

THE AMELIORATIVE EFFECT OF NANO-SELENIUM SUPPLEMENTATION ON GROWTH, BODY COMPOSITION, LEAD BIOACCUMULATION, AND BLOOD COMPONENTS OF NILE TILAPIA FED LEAD-CONTAMINATED DIET.

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(Received 12/7/2022, accepted 24/8/2022)

SUMMARY

This study was intended to investigate whether dietary supplementation with different levels of nano-selenium (NSE) may reverse the negative influences of lead (Pb) contamination on growth, whole body composition, Pb residue in the tissues, and blood components in *Oreochromis niloticus*. The fish (n=180; 16.97±0.71 g) were allocated randomly into six equal groups as follows; the first group was fed the control diet, and the second was fed on the control diet contaminated with 80 ppm Pb (LW). From the third to the sixth groups (NSE0.2, NSE0.3, NSE0.4, and NSE0.5) were fed LW diet fortified with NSE at 0.5, 1.0, 2.0, and 4.0 mg nano-selenium/kg diet, respectively for 112 days. Results showed that fish LW group displayed significantly impaired growth performance, feed intake, feed conversion, and blood biochemical ($P \leq 0.01$) compared with fish in the control group. In contrast, fish groups NSE0.2, NSE0.3, NSE0.4, and NSE0.5 had significantly increased final body weight, daily weight gain, relative growth rate, survival rate, and daily feed intake compared with fish in the LW group. Furthermore, feed conversion and Pb bioaccumulation in fish tissues as well as serum contents of creatinine, AST, and ALT were significantly decreased ($P \leq 0.01$) in enriched NSE groups compared to the LW group. However, a significant increase in the values of hemoglobin, total protein, and albumin was observed in all NSE supplemented groups. The maximum enhancement was noted in NSE0.5 in most of the tested parameters. In conclusion, supplementing *O. niloticus* diet with NSE at 0.2 to 0.5 mg/ kg diet ameliorated the negative impacts of Pb toxicity on growth indicators, Pb residue in the tissues, and blood components.

Keywords: Nile tilapia, lead, selenium, growth, blood biochemistry, bioaccumulation of lead.

INTRODUCTION

Aquatic pollution is a major source of concern around the world since it causes several significant health problems for humans and aquatic organisms (Sthanadar *et al.*, 2013). Heavy metals are responsible for the pollution of rivers and freshwater bodies in ecosystems. Heavy metals are dense, non-biodegradable metallic elements having persistently harmful properties (Sthanadar *et al.*, 2015), that accumulate in aquatic environments and are passed to aquatic biota via several pathways (Khalifa *et al.*, 2010). This has a particular effect on ecotoxicity and can alter species diversity and the environment (Storelli *et al.*, 2005 and Türkmen *et al.*, 2009).

Pollution refers to any action that reduces the quality of the environment to the point that it is unsafe for human or animal habitation. Human populations and a wide range of ecosystems have both been negatively impacted by heavy metal contamination due to the improper disposal of the trash containing these substances (Ayyat *et al.*, 2020, a). Heavy metals pollution is a widespread problem in the aquatic environment, caused by sources as varied as agricultural runoff, industrial effluents, and untreated sewage systems (Bhuvaneshwari *et al.*, 2012). The pollution of Nile tilapia diets with heavy metals

causes a significant decrease in growth rate and feed efficiency, resulting in substantial economic losses for the aquaculture industry (Ayyat *et al.*, 2017).

Nanoparticles have gained a great deal of attention in terms of treatments due to their unique physicochemical characteristics that revolutionize the feed additives industry with more effective, less toxic, and creative outputs (Yin and Zhong, 2020). It is worth noting that physicochemical methods for producing nanomaterials are capital-intensive, inefficient in terms of material and energy balance, and may produce/require harmful substances. Microbial nanomaterial production is a bottom-up strategy in which the oxidation or reduction of metal forms nanomaterials, and the agents primarily responsible for such processes are the various enzymes released by microbial systems. Versatile nanoparticles (NPs) offer distinct and biocompatible features, prompting scientists to investigate biogenic routes of NP generation from many types of microorganisms, including fungi and bacteria (Prasad *et al.* 2016 and Aziz *et al.* 2019). Selenium is recognized as one of the most contentious trace minerals. Selenium is an essential component of the enzyme glutathione peroxidase (GSH-Px), which prevents oxidative damage to tissues and cell membranes. The incorporation of selenium into the diets of livestock and aquatic animals has sparked considerable interest because it has been found to improve product quality, productivity, and reproduction (Arteel and Sies, 2001). Selenium deficiency is a global concern associated with an increase in animal and human vulnerability to a variety of diseases and a decline in the reproductive and productive performance of domestic animals. (Lyons *et al.*, 2007). In fish, selenium was required for normal development, growth and flesh quality. Selenium is essential to maintaining fish health, mainly fish immunity (Nastova *et al.*, 2014).

