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REDUCTION OF LEAD OXIDE TOXICITY BY USING BENTONITE IN MONO-SEX NILE TILAPIA *Oreochromis niloticus* DIETS

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Abstract

The present study designed to used of bentonite as an adsorbent agent with levels (0, 1 and 2%) to alleviate the toxic effects of dietary lead oxide with levels (0, 100 and 1000 ppm) on growth performance and survival rate, carcass composition and its residues in fish muscles, and blood hematological and biochemical parameters of mono-sex Nile tilapia *Oreochromis niloticus* for 16 weeks. So, the factorial design analysis (3X3) was used in the present study. The obtained results showed that contaminated diets with lead oxide led to significantly ($P \leq 0.05$) decreased of growth performance (final weight, weight gain, average daily gain, specific growth rate and feed intake), carcass composition (crude protein), blood hematological (hemoglobin, red blood cells, packed cell volume, MCHC, blood platelets, white blood cells and the percentage of lymphocytes), blood biochemical (total protein, albumin, globulin, albumin/globulin ratio and total cholesterol) compared with control group. While, survival rate and blood indices (MCH) insignificantly ($P \geq 0.05$) decreased. But, feed conversion ratio impaired significantly compared with control group. However, dry matter, ether extract, ash, bioaccumulation of total lead in fish muscles, MCV, the percentage of monocytes, neutrophils, eosinophils and aspartate aminotransferase, alanine aminotransferase and uric acid concentrations were increased significantly ($P \leq 0.05$) compared with the control group. As well as, these drastic effects were increased by increasing level of dietary lead oxide. On the other side, dietary supplemented by bentonite as an adsorbent agent reduced the toxic effects of lead oxide on mentioned measured parameters. Consequently, it could be recommended that the safety and useful addition of bentonite with levels 1 or 2% to alleviate the toxic effects of dietary contaminated by lead oxide of *O. niloticus* fish.

Keywords: Nile tilapia – Lead oxide – Bentonite – Blood parameters - Residues.

INTRODUCTION

According to the Codex Committee for Food Additives and Contaminants, dietary intakes of heavy metals with high public concern need to be monitored on a regular basis and rapidly updated to identify recent dietary intakes of heavy metals in developing countries (Kim *et al*., 1999 and Yoon *et al*., 2000). Contamination of food products by heavy metals is becoming an unavoidable problem these days. Air, soil, and water pollution are contributing to the presence of harmful elements, such as cadmium, lead, mercury, and arsenic in foodstuff (Zukowska and Biziuk, 2008).

Lead (Pb) is common in the general population and has been well documented around the world (WHO, 2000). Environmental levels of lead have increased more than 1000-fold over the past three centuries as a result of human activity; the greatest increase occurred between the years 1950 and 2000 (ATSDR, 2005). Lead has been recognized as a poison for millennia and has recently been the focus of public health regulations in most of the developed world. Consequently, fatalities and symptomatic lead poisoning have declined dramatically during the latest decades and are continuing to decline (Kaufmann *et al*., 2003). It is harmful to humans, plants and animals. The lead poisoning can cause hypertension, nephritis, abdominal pain, constipation, cramps, nausea, vomiting, behavioral changes, learning disabilities, reading problems, development defects and language difficulties. Major lead pollution has been through in the manufacture of storage batteries, painting pigments, ammunition, solder, plumbing fixtures, automobiles, cable coverings, radioactivity shields, caulking and bearings (Ake *et al*., 2001 and Tunali *et al*., 2006b).

Although the traditional treatment methods such as precipitation, oxidation, reduction, electrochemical treatment, reverse osmosis, solvent extraction, adsorption, ion-exchange and evaporation can be used for the metal bearing effluents, most of these methods are expensive and difficult to apply (Volesky, 2001). Among these methods, adsorption has proved to be one of the most feasible, simple, selective, cost-effective, ease of operation and high efficient process for the removal of heavy metals from polluted sources. The most popular adsorbent for the adsorption process is activated carbon. It has a high surface area, high adsorption capacity and high degree of surface reactivity, whereas it is very expensive and there is a need for regeneration after each adsorption experiment (Özcan and Özcan, 2004 and Özcan *et al*., 2004). In order to decrease the cost of treatment process, the scientists have been attempted to investigate inexpensive, efficient and easily available adsorbents. In this manner, biological-based materials such as *Cephalosporium aphidicola* (Tunali *et al*., 2006b), *Saccharomyces cerevisiae* (Huang *et al*., 1990), *Aspergillus niger* (Jianlong *et al*., 2001) and *Bacillus* sp. (Tunali *et al*., 2006a); natural clay materials such as kaolinite (Gupta and Bhattacharyya, 2005), illite (Echeverria *et al*., 2005), bentonite (Naseem and Tahir, 2001 and Donat *et al*., 2005), montmorillonite (Barbier *et al*., 2000 and Gupta and Bhattacharyya, 2005), zeolite (Zamzow *et al*., 1990 and Ouki *et al*., 1993) and sepiolite (Brigatti *et al*., 2000 and Bektaş *et al*., 2004) have been used to remove lead (II) ions by adsorption.

Bentonite is natural clay that comes from volcanic ash. Because of properties and accessibility, bentonite is widely used as a feed additive (Abehsera, 1979). Sodium bentonite exhibited a high affinity for the toxin and could reduce the percentage of fish developing tumors after consuming toxins. Therefore, bentonite is commonly employed as a feed binder (Ellis *et al*., 2000). Consequently, the present study was implemented to overcoming the dietary lead (as lead oxide), toxicity affect at low (100 ppm) and high levels (1000 ppm) on growth performance, carcass composition, its residues in muscles and blood hematological and biochemical parameters of mono-sex Nile tilapia *Oreochromis niloticus* by using bentonite as an adsorbent agent at levels 1 and 2% for 16 weeks.

