

## Effect of Dietary Supplementation of Selenium and Copper Nanoparticles on The Growth Performance and Feed Utilization of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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**ABSTRACT:** The present study was conducted to evaluate the effect of dietary supplementation of selenium and copper alone or in combination on growth performance, feed utilization and survival rate of Nile tilapia (*O. niloticus*) fingerlings in fresh water for 15 weeks. Apparent healthy 420 fingerlings ( $3.0 \pm 0.1$ g /each) were distributed randomly into seven experimental groups each treatment was duplicated (30 fish each) as follow: D<sub>1</sub>.control diet(32%crude protein); D<sub>2</sub>.basal diet plus 1 mg Se-NP/kg<sup>-1</sup>; D<sub>3</sub>.basal diet plus 0.3 mg NaSe/kg<sup>-1</sup> diet; D<sub>4</sub>.basal diet plus 2 mg Cu-NP/kg<sup>-1</sup>; D<sub>5</sub>.basal diet plus 3 mg CuSO<sub>4</sub>/kg<sup>-1</sup> diet; D<sub>6</sub>.basal diet plus 1 mg Se-NP plus 2 mg Cu-Np/kg<sup>-1</sup> diet; D<sub>7</sub>.basal diet plus 0.3 mg NaSe plus 3 mg CuSO<sub>4</sub>/kg<sup>-1</sup> diet. Tanks (1m<sup>3</sup>/each) were aerated and supplied with underground water sources maintained at a constant temperature 24 °C. All fish groups were hand-fed three meals per day six days a week at feeding level of 6% of live body weight for the first four weeks, 5% for the second four weeks and 4% until the end of the experimental period. Fish in each tank were counted and weighed (collectively) biweekly, and the total amount of feed consumed during the study period was determined and calculated accordingly. The results showed that growth performance, feed utilization parameters, survival rate (SR) and condition factor(CF) for fish fed control diet were significantly( $p < 0.05$ ) higher compared to those fed diets supplemented with different forms of selenium and copper. There were no significant ( $p > 0.05$ ) differences in growth performance on diets containing copper sulphate and copper nanoparticles respectively. The effect of mixture sodium selenite and copper sulphate on all previous mentioned parameter was more pronounced than the effect of those fed diets containing mixture selenium nanoparticles and copper nanoparticles. Survival rate didn't exhibit any significant ( $p > 0.05$ ) differences among all studied treatments. Fish fed on diets supplemented with different forms of selenium and copper exhibited a significant ( $p < 0.05$ ) effect on all estimated feed and nutrients utilization traits. Feed intake significantly ( $p < 0.05$ ) increase with control compared to the other treatments however, the bulk of selenium and copper alone or in combination showed a satisfactory results near to the control Feed conversion ratio (FCR) improved with control compared to the other treatments however, the bulk of selenium and copper alone or in combination showed a satisfactory results as well. However, protein efficiency ratio (PER) and protein productive value (PPV) increased significantly ( $p < 0.05$ ) with control and bulk of selenium and copper alone or in combination and did not differ significantly ( $p > 0.05$ ) with all other treatments. Therefore, the present results recommend that the basal diet improve growth performance, feed utilization and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings.

**Keywords:** selenium nanoparticles, copper nanoparticles, growth performance, feed utilization, Nile tilapia

## INTRODUCTION

The Egyptian aquaculture sector is the largest producer of farmed fish in Africa (1.14 million ton in 2014) and the third largest global producer of farmed tilapia after China and Indonesia (FAO, 2016; Fitzsimmons, 2016). Tilapia is the second largest consumers of commercial aqua feed after carp (6.67 and 11.03 million ton respectively) and consume approximately 3% of the global supply of fishmeal (Tacon *et al.*, 2006; Tacon and Metian, 2015).

