 50 μg lead was added per g of feed. Liver lead levels were significantly higher 48 h from the start ($p<0.05$).

Seven days after lead was withdrawn from feed, blood and tibia lead levels were still significantly higher than at the start. Lead levels in bursa and liver returned in 5 days to the same level as at the start; in brain by 7 days and in kidney by 3 days. The correlation matrix from our earlier experiment (Bakalli *et al.*, 1995a) and this experiment are presented in Table 3. Correlations between blood ALAD activity and blood, liver and tibia lead levels in Bakalli *et al.* (1995a) experiment were very high and significant. The same relations were found in the first phase of the current experiment. After withdrawing lead from feed (second phase), the correlation between ALAD activity and lead levels in liver, tibia and especially in kidney were reduced.

In the previous experiment (Bakalli *et al.*, 1995a), even 1 μg lead/g feed reduced blood ALAD activities to 75%, 10 μg lead reduced them to 56% and 100 μg to 12% of normal values. There were significant correlations between body weight and feed conversion ratio, and blood ALAD (Bakalli *et al.*, 1995a; Table 3). The correlations between blood ALAD and tissue Pb in the longer feeding trial were very similar to what was observed in the current experiment where feeding and withdrawal gave a wider range of values in a shorter period of time. Stone *et al.* (1977) observed similar results in experiments with Japanese Quail. We have shown the depressed and then increased activity of ALAD following a very low lead level exposure (1 μg/g feed; Bakalli *et al.*, 1995a), and exposure for a short time (50 μg/g, 24 h; Table 2). This

Table 2. Blood ALAD activity and tissue lead levels.

	ALAD U/LE Mean±SE	Blood Lead μg/g DL Mean±SE	Bursa μg/g DM Mean±SE	Brain μg/g DM Mean±SE	Liver μg/g DM Mean±SE	Kidney μg/g DM Mean±SE	Tibia μg/g Ash Mean±SE
Start (n=5)	58.55±2.83^{a}	3.84±0.25^{a}	1.62±0.20^{a}	1.67±0.06^{a}	1.39±0.19^{a}	2.13±0.26^{a}	36.47±1.58^{a}
Days with added Pb (50 μg/g feed) (n=4):							
1	36.41±3.77^{bc}	25.00±1.82^{ef}	2.33±0.15^{b}	3.03±0.13^{bc}	1.87±0.07^{abc}	3.80±0.28^{cd}	44.90±1.81^{b}
2	33.16±1.14^{c}	26.51±3.22^{fg}	3.77±0.31^{b}	2.65±0.23^{bc}	2.24±0.18^{bcd}	4.09±0.30^{dc}	49.91±1.59^{bc}
3	33.25±1.49^{c}	21.40±1.40^{dc}	3.26±0.19^{b}	3.10±0.49^{c}	2.62±0.17^{cd}	4.39±0.17^{cd}	60.97±3.81^{ef}
5	18.58±2.60^{d}	29.80±1.00^{fg}	5.89±0.41^{c}	3.39±0.26^{cd}	3.03±0.16^{de}	4.36±0.28^{de}	73.62±3.50^{fg}
7	18.16±1.88^{d}	32.80±0.57^{h}	7.61±0.78^{d}	4.05±0.51^{d}	3.56±0.15^{e}	4.90±0.16^{e}	77.81±1.09^{h}
Days after withdrawal of Pb from feed (n=4):							
1	24.10±3.46^{d}	24.00±0.49^{ef}	5.29±0.41^{c}	3.04±0.22^{bc}	2.95±0.47^{d}	3.14±0.13^{bc}	67.61±2.85^{fg}
2	41.83±1.61^{b}	18.70±0.85^{cd}	3.71±0.54^{b}	2.63±0.24^{bc}	2.46±0.15^{bcd}	3.00±0.29^{abc}	61.30±1.36^{ef}
3	33.27±1.22^{c}	17.00±0.99^{c}	3.49±0.72^{b}	3.00±0.20^{bc}	2.46±0.12^{bcd}	2.78±0.33^{ab}	57.33±1.60^{de}
5	40.46±0.91^{b}	15.80±0.74^{c}	2.76±0.50^{ab}	2.50±0.20^{bc}	1.80±0.11^{ab}	2.74±0.23^{ab}	52.86±2.55^{cd}
7	52.77±0.94^{a}	10.20±0.38^{b}	1.72±0.32^{a}	2.16±0.19^{ab}	1.76±0.07^{ab}	2.49±0.18^{ab}	52.72±1.09^{cd}

$^{a\text{-}h}$Means in the same column with different letter superscripts are different at $P<0.05$.

Table 3. Correlation matrix between ALAD activity and body gain (BG), feed conversion ratio (FCR) and tissue lead level[1].

	BG	FCR	Blood Pb	Bursa	Brain	Liver	Kidney	Tibia
After added 50 μg Pb/g feed:								
r	ND	ND	-0.956	-0.881	-0.939	-0.925	-0.942	-0.921
P	ND	ND	0.003	0.020	0.005	0.008	0.005	0.009
After withdrawal Pb from feed:								
r	ND	ND	-0.934	-0.937	-0.928	-0.869	-0.792	-0.866
P	ND	ND	0.003	0.020	0.005	0.008	0.005	0.009
				Bakalli et al. (1995a)				
r	0.977	-0.975	-0.975	ND	ND	-0.984	-0.984	-0.0965
P	0.023	0.027	0.025	ND	ND	0.016	0.016	0.035

[1]r=correlation coefficient; P=probability that the correlation coefficient is not significantly different from zero; ND=not determined.

demonstrates the ALAD sensitivity to lead and extends the conclusions of Stone *et al.* (1977), Tomokuni (1974), deBruin and Hoolboom (1967), Weisberg *et al.* (1971), and Bakalli et al. (1990).