Consequently, based on its beneficial properties, the current study hypothesized that if it is used as a feed additive at the different levels in *Oreochromis niloticus* diets, nano selenium could alleviate the negative influences of lead contamination. The current study aimed to investigate the efficacy of various levels of nano-selenium in ameliorating the negative influences of lead contamination on growth, whole body composition, Pb residue in the tissues, and blood components in *O. niloticus*.

MATERIALS AND METHODS

The current experiment was carried out in the Fish Rearing Laboratory at the National Institute of Oceanography and Fisheries (NIOF), Gulfs of Suez & Aqaba's Branch, Suez, Egypt. Mono-sexed *O. niloticus* fingerlings were purchased commercially from a private farm to be used in this study. For two weeks, the experimental fish was acclimated to lab conditions. During the acclimation period, all fish were fed the control feed three times a day until they showed signs of satiety.

The acclimated fish (n=180; average initial weight 16.97±0.71 g) were divided into six groups, each containing three replicates. Eighteen glass aquariums (10 fish/aquarium) with dimensions 80 × 30 × 40 cm (containing = 0.08 m³ of water) were used and supplied with well-aerated and dechlorinated tap water. To provide adequate aeration, all aquaria were equipped with two air stones connected to air pumps. Every two days, 30% of each aquarium's water capacity was changed with well-aerated water from the storage tanks. Siphoning was used to remove fish wastes and uneaten diet. The lighting scheme followed the natural light cycle of the day (about 10 hrs D: 14 hrs L).

The experimental groups were divided as follows; C, the negative control fish group, was fed on the basal diet; the second group (LW) the positive control fish group, was fed on the basal diet and contaminated with 80 mg lead/kg diet; the other fish groups (LW+0.2, LW+0.3, LW+0.4 and LW+0.5) were fed on the diet supplemented with nano-selenium (microbial synthesis of nano selenium) at 0.2, 0.3, 0.4, and 0.5 mg nano-selenium/kg diets. All fish groups were fed on a basal pelleted diet consisting of soybean meal 28.0%, Maize grain 35.0%, fish meal 10.0%, corn gluten meal (60%) 12.0 wheat bran 10.0%, sunflower oil 1.0%, vitamin mixture 1.0%, mineral mixture 1.0% and dicalcium phosphate 1.0%. The chemical composition of the diet was crude protein 35.08%, ether extract 5.32%, crude fiber 6.70% and gross energy (MJ/kg) 18.47. The experimental diets were provided to the fingerlings three times daily, at 9:00 a.m., 12:00 p.m., and 4:00 p.m., until indicated satiety. Unconsumed feed was quickly collected to prevent water contamination, dried, and weighed to estimate the amount of feed consumed. The length of the experiment was 112 days.

The number of successively surviving fish was recorded for each aquarium, and their survival rate was computed. To calculate the live weight biomass, fish were weighed every two weeks. The growth performance indicators and feed utilization were computed as follows:

1. Average daily gain = $(W1 - W0)/\text{trial period in days}$.
2. Daily gain weight/100 g of body weight (relative growth rate; Brody, 1945) = $((W1-W0)/\frac{1}{2}(W1+W0)) \times 100$, where $W1$ = Final weight and $W0$ = Initial weight.
3. Daily feed intake was estimated as g/fish/day by dividing the feed consumed daily by the number of fish in the aquarium.
4. Feed conversion ratio (FCR; g feed to g gain) = Total feed provided to each aquarium/fish biomass gain according to Berger and Halver (1987).

Every two weeks, water samples were taken from the 20-cm subsurface of each aquarium to assess the quality of the water. Water temperature and dissolved oxygen were evaluated using a temperature and oxygen portable meter (model HI9146, Hanna Company, Romania). A multiparameter bench meter (Hach kit model HI 83205, Hanna Instruments, Romania) was used to estimate the pH, nitrate and nitrite levels in the sampled water.