MATERIALS AND METHODS

The present study was conducted during the summer season 2008 in Fish laboratory research, Animal production department, Faculty of Agriculture, Mansoura University, Al-Dakahlia Governorate, Egypt. Fish were stocked in rearing tank for two weeks as adaptation period on the wet lab. conditions and feeding on basal experimental diet. After that a total number of 135 apparently-healthy fish at average initial body weights of (5.0-6.0 g) were distributed randomly. Then fish were stocked at rates of 5 fish/glass aquarium (90 x 40 x 50 cm). Each aquarium was supplied with 108 l dechlorinated tap water and an air stone connected with small electric compressor. The replacement of the aquaria water was done partially every day to re-new the tap water and to remove the wastes. Light period was controlled to provide a 14h light: 10h dark as a daily. The basal diet as chemical composition (89.19% dry matter, 27.24% crude protein, 6.42% ether extract, 55.4% carbohydrates, 10.91% ash), but gross energy 439.94 Kcal/100g DM and Protein/Energy ratio 61.91 mg CP/Kcal GE, which was calculated according (Macdonald *et al.*, 1973). It was formulated from the commercial ingredients (fish meal 12%, soybean meal 31%, yellow corn 20%, wheat bran 25%, corn oil 5%, vit. & mineral mixture 2% and molasses 5%). The dietary ingredients, lead oxide and bentonite were bought from the local market. Feed ingredients were grinded and the different ingredients mixed with lead oxide and bentonite at different levels by warm water and molasses. Diets were pressed by manufacturing machine (pellets size 1mm). During the experimental period (16 weeks), the fish were fed on the experimental diets at a rate of 4% of the live body weight daily, for six days a week. Experimental diets were introduced by hand twice daily, at 8 a.m. and 2 p.m. All fish were divided at 9

treatments (each three aquaria were refereed as a treatment). The experimental design of treatments showed in Table (1).

Table 1. The experimental design and treatments

Treat.	Details
T_1	0.00 ppm lead oxide + 0 % bentonite
T_2	0.00 ppm lead oxide + 1 % bentonite
T_3	0.00 ppm lead oxide + 2 % bentonite
T_4	100 ppm lead oxide + 0 % bentonite
T_5	100 ppm lead oxide + 1 % bentonite
T_6	100 ppm lead oxide + 2 % bentonite
T_7	1000 ppm lead oxide + 0 % bentonite
T_8	1000 ppm lead oxide + 1 % bentonite
T_9	1000 ppm lead oxide + 2 % bentonite

At the end of the experiment, the remained fish and its muscles were sampled from each treatment and kept frozen for chemical analysis. The chemical analyses of the basal diet and whole fish body were carried out according to the AOAC (2000). However, the residues of total lead content was determined by digesting the fish muscles using a mixture of sulfuric and perchloric acids according to Jackson (1973), which determined by using the atomic absorption spectrophotometer – Model PERKIN ELMER 2380. Body weight of individual fish was measured biweekly to point feed quantity and to calculate growth performance according to Abdelhamid (2000) in form of: average weight gain (g/fish) AWG = average final weight (g) – average initial weight (g), average daily gain, (g/fish/day) ADG = AWG (g)/experimental period (days), specific growth rate (SGR, %/day) = [ln final weight – ln initial weight] x 100/Experimental period (d), feed conversion ratio (FCR) = feed intake (g)/live weight gain (g) and survival rate (SR%) = end number of the alive fish/the beginning number of the fish x 100.

At the end of the experiment, blood samples were collected from the fish caudal peduncle of the different groups. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt) and the hematocrit (PCV%) was measured according to Stoskopf (1993). Also, total erythrocytes (RBCs), platelets and total leukocytes (WBCs) were counted according to Dacie and Lewis (1995) on an A_o Bright – Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for

determination of total protein (TP) (Gornall *et al*., 1949); albumin (Al) (Weichsebum, 1946); globulin (Gl) by difference (Doumas and Biggs, 1972); uric acid (Schultz, 1984); aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman and Frankel, 1957) and total cholesterol (Ellefson and Caraway, 1976) were assayed following commercial test kits using a spectrophotometer (model 5010, Germany).

The data collected were statistically analyzed by using SAS (1997), with factorial design (3 X 3) and evaluated by using the following model:

$$Y_{ijk} = \mu + L_i + B_j + LB_{ij} + e_{ijk}$$

Where, Y_{ijk} is the data of growth performance, carcass composition, residues in muscles and blood hematological and biochemical parameters of mono-sex Nile tilapia, μ is the overall mean, L_i is the fixed effect of the dietary lead oxide levels, B_j is the fixed effect of the dietary supplementation of bentonite levels, LB_{ij} is the interaction effect between dietary lead oxide levels with dietary supplementation of bentonite levels and e_{ijk} is the random error. The differences between means were statistically compared for the significance ($P \leq 0.05$) using Duncan (1955) multiple range test.

RESULTS

1- Growth performance

Mono-sex *O. niloticus* fed on contaminated diet by lead oxide showed significant ($P \leq 0.05$) decreased in growth performance parameters, but survival rate was insignificantly ($P \geq 0.05$) decreased while, feed conversion ratio was impaired significantly compared with the control group (Table 2). These drastic effects increased significantly by increased level of lead oxide from 100 to 1000 ppm. On the other side, dietary supplementation by 1% bentonite led to insignificantly ($P \geq 0.05$) decreased in growth performance parameters and survival rate but, feed conversion ratio was not affected compared to the control treatment (Table 2).

Addition of bentonite with 1 and 2% levels to the untreated diet with lead oxide (0 ppm) or the contaminated diet with low level (100 ppm) did not improved growth performance parameters and survival rate of *O. niloticus*, whereas all growth performance parameters significantly or not significantly decreased compared with the control group. However, dietary supplemented by 1% bentonite to contaminated diet with high level (1000 ppm) of lead oxide led to significantly ($P \leq 0.05$) improved of final weight, weight gain,

average daily gain, specific growth rate, feed intake and feed conversion ratio, but survival rate was decreased insignificantly ($P \geq 0.05$) when compared with the control treatment.