Selenium (Se) is an essential trace element for animals including fish, and has been considered as an excellent essential nutrient for aquaculture product enhancement.(Rotruck *et al.*, 1973; Watanabe *et al.*, 1997). Administration of dietary selenium has been reported to enhance growth performance and feed utilization in various fish species (Jaramillo and Gatlin, 2004; Zhou *et al.*, 2009). As the chemical form in which Se is supplemented is an important consideration (Han *et al.*, 2011), various forms of Se supplementation have been studied in fish species including hybrid striped bass (Cotter *et al.*, 2008), common carp (Jovanovic *et al.*, 1997), crucian carp(Wang *et al.*, 2007) and common barbell (Kouba *et al.*, 2014). The different Selenium forms (nano-Se and selenomethionine) supplemented in diet could improve the final weight, relative gain rate of crucian carp, *Carassius auratus gibelio*(Zhou *et al.*, 2009). Copper (Cu) is an essential nutrition trace element for Tilapia (Tang *et al.*, 2017). Administration of dietary Cu has been reported to enhance growth performance and feed utilization in various fish species (Mohseni *et al.*, 2014; Sabatini *et al.*, 2009).However, high dosage of dietary Cu is toxic to organisms (Fokina *et al.*, 2013; Oguz *et al.*, 2014). Dietary Cu can bind proteins and nucleic acids and cause the oxidation of lipids and proteins. Therefore, Copper deficiency can cause growth retardation and low feed efficiency, such as *Epinephelus malabaricus* (Lin *et al.*, 2008) and *Pelteobagrus fulvidraco* (Tan *et al.*, 2011). Furthermore, the deficiency of Cu will cause various diseases (Angelova *et al.*, 2014).

Nanotechnology holds promise for both medication and nutrition, because materials at the nanometer dimension exhibit novel properties different from those of isolated atom and bulk material (Wang *et al.*, 2007). Over the last decade, several studies have reported that metal oxide NPs are potentially toxic, but few attempts have been done to assess the ecotoxicity of nano-metal oxides in aquatic systems (Miller *et al.*, 2010; Adam *et al.*, 2015).Therefore, the present study was conducted to evaluate the effect of dietary supplementation of Se, Cu forms, and their mixture on growth performance, feed utilization, survival rate and whole body composition of Nile tilapia (*O. niloticus*) fingerlings in fresh water.

## MATERIAL AND METHODS

The present study was performed at the Laboratory of Fish Nutrition, Faculty of Agriculture (Saba-Basha) in cooperation with Reproductive Toxicity Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, throughout the period from August to November, 2016 to investigate the effect of supplementation dietary mixture of selenium and copper nanoparticles on growth performance of Nile tilapia (*O. niloticus*).

### 1. Experimental fish

Apparent healthy 420 Nile tilapia (*O. niloticus*) fingerlings, with an average initial body weight of  $3.0\pm 0.1$ g. Fish were obtained from a private commercial fresh water fish farm sited in the city of Motobas at Kafr–El Sheikh Governorate, Egypt. Fish were kept for two weeks in circular fiberglass tanks (1 cubic meter) as an acclimation period and fed on a control basal diet Table (1).

## 2. Experimental Design

All fish were weighed, and then distributed randomly into seven experimental groups duplicated (30 fish each) as follow: 1) Treatment fed on a control diet (D1); 2) Treatment fed on a diet with 1 mg Se-NP/kg<sup>-1</sup> (D2); 3) Treatment fed on a diet with 0.3 mg NaSe/kg<sup>-1</sup> diet(D3); 4) Treatment fed on a diet with 2 mg Cu-NP/kg<sup>-1</sup> (D4); 5) Treatment fed on a diet with 3 mg CuSO<sub>4</sub>/kg<sup>-1</sup> diet(D5); 6) Treatment fed on a diet with 1 mg Se-NP plus 2 mg Cu-Np/kg<sup>-1</sup> diet(D6); 7) Treatment fed on a diet with 0.3 mg NaSe plus 3 mg CuSO<sub>4</sub>/kg<sup>-1</sup> diet (D7). The experimental diets (32%) Diets were stored in labeled black plastic bags and stored in at -20°C freezer to avoid oxidation and rancidity, the chemical composition (g kg<sup>-1</sup>) of the formulated diets were analyzed according to AOAC (2000). Fish samples were collected at the beginning and the end of the experiment and store frozen at -20°C until whole body chemicals analysis. Appropriate pellet sized diets were fed to each group during the 15 weeks experimental period. All fish groups were hand-fed three meals per day six days a week at feeding level of 6% for the first four weeks, 5% for the second four weeks and 4% until the end of the experimental period. Fish in each tank were counted and weighed (collectively) biweekly, and the total amount of feed consumed during the study period was determined and calculated accordingly.

