Journal of Animal & Plant Sciences, 33(1): 2023, Page: 33-39
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.1.0591

EFFECTS OF DIETARY SELENIUM NANOPARTICLES SUPPLEMENTATION ON GROWTH PERFORMANCE, HEMATOLOGY AND BODY COMPOSITION OF *OREOCHROMIS NILOTICUS* FINGERLINGS

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ABSTRACT

Selenium (Se) is an essential nutrient that plays important role in numerous biological processes and fish feed is the major route of Se supply to fish. A 70-days feeding trial was conducted to evaluate the effects of selenium nanoparticles (Se-NPs) on growth, hematology, and body composition of *Oreochromis niloticus* fingerlings. A total of 270 fingerlings were assigned into 6 treatment groups. Se-NPs (%) were added to formulate six test diets *viz.* D-0 (control), D-0.1, D-0.2, D-0.3, D-0.4 and D-0.5, respectively and fed to fingerlings under completely randomized design (CRD). Growth parameters such as weight gain % and FCR started to improve ($p \leq 0.05$) when fingerlings were fed on test diet D-0.1 and reached to maximum (229.74%, 1.29) when fish fed on diet D-0.3 as compared to fish fed on control diet (D-0). The values of hematological parameters such as red blood cells ($2.90 \times 10^6 mm^{-3}$) and white blood cells ($7.89 \times 10^3 mm^{-3}$) were found to be the maximum ($p \leq 0.05$) at D-0.3, while hemoglobin (7.95 and 8.47) was found to be improved significantly at D-0.2 and D-0.3, respectively. Similarly, parameters of body composition i.e. crude protein (20.86%) was improved significantly at D-0.3 while crude fat showed non-significant improvement at D-0.2 and D-0.3 (3.10% and 2.17%) respectively as compared to the control diet. Above D-0.3 level, a significant increase was observed. Fat and moisture contents were found to be maximum at control and D-0.5. So, the results suggested that Se-NPs have the potential to improve the fish body composition and are recommended to be used in fish feed at an optimized level.

Keywords: Selenium, nanoparticles, Nile tilapia, hematology, aquaculture
Published first online September 20, 2022 Published final February 22, 2023

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) has been recognized as the economically important fish species in the aquaculture industry (Huang *et al.,* 2012). It is known as the second most farmed fish species throughout the world (Guyon *et al.,* 2012). The culture of this species has expanded in recent decades because it is easy to grow. It is consumed as a traditional food in Africa and Asia (Wang and Lu, 2016) and is best suitable in aquaculture because it can tolerate harsh environmental conditions (Shoko *et al.,* 2016). To improve the production of tilapia, suitable feed production is required for both intensive and extensive feeding in tanks and ponds, respectively (Köprücü and Özdemir, 2005).

The commonly used protein sources for tilapia include poultry by-products (Gaber, 1996), sunflower cake, wheat bran, fish meal (Maina *et al.,* 2002), cottonseed meal as well as sunflower meal (El-Saidy and Gaber, 2003). Because of the presence of 45-48% crude protein, sunflower meal is known as an important protein source (Mushtaq *et al.,* 2006) and is widely used in animal feed as it consists of many proteolytic enzymes which act as protein digesting machinery (Kocher *et al.,* 2000).

Selenium (Se) is one of the important trace elements and is considered an essential nutrient that is inevitable for the better health of aquatic animals (Ashouri *et al.,* 2015). The main pathway of Se supply to fish is via diet (Fotedar and Munilkumar 2016). Administration of Se through diet was observed to improve the growth performance of different fish species (Jaramillo and Gatlin, 2004; Zhou *et al.,* 2009). Selenium in a variety of forms have been used in the diet of various fish species including hybrid striped bass (*Morone chrysops* × *Morone saxatilis)* (Cotter *et al.,* 2008), Common carp (*Cyprinus carpio)* (Jovanovic *et al.,* 1997) and *Barbus barbus* (Kouba *et al.,* 2014). Previous feed experiments concluded that Se in its organic form was easily digestible and showed higher biological activity comparable to its inorganic forms (Zhou *et al.,* 2009). However, Se-NPs have lately been regarded as a new form of Se (Saffari *et al.,* 2017) because the materials at nanometer scale show unique properties (Yang *et al.,* 2014). Scientists have confirmed that various forms of Se from different sources added in basal diet improved the final weight (FW), the activity of antioxidant enzymes,

