Effects of dietary lead acetate and aluminosilicates on the antioxidative defense system of broilers' muscle tissues

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Summary

The objective of this study was to evaluate the effect of dietary supplements with lead acetate and aluminosilicates (ATN) on antioxidative enzyme activities and lipid peroxidation of the heart and skeletal muscle tissues of broiler chickens. Broilers were allotted to four diets including the control group, the Pb group, the aluminosilicate-ATN (antitoxic nutrient) group and the Pb+ATN group, in a 3-week feeding experiment. Dietary intake of lead acetate induces oxidative stress and promotes lipid peroxidation in the heart muscle tissue. The combined data showed that chickens fed with ATN received significant protection against the effects of lead acetate for most of the measured parameters.

Key words: Aluminosilicates, Heart, Muscle tissue, Oxidative stress, Lead acetate

Introduction

Lead (Pb) is a toxic heavy metal widely distributed in the environment due to its role in modern industry. Both environmental and occupational exposure remain serious problems in many countries. Effectively, Pb constitutes the most abundant non-essential element in animal and human organisms and is related to a broad range of physiologic, biochemical and behavioral dysfunctions (Erdoğan *et al*., 2005; Sharma *et al*., 2011). Many heavy metals, including Pb, are known to induce over-production of reactive oxygen species (ROS) and consequently, enhance lipid peroxidation of membranes. Reactive oxygen species are byproducts of degenerative reactions in many tissues, affecting regular metabolism by damaging cellular components (Ahamed and Siddiqui, 2007; Khaki and Khaki, 2010; Sharma *et al*., 2011).

Clays and zeolites belong to the group of aluminosilicates (ATN), are hydrated and composed mostly of aluminium and silica. Phyllosilicate clays are hydrated, crystalline ATNs containing alkali and alkaline earth cations and have layered structures (Serwicka and Bahranowski, 2004). Natural zeolites are hydrated aluminosilicate minerals characterized by cage-like structures with high internal and external surface areas, and high cation-exchance capacities (Matijašević *et al*., 2006). Because of its functional properties and accessibility, bentonite is widely used as a feed additive. Numerous studies have demonstrated that dietary inclusion of ATN did not disturb normal biochemical and physiological processes in animals (Prvulović *et al*., 2007, 2009, 2014; Demirel *et al*., 2011; Damiri *et al*., 2012; Eleroglu and Yalcin, 2012).

Due to their high ion-exchange capacity, alumino-silicates have been used effectively to prevent heavy metal toxicity in animals (Papaioannou *et al*., 2005; Yu *et al*., 2006).

The present study was performed to investigate the effect of lead acetate and ATN on the activities of antioxidative defense enzymes and lipid peroxidation in the heart and skeletal muscle tissues of broiler chickens.

Materials and Methods

Eighty four 1-day-old, unvaccined broiler chicks of both sexes were obtained from a commercial hatchery. Individually weighted chicks were divided randomly into four groups, housed in electrically heated batteries under fluorescent lighting and given a commercial basal diet (maize and soybean meal diet 220 g protein, 13.00 MJ ME/kg) formulated to contain National Research Council (1994) requirements. Lighting was continuous and food and water were available *ad libitum*. The experimental design consisted of four dietary treatments with seven replicates of three broiler chicks for each dietary treatment.

1. Control: Basal diet
2. ATN: Basal diet + 5.0 g ATN/kg diet
3. Pb: Basal diet + 500 mg lead acetate/kg diet
4. Pb + ATN: Basal diet + 500 mg lead acetate + 5 g ATN/kg diet

ATN (antitoxic nutrient) is a fine powder containing mostly zeolitic ore (with >90% of clinoptilolite) and bentonite (with >83% of montmorillonite), with small amounts of activated charcoal (ratio 60:20:1/zeolite: bentonite:charcoal). The study was approved by the Ethical Committee for Animal Use in Experiments of the University of Novi Sad.

The feeding trial was terminated when the chicks reached 3 weeks of age. According to the following recommendations for the euthanasia of experimental animals (Close *et al*., 1997), all 84 broilers were sacrificed without stress by cervical dislocation and their hearts, breast muscles and drumsticks were removed. Homogenates of these organs were used with phosphate buffers (pH=7.0) for further biochemical analysis. As measures of lipid peroxidation intensity, activities of the antioxidant enzymes superoxide dismutase (SOD-1), catalase (CAT), pyrogallol peroxidase (PPx), guaiacol peroxidase (GPx), and levels of malonyldlialdehyde (MDA) were measured in selected muscle tissues. Superoxide dismutase activities were determined in samples according to McCord and Fridovich (1968). CAT activity was assayed by the Clairborne method (1986). Utilization of hydrogen peroxide by CAT was measured spectrophotometrically in the samples as the decrease in optical density at 240 nm. PPx activity was measured using pyrogallol as substrate according to Chance and Maehly (1955). The formation of purpurogallin was followed at 430 nm. GPx activity was measured by following the H_2O_2 dependent oxidation of guaiacol at 470 nm according to Agrawal and Laloraya (1977). MDA level was analyzed with 2-thiobarbituric acid and the change of absorbance was monitored at 532 nm with the spectrophotometer (Placer *et al*., 1966). Protein content in the homogenate of selected organs was determined according to the Bradford method (1976), using bovine serum albumin as the protein standard.