Table 2. Effect of lead oxide, bentonite concentrations in diets and their interaction on growth performance of mono-sex *O. niloticus* (means ±SE)

Treat.	Initial weight (g)	Final weight (g)	Total weight gain (g)	ADG	SGR	SR	FI	FCR
Lead oxide levels, ppm (L)								
0	5.57 ±0.02	40.5 ^{a} ±0.91	34.9 ^{a} ±0.90	291.2 ^{a} ±7.52	1.65 ^{a} ±0.02	96.67 ±3.33	64.2 ^{a} ±0.93	1.84 ^{b} ±0.04
100	5.68 ±0.04	41.2 ^{a} ±0.74	35.5 ^{a} ±0.73	295.7 ^{a} ±6.05	1.65 ^{a} ±0.02	93.33 ±6.66	62.9 ^{ab} ±1.09	1.77 ^{b} ±0.02
1000	5.62 ±0.04	31.9 ^{b} ±1.27	26.3 ^{b} ±1.29	218.8 ^{b} ±10.7	1.44 ^{b} ±0.04	76.67 ±9.54	61.2 ^{b} ±2.20	2.35 ^{a} ±0.07
Bentonite levels, % (B)								
0	5.64 ±0.04	38.8 ^{a} ±2.49	33.2 ^{a} ±2.48	276.3 ^{a} ±20.7	1.59 ^{a} ±0.06	80.00 ±7.30	62.7 ^{ab} ±1.98	1.96 ±0.12
1	5.58 ±0.02	38.1 ^{ab} ±0.75	32.6 ^{ab} ±0.73	271.5 ^{ab} ±6.11	1.60 ^{ab} ±0.01	86.67 ±9.88	65.0 ^{a} ±1.42	2.01 ±0.07
2	5.65 ±0.05	36.6 ^{b} ±1.63	30.9 ^{b} ±1.63	257.8 ^{b} ±13.5	1.55 ^{b} ±0.04	100.00 ±0.00	60.5 ^{b} ±0.51	2.00 ±0.10
Interaction (L*B)								
0*0	5.58 ±0.03	43.6 ^{a} ±0.20	38.0 ^{a} ±0.23	316.9 ^{a} ±1.96	1.71 ^{a} ±0.01	90.00 ±9.99	67.5 ^{a} ±0.75	1.77 ±0.03
0*1	5.63 ±0.01	38.2 ^{b} ±0.72	32.6 ^{b} ±0.72	271.8 ^{b} ±6.03	1.60 ^{b} ±0.01	100.00 ±0.00	63.2 ^{b} ±0.95	1.94 ±0.07
0*2	5.50 ±0.02	39.7 ^{b} ±1.21	34.2 ^{b} ±1.21	284.9 ^{b} ±9.96	1.65 ^{b} ±0.02	100.00 ±0.00	61.8 ^{b} ±0.06	1.81 ±0.06
100*0	5.69 ±0.06	43.6 ^{a} ±0.00	37.9 ^{a} ±0.06	315.6 ^{a} ±0.52	1.70 ^{a} ±0.01	80.00 ±19.99	64.6 ±0.29	1.71 ±0.00
100*1	5.60 ±0.00	40.4 ^{b} ±0.92	34.8 ^{b} ±0.92	290.2 ^{b} ±7.71	1.65 ^{b} ±0.02	100.00 ±0.00	62.9 ±3.29	1.80 ±0.05
100*2	5.76 ±0.10	39.5 ^{b} ±1.04	33.7 ^{b} ±0.95	281.2 ^{b} ±7.79	1.60 ^{b} ±0.01	100.00 ±0.00	61.1 ±0.52	1.81 ±0.03
1000*0	5.66 ±0.10	29.2 ^{b} ±2.31	23.6 ^{b} ±2.28	196.5 ^{b} ±18.97	1.36 ^{b} ±0.06	70.00 ±9.99	56.0 ^{b} ±3.23	2.41 ±0.19
1000*1	5.51 ±0.03	35.8 ^{a} ±0.00	30.3 ^{a} ±0.03	252.6 ^{a} ±0.23	1.56 ^{a} ±0.01	60.00 19.99	69.0 ^{a} ±0.75	2.28 ±0.03
1000*2	5.70 ±0.00	30.6 ^{b} ±1.42a	24.9 ^{b} ±1.42	207.4 ^{b} ±11.73	1.40 ^{b} ±0.04	100.00 ±0.00	58.6 ^{b} ±0.06	2.37 ±0.14

a-b: Means in the same column having differ small letters are significantly differ ($P \leq 0.05$).

ADG = Average daily gain (mg/fish/day) SGR = Specific growth rate (%/d)

SR = Survival rate (%) FI = Feed intake (g/fish) FCR = Feed conversion ratio

2- Carcass composition and residues of lead in muscles of fish

A. Carcass composition of fish

Dietary contaminated with lead oxide led to significantly ($P \leq 0.05$) increased of dry matter, ether extract and ash while, crude protein was significantly decreased compared with the control group. On the other side, dietary supplemented by 2% bentonite led to significantly increased of ash and significantly decreased of ether extract while, both of dry matter and crude protein not affected compared with the control treatment (Table 3).

Addition of 2% bentonite to the uncontaminated diet with lead oxide (0 ppm) resulted in significantly increased in crude protein and significantly decreased in ether extract, but the dry matter and ash not affected compared with the control. On the other side, addition of 2% bentonite to contaminated diet with low level (100 ppm) of lead oxide led to significantly increased of ash and insignificantly ($P \geq 0.05$) increased of dry matter, meanwhile the ether extract and crude protein were decreased significantly compared to the control group. However, dietary supplemented by 2% bentonite in case of high level of lead oxide (1000 ppm) caused significantly increased of dry matter, ash and crude protein, since ether extract only was decreased significantly compared with the control treatment.

B. Residues of lead in fish muscles

Results in Table (3) showed that bioaccumulation of lead in fish muscles which was increased significantly ($P \leq 0.05$) for *O. niloticus* fed on contaminated diet compared with the control treatment. Whereas, this bioaccumulation was increased by increasing level of lead oxide (1000 ppm) in diets. However, not significant ($P \geq 0.05$) differences of lead residues in fish muscles were recorded by addition of 1 or 2% bentonite compared with the control treatment.