and muscles concentration of Se for *Carassius auratus gibelio* (Zhou *et al.,* 2009; Chris *et al.,* 2018).

Recently, Se-NPs have drawn the attention of researchers, to overcome the problems associated with Se deficiency. It is helpful to boost up the dietary availability as well as the retention of Se for improved health and growth of fish (Prabhu *et al.,* 2020). The use of Se-NPs have been preferred to be used in fish feed because of their increased bioavailability and decreased toxicity rate (Saffari *et al.,* 2017). But it is important to focus on the optimized utilization of Se-NPs as the excessive accumulation of Se might be harmful to the organisms (Hamilton, 2004). Therefore, the current study was aimed to investigate the effect of different doses of Se-NPs supplementation on growth, hematology, and body composition of *O. niloticus* fingerlings.

MATERIALS AND METHODS

The experimentation was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad, Pakistan.

Experimental Animal and Feeding Trial: *O. niloticus* fingerlings were transported from Government Fish Seed Hatchery, Faisalabad. A feeding trial was conducted in an indoor experimental setup. V-shaped steel tanks with a 70 L water capacity were used. Apparently healthy fingerlings (7.41±0.098 g $fish^{-1}$) were subjected to 0.5% saline solution treatment, for a few minutes, to remove the ectoparasites (Francis-Floyd, 1993) and then acclimatized for two weeks. During the acclimatization period fingerlings were fed basal diet once per day (Allan and Rowland, 1992). To maintain the quality of water, parameters were checked by using specialized apparatus i.e., pH meter, thermometer, and DO meter. Water q ality parameters were maintained at specific levels, such as pH 7-7.5, temperature 26-29 ºC, and dissolved oxygen above 5 mg/L. Oxygen was supplied through the capillary system. The feeding trial lasted for 70 days. There were 6 treatments, each treatment with three replicates and each replicate consisted of 15 fingerlings**.**

Experimental Diets: All the ingredients were purchased from a local feed mill and then finely ground into powder form to be sieved through a 0.3 mm sieve size (Table 1). The proximate chemical composition of all the ingredients was analyzed by using standard techniques (Table 2) (Association of Official Analytical Chemists, 1995). The powdered feed ingredients were mixed in a mixer and fish oil (6%) was added gradually. Water (10-15%) was added to blend the ingredients which ended in fine-textured dough to be processed in a pelleting machine (Lovell, 1989).

Table 1. Ingredient composition (%) of control and test diets.

Ingredients	D-0	D-0.1	D-0.2	D-0.3	D-0.4	D-0.5
Se-NPs	0	0.1	0.2	0.3	0.4	0.5
Sunflower meal	55	55	55	55	55	55
Fish meal	14	14	14	14	14	14
Wheat flour[$]	12	11.9	11.8	11.7	11.6	11.5
Canola meal	9	9	9	9	9	9
Fish oil	6	6	6	6	6	6
Vitamin Premix[*]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral Premix[**]	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0

[$]Nanoparticles were added at the expense of wheat flour

[*]Vitamin Premix: D_3: 3,000,000 IU, Vitamin A: 15,000,000 IU, Vitamin E: 30000 IU, Vitamin B_1: 3000 mg, Vitamin B_6: 4000 mg, Vitamin B_{12}: 40 mg, Vitamin B_2: 7000 mg, Vitamin C: 15,000 mg, Vitamin K_3: 8000 mg, Folic acid: 1500 mg, Calcium pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg.