The chemical composition including moisture, crude fat, crude protein, and crude ash of whole-body composition was determined according to AOAC (2005) methods. Residual lead was analyzed according to Kiflom and Tarekegn (2015). In brief, fish samples were dried at 105 °C and then dry matter content was calculated. After that, it was dried and powdered to be ready for the digestion step. About 0.5 g of the sample was added with a mixture of 2.0 ml perchloric acid 70%, 6.0 ml nitric acid 70%, and 4.0-ml hydrogen peroxide 35% and boiled until the solution was clear. The solution was transferred to a 25 ml volumetric flask and diluted with distilled water to the desired volume. The lead was evaluated using flame atomic absorption spectrophotometer Varian specter AA photometry and Varian AA 240 FS fast sequential atomic absorption photometry.

Before blood sampling, fish were anaesthetized with 120 mg/l amino-benzoic acids (Sigma-Aldrich). At the end of experiment, blood samples were obtained from the caudal blood vessels using a disposable 1 cc tuberculin syringe. The obtained blood samples were divided into two tubes: the first containing EDTA as an anticoagulant agent for hematological examination. The second tube was without anticoagulant for serum separation for biochemical analysis and centrifuged at 1075 xg for 15 minutes, which was then stored at 20°C. The white blood cell (WBCs) and red blood cell (RBCs) were counted as described by Jain (1993) using Bright-Line Hemocytometer (Neubauer improved, Precicolor HBG, Germany) procedure. The hemoglobin (Hb) level was measured according to Drabkin and Austin (1932) by using the colorimetric method at 540 nm. Total protein and albumin; Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT); creatinine, and urea-N were determined according to the methods of Coz-Rakovac *et al.* (2008), Tripathi *et al.* (2003) and Holloway *et al.* (1993) respectively by using commercial kits obtained from Diamond Diagnostics Company in Egypt.

The data were statistically analyzed by a completely randomized design with SAS (2004) according to the following model: $Y_{ij} = \mu + T_i + \epsilon_{ij}$. Where μ is the overall mean, T_i is the fixed effect of i^{th} treatments, and ϵ_{ij} is the random error. All data are expressed as the mean \pm standard error. After conducting a one-way ANOVA, the orthogonal comparison was conducted to identify whether there were significant differences between treatments at $P < 0.05$.

RESULTS AND DISCUSSION

Throughout the experiment, the average water temperature was 25.19°C, pH was 7.27, nitrite was 0.041 mg/l, nitrate was 0.11 mg/l, and dissolved oxygen was 6.85 mg/l. All of the water parameters remained stable and within adequate limits (Boyd, 1990 and Boyd and Tucker, 1998). In all experimental groups, there was no discernible effect of dietary lead contamination and its amelioration on water quality.

Insignificant differences in live body weight between experimental groups indicated that the groups were homogeneous at the start of the experiment (Table 1). The lead contamination had a significant ($P < 0.001$) effect on Nile tilapia's final live body weight (at 16 weeks) and daily body gain throughout the entire experimental period (0-16 weeks) (Table 1). When compared to the control group that was fed a basic diet, the fish fed a lead-contaminated diet had 19.14 and 57.41% lower final body weight and daily gain weight at 0-16 weeks, respectively (Figure 1). On the other hand, the fish fed a lead-contaminated diet fortified with diverse nano-selenium concentrations displayed a significantly ($P < 0.001$) higher daily gain weight (0-16 weeks) and final body weight than those fed lead-contaminated non-fortified diets

(Table 1). In view of this, 15.11, 19.89, 23.11 and 22.61% increases in final body weight were recorded in fish fed a lead-contaminated diet fortified with 0.2, 0.3, 0.4 and 0.5 mg nano-selenium, respectively, relative to fish fed lead-contaminated non-fortified diets.

