

Inhibitory Effects of Aluminosilicates on Lead Acetate Toxicity in Selected Organs of Broilers

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Abstract

The aim of this study was to determine the effect of dietary supplements of lead acetate and aluminosilicates on oxidative status in brain, kidney and duodenum of broiler chickens. In this regard, activity of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPx), pyrogallol peroxidase (PPx) and lipid peroxidation was determined in examined organs. The experiment was performed with eighty-four broiler chickens of both sexes randomly allotted to four diets including the control group, the Pb group containing 500 mg lead acetate/kg diet, the ATN (Antitoxic nutrient) group with 5 g mixture of aluminosilicates (zeolite and montmorillonite)/kg diet and the Pb + ATN group containing 500 mg lead acetate and 5 g mixture of aluminosilicates/kg diet, in a 3-week feeding experiment. Each of these groups consisted of seven replications with 3 chickens per replication. Results suggest that aluminosilicates alone did not provoke any adverse effects and did not disturb normal biochemical and physiological homeostasis in the broilers. The dietary intake of lead acetate induced oxidative stress and promoted increase in level of malondialdehyd (MDA), a lipid peroxidation marker, in all examined organs. The lead intake induced increase in CAT activity in all organs, while it decreased the activity of GPx and PPx in the brain and duodenum but did not affect that in the kidneys. The activity of SOD increased in the kidneys and duodenum but did not significantly change in the brain. The combined data showed that the chickens fed aluminosilicates received significant protection against the effects of lead acetate for most parameters measured which remained at control level.

Keywords: antitoxic nutrient, brain, duodenum, kidney, lipid peroxidation, oxidative status

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Introduction

The most common form of heavy metal intoxication is probably lead toxicity. It is well-documented as one of the most dangerous and insidious poisons. Lead is classically a chronic or cumulative toxin due to its slow rate of elimination. The harmful levels of lead can accumulate in tissues after prolonged exposure to low quantities. Lead, like many other heavy metals, is known to induce overproduction of reactive oxygen species (ROS) including hydroperoxides, singlet oxygen, and hydrogen peroxide and disruption of prooxidant/antioxidant balance in blood and other soft tissues. Lead is a poison that affects virtually every system in the body. It causes oxidative stress by increasing the level of lipid peroxidation and disturbing the activity of many antioxidant enzymes with deleterious effect on erythropoiesis, kidney function, immune system and the central nervous system (Gautam and Flora, 2010; El-Newehy and El-Sayed, 2011; Mobarak and Sharaf, 2011; Sharma et al., 2011).

Zeolites and clays are hydrated and composed mostly of aluminium and silica and belong to the group of aluminosilicates. Aluminosilicates have found multifarious applications as adsorbents, ion exchangers and catalyst in industry, veterinary medicine, environmental protection, agriculture and sanitation. Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations consisting of three-dimensional framework of SiO_4^{4-} and AlO_4^{5-} tetrahedral linked through the shared oxygen atoms. They are porous materials, characterized by the ability to lose and gain water reversibly, to adsorb molecules and act as molecular sieves and to exchange their constituent cations without major change in their structures (Mumpton, 1999). Zeolites, due to their high ion-exchange capacity, have been used effectively for the prevention of heavy metal toxicity in animals (Papaioannou et al., 2005). Phyllosilicate clays are hydrated, crystalline aluminosilicates containing alkali and alkaline earth cations and have layered structure. Montmorillonite, the main constituent of phyllosilicate ore bentonite, is trimorphic phyllosilicate formed by a 2:1 condensation of layers with aluminium sandwiched between two layers of silica. Montmorillonite possesses exchangeable sodium or calcium cations and has expandable sheets (Serwicka and Bahrnowski, 2004). Because of functional properties and accessibility, bentonite is widely used as a feed additive. Numerous studies demonstrated that dietary inclusion of aluminosilicates did not disturb normal biochemical and physiological processes in animals (Pruvulović et al., 2008, 2012; Safaiekatouli et al., 2011; Slamova et al., 2011).