[**]Mineral premix/kg: Mn (Manganese): 2000 mg, Ca (Calcium): 155 gm, Zn (Zinc): 3000 mg, Cu: (Copper), 600 mg: Co: (Cobalt), 40 mg, I (Iodine): 40 mg, P (Phosphorous): 135 gm, Fe (Iron): 1000 mg, Mg (Magnesium): 55 gm, Se (Selenium): 3 mg, Na (Sodium): 45 gm.

Experimental diets were formulated according to the % level of Se-NPs (0, D-0.1, D-0.2, D-0.3, D-0.4, D-0.5). The stock solution of Se-NPs (SIGMA ALDRICH, Lot# MKBT0973V, Pcode: 1002048682) was prepared and sonicated for 8 hours (Federici *et al.,* 2007) which was then sprayed to the test diets. Spraying the test diets with metal solutions was considered a well-established method (Shaw and Handy, 2006; Ramsden *et al.,* 2009). An equal volume of deionized water was also sprinkled into the control diet, so to keep the moisture content similar. Drying the test diets was done under shady place and then stored at 4°C until further use. Triplicate groups were fed quantitatively one of the assigned diets twice a day at 4% rate of their body weight. The fish were allowed to feed for two hours and then the residual feed was collected from the entire tank and dried to calculate

the feed intake (FI) (Hussain *et al.*, 2018). The tanks were washed and refilled again on daily basis, to remove any leftover feed particles.

Table 2. Chemical composition (%) of feed ingredients.

Ingredients	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Gross Energy (kcal/g)	Carbohydrates
Sunflower meal	93.74	35.41	4.28	2.03	9.63	3.37	45.28
Fish meal	91.67	48.17	7.12	1.12	24.66	2.65	16.28
Canola meal	94.06	12.38	13.46	12.74	10.17	3.18	48.07

Growth Study: To examine the effect of the feeding trial on growth performance, the weight was measured initially at the start and final weight at the end of the trial. The values of growth parameters such as feed conversion ratio (FCR), specific growth rate (SGR), and weight gain percentage were determined by using the standard formulae.

$$\text{Weight gain \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{SGR\%} = \frac{\text{ln. Final weight of fish} - \text{ln. Initial weight of fish}}{\text{Trial day}} \times 100$$

Haematological study: About 300-500 µl of the blood was taken from the caudal vein of the fish by inserting a heparinized syringe and then blood samples were transferred to the vials containing heparin and transported to the Molcare Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan for further analysis of blood indices. To determine the haematocrit/packed cell volume (PCV), the micro-haematocrit technique was used (Brown, 1980). Haemo-cytometer with an approved Neubauer counting chamber was used to count Red Blood Cells (RBCs) and White Blood Cells (WBCs) (Blaxhall and Daisley, 1973). The concentration of Haemoglobin (Hb) was determined as described by Wedemeyer and Yasutake (1977). To calculate mean corpuscular haemoglobin concentration (MCHC); mean corpuscular haemoglobin (MCH) and mean cell volume (MCV) the following equations were used:

$$\text{MCHC} = \text{Hb/PCV} \times 100$$
$$\text{MCV} = \text{PCV/RBC} \times 10$$
$$\text{MCH} = \text{Hb/RBC} \times 10$$

Chemical Analysis: At the end of the feed trial, three fingerlings from all replicates were randomly selected, sacrificed and then dried at room temperature to measure the body composition. The moisture content of the whole body was calculated after oven-drying of homogenized samples at 105°C for 12 hours. Micro Kjeldahl Apparatus (InKjel M Behr Labor Technik GmbH D-40599 Dusseldorf) was used to measure the crude protein (N × 6.25) while the Soxhlet method (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to determine the amount of crude fat using petroleum ether extraction (EE) method. Furthermore, Ash was determined through an electric furnace by combustion at 650°C for 12 h (Naberthern B170) to constant weight.