Addition of 1% or 2% of bentonite to contaminated diet with high level (1000 ppm) of lead oxide result in significantly ($P \leq 0.05$) decreased the residues of lead in fish muscles when compared with the control treatment. While, no significant differences in case of the interaction between dietary contaminated by lead oxide with low level (100 ppm) (Table 3).

Table 3. Effect of lead oxide, bentonite concentrations in diets and their interaction on carcass composition and lead residues of mono-sex *O. niloticus* (means ±SE)

Treat.	Dry matter %	Ash %	Ether extract %	Crude Protein %	Residues of lead (ppm)
Lead oxide levels, ppm (L)					
0	24.5±0.20^{b}	15.9±0.27^{a}	21.7±0.88^{b}	62.5±0.81^{b}	0.38±0.02^{c}
100	24.4±0.17^{b}	14.4±1.05^{b}	21.8±0.71^{b}	63.7±0.87^{a}	0.45±0.01^{b}
1000	26.0±0.15^{a}	15.8±0.20^{a}	26.0±0.63^{a}	58.2±0.51^{c}	0.49±0.02^{a}
Bentonite levels, % (B)					
0	25.0±0.18	13.7±0.84^{b}	24.9±0.77^{a}	61.4±1.24	0.43±0.04
1	25.0±0.32	15.9±0.16^{a}	23.1±1.04^{b}	61.0±1.17	0.45±0.01
2	24.9±0.39	16.6±0.24^{a}	21.4±0.87^{c}	61.9±0.90	0.45±0.01
Interaction (L*B)					
0*0	24.6±0.27	15.3±0.55	22.9±0.14^{a}	61.9±0.41^{b}	0.29±0.01^{b}
0*1	24.9±0.28	16.0±0.16	23.9±0.17^{a}	60.1±0.33^{c}	0.42±0.00^{a}
0*2	24.0±0.29	16.4±0.44	18.2±0.49^{b}	65.4±0.58^{a}	0.45±0.02^{a}
100*0	24.8±0.38^{a}	10.6±1.05^{b}	24.0±0.47^{a}	65.4±0.74^{a}	0.46±0.00
100*1	23.9±0.03^{b}	15.3±0.01^{a}	19.3±0.55^{c}	65.3±0.57^{a}	0.46±0.01
100*2	24.3±0.02^{ab}	17.4±0.17^{a}	22.1±0.26^{b}	60.5±0.41^{b}	0.44±0.00
1000*0	25.4±0.03^{c}	15.2±0.29^{b}	27.9±0.12^{a}	57.0±0.16^{b}	0.55±0.01^{a}
1000*1	26.1±0.02^{b}	16.3±0.19^{a}	26.1±0.91^{a}	57.6±0.72^{b}	0.46±0.00^{b}
1000*2	26.5±0.01^{a}	16.1±0.08^{a}	24.0±0.38^{b}	59.9±0.42^{a}	0.46±0.01^{b}

a-c: Means in the same column having differ small letters are significantly differ ($P \leq 0.05$).

3- Blood hematological and biochemical parameters

Results in Table (4) illustrated that dietary contaminated with low 100 ppm and high level 1000 ppm of lead oxide caused significantly ($P \leq 0.05$) decreased in overall means of hemoglobin concentration, red blood cells count, paced cell volume, MCHC and blood platelets but not significantly ($P \geq 0.05$) decreased in MCH. While, MCV percentage was increased significantly compared with the control group. However, dietary supplementation of 2% bentonite led to significantly increased in hemoglobin concentration, red blood cells count, paced cell volume, MCHC and blood platelets, but it was not significantly ($P \geq 0.05$) increased in MCH. While, MCV percentage was decreased significantly compared with the control treatment.

The interaction between lead oxide levels and bentonite for all levels did not show any significant differences in hemoglobin concentration, red blood cells count and blood indices (MCV, MCH and MCHC). On the other side, addition of 2% bentonite for uncontaminated diet with lead oxide (0 ppm) led to significantly ($P \leq 0.05$) increased in blood platelets compared with the control treatment. As well as, addition of 2% bentonite to contaminated

diet with low (100 ppm) and high (1000 ppm) levels of lead oxide led to significantly increase in paced cell volume and blood platelets compared with the control (Table 4).

Table 4. Effect of lead oxide, bentonite concentrations in diets and their interaction on blood hematological parameters of mono-sex *O. niloticus* (means ±SE)