## 3. Experimental facilities

The experimental fish were equally distributed into 14 circular fiberglass 1m<sup>3</sup>tanks at a stocking density of 30 fish per tank representing seven experimental treatments (in duplicates each). Tanks were aerated and supplied with underground water sources maintained at a constant temperature. Water temperature, dissolved oxygen and pH were monitored daily. The mean water quality parameters were recorded as follows: Temperature 24 °C, Dissolved oxygen 6.4 mg L<sup>-1</sup> and pH 7.8. A photo period of 14 h light (06:00–20:00 h) and 10 h dark was used. All tanks were cleaned daily before the first feeding by siphoning off accumulated waste materials. Approximately one-third of water in each tank was replaced.

## 4. Experimental diets formulation

The experimental diets composed mainly of fish meal, yellow corn meal, soybean meal, corn gluten meal, wheat bran, wheat flour, sunflower oil, minerals and vitamin mixture. Diet formulation and proximate composition of the basal diet were shown in Table 1. All dry ingredients were finely ground in a hammer mill and passed through a 250-µm mesh sieve and then mixed mechanically to realize homogeneity of ingredients and thoroughly mixed with 150 ml/kg<sup>-1</sup> worm distilled water using a dry, steamless pelleting machine (Regina Supernova, Italy) through a 0.3 mm dye. Diets were stored in labeled black plastic bags and stored in the freezer at -20°C to avoid oxidation and rancidity, the chemical composition (g kg<sup>-1</sup>) of the formulated diets was analyzed according to AOAC (2000).

Selenium and copper sources were provided by Sigma-Aldrich Chemical (St. Louis, MO, USA). The sizes of the elemental Se and Cu nanoparticles ranged from 30 to 40 nm. The levels were selected according to the literature data (Ashouri *et al.*, 2015; El-Basuini *et al.*, 2016).

**Table (1). Formulation and chemical proximate composition of experimental diets (g kg<sup>-1</sup> dry matter)**

Ingredients	Amount (g kg <sup>-1</sup> )	Proximate composition <sup>2</sup>	(%)
Soybean meal (48 %CP)	300	Dry matter (DM)	93.1
Yellow corn meal (7.5%CP)	250	Crude protein	32.3
Fish meal (62% CP, 9% fat)	150	Crude lipid	6.42
Corn gluten meal (60% CP)	100	Crude fiber	1.78
Wheat bran (12% CP)	110	Ash	6.62
Wheat flour	50	NFE	51.7
Sun flower oil	15	Gross energy(Kcal/100g diet) <sup>3</sup>	455.65
Dicalcium Phosphate	5		
Vitamins mixture <sup>1</sup>	10		
Minerals mixture <sup>1</sup>	10		

(1) Premix Composition:-

Vit A (1200000 i.u.), Vit D (300000 i.u.), Vit E (700 mg,) Vit K3 (500 mg,) Vit B1 (500 mg), Vit B2 200mg, Vit B6 (600mg), Vit B12 (3mg), Vit C 450mg, Niacin 3000mg, Methionine3000mg, Cholin chloride 10000mg, Folic acid 300mg, Biotin 6mg, Panthonicacid 670mg, Magnesiam sulphate 3000mg, Iron sulphate 10000mg, Zinc sulphate , 1800mg, Cobalt sulphate 300mg, Carrier up to 3000mg.

(2) Dry matter basis.

(3) Gross energy (Kcal/100g diet)<sup>3</sup> was calculated as 5.65, 9.45 and 4.11 kcal/g for protein, lipid and NFE, respectively (NRC,1993).

## 5 Sampling and analytical methods

### 5.1 Growth performance and feed utilization parameters

At the end of the feeding trials, all fish were fasted for 24hrs prior to final sampling. Then the total number, individual body weight and length of fish from each tank were measured. Fish growth performance and feed utilization parameters were calculated according to Cho and Kaushik, (1985).

Weight gain (g/fish):  $WG = Wt - W0$

Where:

W0: initial mean weight of fish in grams.

Wt: final mean weight of fish in grams.

Average daily gain (mg/fish/day):  $ADG = (Wt - W0)/n$

Where:

n: duration period.

Specific growth rate (%/day):  $SGR = 100 \times (\ln Wt - \ln W0) / \text{days}$

Where:

ln: natural logarithm.