Lead from feed significantly accumulated in all analyzed tissues. After ingestion, bursa accumulated Pb the fastest, followed by liver > brain > kidney > tibia (based on % increase). After lead was withdrawn from the feed tibia retained lead the longest, followed by brain < kidney < liver < bursa (based on % decrease).

Enzyme activity and tissue lead levels had significant inverse relationships (Table 3). The most significant and strongest negative correlations were between blood ALAD activity and blood lead level. The correlations with lead levels in liver and tibia were higher than in experiments with Japanese Quail (Stone *et al.*, 1977).

The chicken is proposed as a model for studying lead toxicity since it is very sensitive with measurable responses to low Pb levels after as little as 24 hours. Serum ALAD in chickens may be used to monitor lead concentration in the environment.

Acknowledgments. This research was supported by funds the Agricultural Experiment Station and the Veterinary Medical Station, University of Georgia, and a United States Information Agency scholarship awarded to R. Novak by the Baltic/East Central European Assistance Awards Program administered by NAFSA: Association of International Educators.

REFERENCES

Bakalli IR, Elezaj I, Mestani N, Demaj A, Isufi S, Markovic D (1990) The laying hens as a monitoring organism of industrial pollution by heavy metals. International Conference on Metals in Soils, Waters, Plants and Animals, Orlando, Florida, 30 April - 3 May.

Bakalli RI, Pesti GM, and Ragland WL (1995a) The magnitude of lead toxicity in broiler chickens. Vet Hum Toxicol 37:15-19.

Bakalli RI, Pesti GM, Ragland WL and Konjufca V (1995b) Dietary copper in excess of nutritional requirements reduces plasma and breast muscle cholesterol of chickens. Poult Sci 74:360-365.

Blanusa M, Breski D, (1981) Comparison of dry and wet ashing procedures for cadmium and iron determination in biological material by atomic absorption spectrophotometry. Talanta 28:631-684.

deBruin A, Hoolboom H (1967) Early signs of lead-exposure. A comparative study of laboratory test. Brit J Industr Med 24:203-212.

Buchet JP, Lauwerys R, Grudner FI (1988) Blood delta aminolevulinate dehydratase (δ-ALAD) activity. In: Methods for Biological Monitoring (Edited by Kneip, TJ, Crable JV). American Public Health Association. Washington, pp. 413-418.

Finelli VN, Murthy L, Peirano WB (1974) δ-aminolevulinate dehydratase, a zinc dependent enzyme. Biochem Biophys Res Commun 60:1418-1424.

Elezaj I, Rozhaja D, Bakalli R, Halili F (1988) Erythrocytes delta-aminolevulinic acid dehydratase activity, and lead level in blood of laying hens exposed to heavy metals pollutions. Proc 1st European Conference on Ecotoxicology, Copenhagen, Denmark, 17-20 October.

Hernberg S, Nikkanen J, Mellin G, Lilius H (1970) δ-Aminolevulinic acid dehydratase as a measure of lead exposure. Arch Environ Hlth 21:141-145.

Hernberg S, Tola D, Nikkanen J, Vetkonen S (1972) Erythrocyte-aminolevulinic acid dehydratase in lead exposure. Arch Environ Hlth 25:109-113.

McIntire MS, Wolf GL, Angle CR (1973) Red cell lead and δ-aminolevulinic acid dehydratase. Clin Toxicol 6:183-188.

Nakao K, Wada O, Yano Y (1968) Delta-aminolevulinic dehydratase activity in erythrocytes for the evaluations of lead poisoning. Clin Chim Acta 19:319-325.

Neiburg PI, Weiner LS, Oski BF, Oski FA (1974) Red blood cell δ-aminolevulinic acid dehydratase activity. An index of body lead burden. Amer J Dis Child 127:348-350.

Prpic-Majic D, Meczner J, Telisman S, Kersenk A (1984) Biological monitoring of lead effects in a smelter community before and after emission control. Sci Environ 32:277-288.

Prpic-Majic D, Karacic V, Skender LJ (1990) A follow-up study of lead absorption in cows as an indicator of environmental lead pollution. Bull Environ Contam Toxicol 45:19-24.

Rozhaja AD, Elezaj I, Jusufi I (1990) Blood lead level of laboratory rats exposed to heavy metal pollution conditions. Environ Contam, 4th International Conference, Barcelona, 154-156.

SAS Institute, Inc. (1985) SAS User's Guide: Statistics, version 6 edition. Cary, NC: SAS Institute, Inc.

Stone CL, Fox MRS, Jones AL, Mahaffey KR (1977) δ-Aminolevulinic acid dehydratase - a sensitive indicator of lead exposure in Japanese Quail. Poult Sci 56:174-181.

Tomokuni K (1974) δ-aminolevulinic acid dehydratase test for lead exposure. Arch Environ Hlth 29:274-281.

Weisberg JB, Lipschutz F, Oski FA (1971) δ-Aminolevulinic acid dehydratase activity in circulating blood cells. A sensitive laboratory test for the detection of childhood lead poisoning. N Engl J Med 284:565-569.