Treat.	Hb (g / dl)	RBCs ($X10^6/mm^3$)	PCV (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	Platelets ($X10^3/mm^3$)
Lead oxide levels, ppm (L)							
0	5.38^{a} ±0.15	1.69^{a} ±0.05	16.3^{a} ±0.16	97.1^{b} ±2.22	32.0 ±0.64	33.0^{a} ±0.79	541.7^{a} ±5.7
100	4.54^{b} ±0.21	1.39^{b} ±0.07	14.8^{b} ±0.46	108.4^{a} ±3.12	33.1 ±1.21	30.5^{b} ±0.89	498.3^{b} ±15.2
1000	4.08^{c} ±0.24	1.28^{c} ±0.06	14.1^{c} ±0.50	109.9^{a} ±1.49	31.6 ±1.01	28.8^{b} ±0.84	465.0^{c} ±18.1
Bentonite levels, % (B)							
0	4.04^{c} ±0.29	1.28^{c} ±0.08	13.7^{c} ±0.57	107.9^{a} ±2.86	31.4 ±0.64	29.3^{b} ±0.97	453.3^{c} ±19.3
1	4.74^{b} ±0.18	1.48^{b} ±0.06	15.5^{b} ±0.20	106.7^{ab} ±3.22	32.5 ±0.95	30.5^{ab} ±0.90	515.0^{b} ±7.64
2	5.21^{a} ±0.18	1.60^{a} ±0.06	16.0^{a} ±0.24	100.8^{b} ±2.63	32.8 ±1.26	32.5^{a} ±0.90	536.7^{a} ±7.82
Interaction (L*B)							
0*0	5.07 ±0.20	1.60 ±0.06	15.9 ±0.20	99.3 ±2.31	31.6 ±0.15	31.8 ±0.87	525.0^{b} ±2.89
0*1	5.30 ±0.23	1.67 ±0.09	16.1 ±0.17	98.6 ±6.21	32.2 ±0.29	33.0 ±1.79	540.0^{b} ±5.77
0*2	5.77 ±0.20	1.80 ±0.06	16.8 ±0.12	93.6 ±2.34	32.2 ±2.17	34.3 ±1.41	560.0^{a} ±5.77
100*0	3.87 ±0.15	1.17 ±0.03	13.1^{c} ±0.20	113.8 ±4.62	33.4 ±0.40	29.6 ±1.56	440.0^{c} ±5.77
100*1	4.67 ±0.20	1.40 ±0.06	15.5^{b} ±0.17	111.4 ±5.83	33.6 ±2.83	30.0 ±0.95	515.0^{b} ±2.89
100*2	5.10 ±0.29	1.60 ±0.06	16.0^{a} ±0.15	100.0 ±2.71	32.2 ±2.97	32.1 ±2.11	540.0^{a} ±5.77
1000*0	3.20 ±0.17	1.07 ±0.03	12.1^{b} ±0.23	110.6 ±3.70	29.1 ±0.06	26.4 ±0.92	395.0^{b} ±8.66
1000*1	4.27 ±0.09	1.37 ±0.03	14.9^{a} ±0.15	110.1 ±1.27	31.6 ±1.30	28.7 ±0.84	490.0^{a} ±5.77
1000*2	4.77 ±0.09	1.40 ±0.06	15.2^{a} ±0.17	109.0 ±3.26	34.2 ±2.02	31.3 ±0.92	510.0^{a} ±5.77

a-c: Means in the same column having differ letters are significantly differ ($P \leq 0.05$).

Hb= Hemoglobin RBCs= Red blood cells (Erythrocytes)

PCV= Packed cell volume MCV= Mean corpuscular volume

MCH= Mean corpuscular hemoglobin MCHC= Mean corpuscular hemoglobin concentration

Platelets= Blood platelets (Thrombocytes)

Mono-sex *O. niloticus* fed on contaminated diet with low (100 ppm) or high (1000 ppm) levels of lead oxide led to significantly ($P \leq 0.05$) decreased in white blood cells (Leukocytes) count and the percentage of lymphocytes (as macrophage cells) while the percentage of monocytes, neutrophils and eosinophils were increased significantly compared with the control group. These drastic effects increased with increasing the level of lead oxide. However, supplementation of 2% bentonite led to significantly ($P \leq 0.05$) increased in white blood cells (Leukocytes) count and the percentage of lymphocytes while, the percentage of monocytes, neutrophils and eosinophils were decreased significantly compared with the control (Table 5).

The interaction between dietary lead oxide and bentonite levels did not show any significant differences in the percentage of lymphocytes, monocytes, neutrophils and eosinophils. However, the interaction between 2% bentonite and the untreated diet with lead oxide (0 ppm) led to insignificantly increased in white blood cells count. But, in case of contaminated diet with low (100 ppm) or high (1000 ppm) levels of lead oxide with addition of 2% bentonite led to significantly increased of the white blood cells count compared to the control treatment (Table 5).

Table 5. Effect of lead oxide, bentonite concentrations in diets and their interaction on total leukocytes and differential count of mono-sex *O. niloticus* (means ±SE)

Treat.	WBCs ($X10^3$/ mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
Lead oxide levels, ppm (L)					
0	852±4.41^a	94.4±0.44^a	1.78±0.28^b	3.11±0.20^b	1.00±0.00^c
100	803±10.6^b	89.8±0.66^b	2.89±0.26^a	6.22±0.36^a	1.33±0.17^b
1000	775±18.1^c	88.4±0.44^c	3.22±0.22^a	6.78±0.22^a	1.89±0.11^a
Bentonite levels, % (B)					
0	773±21.6^c	89.2±0.98^c	3.44±0.24^a	5.89±0.59^a	1.67±0.17^a
1	820±7.31^b	91.0±0.94^b	2.56±0.24^b	5.44±0.63^a	1.33±0.17^b
2	837±6.67^a	92.4±0.90^a	1.89±0.26^c	4.78±0.62^b	1.22±0.15^b
Interaction (L*B)					
0*0	850±11.5	93.0±0.58	2.67±0.33	3.67±0.33	1.00±0.00
0*1	845±2.89	94.7±0.33	1.67±0.33	3.00±0.00	1.00±0.00
0*2	860±5.77	95.7±0.33	1.00±0.00	2.67±0.33	1.00±0.00
100*0	765±8.66^b	87.7±0.33	3.67±0.33	7.00±0.00	2.00±0.00
100*1	815±8.66^a	89.7±0.33	3.00±0.00	6.67±0.33	1.00±0.00
100*2	830±5.77^a	92.0±0.58	2.00±0.00	5.00±0.58	1.00±0.00
1000*0	705±8.66^b	87.0±0.58	4.00±0.00	7.00±0.58	2.00±0.00
1000*1	800±5.77^a	88.7±0.33	3.00±0.00	6.67±0.33	2.00±0.00
1000*2	820±5.77^a	89.7±0.33	2.67±0.33	6.67±0.33	1.67±0.33

a-c: Means in the same column having differ small letters are significantly differ ($P \leq 0.05$).

WBCs= White blood cells (Leukocytes).

Means of plasma proteins (total protein, albumin, globulin and albumin/globulin ratio) were decreased significantly ($P \leq 0.05$) of mono-sex *O. niloticus* fed on diets contaminated with lead oxide at low (100 ppm) or high (1000 ppm) levels compared with the control. These drastic effects were increased by increasing level of lead oxide. However, dietary supplementation with 2% bentonite led to significantly ($P \leq 0.05$) increased in total protein, albumin and globulin but albumin/globulin ratio was increased insignificantly ($P \geq 0.05$) compared with the control treatment (Table 6).