Survival rate (%) =  $100 \times (\text{initial number of fish} / \text{final number of fish})$

Length gain (cm) =  $Lt - L0$

Where:

L0: initial mean length of fish in cm.

Lt: final mean length of fish in cm.

Condition factor (K) =  $100 \times (BW (g) / L^3 (cm))$ .

Feed conversion ratio (FCR) = dry matter intake (g)/weight gain (g)

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)

Protein productive value (PPV %) =  $100 \times (Pt - P0) / \text{protein intake (g)}$

Where:

P0: protein content in fish body at the start (g).

Pt: protein content in fish body at the end (g).

Energy gain (Kcal) =  $Et - E0$

Where:

E0: energy content in fish body (Kcal) at the start.

Et: energy content in fish body (Kcal) at the end.

Energy utilization (EU %) =  $100 \times (Et - E0) / \text{Energy intake (Kcal)}$

## 5.2 Fish whole body chemical composition (%):

Proximate chemical analyses (%; protein, ether extract, ash and dry matter) of fish whole body (before and after the experiment) were performed according to the official methods of analysis of the AOAC (2000).

## 6. Statistical analysis

Experimental data were statistically analyzed with one-way ANOVA and Duncan (1955) expressed as mean values  $\pm$  SE. Statistical analyses were performed using SPSS for Windows (Standard Version 17 SPSS Inc. Chicago, Illinois)

## RESULTS AND DISCUSSION

The current study was conducted to evaluate the effect of dietary supplementation of selenium, copper and their mixture on growth performance, feed utilization and survival rate of Nile tilapia (*O. niloticus*) fingerlings in fresh water.

### Growth performance:

Growth performance and survival rate of fish fed control diet showed significantly ( $p < 0.05$ ) higher growth performance survival rate (SR) and Condition factor (CF) compared to those fed diets supplemented with different forms of selenium and copper. There were no significant ( $p < 0.05$ ) differences in growth performance on diets containing copper sulphate and copper nanoparticles respectively. The effect of mixture sodium selenite and copper sulphate on all previous mentioned parameter was more pronounced than the effect of those fed diets containing mixture selenium nanoparticles and copper nanoparticles. Furthermore, fish fed the basal diets encompasses Se and Cu at levels of  $2 \text{ mg kg}^{-1}$  and  $29 \text{ mg kg}^{-1}$  respectively in the actual concentration had the highest values of growth performance when compared with the other groups. The present study agrees with many authors who cited that the dietary of selenium and copper are an essential element for fish normal growth and development. (Mohseni *et al.*, 2014; Elia *et al.*, 2011; Lin, 2014). Similar results have been reported for increasing growth performance and afterwards gradual decline suggested, a dietary inclusion up to  $2.06 \text{ mg Se/kg}$  diet could be beneficial in this species (Lee *et al.*, 2016) Also observations are in agreement with those of previous reports suggesting a beneficial effects of dietary Se inclusion in grouper (Lin and Shiau, 2005), cobia (*Rachy-centroncanadum*) (Liu *et al.*, 2010) and gibel carp (*Carassius auratus gibelio*), (Han *et al.*, 2011).

Survival rate didn't exhibit any significant ( $P > 0.05$ ) differences among all studied treatments and ranged from 92% to 97%, without any obvious dose related drastic effect of all treatments. This work is similar with Lee *et al.* (2016) who found that the dietary Se requirement level for the maximum growth of Nile tilapia could be  $>1$  but  $< 2 \text{ mg Se-Met/kg}$  using organic Se-methionine as the source of Se. Whereas, a dietary Se level of  $6\text{--}15 \text{ mg Se-Met/kg}$  could have toxicity effects characterized by reduced growth performance, survival rate and nonspecific immune responses, in juvenile Nile tilapia, *O. niloticus*. On the other