Statistical analysis: All the study parameters were analyzed through one-way Analysis of variance (ANOVA) (Steel *et al*., 1997). Tukey's HSD test was used to compare the differences between all the treatments and the results were considered significant at $p<0.05$. To perform the statistical analysis, CoStat Computer Software (version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

RESULTS

Growth Performance: The results of growth performance of *O. niloticus* fingerlings fed SFM based diets supplemented with Se-NPs were improved significantly (Table 3). The results showed that weight gain (WG) (9.95g), Weight gain % (134.34%) and feed intake (FI) (0.25g) started increasing at D-0.1 supplemented with 0.1% Se-NPs and found to be maximum (16.98g; 229.74%; 0.31g) at D-0.3 as compared to the control diet. Significant reduction ($p<0.05$) in FCR (1.29) and increase in SGR was observed at D-0.3 supplemented with 0.3% Se-NPs supplemented SFM based diet. Above and below this level, the FCR was found to be increased significantly. Furthermore, the maximum survival rate was observed at D-0.2, but it was statistically same among all the study groups.

Table 3. Growth performance of *O. niloticus* fingerlings fed on different doses of Se-NPs.

Experimental Diets (Se-NPs %)	IW (g)	FW (g)	WG (g)	WG (%)	FI (g)	FCR	SGR	Survival (%)
Control	7.41±0.09	17.35±0.43^{d}	9.95±0.48^{d}	134.34±7.63^{d}	0.25±0.014b^{c}	1.78±0.010^{a}	0.95±0.04^{d}	96.18
D-0.1	7.41±0.10	19.12±0.49^{c}	11.71±0.50^{c}	158.06±7.47^{c}	0.27±0.028^{abc}	1.59±0.123^{ab}	1.05±0.03^{c}	96.40
D-0.2	7.40±0.11	22.50± 0.37^{b}	15.10±0.34^{b}	204.17±5.33^{b}	0.30±0.016^{ab}	1.40±0.091^{bc}	1.24±0.02^{b}	99.39
D-0.3	7.39±0.07	24.38± 0.44^{a}	16.98±0.47^{a}	229.74±7.39^{a}	0.31±0.026^{a}	1.29±0.071^{c}	1.33±0.02^{a}	98.98
D-0.4	7.40±0.17	19.23± 0.54^{c}	11.83±0.67^{c}	159.96±12.19^{d}	0.27±0.010^{abc}	1.62±0.036^{a}	1.06±0.05^{c}	96.65
D-0.5	7.40±0.05	16.98± 0.33^{d}	9.58± 0.28^{d}	129.49±2.96^{d}	0.24±0.005^{c}	1.77±0.034^{a}	0.92±0.01^{d}	96.11

Means within columns having different superscripts are significantly different at $p<0.05$. Data are means of three replicates.
IW= Initial Weight, FW= Final Weight, WG= Weight gain, FI= Feed Intake, FCR= Feed Conversion Ratio, SGR= Specific Growth Rate.

The results suggested that Se-NPs supplementation in SFM based diet at the level of 0.3% had a growth-promoting role. Weight gain and FCR are the two main parameters to evaluate the beneficial effects of feed. The dietary supplementation of Se had previously been reported to improve the growth parameters of *O. niloticus* (Lee *et al.,* 2016). Ashouri *et al*. (2015) reported that the supplementation of Se-NPs in diet enhanced the growth parameters of *C. carpio*. Weight gain of fish showed a significant increase at the levels of ≤1 mgkg^{-1} of diet and no effects were observed above this level. Similarly, Han *et al*. (2011) showed that the optimum Se requirement was 1.18 mgkg^{-1} for *C. gibelio*. Another study suggested that the use of 1mg/kg Se-NPs improved the WG and SGR of *O. niloticus* fingerlings (Hussain *et al.,* 2019). In contradiction, it was found that the above values are quite higher than that examined for loach, fed on 0.48-0.50 mgkg^{-1} of Se-NPs supplemented diet (Hao *et al.,* 2014). Similarly, Lin and Shiau (2005) reported that Se-NPs supplementation for grouper showed increased growth at the level of 0.77 mg Se/kg. In another study, it was found that Se supplemented diet can improve the growth of *C. carassius,* thus dietary Se supplementation is necessary (Wang *et al*., 2007). Very different results were found by Ramsden *et al*. (2009), who concluded that *Oncorhynchus mykiss* fed on Se supplemented diet showed a very steady weight gain. Also, no effect on FCR and SGR was observed throughout the experiment.