The present investigation focused on the lead-induced oxidative stress in duodenum, brain and kidney in chickens and further examined the protective effects of aluminosilicates supplementation on lead-induced oxidative stress.

Materials and Methods

Eighty-four 1-day-old, unvaccinated broiler chicks of both sexes were obtained from a commercial hatchery. Individually weighed chicks were divided at random into four groups. There were seven replicates of three broiler chicks for each dietary treatment. The chicks were housed in electrically heated batteries under fluorescent lighting and received a commercial basal diet (maize and soybean meal diet 220 g protein, 13.00 MJ ME/kg) formulated to meet the National Research Council (1994) requirements. Food and water were available *ad libitum* and lighting was continuous. The experimental design consisted of four dietary treatments: 1. Control: basal diet; 2. ATN: basal diet plus 5.0 g ATN/kg diet; 3. Pb: basal diet plus 500 mg lead acetate/kg diet; and 4. Pb + ATN: basal diet plus 500 mg lead acetate plus 5 g ATN/kg. ATN (Antitoxic nutrient) is a fine powder containing mostly zeolitic ore (with > 90% of clinoptilolite) and bentonite (with > 83% of montmorillonite), together 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, Serbia.

When the chicks reached 3 weeks of age, the feeding trial was terminated. 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. Selected organs as kidneys, brains and duodenum from three animals were removed and collected in seven replicates per group. After rinsing with saline the organs were homogenized in an ice-cold 50 mM phosphate buffer pH 7.0 (10 w/v%). The homogenates were centrifuged at 3000 g for 15 min, supernatants were aliquot and stored at -20°C for further biochemical analysis. Activity of antioxidant enzymes, i.e. superoxide dismutase (SOD-1), catalase (CAT), guaiacol peroxidase (GPx), pyrogallol peroxidase (PPx), and lipid peroxidation, was measured in the homogenates of kidney, brain and duodenum. Protein content in the homogenates of selected organs was determined according to the method of Bradford (1976), using bovine serum albumin as the protein standard. The SOD activity was determined in the samples according to McCord and Fridovich (1968). The CAT activity was assayed by the method of Clairborne (1986). The utilization of hydrogen peroxide by CAT in the samples was measured spectrophotometrically as decrease in optical density at 240 nm. The GPx activity was measured by following the H_2O_2 depend oxidation of guaiacol at 470 nm of Agrawal and Laloraya (1977). The activity of PPx was measured using pyrogallol as the substrate according to Chance and Maehly (1955). The formation of purpurogallin was followed at 430 nm. Malondialdehyd (MDA) level was analyzed with 2-thiobarbituric acid, monitoring the change of absorbance at 532 nm with a spectrophotometer (Placer et al., 1966).

Results were expressed as mean of determinations \pm standard error (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 12.0 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

Results

Table 1 shows the activity of antioxidant enzymes SOD-1, CAT, GPx and PPx in brain tissue. Results of our study demonstrated significant ($p < 0.05$) changes in the activity of CAT, GPx and PPx while the activity of SOD-1 stayed unchanged in the Pb group compared to the control and ATN groups. Our study showed an increase in the MDA content in the brain of broilers treated with the lead acetate, suggesting an increase in lipid peroxidation in brain cells. ATN alone

did not provoke lipid peroxidation and did not induce any significant changes in the activity of measured enzymes in the brain tissue ($p > 0.05$).

The effects of lead acetate and ATN on the activity of endogenous antioxidant enzymes and lipid peroxidation in the kidneys of broilers are shown in Table 2. A significant increase ($p < 0.05$) in the SOD-1 and CAT activity was observed in the lead-treated broilers' kidney homogenates. There was no significant difference in the activity of peroxidases (GPx and PPx) in the kidneys of the control group and those of the animals treated with Pb, ATN or ATN along with Pb ($p > 0.05$). The MDA level in kidneys was increased significantly ($p < 0.05$) in the group of animals treated with Pb compared with the control and ATN groups. There were no significant changes in the levels of MDA and endogenous antioxidants (SOD-1, CAT and peroxidases) in the animals exposed to Pb in combination with ATN compared to the control or ATN groups.