hand, the present study disagrees with the results showed by Ashouri *et al.* (2015) who demonstrated that fish were fed diets supplemented with 1 mg nano-Se/kg improved the final weight (FW) and weight gain (WG) of fish significantly. Thus, the difference in Se requirement could also be related to species and age of fish, chemical forms of Se and dietary factors (Hao *et al.*, 2014). Regarding to feeding with copper of the present study these results are in agreement with Tang *et al.* (2017) who reported that fish were fed diets supplemented with (17-65mg/kg<sup>-1</sup>) of dietary Cu improved the growth performance, SR, WG and CF of hybrid tilapia ( $p < 0.05$ ). Similarly, Wang *et al.* (2015) reported that grouper, *Epinephelus coioides* fed diets supplemented with CuSO<sub>4</sub> or Cu-NPs at 20 mg or 100 mg Cu L<sup>-1</sup> rearing water showed lower WG and SGR than the fish fed the basal diet. Also, Muralisankar *et al.* (2015) reported that *M. rosenbergii* fed diets supplemented with Cu-NPs at 20 mg/kg of dietary improved the growth rate and better survival. On the other hand, our results disagree with El-Basuini *et al.* (2016) who demonstrated that fish fed diets supplemented with Cu-NPs at 2 mg kg<sup>-1</sup> had the highest values of final body weight, weight gain and specific growth rate of red sea bream (*Pagrus major*).

**Table (2). Effect of dietary exposure of nanoparticles and bulk of selenium and copper alone or in combination on final weight (FBW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), survival rate (%) and Condition factor (CF) (%) of Nile tilapia, *O. niloticus*, fingerlings ( $\bar{X} \pm SE$ )**

Diet NO	IW (g/fish)	FW (g/fish)	WG (g /fish)	ADG (g/fish/day)	SGR (%/day)	SR (%)	CF (%)
D1	3.09 <sup>c</sup> ±0.01	49.55 <sup>a</sup> ±1.54	46.46 <sup>a</sup> ±1.53	0.44 <sup>a</sup> ±0.01	2.64 <sup>a</sup> ±0.03	92.23 <sup>a</sup> ±3.99	2.257a±0.13
D2	3.10 <sup>abc</sup> ±0.0	37.79 <sup>c</sup> ±1.55	34.69 <sup>c</sup> ±1.55	0.33 <sup>c</sup> ±0.01	2.38 <sup>c</sup> ±0.04	96.67 <sup>a</sup> ±1.93	1.723c±0.02
D3	3.13 <sup>a</sup> ±0.00	44.27 <sup>b</sup> ±1.62	41.14 <sup>b</sup> ±1.63	0.39 <sup>b</sup> ±0.02	2.52 <sup>b</sup> ±0.04	95.57 <sup>a</sup> ±1.13	1.967b±0.01
D4	3.10 <sup>abc</sup> ±0.0	41.81 <sup>bc</sup> ±2.49	38.71 <sup>bc</sup> ±2.49	0.37 <sup>bc</sup> ±0.02	2.47 <sup>bc</sup> ±0.06	95.57 <sup>a</sup> ±1.13	1.710c±0.04
D5	3.09 <sup>bc</sup> ±0.01	43.10 <sup>bc</sup> ±1.69	40.01 <sup>bc</sup> ±1.69	0.38 <sup>bc</sup> ±0.0	2.51 <sup>b</sup> ±0.04	94.43 <sup>a</sup> ±1.13	1.847bc±0.02
D6	3.10 <sup>abc</sup> ±0.01	40.07 <sup>bc</sup> ±1.64	36.97 <sup>bc</sup> ±1.63	0.35 <sup>bc</sup> ±0.02	2.44 <sup>bc</sup> ±0.03	95.57 <sup>a</sup> ±2.94	1.727c±0.03
D7	3.12 <sup>ab</sup> ±0.01	43.57 <sup>b</sup> ±1.21	40.45 <sup>b</sup> ±1.22	0.39 <sup>b</sup> ±0.01	2.51 <sup>b</sup> ±0.03	93.33 <sup>a</sup> ±1.93	1.963b±0.03

Values superscripted by different alphabets within the same column are significantly different ( $P < 0.05$ ).

D1= basal diets; D2 = selenium nanoparticles; D3 = sodium selenite; D4 = copper nanoparticles; D5 = copper sulphate; D6 = mixture selenium nanoparticles and copper nanoparticles; D7 = mixture sodium selenite and copper sulphate.