Results were expressed as means±SE. Statistical significance was tested by analysis of variance followed by comparison of means by Duncan's multiple range test ($P<0.05$) calculated using STATISTICA for Windows version 9.0 (StatSoft, Tulsa, OK, USA).

Results

Effects of lead acetate and ATN on the activities of endogenous antioxidant enzymes and lipid peroxidation in the heart tissue of broilers are shown in Table 1. ATN alone did not induce any significant change of activity of the measured enzymes and did not provoke lipid peroxidation in the heart muscle tissue ($P>0.05$). Significant decreases in SOD-1 and CAT activities were observed in the lead-treated broilers' heart homogenate ($P<0.05$). There were no significant differences in the heart activities or peroxidases (GPx and PPx) of the control group and animals treated with Pb, ATN or ATN along with Pb ($P>0.05$). MDA level in the heart tissues increased significantly in the group treated with Pb alone or in combination with ATN compared with the control and ATN groups ($P<0.05$). Generally, there was no significant change in the levels of endogenous antioxidants (SOD-1, CAT and peroxidases) in animals exposed to Pb in combination with ATN compared to the control or ATN groups.

Table 2 represents the activity of the measured antioxidant enzymes and MDA levels in breast muscle tissues (white meat). Antioxidative protection enzyme activities and MDA levels in the drumstick muscle tissues of broilers (red meat) are presented in Table 3.

Table 1: Effect of Pb and ATN on antioxidant enzyme activities and lipid peroxidation in broilers' heart tissues

Parameter	Experimental group			
	Control	ATN	Pb	Pb + ATN
SOD-1 (IU/mg protein)	1.27 ± 0.02^{a}	1.33 ± 0.03^{a}	0.56 ± 0.02^{b}	1.22 ± 0.02^{a}
CAT (IU/mg protein)	$5.04 \pm 0.53^{a, b}$	5.09 ± 0.27^{a}	4.52 ± 0.15^{b}	$4.77 \pm 0.14^{a, b}$
GPx (IU/mg protein)	1.21 ± 0.02^{a}	1.33 ± 0.02^{a}	1.35 ± 0.02^{a}	1.30 ± 0.02^{a}
PPx (IU/mg protein)	10.03 ± 0.25^{a}	11.27 ± 0.28^{a}	9.85 ± 0.27^{a}	10.34 ± 0.29^{a}
Lipid peroxidation (nmol MDA/mg protein)	5.11 ± 0.20^{a}	5.08 ± 0.10^{a}	8.25 ± 0.48^{b}	7.46 ± 0.67^{b}

The data are mean values ± SE. $^{a, b}$ Values without the same superscripts differ significantly ($P<0.05$). SOD-1: Superoxid dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, and PPx: Pyrogallol peroxidase

Table 2: Effect of Pb and ATN on antioxidant enzyme activities and lipid peroxidation in broilers' breast muscle tissues

Parameter	Experimental group			
	Control	ATN	Pb	Pb + ATN
SOD-1 (IU/mg protein)	0.46 ± 0.01^{a}	0.42 ± 0.01^{a}	0.44 ± 0.01^{a}	0.40 ± 0.01^{a}
CAT (IU/mg protein)	0.91 ± 0.08^{a}	0.95 ± 0.07^{a}	0.68 ± 0.07^{b}	0.92 ± 0.06^{a}
GPx (IU/mg protein)	1.05 ± 0.07^{a}	1.16 ± 0.08^{a}	0.98 ± 0.06^{a}	0.99 ± 0.07^{a}
PPx (IU/mg protein)	4.15 ± 0.10^{a}	3.46 ± 0.06^{a}	3.11 ± 0.15^{a}	3.32 ± 0.15^{a}
Lipid peroxidation (nmol MDA/mg protein)	1.37 ± 0.04^{a}	1.64 ± 0.03^{a}	1.40 ± 0.02^{a}	1.40 ± 0.05^{a}