Dietary supplementation of 2% bentonite to the uncontaminated (0 ppm) or contaminated diet with low (100 ppm) or high (1000 ppm) levels of lead oxide led to significantly ($P \leq 0.05$) increased in total protein compared with the control treatment (Table 6).

Table 6. Effect of lead oxide, bentonite concentrations in diets and their interaction on plasma proteins of mono-sex *O. niloticus* (means ±SE)

Treat.	TP (g/dl)	AL (g/dl)	GL (g/dl)	AL/GL*
Lead oxide levels, ppm (L)				
0	7.63±0.04[a]	3.60±0.04[a]	4.04±0.05[a]	0.89±0.02[a]
100	6.76±0.05[b]	2.77±0.05[b]	3.99±0.04[a]	0.69±0.02[b]
1000	6.01±0.12[c]	2.26±0.06[c]	3.75±0.08[b]	0.60±0.01[c]
Bentonite levels, % (B)				
0	6.62±0.28[c]	2.76±0.23[b]	3.86±0.08[b]	0.71±0.05
1	6.73±0.23[b]	2.88±0.19[a]	3.85±0.07[b]	0.75±0.04
2	7.04±0.19[a]	2.98±0.17[a]	4.07±0.06[a]	0.73±0.04
Interaction (L*B)				
0*0	7.62±0.02[b]	3.60±0.11	4.03±0.10	0.90±0.05
0*1	7.52±0.02[c]	3.58±0.05	3.94±0.07	0.91±0.03
0*2	7.76±0.04[a]	3.61±0.05	4.15±0.10	0.87±0.03
100*0	6.58±0.03[c]	2.63±0.06	3.95±0.10	0.67±0.03
100*1	6.77±0.02[b]	2.79±0.06	3.99±0.08	0.70±0.03
100*2	6.92±0.02[a]	2.88±0.05	4.04±0.07	0.71±0.03
1000*0	5.67±0.03[b]	2.06±0.03	3.61±0.07	0.57±0.02
1000*1	5.90±0.06[b]	2.28±0.01	3.62±0.05	0.63±0.01
1000*2	6.46±0.10[a]	2.44±0.04	4.02±0.14	0.61±0.03

a-c: Means in the same column having differ small letters are significantly differ ($P \leq 0.05$).

TP = Total protein AL = Albumin GL = Globulin * Al / Gl ratio = Albumin / Globulin

Results in Table (7) showed that contaminated diet with lead oxide at low (100 ppm) or high (1000 ppm) levels led to significantly ($P \leq 0.05$) increased in liver enzymes (AST and ALT) and uric acid concentrations, except plasma total cholesterol which was decreased significantly compared with the control treatment, these drastic effects on blood biochemical parameters increased by increasing of lead oxide level compared with the control. However, addition of 2% bentonite led to significantly ($P \leq 0.05$) decreased in AST, ALT and uric acid concentrations while plasma total cholesterol was increased significantly compared with the control treatment.

On the other side, the interaction between dietary lead oxide levels and bentonite levels did not show any significant differences in all above blood biochemical parameters (Table 7).

Table 7. Effect of lead oxide, bentonite concentrations in diets and their interaction on blood biochemical parameters of mono-sex *O. niloticus* (means ±SE)

Treat.	AST (U/L)	ALT (U/L)	Uric acid (mg/dl)	Total cholesterol (mg/dl)
Lead oxide levels, ppm (L)				
0	21.4± 0.87^{c}	19.8±0.89 ^{c}	1.75±0.04^{c}	67.5±0.55^{a}
100	39.3±1.53^{b}	28.6±0.93 ^{b}	2.41±0.04 ^{b}	56.6±1.28^{b}
1000	52.8±1.55 ^{a}	38.9±0.92 ^{a}	2.78 ±0.03 ^{a}	51.8±1.36^{c}
Bentonite levels, % (B)				
0	41.6±5.08 ^{a}	30.8±3.22 ^{a}	2.37±0.17 ^{a}	56.5±3.09^{b}
1	38.1±4.57 ^{b}	29.4±2.53 ^{a}	2.32±0.16 ^{ab}	58.7±2.49^{ab}
2	33.9±4.11 ^{c}	27.0± 2.77 ^{b}	2.25±0.13 ^{b}	60.6±1.80^{a}
Interaction (L*B)				
0*0	23.0±1.15	19.7±2.03	1.73±0.07	67.5±0.64
0*1	21.7±2.03	21.7±0.88	1.74±0.10	67.7±1.77
0*2	19.7±0.88	18.0±1.15	1.79±0.05	67.2±0.22
100*0	44.0±1.15	31.7±0.88	2.49±0.05	54.6±2.65
100*1	40.0±1.15	28.0±1.15	2.43±0.08	56.4±2.27
100*2	34.0±1.15	26.0±1.15	2.30±0.05	58.7±1.80
1000*0	57.7±1.45	41.0±2.31	2.89±0.02	47.3±1.39
1000*1	52.7±1.45	38.7±0.88	2.79 ±0.01	52.1±0.97
1000*2	48.0±1.15	37.0±0.88	2.67±0.02	55.9±1.00

a-c: Means in the same column having differ small letters are significantly differ ($P \leq 0.05$).