### Feed and nutrients utilization:

The obtained results, given in Table (4) clearly show that fed diet supplemented with different forms of selenium and copper exhibited a significant ( $P < 0.05$ ) effect on all estimated feed and nutrients utilization traits. Feed intake significantly ( $P < 0.05$ ) increased with control compared to the other treatments however, the bulk of selenium and copper alone or in combination showed a satisfactory results near to the control feed conversion ratio (FCR) improved with control compared to the other treatments however, the bulk of selenium and copper alone or in combination showed a satisfactory results as well. From the nutrients utilization point of view, protein efficiency ratio (PER) and protein productive value (PPV) were increased significantly ( $P < 0.05$ ) with control and bulk of selenium and copper alone or in combination and insignificantly ( $P > 0.05$ ) with other treatments. The same trend was observed

with energy gain, meanwhile, energy utilization increased significantly ( $P < 0.05$ ) only with copper sulphate. The percent of energy gain increase more than control were 20 and 19% with sodium selenite and copper sulphate (bulk) respectively. According to the current study it was revealed that selenium and copper are essential micronutrients for aquatic animals. This is in agreement with some other reports (Lin 2014 and Bell *et al.*, 1985). Regarding the feed utilization, the present study revealed a highest selenium level (2 mg/kg), comparing to the other studies like results reported for Nile tilapia 4.6 mg Se/kg; (Ahmad *et al.*, 2006) and African catfish 3.67 mg Se/kg (Abdel-Tawwab *et al.*, 2007), and higher than that reported for rainbow trout 0.38 mg Se/kg, (Hilton *et al.*, 1980), channel catfish 0.25 mg Se/kg, (Gatlin and Wilson, 1984), and grouper 0.77 mg Se/kg (Lin and Shiau, 2005). The differences among the previous results may be due to the differences in Se sources, forms, concentration, size and aquatic animal species. Selenium supplementation played a role in enhancing feed intake with a subsequent enhancement of the fish body composition. On the other hand, Our results disagree with Ashouri *et al.* (2015) who reported that fish were fed diets supplemented with 1 mg/kg<sup>-1</sup> selenium nanoparticles no significant differences ( $P > 0.05$ ) in feed conversion ratio (FCR) of common carp. Regarding to feeding with copper these results in the same line with Tang *et al.* (2017) who reported that fish were fed diets supplemented with (17-65 mg/kg<sup>-1</sup>) of dietary Cu improved the feed conversion ratio (FCR) of hybrid tilapia ( $p < 0.05$ ).

**Table (3). Effect of dietary exposure of nanoparticles and bulk of selenium and copper alone or in combination (mg/kg diet) on feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), energy gain (EG) and energy utilization (EU) of Nile tilapia, *O. niloticus*, fingerlings ( $\bar{X} \pm SE$ )**

Diet NO	FI(g/fish)	FCR (g)	Protein utilization		EG (Kcal)	EU(%)
			PER	PPV (%)		
D1	62.21 <sup>a</sup> ± 1.68	1.34 <sup>c</sup> ± 0.01	2 <sup>a</sup> ± 0.01	27.39 <sup>c</sup> ± 0.24	76.72 <sup>a</sup> ± 2.55	26.35 <sup>bc</sup> ± 0.16
D2	54.54 <sup>bc</sup> ± 1.91	1.57 <sup>a</sup> ± 0.02	1.70 <sup>c</sup> ± 0.02	28.42 <sup>c</sup> ± 0.52	63.05 <sup>b</sup> ± 2.98	24.69 <sup>c</sup> ± 0.33
D3	58.98 <sup>ab</sup> ± 0.76	1.44 <sup>b</sup> ± 0.05	1.87 <sup>b</sup> ± 0.06	32.03 <sup>a</sup> ± 0.96	78.76 <sup>a</sup> ± 1.99	28.55 <sup>a</sup> ± 0.77
D4	57 <sup>bc</sup> ± 2.19	1.48 <sup>b</sup> ± 0.04	1.82 <sup>bc</sup> ± 0.05	29.58 <sup>bc</sup> ± 1.04	72.74 <sup>ab</sup> ± 5.15	27.21 <sup>ab</sup> ± 0.89
D5	57.90 <sup>abc</sup> ± 1.38	1.45 <sup>b</sup> ± 0.03	1.85 <sup>b</sup> ± 0.04	31.18 <sup>b</sup> ± 0.62	78.47 <sup>a</sup> ± 2.84	28.95 <sup>a</sup> ± 0.43
D6	53.71 <sup>c</sup> ± 0.87	1.46 <sup>b</sup> ± 0.04	1.84 <sup>b</sup> ± 0.05	29.14 <sup>bc</sup> ± 0.74	71.76 <sup>ab</sup> ± 3.05	28.54 <sup>a</sup> ± 0.77
D7	58.89 <sup>ab</sup> ± 0.93	1.47 <sup>b</sup> ± 0.02	1.84 <sup>b</sup> ± 0.03	33.43 <sup>a</sup> ± 0.52	75.89 <sup>a</sup> ± 2.24	27.53 <sup>ab</sup> ± 0.38

Values superscripted by different alphabets within the same column are significantly different ( $P < 0.05$ ).