Table (1): Body weight (g) and daily gain weight (g/day) of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatment	Body weight at			Daily weight gain at			
	0 Week	8 Week	16 Week	0-8 Weeks	8-16 Weeks	0-16 Weeks	
Control (C)	16.967±0.00	41.017±0.55	55.065±1.19	0.249±0.00	0.251±0.01	0.340±0.01	
LW	16.967±0.03	33.733±0.41	46.217±1.70	0.299±0.00	0.223±0.02	0.216±0.01	
LW+0.2SE	16.933±0.03	38.513±0.28	53.201±0.37	0.386±0.00	0.262±0.00	0.324±0.00	
LW+0.3SE	17.033±0.03	39.874±0.59	55.411±0.76	0.408±0.01	0.277±0.02	0.343±0.00	
LW+0.4SE	16.933±0.03	40.766±1.07	56.898±1.09	0.425±0.02	0.288±0.02	0.357±0.01	
LW+0.5SE	16.967±0.03	40.262±0.75	56.667±0.44	0.416±0.01	0.293±0.01	0.355±0.00	
SOV	Df						
Treatment	5	0.004 ^{NS}	22.513 ^{***}	47.882 ^{***}	0.0072 ^{***}	0.0021 ^{NS}	0.0038 ^{***}
Control vs. LW	1	---	79.592 ^{***}	117.431 ^{***}	0.0254 ^{***}	---	0.0094 ^{***}
LW vs. LW+0.2SE	1	---	34.282 ^{***}	73.171 ^{***}	0.0112 ^{***}	---	0.0059 ^{***}
LW vs. LW+0.3SE	1	---	56.574 ^{***}	126.813 ^{***}	0.0177 ^{***}	---	0.0100 ^{***}
LW vs. LW+0.4SE	1	---	74.209 ^{***}	171.126 ^{***}	0.0238 ^{***}	---	0.0137 ^{***}
LW vs. LW+0.5SE	1	---	63.948 ^{***}	163.804 ^{***}	0.0204 ^{***}	---	0.0132 ^{***}
Error	12	0.005	1.315	3.244	0.0004	0.0010	0.0003

*** $P < 0.001$ and NS = Not significant.

Control = Basic diet, LW = Basic contaminated diet; LW+0.2SE = lead contaminated diet supplemented with 0.2 mg Selenium; LW+0.3SE = lead contaminated diet supplemented with 0.3 mg Selenium; LW+0.4SE = lead contaminated diet supplemented with 0.4 mg Selenium; LW+0.5SE = lead contaminated diet supplemented with 0.5 mg Selenium.

Additionally, daily weight gain increased by 50.00, 58.80, 65.28 and 64.35% for (LW+0.2SE), (LW+0.3SE), (LW+0.4SE) and (LW+0.5SE), respectively. Of note, among different tested selenium concentrations, the group fed a lead-contaminated diet containing 0.4 mg nano-selenium showed the highest final body weight and daily weight gain (Figure 1). Ayyat *et al.* (2020, b) established that lead contamination of fish diets could have a wide range of negative effects on fish health and growth. According to Vodela *et al.* (1997), adding a heavy metal mixture containing lead to the fish diet reduced their growth. The lead associated retarded growth could be highly linked to the lead-related metabolic disorders like inhibiting of the enzymes responsible for the oxidase system and heme synthesis. Yiin and Lin (1995) revealed that lead has the potential to cause oxidative stress, which may result in the loss of cellular functions and tissue damage, potentially retarding growth and causing health problems. On the other hand, Alina *et al.* (2009) showed that a Se-enriched diet can be converted into enzymes and antioxidants, which are essential for body immunity and development. Moreover, selenium supplementation improved Nile tilapia growth performance in the study of Lee *et al.* (2016). Nano-selenium could easily access to the biological system, and its effectiveness is quickly reflected in the digestive organs and intestine, increasing absorption and feed utilization and, as a result, growth (Ashouri *et al.*, 2015 and Saffari *et al.*, 2017).

As displayed in Table (2), Nile tilapia's relative growth rate was significantly ($P < 0.001$) affected by experimental treatments at weeks 0-8 and 0-16, but only minimally changed with no significant differences at weeks 8-16. Compared to the other experimental groups, the fish fed a lead contaminated diet recorded a lower rate of growth. On the contrary, the relative growth rate of fish given lead-contaminated diets and nano-selenium supplements was enhanced.

The Nile tilapia group fed diets containing lead had the lowest survival rates at 0-8, 8-16, and 0-16 weeks. A higher survival rate was seen in the fish group fed a contaminated diet and given a dietary supplement of 0.5 mg selenium than other experimental groups (Table 2).

Nile tilapia's daily feed intake was significantly ($P < 0.01$ or 0.001) impacted by experimental treatments at the 0-8, 8-16, and 0-16 week experimental periods. The fish group fed a basic diet (control group) recorded higher feed intake when compared with the other experimental fish groups. In contrast, the fish group fed a lead contaminated diet recorded lower feed intake (Table 3). Feed conversion rate of Nile tilapia was affected significantly ($P < 0.01$ or 0.001) by experimental treatments at 0-8 and 0-16 weeks, but not at 8-16 weeks.