The data are mean values ± SE. $^{a, b}$ Values without the same superscripts differ significantly ($P<0.05$). SOD-1: Superoxid dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, and PPx: Pyrogallol peroxidase

Table 3: Effect of Pb and ATN on antioxidant enzyme activities and lipid peroxidation in broilers' drumstick muscle tissues

Parameter	Experimental group			
	Control	ATN	Pb	Pb + ATN
SOD-1 (IU/mg protein)	0.84 ± 0.03^{a}	0.95 ± 0.04^{a}	0.94 ± 0.02^{a}	0.95 ± 0.01^{a}
CAT (IU/mg protein)	1.65 ± 0.12^{a}	1.53 ± 0.09^{a}	1.50 ± 0.13^{a}	1.68 ± 0.16^{a}
GPx (IU/mg protein)	1.58 ± 0.09^{a}	$1.80 \pm 0.01^{a, b}$	2.03 ± 0.15^{b}	$1.81 \pm 0.14^{a, b}$
PPx (IU/mg protein)	$6.21 \pm 0.39^{a, b}$	4.94 ± 0.37^{b}	6.56 ± 0.47^{a}	5.00 ± 0.21^{b}
Lipid peroxidation (nmol MDA/mg protein)	2.40 ± 0.09^{a}	2.72 ± 0.12^{a}	2.30 ± 0.08^{a}	2.38 ± 0.10^{a}

The data are mean values ± SE. $^{a, b}$ Values without the same superscripts differ significantly ($P<0.05$). SOD-1: Speroxid dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, and PPx: Pyrogallol peroxidase

Compared to the control group, treatment with Pb significantly decreased CAT activity in the white meat and increased GPx activity in the red meat of broilers ($P<0.05$). It was observed that the oral intake of ATN and lead acetate alone or in combination did not cause impairments of the other selected enzymes' activity or lipid peroxidation levels in the broilers' skeletal muscles ($P>0.05$).

Discussion

Toxic effects of Pb have been recognized and remain a major public health problem. Primary sources responsible for Pb exposure include food, water, inhalation, and an ecosystem contaminated with Pb. Exposure to environmental Pb is known to affect various organ systems. Absorption of inorganic Pb causes biochemical and metabolic toxicity by inhibiting many processes (Sharma *et al*., 2011).

One of the causes of Pb toxicity is thought to be its impact on the release of hydroxyl radicals. Superoxide dismutase metabolizes the superoxide free radical anion to hydrogen peroxide, and is an effective cell defense against the endogenous and exogenous generations of superoxide (Sharma *et al*., 2012). Under oxidative stress, SOD-1 can behave in two different ways: initially and when stress is moderated, the cells act by suppressing SOD-1, but if the stress lasts for a long time and favors the increased production of ROS, the enzyme is exhausted and its concentration falls (Levine and Kidd, 1996). In the present study, SOD-1 levels in the heart decreased in lead-intoxicated animals. This could be explained by the massive production of superoxide anions which override enzymatic activity in the heart tissue. These results are in agreement with those of other studies on rats (Upasani and Balaraman, 2003). However, in the ATN treated animals, SOD-1 levels were restored to normal even after Pb exposure.

Catalase has been reported to be responsible for the detoxification of significant amounts of hydrogen peroxide which is formed during various biochemical and metabolic reactions (Ahamed and Siddiqui, 2007). Seven *et al*. (2010) reported increased CAT activities in the hearts of broilers orally exposed to Pb. In the present study, CAT levels in the heart tissue slightly decreased in animals with Pb exposure compared to those of the ATN group. The co-administration of ATN partially restored CAT levels to normal.

Roshan *et al*. (2012) also found chronic lead acetate administration to induce imbalances in myocardial antioxidant defenses, promoting lipid peroxidation in rats. On the contrary, other authors (Patra and Swarup, 2004) did not detect any changes in lipid peroxidation levels in the hearts of Pb-treated rats. The present study shows ATN to have a protective effect against Pb-induced alteration in MDA levels and endogenous antioxidants in the heart.

Treatment with different dosage of Pb did not significantly affect concentrations of Pb in skeletal muscles of American kestrels (Custer *et al*., 1984) and broilers (Erdoğan *et al*., 2005). In the present study, it was observed that chronic low level Pb exposure did not induce oxidative stress in the experimental animals' skeletal muscles.

In conclusion, the co-administration of natural occurring ATN offers partial protection against Pb-induced oxidative stress in heart muscle tissues of broilers. Lead did not induce any adverse effect on skeletal muscle tissues. Aluminosilicates have the ability to absorb Pb in the lumen of the digestive tract and could thus be used as supplementary agents in animal feeds.

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