D1 = basal diets; D2 = selenium nanoparticles; D3 = sodium selenite; D4 = copper nanoparticles; D5 = copper sulphate; D6 = mixture selenium nanoparticles and copper nanoparticles; D7 = mixture sodium selenite and copper sulphate.

### Fish whole body chemical composition:

The results in Table (5) showed the effect of fed diet supplemented with different forms of selenium and copper treatments on final body composition and gross energy expressed as percent on dry weight of Nile tilapia, *O. niloticus*, and fingerlings at the end of the experiment. The obtained results showed that dry matter content and ash of treated fish there were no significant ( $P > 0.05$ ) difference. On the other side, ether extract and gross energy decreased numerically with all fed diet supplemented with different forms of

selenium and copper in high dose and the lowest values recorded with bulk of selenium and copper alone or in combination as well the control .

The present findings declared that crude protein showed significant ( $P < 0.05$ ) differences due to supplementation treatments. The highest protein contents gained by fish treated with control following to it bulk of selenium and copper alone or in combination and the lowest value showed by fish fed diet supplemented with the rest of treatments . Our results disagree with Ashouri *et al.* (2015) who reported that fish were fed diets supplemented with  $1 \text{ mg/kg}^{-1}$  selenium nanoparticules no significant differences ( $P > 0.05$ ) in proximate composition of common carp. Also observations are in agreement with results obtained by Zhou *et al.* (2009) for crucian carp and Le and Fotedar (2014) for juvenile yellowtail kingfish. Regarding to feeding with copper these results are in agreement with Muralisankar *et al.* (2015) The significant ( $P < 0.05$ ) increments recorded in biochemical compositions such as total nitrogen, crude protein, total protein, amino acid, carbohydrate, lipid, and ash suggests that dietary inclusion of  $20 \text{ mg Cu-NPs kg}^{-1}$  have promoted the absorption of nutrients and it led to increase the synthesis of muscle biochemical composition of *M. rosenbergii*. The optimal supplementation of Cu showed elevated level of biochemical compositions such as protein, amino acids, carbohydrate, lipid, and ash in *M. rosenbergii* and *H. discushannai* has been reported (Wang *et al.*, 2009 ; Muralisankar *et al.*, 2015. Conversely, our results disagree with El-Basuini *et al.* (2016) who demonstrated that whole body protein of fish fed diet Cu-free diet was significantly lower than those of fish fed other diets on red sea bream (*Pagrus major*).

**Table (4). Effect of dietary exposure of nanoparticles and bulk of selenium and copper alone or in combination (mg/kg diet) on whole body chemical composition of Nile tilapia, *O. niloticus*, fingerlings ( $\bar{X} \pm \text{SE}$ )**

Diet NO	On dry matter bases %				
	Dry matter(%)	Crude protein (%)	Ether extract (%)	Ash (%)	Gross energy (kcal/100g) <sup>1</sup>
D1	30.97a±0.00	57.54a±0.17	28.04f±0.09	14.42a±0.22	589.19e±1.52
D2	30.34ab±0.09	51.46g±0.06	34.92a±0.18	13.62b±0.20	619.88a±1.82
D3	29.53b±0.13	55.53b±0.22	30.06e±0.18	14.41a±0.05	596.94d±0.48
D4	30.47ab±0.42	53.03e±0.03	33.12c±0.14	13.59b±0.08	612.04b±1.12
D5	30.53ab±0.36	53.84d±0.06	32.82c±0.12	13.34b±0.06	613.51b±0.77
D6	26.13c±0.02	52.29f±0.15	34.45b±0.13	13.26b±0.05	620.11a±0.49
D7	30.93a±0.78	54.58c±0.13	31.22d±0.13	14.20a±0.01	602.58c±0.47

<sup>1</sup>Gross energy, calculated on the basis of 5.64 and 9.44 Kcal GE/g protein and ether extract respectively (NRC, 1993).