Supplemented lead-contaminated diet with nano-selenium improved feed conversion to fish fed lead-contaminated diet with and without selenium supplement (Table 3). When compared to the control group that was fed a basal diet, the fish fed a lead-contaminated diet had a 12.63% lower feed conversion. Also, the fish fed a lead contaminated diet and fortified with 0.2, 0.3, 0.4 and 0.5 mg nano-selenium showed an improved feed conversion at 0-16 weeks by 13.29, 15.01, 16.42 and 18.06% respectively, respectively relative to those fish fed a lead contaminated basal diet (Figure 1).

As presented in Tables (4 and 5), a significant ($P < 0.05$ or 0.01) effect of the dietary treatments was recorded on the levels of hemoglobin (Hb), albumin, total protein, AST, ALT, and creatinine. On the other hand, white blood cell (WBC), red blood cell (RBC), and urea-N were not significantly affected by the dietary treatments. Instead, the fish fed a lead-contaminated diet but contained various levels of nano-selenium recorded high values of hemoglobin, red blood cells, total protein, and albumin relative to the fish fed a lead-contaminated non-supplemented diet.

Conversely, the white blood cells, ALT, AST and creatinine concentrations decreased. Blood hematological and biochemical indices are thought to be useful measuring tools for assessing fish health, physiological responses to their surroundings, and nutrient absorption (Peres *et al.*, 2013). Ayyat *et al.* (2020, b) stated that serum total protein and albumin, hemoglobin and total erythrocyte concentrations were reduced, while serum urea-N, creatinine, ALT, AST, and leukocytes increased in fish fed lead contaminated diet relative to the fish group fed diets without lead contamination.

Ayyat *et al.* (2020, a) also verified that increasing the transaminase enzymes (ALT and AST) level in the serum of fish-fed lead contaminated diets may reveal the liver functions impairment because of its role in lead detoxification and reducing the hepatic protein synthesis. The decrease in serum total protein could be due to protein loss, which could be caused by kidney dysfunction and consequently protein excretion in the urine. According to Ozkan-Yilmaz *et al.* (2013), dietary selenium supplementation improves liver and kidney function. This could be attributed to its active role in breaking the free radical reactions chain by allowing free radicals to eliminate the hydrogen atom from the antioxidant molecule rather than polyunsaturated fatty acids, resulting in the formation of relatively unreactive radical species that protect kidney tissue from peroxidative damage.

As demonstrated in Table (6), Dietary treatments had no significant effect on the body composition of tested Nile tilapia. Comparable findings were recorded in the study of Ayyat *et al.* (2020, a). The experimental treatments had a significant ($P < 0.001$) effect on the accumulation of lead in Nile tilapia body. Lead residues were significantly ($P < 0.001$) higher in fish fed lead-contaminated diets than in fish fed a basal diet. On the contrary, lead-contaminated diets containing nano-selenium significantly ($P < 0.001$) reduced the lead accumulation in fish tissues compared to fish fed lead-contaminated non-supplemented diets. Lead residual in the fish control group was 0.004 mg/kg and vice versa 2.817 mg/kg in fish fed group contaminated diet. Ayyat *et al.* (2020, b) reported that lead was considerably accumulated in the body of fish fed lead-contaminated diets relative to the fish fed basal diets (control group) and feed additive supplemented diets (treated groups). Jelinek (1982) reported that the lead content of unprocessed fish and shellfish ranged from 0.02 to 0.4 ppm on average.

Table (2). Relative growth rate (g gain/100 g body weight) and survival rate (%) of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatment		Relative growth rate at			Survival rate at		
		0-8 Weeks	8-16 Weeks	0-16 Weeks	0-8 Weeks	8-16 Weeks	0-16 Weeks
Control		82.939±0.780	29.211±0.886	105.373±1.273	96.67±3.33	100.00±0.00	96.67±3.33
LW		66.121±1.012	31.092±3.207	92.432±2.833	93.33±3.33	90.00±0.00	83.33±3.33
LW+0.2SE		77.834±0.650	32.029±0.845	103.417±0.653	100.00±0.00	96.67±3.33	96.67±3.33
LW+0.3SE		80.249±1.082	32.603±2.668	105.928±1.140	96.67±3.33	100.00±0.00	96.67±3.33
LW+0.4SE		82.526±2.346	33.056±2.322	108.216±1.473	96.67±3.33	96.67±3.33	93.33±3.33
LW+0.5SE		81.372±1.423	33.872±1.855	107.825±0.543	100.00±0.00	100.00±0.00	100.00±0.00
SOV	df			Mean Square			
Treatment	5	120.540***	8.149 ^{NS}	103.968***	18.889 ^{NS}	45.556*	102.222*
Control vs. LW	1	424.251***	---	265.561***	---	150.000*	266.667**
LW vs. LW+0.2SE	1	205.792***	---	181.016***	---	66.667*	266.667**
LW vs. LW+0.3SE	1	299.401***	---	273.227***	---	150.000**	266.667**
LW vs. LW+0.4SE	1	403.686***	---	373.734***	---	66.667*	150.000*
LW vs. LW+0.5SE	1	348.890***	---	355.448***	---	150.000**	416.667**
Error	12	5.377	13.868	6.919	2.222	11.111	27.778

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS = Not significant.