AST= Aspartate aminotransferase ALT= Alanine aminotransferase

DISCUSSION

Data in the present results confirmed that the drastic effects of dietary lead oxide on the growth performance parameters of mono-sex Nile tilapia agreement of those recorded by Hussein and Mekkawy (2001) for *Tilapia zillii* and Ayyat *et al*. (2003) for *O. niloticus*. Moreover, Burdena *et al*. (1998) found that lead enters aquatic systems from anthropogenic and natural sources and is responsible for death or sublethal changes in reproduction, growth, and behavior of fish. In addition, Ayyat *et al.* (2003) stated that live body weight and daily body gain of Nile tilapia decreased significantly ($P < 0.001$) with increasing dietary lead level (200 ppm). Also, the same authors added that daily feed intake decreased significantly with increasing dietary lead level (200 ppm). Also, increasing dietary lead level impaired the feed conversion ratio during the whole experimental period (4 months). This reduction in body weight gain, feed intake and feed conversion ratio may be due to nutritional disturbance and also related to appetite depression induced by lead exposure. Whereas, lead may depress appetite through its action on sites influencing gastrointestinal tract e.g. brain centers involved in the regulation of food consumption (Zaki El-Dean *et al*., 1996). On the other side, the positive effects of bentonite on growth performance parameters and also significantly interaction between dietary supplementation of 1% bentonite and high level (1000 ppm) of lead oxide may be due to its high adsorbent capability of smectite minerals have found wide-range applications not only in industry, also as excellent adsorptive materials of heavy metals and bacteria (Hassen *et al*., 2003 and Katsumata *et al*., 2003) and toxic and antinutritive agents (Abdel-Wahhab *et al*., 1999; Ibrahim *et al*., 2000 and Phillips *et al*., 2002). Moreover, Oscarson *et al.* (1994) confirmed the protective actions of bentonite may result from a great number of sorption sites on the surface of this agent.

Contaminated diet with lead oxide in the present study led to hazard effects of fish carcass composition and its accumulation in fish muscles these drastic effects and lead bioaccumulation in fish muscles were increased by increasing level of dietary lead oxide (1000 ppm) which, may be related with the reduction of fish growth performance, feed intake and feed conversion ratio. The same drastic effects of lead oxide in fish carcass composition were recorded by Ayyat *et al.* (2003) whom reported that body moisture and ether extract contents were significantly ($P < 0.001$) decreased, while protein content was significantly ($P < 0.05$) increased by increasing dietary lead level. Body ash did not affected by dietary lead level, but ether extract contents were significantly ($P < 0.001$) increased. In

addition, they added that body lead residues increased significantly ($P < 0.001$) by increasing lead level (200 ppm) in fish diets. The obtained results reflexes the positive effects of addition 1 or 2% of dietary bentonite as adsorbent agent to alleviate the toxic effects of dietary lead oxide on *O. niloticus* fish, which were coincided with the findings of Carson and Smith (1983) whom stated that dietary clay reduce residual of T-2 toxin in the muscles, whereas natural clays adsorb the toxic material and excrete it in feaces. Yet, Hussein and Mekkawy (2001) added that the fish groups exposed to various lead levels and fed 2 and 3% dietary clay had significantly ($P < 0.05$) lower lead accumulation than the clay-free groups, whereas the low level of residue in the lead-clay treatments emphasizing on the validity of dietary clay in the adsorption of the lead in intestine. Moreover, Andreji *et al.* (2006) determined lead concentrations which exceeded the limit for lead content in fish muscle, 0.2 mg/kg wet weight; otherwise a determination of lead in six fish species from the Adriatic Sea showed that concentrations of lead in all species examined were below the maximum levels recommended by the European Community for this element in seafood, which would lead to exposure levels lower than the provisional tolerable daily intakes (Sepe *et al*., 2003). From the other side, the positive effects and significantly interaction between the dietary addition of 2% bentonite and untreated or treated diets with low or high levels of lead oxide may be due to its adsorbabilities of bentonite and/or montmorillonite were found to be higher than other clay minerals. This reason may be due to the bigger surface area of these clays, which characteristically undergoes larger swelling (Sposito *et al*., 1999).

Results, in the present study showed that dietary lead oxide led to drastic effects on blood hematological parameters (hemoglobin, red blood cells, packed cell volume, blood indices and blood platelets), and also in total white blood cells count and its differentiation (the percentage of lymphocytes, monocytes, neutrophils and eosinophils) of mono-sex *O. niloticus*, as well as the obtained results confirmed that these drastic effects were increased by increasing level of dietary lead oxide (1000 ppm) compared with fish fed on free diet from lead oxide which due to chronic lead toxicity affects on gastrointestinal, neuromuscular, renal and haematological systems (ATSDR, 2005). These findings were agreement with those reported by Hussein and Mekkawy (2001) who stated that the toxic effects of lead in *Tilapia zillii* treated fish led to significant ($P < 0.05$) decreases in hematological components (Hb and PCV%), as well as Shabana (1983) in *Clarias lazera*; Tewari *et al*. (1987) in *Barbus conchonius* and Amin (1992) in European eel. In this respect,

Gossel and Bricker (1989) added that lead had a suppressive effect on both haem and globin formation. It inhibits amino levulinic acid synthetase (ALA-S), amino levulinic acid dehydratase (ALA-D) and ferrocheltase enzymes resulting in reduction of haem formation. Lead also prevents the incorporation of glycine into globin. Moreover, results referred to the decrease in Hb concentration, RBCs count and PCV% which is responsible to the case of hypochromic anemia induced. However, supplementation of 2% bentonite alleviated these toxic effects of dietary lead oxide on blood hematological components and led to significantly interaction between the dietary addition of bentonite and untreated or treated diets with low (100 ppm) or high (1000 ppm) levels of lead oxide may be due to the high specific area, high chemical and mechanical stability, and variety of surface and structural properties. The chemical nature and pore structure generally determine the adsorption ability of clays (Kubilay *et al*., 2007). Also in this topic, the improvement effect of bentonite on all hematological component parameters may be emphasizing on the counteract role of clay in the removal of lead-impacts (Winfree and Allred, 1992 and El-Gendy *et al*., 1993).

Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al*., 2004). In this topic, obtained results revealed that fish fed contaminated diet with lead oxide led to significantly decreased of plasma biochemical constituents (total protein, albumin, globulin, albumin/globulin ratio and total cholesterol), while, other plasma constituents were increased significantly (AST, ALT and uric acid concentrations) compared with the control group. These drastic effects were increased by increasing level of dietary lead oxide as well as, may be due to toxicity of lead, which is widely spread throughout the environment, reveals relatively high reactivity and strongly affects many physiological processes (Lukaszek and Poskuta, 1998) and also related to AST and ALT secretion which play an important role in protein synthesis. These findings are accordance with those reported by Hussein and Mekkawy (2001). Also, Ayyat *et al.* (2003) reported that serum total protein and albumin were significantly ($P < 0.001$) decreased, while urea-N, creatinine, AST and ALT significantly ($P < 0.001$) increased with increasing dietary lead level (200 ppm). In this topic, Holcombe *et al*. (1976) explained the toxic role of lead on liver concerning the cholesterol synthesis, estrification and excretion. Yet, Hussein and Mekkawy (2001) revealed that the significant ($P \leq 0.01$) increased in AST, ALT, total cholesterol ($P \leq 0.05$) and significant ($P \leq 0.05$) decreased in serum total protein of lead-

treated fish referred to the lead-induced liver dysfunction related to AST and ALT secretion which play an important role in protein synthesis. As well as, Barnhoorn and Van Vuren (2004) reported that biochemical and physiological indicators, such as enzymes, could be used (as biomarkers) to identify possible environmental contamination before the health of aquatic organisms is seriously affected. Moreover, recently Newairy and Abdou (2009) reported that total lipids, cholesterol, triglycerides and LDL-c were significantly increased by lead acetate treatment, while HDL-c levels were decreased in the serum and liver extracts. In contrary, Santos and Hall (1990) didn't find any changes in the total cholesterol level in lead exposed fish as compared with the control. Recently, Herman *et al.* (2009) confirmed that lead can cause adverse effects to hepatic cells owing to its storage in the liver after lead exposure. Liver, being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances. Such disturbance is detected by the inhibition of protein synthesis and general decrease in amino acids concentration (Tolba *et al*., 1997 and Rizk *et al*., 1999). On the other hand, the positive effects of dietary addition of 2% bentonite to alleviated these toxic effects of dietary lead oxide on plasma constituents and also led to significantly interaction between the dietary addition of bentonite and untreated or treated diets with lead oxide may be due to the role of bentonite to bind lead and thus protects the intestinal mucosa in the treated *Tilapia zillii* fish (Hussein and Mekkawy, 2001). Also, El-Zait (2003) reported that natural clay supplementation reduced the lead toxicity. Moreover, Ellis *et al*. (2000) revealed that sodium bentonite exhibited a high affinity for the toxin and could reduce the percentage of fish developing tumors after consuming toxins. Therefore, bentonite is commonly employed as a feed binder.

CONCLUSION

Consequently, from the obtained results it could be established the drastic significant effects of contaminated diet by lead oxide on mono-sex *O. niloticus* fish, which is reflected in defect of growth performance parameters, its high residue in the muscles, decreased crude protein in body composition and impaired effects of blood hematological and biochemical parameters. So, from the healthy point of view no doubt in the usefulness and safetyness addition of dietary bentonite with levels 1 or 2% as an adsorbent agent to alleviated the toxic effects of dietary lead oxide on mono-sex *O. niloticus* fish and prohibited the lead toxicity on human health.

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تقليل سمية أكسيد الرصاص باستخدام البنتونيت فى علائق أسماك البلطى النيلى وحيد الجنس

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صممت الدراسة الحالية لاستخدام البنتونيت كمادة مدمصة بمستويات (صفر،1،2%) لتخفيف التأثيرات السامة لأكسيد الرصاص بمستويات (صفر،100، 1000 جزء فى المليون) على كفاءة النمو، التحليل الكيماوى لجسم الأسماك، متبقيات الرصاص فى عضلات الأسماك، القياسات الهيماتولوجية و البيوكيميائية لدم أسماك البلطى النيلى وحيد الجنس لمدة 16 أسبوع. لذلك استخدم التصميم التجريبى العاملى (3X3) للدراسة الحالية. **حيث أوضحت النتائج المتحصل عليها** أن العلائق الملوثة بأكسيد الرصاص أدت إلى حدوث انخفاض معنوى فى قياسات النمو للأسماك (الوزن النهائى، الزيادة الوزنية، معدل الزيادة اليومية، معدل النمو النوعى، استهلاك الغذاء)، التحليل الكيماوى لجسم الأسماك (البروتين الخام)، قياسات الدم الهيماتولوجية (الهيموجلوبين، عدد خلايا الدم الحمراء، حجم كرات الدم الحمراء المضغوطة ، الصفائح الدموية، عدد خلايا الدم البيضاء، النسبة المئوية لخلايا الدم البيضاء الليمفاوية)، قياسات الدم البيوكيميائية (البروتين الكلى، الألبيومين، الجلوبيولين، النسبة بينهما، الكلسترول الكلى) مقارنة بالمجموعة الضابطة. بينما حدث انخفاض غير معنوى لكل من معدل الإعاشة، دلائل الدم (متوسط وزن الهيموجلوبين فى الخلية MCH)، أما كفاءة تحويل الغذاء للأسماك ساءت معنوياً مقارنة بالمجموعة الضابطة. بينما زاد معنوياً كل من المادة الجافة، المستخلص الأثيرى ، الرماد فى جسم الأسماك، التراكم الحيوى للرصاص فى عضلات الأسماك، متوسط حجم الخلية (MCV) ، النسبة المئوية لخلايا الدم البيضاء الأحادية،المتعادلة،الحامضية، تركيز إنزيمات الألانينين أمينوترانسفريز ، الأسبرتيت أمينوترانسفريز وحامض اليوريك مقارنة بالمجموعة الضابطة ، حيث أن هذه التأثيرات السيئة زادت مع زيادة مستوى أكسيد الرصاص فى العليقة. و من ناحية أخرى وجد أن إضافة البنتونيت للعلائق كمادة مدمصة خففت من التأثيرات السامة لأكسيد الرصاص على القياسات السابقة. وبالتالى يمكن أن توصى الدراسة بالإضافة المفيدة والآمنة للبنتونيت بالمستويين1،2% لتخفيف التأثيرات السامة لعليقة أسماك البلطى النيلى الملوثة بأكسيد الرصاص.

الكلمات الدالة: أسماك البلطى النيلى – أكسيد الرصاص – البنتونيت – المتبقيات – قياسات الدم.