Hematological Studies: Results of hematological studies showed that Se-NPs supplementation improved the blood indices as compared to the control diet (Table 4). The maximum values of RBCs (2.90×10^{6}mm^{-3}), WBCs (7.89×10^{3}mm^{-3}), Hb (8.47g/100ml), and PLT (68.35%) were observed at D-0.3, a diet supplemented with 0.3% Se-NPs and these values differed significantly ($p<0.05$) from control as well as other Se-NPs supplemented test diets. PCV (24.91%) and Hb were found to be maximum at D-0.2 and D-0.3, which differed from the control diet. In contrast, the values of MCHC (35.24%) and MCV (fl) (188.36%) increased significantly at D-0.4.

Analysis of the blood parameters can provide important information about the internal health of the organism, so, hematology is an important subject for understanding normal and pathological impacts. The present study indicated that Se-NPs supplementation at the level of 0.3% improved the blood indices of *O. niloticus* fingerlings. Similarly, it was concluded by Hao *et al*. (2014) that Se-NPs supplementation increased all hematological parameters. The blood profile showed improvements for loach (*Paramisgurnus dabryanus*), which increased at first and then lowered, as the Se content in diets increased above 0.50-0.62 except for WBCs. These results were also supported by Behera *et al*. (2014), who found a significant increase in RBCs and Hb when *Labeo rohita* fed Fe-NPs supplemented diets. It was observed that the supplementation of Se-NPs improved WBC and Hb in *Seriola lalandi* (Le and Fotedar, 2014). Another study concluded that fishmeal-based diet supplemented with 1.2 mgkg^{-1} Se meets the Se requirement of *Salmo salar* (Lorentzen *et al.,* 1994). However, unlike our findings, Dawood *et al*. (2019) showed that the Se-NPs supplementation did not affect the blood biochemical parameters.

Proximate Body Composition: Proximate body composition of fish fed Se-NPs supplemented SFM based diet is shown in table 5. Supplementation of Se-NPs played a significant role in improving carcass composition of fish fed sunflower meal-based diets as compared to the control diet. The values of crude protein and ash began to increase from D-0.2 and were found to be maximum ($p<0.05$) at D-0.3 (20.86% and 3.13%) respectively, as compared to the control diet. However, the values for fat (6.75%) and moisture content (75.12%) were noted to be maximum at control and D-0.5 diet. The supplementation of Se-NPs at 0.2 and 0.3% was the most suitable level among all the treatments, as it increased the protein in fish body as compared to control diet.

Table 4. Hematological parameters of *O. niloticus* fingerlings fed on different doses of Se-NPs