Table (3): Daily feed intake (g) and feed conversion (g food/g gain) of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatment		Daily feed intake (g) at			Feed conversion (g food/g gain) at		
		0-8 Weeks	8-16 Weeks	0-16 Weeks	0-8 Weeks	8-16 Weeks	0-16 Weeks
Control		0.455±0.004	0.891±0.017	0.672±0.009	1.059±0.020	3.559±0.099	1.979±0.032
LW		0.431±0.006	0.746±0.010	0.588±0.007	1.440±0.049	3.448±0.418	2.265±0.114
LW+0.2SE		0.444±0.003	0.827±0.004	0.636±0.001	1.153±0.021	3.159±0.082	1.964±0.021
LW+0.3SE		0.446±0.003	0.872±0.010	0.659±0.004	1.095±0.033	3.198±0.329	1.925±0.051
LW+0.4SE		0.451±0.003	0.898±0.032	0.675±0.017	1.065±0.044	3.161±0.327	1.893±0.060
LW+0.5SE		0.448±0.000	0.868±0.011	0.657±0.006	1.078±0.035	2.979±0.179	1.856±0.025
SOV	Df			Mean Square			
Treatment	5	0.00021**	0.0096***	0.0032***	0.0646***	0.1365 ^{NS}	0.0646**
Control vs. LW	1	0.00086***	0.0314***	0.0107***	0.2174***	---	0.1230**
LW vs. LW+0.2SE	1	0.00027**	0.0099**	0.0034**	0.1236***	---	0.1365**
LW vs. LW+0.3SE	1	0.00037**	0.0238***	0.0076***	0.1782***	---	0.1734**
LW vs. LW+0.4SE	1	0.00064**	0.0347**	0.0113**	0.2106***	---	0.2076***
LW vs. LW+0.5SE	1	0.00045**	0.0222***	0.0072***	0.1966***	---	0.2517***
Error	12	0.00004	0.0008	0.0002	0.0037	0.2191	0.0106

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS = Not significant.

Table (4): Hemoglobin (Hb), total erythrocyte (RBCs) and leukocytes (WBCs) of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatment		Hb (g/100 ml)	RBC (10 ⁶ mm ²)	WBC (10 ³ mm ²)
Control		6.300±0.462	5.030±0.142	10.233±0.0829
LW		5.543±0.353	4.880±0.174	12.433±1.097
LW+0.2SE		6.900±0.252	5.363±0.086	10.633±0.296
LW+0.3SE		6.600±0.115	5.097±0.087	10.267±0.067
LW+0.4SE		6.800±0.306	5.223±0.155	10.567±0.328
LW+0.5SE		6.467±0.145	5.173±0.084	10.567±0.636
SOV	Df		Mean Square	
Treatment	5	0.8383*	0.0832 ^{NS}	2.045 ^{NS}
Control vs. LW	1	1.1267 ^{NS}	---	---
LW vs. LW+0.2SE	1	3.2267**	---	---
LW vs. LW+0.3SE	1	2.0417*	---	---
LW vs. LW+0.4SE	1	2.8017*	---	---
LW vs. LW+0.5SE	1	1.6027*	---	---
Error	12	0.2644	0.0482	1.248