Table 1 Effect of lead acetate (Pb) exposure alone and in combination with ATN on the activity of endogenous antioxidant enzymes and lipid peroxidation in the brain of broilers

Parameter	Experimental group							
	Control		ATN		Pb		Pb + ATN	
	mean \pm SE	range	mean \pm SE	range	mean \pm SE	range	mean \pm SE	range
SOD-1 (IU/mg protein)	1.13 ^a \pm 0.03	0.95-1.29	1.21 ^a \pm 0.03	1.10-1.36	1.13 ^a \pm 0.02	0.94-1.26	1.22 ^a \pm 0.03	1.14-1.32
CAT (IU/mg protein)	2.25 ^a \pm 0.08	1.72-2.54	2.45 ^a \pm 0.11	2.00-2.63	2.93 ^b \pm 0.12	2.65-3.50	2.50 ^{a,b} \pm 0.22	1.60-2.61
GPx (IU/mg protein)	4.00 ^{a,c} \pm 0.32	2.39-4.85	3.28 ^a \pm 0.37	2.15-4.66	1.98 ^b \pm 0.12	1.32-2.50	4.28 ^c \pm 0.33	2.73-5.97
PPx (IU/mg protein)	12.85 ^a \pm 0.55	9.84-16.29	12.42 ^a \pm 0.46	10.18-16.03	8.96 ^b \pm 0.16	8.31-9.86	12.68 ^a \pm 0.68	9.89-17.21
Lipid peroxidation (nmol MDA/mg protein)	6.97 ^{a,c} \pm 0.29	5.60-7.91	7.65 ^a \pm 0.37	5.95-7.99	10.68 ^b \pm 0.54	7.54-12.07	6.37 ^c \pm 0.27	5.10-7.38

SE - standard error

^{a,b,c} Values without the same superscripts within each row differ significantly ($p < 0.05$).

SOD-1: Superoxid dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, PPx: Pyrogallol peroxidase

Table 2 Effect of lead acetate (Pb) exposure alone and in combination with ATN on the activity of endogenous antioxidant enzymes and lipid peroxidation in the kidneys of broilers

Parameter	Experimental group							
	Control		ATN		Pb		Pb + ATN	
	mean \pm SE	range	mean \pm SE	range	mean \pm SE	range	mean \pm SE	range
SOD-1 (IU/mg protein)	10.56 ^a \pm 0.33	9.61-13.22	10.98 ^a \pm 0.42	9.28-12.98	19.71 ^b \pm 0.37	16.87-23.55	11.87 ^a \pm 0.29	10.00-13.87
CAT (IU/mg protein)	62.17 ^a \pm 3.05	55.11-66.93	62.08 ^a \pm 2.63	49.90-70.99	89.55 ^b \pm 5.94	86.52-113.95	66.82 ^a \pm 3.19	47.76-75.98
GPx (IU/mg protein)	15.01 ^a \pm 1.18	10.96-19.98	16.85 ^a \pm 1.26	11.80-20.89	12.75 ^a \pm 0.85	10.05-17.71	14.72 ^a \pm 1.81	12.61-19.37
PPx (IU/mg protein)	49.38 ^a \pm 2.41	40.92-55.48	46.85 ^a \pm 2.44	45.22-56.75	43.51 ^a \pm 1.23	38.41-54.88	42.94 ^a \pm 2.69	33.34-56.15
Lipid peroxidation (nmol MDA/mg protein)	2.30 ^a \pm 0.09	1.82-2.52	2.61 ^a \pm 0.09	1.96-3.15	3.80 ^b \pm 0.07	3.51-4.32	2.69 ^a \pm 0.10	2.26-3.11

SE - standard error

^{a,b} Values without the same superscripts within each row differ significantly ($p < 0.05$).