Values superscripted by different alphabets within the same column are significantly different ( $P < 0.05$ ).

D1=basal diets; D2 = selenium nanoparticles; D3 = sodium selenite; D4 = copper nanoparticles; D5 = copper sulphate; D6 = mixture selenium nanoparticles and copper nanoparticles; D7 = mixture sodium selenite and copper sulphate.

## CONCLUSION

Effect of dietary supplementation of nanoparticles and bulk of selenium and copper alone or in combination on growth performance, feed utilization and survival rate of Nile tilapia (*O. niloticus*) fingerlings in fresh water was studied. This research demonstrated that the basal diet improves growth performance, feed utilization and survival rate of Nile tilapia fingerlings.



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## الملخص العربي

# تأثير اضافة جزيئات السيلينيوم والنحاس النانوية بالعليقة على أداء النمو والاستفادة من الغذاء في أصبغيات البلطي النيلي

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الدراسة الحالية تهدف الى بيان تأثير اضافة جزيئات السيلينيوم والنحاس النانوية فى العلائق على معدلات النمو و نسبة الاعاشة مدى الاستفادة من الغذاء ليرقات البلطي النيلي.

تم توزيع ٤٢٠ اصبغية بلطى نيلي متوسط وزنها الأبتدائى ٣جم/ سمكه عشوائيا على ٧معاملات (٢ تانك/ معاملة) (٣٠ سمكه/ تانك) فى ١٤ تانك مصنع من الالياف الزجاجية سعته ١م<sup>٣</sup> ومزوده بمصدر للتهويه والمياه الجوفيه. تم ضبط درجه حرارة المياه طول فتره التجريه على ٢٤ درجه مئوية واستمرت التجريه ١٥ أسبوع . وغذيه الأسماك على ٧ علائق فى سبعة معاملات: المعاملة الأولى تغذت على العليقه القياسيه ( ٣٢% بروتين)؛ المعاملة الثانيه العليقه القياسيه مضاف إليها ١مجم سيلينيوم / كجم علف، المعاملة الثالثه العليقه القياسيه مضاف إليها ٠.٣مجم سيلينات الصوديوم /كجم علف، المجموعه الرابعه المعاملة القياسيه مضاف إليها ٢ مجم نحاس / كجم علف ، المعاملة الخامسه العليقه القياسيه مضاف إليها ٣ مجم كبريتات النحاس / كجم علف ، المعاملة السادسه العليقه القياسيه مضاف إليها ١ مجم جزيئات النانو من السيلينيوم و ٢مجم جزيئات نانو نحاس/ كجم علف ، المعاملة السابعه العليقه القياسيه مضاف إليها ٠.٣ سلينات الصوديوم و ٣مجم كبريتات نحاس / كجم علف . وكان معدل التغذيةه اليومي ٦% من وزن جسم السمكه فى الأسابيع الأربع الأولى ثم ٥% فى الأربع اسابيع التاليه وأخيرا ٤% حتى نهاية التجريه، وكانت التغذيةه مرتين فى اليوم لمدة ٦ أيام فى الاسبوع .وتم قياس كلا من درجة الحراره ومستوى الأوكسجين الذائب و pH يوميا بينما كان يتم قياس وزن وعدد الأسماك فى كل معاملة مره كل اسبوعين. وكانت النتائج كالتالى :

أظهرت العليقه القياسيه أفضل وزن نهائى، وأفضل معدل للأستفاده من الغذاء لاسماك البلطي عن جميع المعاملات المختبره، كما انها كانت المعامله الأفضل فى معدل البقاء على الحياة ومعامل الحاله. كما سجلت العليقه الثانيه المحتويه على جزيئات السيلينيوم النانوية أقل معدلات نمو واقل وزن نهائى، بينما لم يكن هناك فروق معنويه بين باقى المعاملات. لذا توصى الدراسه باستخدام العليقه الكنترول الغير مضاف إليها السيلينيوم والنحاس حيث أدت الى الحصول على أفضل معدلات نمو وإستفاده من الغذاء ونسبة بقاء على الحياة لاصبغيات البلطي النيلي .