** $P < 0.01$, * $P < 0.05$ and NS = Not significant.

Table (5): Some blood components of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatments		Total protein (g/100 ml)	Albumin (g/100 ml)	ALT (U/L)	AST (U/L)	Creatinine (mg/100 ml)	Urea-N (mg/100 ml)
Control		4.987±0.07	3.203±0.16	15.733±0.75	18.667±1.59	0.187±0.01	2.633±0.08
LW		4.137±0.31	2.767±0.06	21.000±2.40	23.367±1.46	0.247±0.00	3.367±0.50
LW+0.2SE		4.903±0.03	3.440±0.03	15.100±0.15	18.033±0.17	0.153±0.00	3.067±0.21
LW+0.3SE		4.777±0.11	3.507±0.17	14.400±0.20	17.067±0.93	0.170±0.01	3.367±0.29
LW+0.4SE		4.880±0.03	3.267±0.21	14.433±0.39	16.667±0.75	0.167±0.03	3.733±0.12
LW+0.5SE		4.84±0.05	3.14±0.110	15.73±0.38	19.63±0.84	0.180±0.017	2.76±0.03
SOV	df			Mean Square			
Treatment	5	0.2895*	0.2070*	18.5640**	17.7859**	0.0032**	0.5129 ^{NS}
Control vs. LW	1	1.0838**	0.2860 ^{NS}	41.6067**	33.1350**	0.0054**	---
LW vs. LW+0.2SE	1	0.8817**	0.6800**	52.2150**	42.6667**	0.01307***	---
LW vs. LW+0.3SE	1	0.6144**	0.8214**	65.3400***	59.5350**	0.0088***	---
LW vs. LW+0.4SE	1	0.8288**	0.3750*	64.6817***	67.3350***	0.0096***	---
LW vs. LW+0.5SE	1	0.7562**	0.2090 ^{NS}	41.6067**	20.9067*	0.0067**	---
Error	12	0.0616	0.0664	3.3533	3.4400	0.0004	0.2050

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS = Not significant.

Table (6): Body composition, lead residue and survival rate of Nile tilapia fish affected by dietary nano-selenium supplementation to prevent lead contamination.

Treatments		Dry matter %	Crud protein %	Ether extract %	Ash %	Lead residue (ppm)
Control		28.80±0.68	64.57±0.72	14.10±0.75	21.33±0.37	0.004±0.003
LW		30.60±0.32	64.58±0.74	13.33±0.62	22.08±0.29	2.817±0.260
LW+0.2SE		29.27±0.39	64.50±0.35	13.80±0.17	21.70±0.23	0.022±0.002
LW+0.3SE		29.62±0.68	64.87±0.78	13.47±0.32	21.67±0.47	0.019±0.003
LW+0.4SE		29.32±0.34	65.20±0.21	13.50±0.21	21.30±0.00	0.019±0.002
LW+0.5SE		29.80±0.00	65.70±0.06	12.97±0.19	21.33±0.15	0.017±0.004
SOV	df			Mean Square		
Treatment	5	1.1190 ^{NS}	0.6615 ^{NS}	0.4552 ^{NS}	0.2848 ^{NS}	3.9211***
Control vs. LW	1	---	---	---	---	11.8667***
LW vs. LW+0.2SE	1	---	---	---	---	11.7152***
LW vs. LW+0.3SE	1	---	---	---	---	11.7404***
LW vs. LW+0.4SE	1	---	---	---	---	11.7376***
LW vs. LW+0.5SE	1	---	---	---	---	11.7544***
Error	12	0.6541	0.9238	0.5800	0.2585	0.0339