SOD-1: Superoxid dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, PPx: Pyrogallol peroxidase

The results of this experiment clearly showed that the oral administration of lead acetate could induce oxidative stress in the duodenum of broilers (Table 3). The content of MDA and the activity of all measured antioxidant enzymes in the Pb group were significantly increased, except PPx, of which the activity was decreased compared to the values of the control group as well as the ATN and Pb + ATN groups ($p < 0.05$). It was observed that the oral intake of ATN alone or in combination with lead acetate did not cause impairment of the selected enzymes' activity or level of lipid peroxidation in the duodenum of broilers ($p > 0.05$).

Discussion

Pollution of the environment by heavy metal through human activity is nowadays serious ecological concern, wherein lead is among the most toxic. Heavy metal ions through air, soil and water enter organisms of plant and animal, causing damage of cells and development of diseases by generation of oxidative stress. Reactive oxygen species as toxic metabolites are generated by aerobic metabolism in the cell which in turn significantly increases pathological conditions, leading to free radical mediated denaturation of

proteins, enzymatic deactivation, base hydroxylation of nucleic acids, cross linking or strand scission, mutation or even cell death (Gautam and Flora, 2010). High affinity of zeolite and mineral clay to heavy metal ions is well documented (Mumpton, 1999; Serwicka and Bahranowski, 2004) based on the use of aluminosilicates for treatment of wastewater and soil fertilizer. Although many studies have reported

dietary supplement aluminosilicates without effects on normal physiological and biochemical parameters, there is limited information about the inhibitory effects of aluminosilicates as dietary supplement on heavy metal toxicity (Papaioannou et al., 2005; Flowers et al., 2009; Prvulović et al., 2014).

Table 3 Effect of lead acetate (Pb) exposure alone and in combination with ATN on the activity of endogenous antioxidant enzymes and lipid peroxidation in the duodenum of broilers

Parameter	Experimental group							
	Control		ATN		Pb		Pb + ATN	
	mean ± SE	range	mean ± SE	range	mean ± SE	range	mean ± SE	range
SOD-1 (IU/mg protein)	2.45 ^a ± 0.05	1.94-2.98	2.67 ^a ± 0.05	2.06-3.11	3.91 ^b ± 0.05	3.18-4.36	2.78 ^a ± 0.06	2.22-3.15
CAT (IU/mg protein)	13.83 ^{a,b} ± 0.26	13.00-14.88	12.14 ^a ± 0.98	10.08-13.82	16.68 ^b ± 0.98	14.34-19.62	13.01 ^a ± 0.29	11.94-13.84
GPx (IU/mg protein)	0.63 ^a ± 0.06	0.43-0.83	0.65 ^a ± 0.07	0.44-0.88	1.04 ^b ± 0.17	0.52-1.78	0.79 ^{a,b} ± 0.12	0.46-1.22
PPx (IU/mg protein)	4.26 ^a ± 0.38	2.91-5.6	4.71 ^a ± 1.14	3.36-6.98	2.58 ^b ± 0.32	1.53-3.16	4.17 ^a ± 0.15	3.54-4.75
Lipid peroxidation (nmol MDA/mg protein)	1.18 ^a ± 0.30	1.08-1.32	1.16 ^a ± 0.30	1.03-1.36	1.98 ^b ± 0.40	1.51-2.19	1.27 ^a ± 0.24	1.09-1.50

SE - standard error

^{a,b} Values without the same superscripts within each row differ significantly ($p < 0.05$).