Experimental diets (Se-NPs %)	RBCs (10^6mm^{-3})	WBCs (10^3mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
Control	1.83±0.14^{c}	6.69±0.21^{c}	53.13±0.27^{e}	6.37±0.11^{c}	21.20±0.50^{c}	24.68±0.69^{e}	37.64±0.09^{d}	90.26±0.10^{e}
D-0.1	2.21±0.17^{bc}	7.09±0.23^{bc}	58.26±0.40^{d}	6.99±0.37^{bc}	22.38±0.10^{bc}	27.69±0.31^{d}	38.61±0.35^{d}	111.30±0.90^{d}
D-0.2	2.43±0.12^{b}	7.60±0.17^{ab}	61.94±0.38^{c}	7.95±0.38^{a}	24.38±0.35^{a}	31.38±0.18^{c}	41.70±0.44^{c}	181.05±0.48^{c}
D-0.3	2.90±0.20^{a}	7.89±0.18^{a}	68.35±0.35^{a}	8.47±0.08^{a}	24.91±0.44^{a}	33.81±0.22^{b}	49.97±0.13^{b}	187.11±0.16^{a}
D-0.4	2.36±0.21^{b}	7.13±0.23^{bc}	63.53±0.46^{b}	7.71±0.40^{ab}	23.24±1.20^{ab}	35.24±0.46^{a}	52.10±1.78^{b}	188.36±0.39^{a}
D-0.5	1.80±0.14^{c}	6.69±0.15^{c}	58.03±0.95^{d}	6.72±0.46^{c}	22.25±0.50^{bc}	33.82±0.71^{b}	56.84±0.30^{a}	183.01±0.27^{b}

Means within columns having different superscripts are significantly different at $p<0.05$.
RBCs = Red Blood Cells, WBCs = White blood cells, PLT = Platelet, Hb = hemoglobin, Packed cell volume= PCV, MCHC= Mean corpuscular hemoglobin=MCH, Mean cell volume= MCV.
Data are means of three replicates.

Table 5. Body composition (%) of *O. niloticus* fingerlings fed on different doses of Se-NPs.

Experimental diets (Se-NPs %)	Crude Protein	Crude Fat	Ash	Moisture
Control	15.27±0.17^{f}	6.75±0.17^{a}	2.86±0.04^{d}	75.12±0.12^{a}
D-0.1	17.50±0.15^{d}	5.14±0.11^{c}	2.95±0.05^{cd}	74.41±0.07^{b}
D-0.2	19.81±0.14^{b}	3.10±0.08^{e}	3.07±0.04^{ab}	74.02±0.05^{bc}
D-0.3	20.86±0.25^{a}	2.17±0.05^{f}	3.13±0.02^{a}	73.84±0.27^{c}
D-0.4	18.54±0.31^{c}	4.29±0.13^{d}	2.98±0.01^{bc}	74.19±0.20^{bc}
D-0.5	16.18±0.39^{e}	6.01±0.39^{b}	2.91±0.03^{cd}	74.89±0.03^{a}

The proximate body composition was significantly affected in fish fed with Se-NPs supplemented diets. Similarly, a significant increase in body composition parameters such as, CP and CF was observed in *Cirrhinus mrigala* fingerlings fed 1.5 mg/kg Se-NPs supplemented diet (Ahmad *et al*., 2021). Srinivasan *et al*. (2016) have found that supplementation of Fe_2O_3 NPs significantly improved whole body composition parameters as compared to control diets in giant freshwater prawn. Similarly, Ismael *et al.,* (2021) reported a significant increase in crude protein in fish body composition fed with the chitosan nanoparticle.

In contradiction to above finding, no significant difference was reported among the body composition parameters in the *Oncorhynchus mykiss* fed with 0.1, 0.2, and 0.3 mg/kg Se-NPs supplemented diet (Harsij *et al.,* 2020). Similar results were reported by Le and Fotedar (2014), who concluded that no significant effect was observed on the muscle composition of juvenile *S. lalandi* fed with different forms of Se.

Conclusion: The current study provided evidence that supplementation of Se-NPs at the level of 0.3% improved almost all the study parameters as compared to the control and other groups. So, the use of Se-NPs supplementation is recommended to improve the production parameters of *O. niloticus*. But there is always a need to optimize the use of a new chemical substance, as a higher dose level may be harmful.

Conflict of Interest: The authors declare that no conflict of interests exists between them.

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