*** $P < 0.001$ and NS = Not significant.

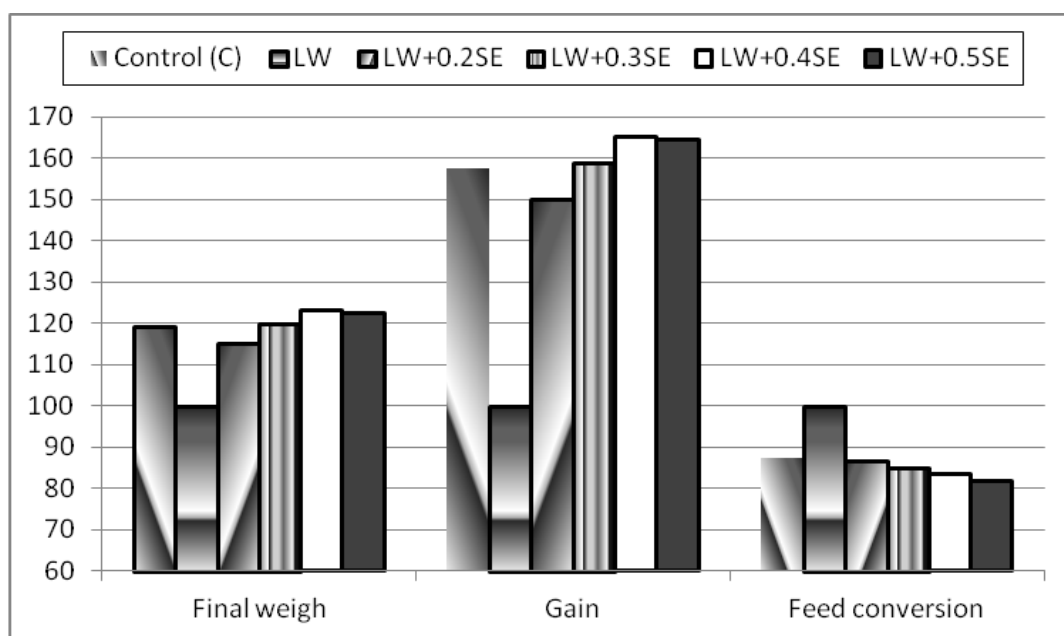


Figure (1): Final body weight, gain and feed conversion index of Nile tilapia fish affected by lead toxicity, when the values of the LW group considered as 100%.

Control = Basic diet, LW = Basic contaminated diet; LW+0.2SE = lead contaminated diet supplemented with 0.2 mg Selenium; LW+0.3SE = lead contaminated diet supplemented with 0.3 mg Selenium; LW+0.4SE = lead contaminated diet supplemented with 0.4 mg Selenium; LW+0.5SE = lead contaminated diet supplemented with 0.5 mg Selenium.

CONCLUSION

Based on our findings, it might be suggested that enriched tilapia diets with 0.2 or 0.5 mg nano-selenium per kg diet could ameliorate the negative effects of on growth indicators, lead residue in the tissues, hematological and biochemical parameters. The maximum enhancement was noted at 0.5 mg nano-selenium per kg diet in most of the tested parameters. Furthermore, future studies are needed on using nano-selenium as a feed additive to counteract the risks of other metal toxicity in fish diets.

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التأثير المحسن لمكملات النانو سيلينيوم على النمو، وتركيب الجسم، ومتبقيات الرصاص في الأنسجة، ومكونات الدم في أسماك البلطي النيلي المغذاه على علائق ملوثة بالرصاص.

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تهدف هذه الدراسة إلى معرفة قدرة المستويات المختلفة من النانو سيلينيوم على تقليل التأثيرات السلبية لتلوث علائق البلطي النيلي (*Oreochromis niloticus*) بالرصاص على النمو، وتركيب الجسم، ومتبقيات الرصاص في الأنسجة، وتركيب الدم. تم تقسيم الأسماك (عدد = 180 ؛ متوسط وزن = 16.97 ± 0.71 جم) بشكل عشوائي إلى ست مجموعات كما يلي: تم تغذية المجموعة الأولى بعليقة الكنترول، بينما تم تغذية المجموعة الثانية على عليقة الكنترول ملوثة بـ 80 جزء في المليون من الرصاص (LW). تم تغذية المجموعات من الثالثة إلى السادسة على العليقة الملوثة بالرصاص مع إضافة النانو سيلينيوم بمستويات 0.2 و 0.3 و 0.4 و 0.5 مجم / كجم على التوالي لمدة 112 يوماً. أظهرت النتائج حدوث تدهور معنوي في أداء النمو، والغذاء المأكول، وتحويل العلف، ومكونات الدم ($P \leq 0.01$) في الأسماك المغذاه على عليقة ملوثة بالرصاص بالمقارنة مع مجموعة الكنترول. في المقابل، حدثت زيادة معنوية في وزن الجسم النهائي، والنمو اليومي، ومعدل النمو النسبي، ومعدل الحياة، والعلف المأكول في أسماك جميع المجموعات المدعمة بالنانو سيلينيوم بالمقارنة مع المجموعة الملوثة بالرصاص. أيضاً، فقد انخفض معدل تحويل الغذاء ومتبقيات الرصاص في أنسجة الأسماك وكذلك محتويات الدم من الكرياتينين و AST و ALT بشكل معنوي ($P \leq 0.01$) في كل المجموعات المدعمة بالنانو سيلينيوم مقارنة بالمجموعة الملوثة بالرصاص. ومع ذلك، لوحظ زيادة كبيرة في قيم الهيموجلوبين والبروتين الكلي والألبومين في جميع المجموعات المكملة بالنانو سيلينيوم. وعموماً لوحظ أكبر معدل من التحسن في معظم القياسات عند مستوى 0.5 مجم نانو سيلينيوم/ كجم علف. إجمالياً، فإن إضافة النانو سيلينيوم بمعدل 0.2 إلى 0.5 مجم / كجم علف في علائق البلطي النيلي يمكن أن يخفف من الآثار السلبية لسمية الرصاص على مؤشرات النمو، ومتبقيات الرصاص في الأنسجة، ومكونات الدم.