SOD-1: Superoxide dismutase, CAT: Catalase, GPx: Guaiacol peroxidase, PPx: Pyrogallol peroxidase

The blood/brain barrier does not for a substantial impediment to lead enter developing or adult brain (Antonio et al., 1996). The deleterious effects of lead on the central nervous system range from behavioral dysfunction to encephalopathy, particularly in developing animals (Adonaylo and Oteiza, 1999; Upasani et al., 2003). Lead induced hyperactivity has been reported in humans and animals (Cervantes et al., 2005; Correa et al., 2005). Lead is a well-known neurotoxin that causes various degrees of edema of the brain and degeneration of white and gray matter of both the central and peripheral nervous system. Chronic Pb toxicity causes poliocephalomacia, laminar necrosis of the cerebrocortex and an accumulation of macrophages. This may be attributed to oxidative damage associated with chronic Pb intoxication in the rat brain (El-Newehy and El-Sayed, 2011). Unlike some other authors who reported that the oral administration of Pb induced inhibition of SOD-1 and CAT activity in the brain of rats (Gautam and Flora, 2010; Prasanthi et al., 2010; Sainath et al., 2011), the results of our experiment showed significant increase in CAT and degree of lipid peroxidation. Moreover, the activity of SOD-1 stayed undisturbed by the oral intake of Pb. However, the activity of peroxidases (GPx and PPx) was decreased by the oral intake of Pb, which is in agreement with the study undertaken by Reckziegel et al. (2011). Studies in experimental animals have reported that lead alters oxidative metabolism and enhances LP directly or indirectly (Wang et al., 2010; Reckziegel et al., 2011; Sainath et al., 2011), agreeing with our results. The result of our experiment indicates that ATN exhibits full protection of broiler's brain against harmful treatment of lead acetate and maintains all examine parameters of oxidative status in the brain at control level.

Interstitial and glomerular damage is characteristic renal lesions due to Pb toxicity (El-Newehy and El-Sayed, 2011). The results of our study demonstrated that the oral intake of lead acetate

induced the activity of antioxidative enzymes and provoked oxidative stress in the kidneys of chickens. In contrast to some recent reports which established the inhibitory effects of Pb on SOD in rat's kidney (Ponce-Canchihuamán et al., 2010; Sainath et al., 2011), in our study the activity of this enzyme in the kidneys of broilers was increased. Other studies (Ponce-Canchihuamán et al., 2010; Sainath et al., 2011) also reported the inhibitory effects of Pb on CAT activity while the activity of CAT in the kidneys of chickens from the Pb group in our experiment was elevated. However, our results are partially in agreement with the experiment performed on rats of Massó-González and Antonio-García (2009). The oxidative stress has also been implicated to contribute to lead-associated tissue injury in the liver. The results of this study showed the increase in MDA content in the kidneys of broilers treated with the lead acetate. This result is in agreement with the studies undertaken by Patra et al. (2000), Ponce-Canchihuamán et al. (2010) and Sainath et al. (2011), who recorded an increase in MDA content in the liver of rats subjected to subchronic exposure to Pb. Furthermore, our study is in agreement with our previous study which demonstrated that the oral intake of ATN did not provoke oxidative stress in the kidney of broiler chicken (Prvulović et al., 2014), further confirming the protective effect of ATN on the oxidative status of kidney during Pb exposure.

Literature data about oxidative process in duodenum of animals under lead toxicity are very limited. The data presented in this manuscript clearly suggest that the oral administration of lead acetate provokes oxidative stress and induces activity of antioxidative enzymes in the duodenum of broiler chickens. Duodenum forms the first line of defense against numerous toxins and the contact with these substances is the most intensive there. Absorption of lead occurs primarily in the duodenum and may involve active transport, transcellular or paracellular diffusion, ionized lead (Pb^{2+}) and/or organic or inorganic complexes. The extent and rate of

gastrointestinal absorption are influenced by physiological conditions of the exposed animals such as age, fasting, presence of nutritional elements, physicochemical characteristics of the ingested medium, including particle size, mineral species, solubility and lead species (Mushak, 1991; Sharma and Barber, 2012). In our study, the oral lead administration induced all examine parameters of oxidative status in the chicken duodenum to increase, except PPx which decreased, indicating obviously the presence of oxidative stress. The protective effect of dietary ATN supplementation was recorded on the oxidative status of duodenum as well as on the brain and kidney in this study.

In conclusion, this study demonstrated that the alterations in biochemical markers of oxidative stress in selected organs of lead-exposed chickens were consistent with the hypothesis that cellular and molecular damage mediated by free radicals may constitute pathogenesis of lead poisoning. This present study reveals that ATN showed a protective effect against lead-induced alteration in the levels of MDA and endogenous elements of antioxidative defense system in the kidney, brain and duodenum of broilers. The beneficial effect of ATN could probably be attributed to the unique physical and chemical properties of natural aluminosilicates, especially to the high cation exchange and ion adsorption capacities.

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บทคัดย่อ

ผลของ Aluminosilicate ต่อการยับยั้งความเป็นพิษของ Lead Acetate ในบางอวัยวะของไก่กระทง

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วัตถุประสงค์ของการศึกษานี้เพื่อวัดผลของอาหารเสริม lead acetate และ aluminosilicate ต่อสถานะของออกซิเดชันในสมอง ไต และลำไส้เล็กส่วนต้นของไก่กระทง การทำงานของเอนไซม์ที่ต้านการเกิดออกซิเดชัน เช่น superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPx), pyrogallol peroxidase (PPx) และ lipid peroxidation ได้ถูกวัดในอวัยวะดังกล่าว การทดลองได้ใช้ไก่กระทงทั้ง 2 เพศ จำนวน 84 ตัว โดยถูกสุ่มแบ่งเป็น 4 กลุ่มตามประเภทอาหาร ได้แก่ กลุ่มควบคุม กลุ่ม Pb ซึ่งมี lead acetate 500 มก./กก. ของอาหาร กลุ่ม ATN (สารอาหารที่มีฤทธิ์ต้านพิษ) ที่มีส่วนผสมของ aluminosilicate (zeolite และ montmorillonite) 5 กรัม/กก. ของอาหาร และกลุ่ม Pb + ATN ที่มีส่วนผสมของ lead acetate 500 มก. และส่วนผสมของ aluminosilicate 5 กรัม/กก. ของอาหาร ในช่วงเวลา 3 สัปดาห์ที่ทดลองให้อาหาร กลุ่มการทดลองเหล่านี้แต่ละกลุ่มประกอบไปด้วย 7 ซ้ำการทดลอง ซึ่งมีไก่ 3 ตัว/การทดลองที่ทำซ้ำ ผลการศึกษาแนะนำว่า aluminosilicate เพียงอย่างเดียวไม่สามารถทำให้เกิดผลข้างเคียง และไม่รบกวนสมดุลปกติของชีวเคมีและสรีรวิทยาในไก่กระทง การกินอาหารที่มี lead acetate เหนี่ยวนำให้เกิด oxidative stress และส่งเสริมให้มีการเพิ่มของระดับ malondialdehyd (MDA) ซึ่งเป็นตัวบ่งชี้ของการเกิด lipid peroxidation ในอวัยวะทั้งหมดที่ทำการตรวจ การกิน lead เหนี่ยวนำให้มีการเพิ่มของการทำงานของ CAT ในทุกอวัยวะ ในขณะที่ทำให้ลดการทำงานของ GPx และ PPx ในสมองและลำไส้เล็กส่วนต้น แต่ไม่ส่งผลดังกล่าวต่อไต การทำงานของ SOD มีเพิ่มขึ้นในไตและลำไส้เล็กส่วนต้น แต่ไม่พบการเปลี่ยนแปลงอย่างมีนัยสำคัญในสมอง จากข้อมูลทั้งหมดได้แสดงให้เห็นว่าไก่ที่ได้รับ aluminosilicate ได้รับการป้องกันอย่างมีนัยสำคัญในการต้านผลของ lead acetate สำหรับตัวชี้วัดทั้งหมดที่ได้มีการวัด ซึ่งยังคงอยู่ในระดับที่ควบคุมได้

คำสำคัญ: สารอาหารต้านพิษ สมอง ลำไส้เล็กส่วนต้น ไต lipid peroxidation สถานะของออกซิเดชัน

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